

10 MEDICINAL PLANTS OF PAKISTAN

A LITERATURE STUDY



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THE FACULTY OF MATHEMATICS AND NATURAL SCIENCES

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THESIS IN PHARMACOGNOSY

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PREFACE

First of all i would like to greatly thank Professor Ph. D. Berit Smestad Paulsen for corrections and all kinds of scientific contribution to complete this work.

I would also like to thank Librarian Bente katrine Rasch for ordering a lot of scientific articles to complete this work. I am also grateful to librarian Kirsten borse Haraldsen librarian at biological library for helping about End-note.

IT consultant Adam babinski gave me a great help, whenever i needed, about computer related problems.

I would like to thank external instructors including Mr. Mohammad Shafiq, Saud Akbar for their efforts to gather traditional information about uses of these plants and other pharmacist friends' district drug inspector Javaid iqbal, Mashood iqbal, Yasir Ayaz and pharmacist and doctor Malik Mohammad Waheed and all other teachers and friends at BZ University Multan for their support.

I am grateful to my wife Alvina and my daughters Waniya and Iqra and my in laws for their support, prays and wishes during this project. I also remember the efforts done for our family by my dear late grandfather. I am greatly thankful to my dear mother and sisters who has always supported me and prayed for my success in this project. I would like to thank my dear uncle and other family members, for their support and wishes to complete this project.

At the end I would like to dedicate this thesis to my late father Mohammad Afzal, he was very kind to all and spent whole of his life to build up the future of his children, may God bless upon his soul and give him higher ranks in paradise.

Mohammad Awais, Oslo, December 2008.

10 SELECTED MEDICINAL PLANTS OF PAKISTAN

1. *Cassia fistula* (L), caesalpinaceae
2. *Trigonella foenum-graecum*(L), leguminosae
3. *Carica papaya* (Linn), caricaceae
4. *Dioscorea floribunda* (M. Martens & Galeotti), dioscoreaceae
5. *Citrullus colocynthis* (L.) Schard , cucurbitaceae
6. *Ferula asafoetida* (H. Karst.), Leguminosae
7. *Caesalpinia crista* (L), caesalpinaceae
8. *Smilax ornata* (Lem.), smilaceae
9. *Styrax benzoin* (Drynad), styraceae
10. *Crocus sativus*(L), iridaceae

INTRODUCTION

In this thesis, I have done research to find any scientific studies, which has been done about these selected 10 medicinal plants of Pakistan, for the Institute of Pharmacy at The University of Oslo. In this thesis I have focused on the chemical, biological and toxicity studies which could give the scientific basis for the traditional usage of these plants.

After getting traditional information about a lot of safe and effective herbal remedies in Pakistan, I and my instructor, at the institute of pharmacy university of Oslo, Berit Smestad Paulsen, chose 10 medicinal plants, which I have tried to find scientific work about.



ISLAMIC REPUBLIC OF PAKISTAN

The areas included in Islamic Republic of Pakistan, has been a part of Asia minor, having hundreds of thousands years old historical background. The Asia minor had been ruled over, by powerful and great emperors in the past.

Pakistan got independence from the British rule on 14th of august 1947 and has been a democratic state since then.

Pakistan is full of natural beauty; greater differences are found in weather depending upon geographical location. There are four weathers in a year. One can easily note the climate variation from warmest areas where temperature raise up to 50 °C to coldest areas where there is snow fall round year.

Capital city= Islamabad

Number of provinces = 4

Largest city= Karachi

Area= 803940 square kilometer.

Population= 172,800,000.

Currency= Rupee

MEDICINAL PLANTS OF PAKISTAN

There are great class differences in Pakistan. Great number of people makes their living through agriculture. All the kinds of treatments are available from smaller to bigger hospitals. However a lot of herbal remedies, known to the herbalist (Hakeem in the local language) and older wise people, mostly in the rural areas, are still being used safely and effectively by both the humans and livestock for various ailments. The traditional medicines are cheaper, especially for those living in the rural areas with lower income. However a lot of people living in the urban areas also believe in the traditional usage of these remedies. A few institutions like Hamdard laboratories has done scientific work about a lot of herbal remedies and is marketing a lot of quality controlled traditional herbal remedies which are both safe and effective.

To get information about 10 most popular medicinal plants, i used the following resources with the help of external instructors:-

1. Hakeem (an herbalist having authorization or knowledge about herbal remedies which has learned at an herbal institution or got it transferred from forefathers.
2. Wise older people.
3. Traditional Literature.
4. Local websites with scientific information about these herbs.

BACKGROUND LITERATURE

Literature search was done in the following authentic scientific databases like Medline, Embase/ Ovid, Biological Abstracts, ISI web of knowledge, International Pharmaceutical Abstracts/ SciFinder. A few articles from the other languages were electronically translated from Japanese, German and Chinese languages to English where it was possible.

Search for the correct plant names was done in www.Ipni.org; a worldwide recognized database for plants.

STRUCTURE OF THESIS

--FAMILY

--LATIN NAME

--ENGLISH NAME

--URDU/ LOCAL NAMES

--OTHER NAMES

--INTRODUCTION

--TRADITIONAL USAGE IN PAKISTAN

--TRADITIONAL USAGE IN THE OTHER COUNTRIES

--CHEMICAL STUDIES

--CHEMICAL STRUCTURES

--BIOLOGICAL STUDIES

--TOXICOLOGICAL STUDIES

--DISCUSSION/ CONCLUSION

--WORDS LIST (Given under the actual paragraphs where necessary)

--REFERENCES

--SYMBOLS AND ABBREVIATIONS (given under the actual articles where necessary)

LITERATURE REFERENCES

Literature references are given in parentheses with writer's name and Year. The complete reference list is given at the end of every chapter. References from internet are given with IP addresses. The photo references are given at the end of literature references.

CHEMICAL STRUCTURES

The chemical structures where necessary are given under the chemical studies done with reference to concerned substances.

WORDS LIST

α = Alfa

β = beta

γ = gamma

M= molar concentration

μ M= micro molar concentration

nM= nano molar concentration

μ g= micro gram

i.p= intraperitoneally

p.o= per oral

i.v= intravenous

ED50= effective dose which gives required effect in the 50 % of population under test.

IC50= Concentration of a substance required to kill 50 % of test organisms.

LD50= lethal dose which causes death in 50% test population.

mg= milli gram

Kg= kilo gram

ml= milli liter

mTOR= Mammalian target of Rapamycin

HER2= Human epidermal growth factor Receptor 2

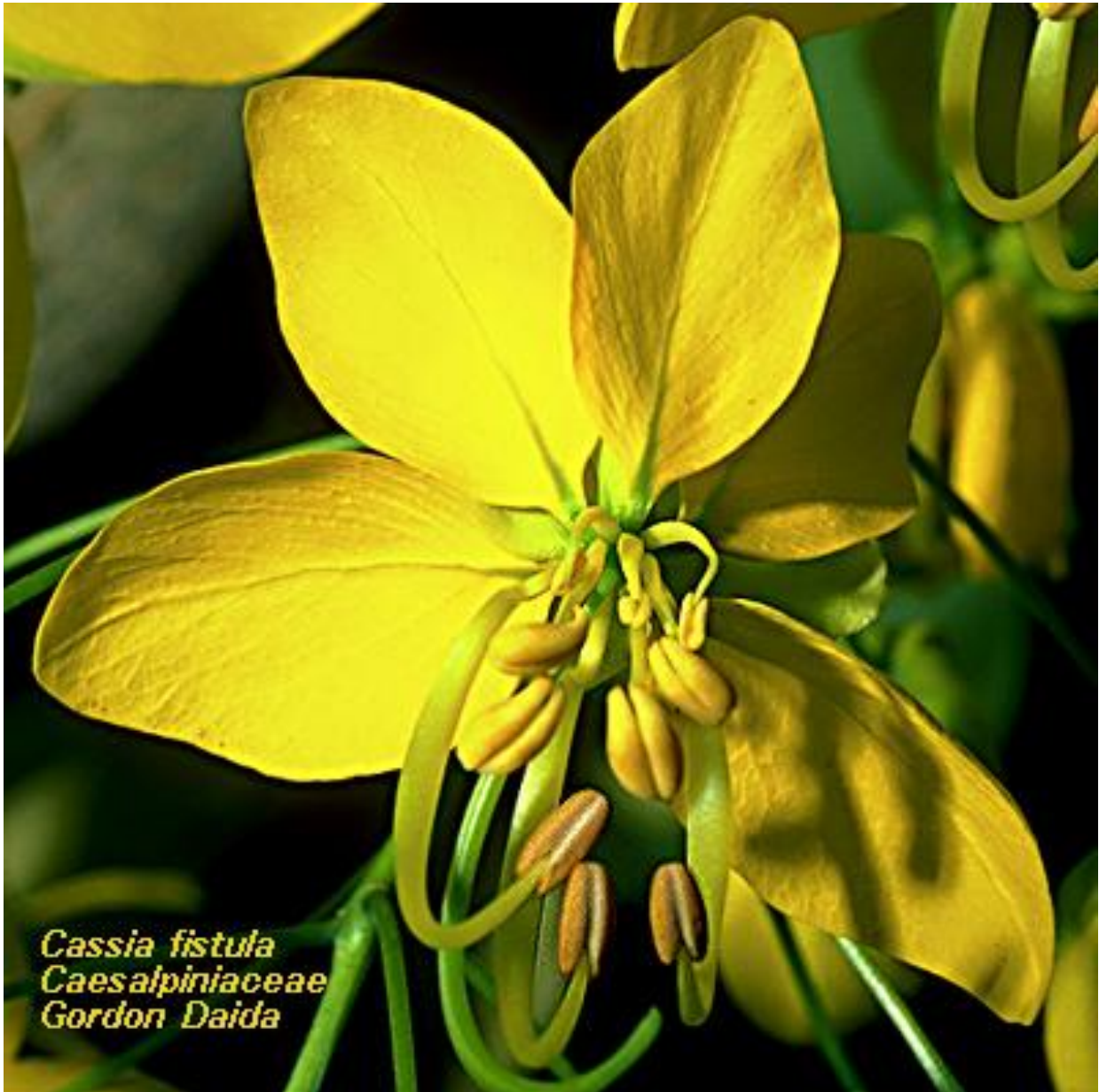
JNK = c-jun N-terminal Kinases; kinases that bind and phosphorylate c-jun[a gene which in combination with c-Fos(a cellular proto-oncogene belonging to the immediate early gene family of transcription factors)forms the activation protein-1(AP-1) early response transcription factor] on ser 63 and ser 73 within its transcriptional activation domain, are mitogen-activated protein kinases which are responsive to stress stimuli, such as cytokines, ultraviolet irradiation, heat shock, and osmotic shock, and are involved in T cell differentiation and apoptosis.

THE WEB REFERENCE TO THE PHOTO OF *Caesalpinia crista* ON FRONT PAGE

<http://homepage3.nifty.com/inagiyasou/photo/iriomote06/jmk2/nantenkazra.jpg>

Retrieved in december 2008.

Cassia fistula



Cassia fistula
Caesalpinaceae
Gordon Daida

BOTANICAL NAME: - *Cassia fistula* (L)

ENGLISH NAME: - Golden Shower

URDU/ LOCAL NAME: - Amaltas

FAMILY: - Leguminosae

PARTS OF PLANT USED:-

Dried leaves and fruit pulp

ETHNOPHARMACOLOGY

USES IN PAKISTAN

1. CATHARTIC
2. ANTI-RHEUMATIC

USES IN INDIA

In the Indian literature *Cassia fistula* has been described to be of the following uses as reported by (Alam et al., 1990; Asolkaret al., 1992):-

1. USEFUL AGAINST SKIN DISEASES like leucoderma and pruritis.
2. LIVER TROUBLES, TUBERCULOSUS GLANDS
3. TREATMENT OF WOUNDS
4. DIABETES

OTHER USES

1. Mild laxative for children and women, recognized by The British pharmacopoeia (Bahorun, Neergheen et al. 2005).
2. Purgative owing to containing aloin and as tonic (Satyavati and Sharma, 1989).
3. Intestinal disorders like ulcer (Biswas et al., 1973; Kirtikar and Basu, 1975).
4. Antipyretic and Analgesic (Patel et al., 1965).
5. Anti-inflammatory and hypoglycemic activity (Bahorun, Neergheen et al. 2005).
6. Inhibits Leukotriene synthesis thus contributing to anti-inflammatory effect (Sunil Kumar and Müller, 1998).
7. Antitussive and wound healing (Bhakta et al., 1998a, b).
8. Against Hypercholesterolemia due to fiber and mucilage content in *Cassia fistula* as suggested by (El-Saadany et al., 1991), also due to presence of β -sitosterol.
9. Other uses include use in diseases and pest control in India (Jaipal et al., 1983; Sharma and Basandrai, 1999; Raja et al., 2000).
10. Callus cultures(derived from young leaves) of *Cassia fistula* could be used to produce a lot of valuable antioxidative and chemopreventive compounds like Flavonoids and Anthraquinone (Bahorun, Neergheen et al. 2005).

CHEMISTRY

The principle ingredients which are therapeutically important are

- 1- Glycosides (Sennoside A, Sennoside B), leaves are rich sources as reported by (Bahorun, Neergheen et al. 2005).
- 2- Phenolic antioxidants like Anthraquinone (Bahorun, Neergheen et al. 2005).
Major Anthraquinone was rhein (1, 8-dihydroxy-3-anthraquinone carboxylic acid) present in pulp as reported (by Modi and Khorana, 1952). Pods are rich in phenolic contents as reported by (Luximon-Ramma et al., 2002).
- 3- Flavonoids(Bahorun, Neergheen et al. 2005).
- 4- Flavan-3-ol derivatives(Bahorun, Neergheen et al. 2005).

Other ingredients having therapeutic importance include:-

- 5- Wax aloin (Satyavati and Sharma, 1989).
- 6- Minerals like heavy metals are also present depending upon the mineral content in the soil (Biswas 2006). It may be exploited both industrially to explore heavy metal content in a geographic area also to find environmental pollution and its potential effects on human health and biodiversity.
- 7- It has been reported that the stem bark of *Cassia fistula* is also a potential source of lupeol, β -sitosterol and hexacosanol (Sen and Shukia, 1968).
- 8- The seeds are rich in glycerides with linoleic, oleic, stearic and palmitic acids as major fatty acids together with traces of caprylic and myristic acids, (Abu Sayeed et al., 1999).

BIOLOGY/ PHARMACOLOGY

1. ANTIOXIDANT ACTIVITY

Cassia fistula contains a lot of phenols i.e. Anthraquinone and flavonoids with proanthocyanidine; that is why it exhibits greater antioxidant activity which is responsible for a lot of pharmacological activities which have therapeutic exploitation. Antioxidant activity of the reproductive parts like pods is higher than non vegetative parts (Luximon-Ramma, Bahorun et al. 2002).

2. ANTICARCINOGENIC ACTIVITY

Mechanisms of anti-carcinogenic action include their binding to carcinogens, their ability to inhibit phase I and induce phase II carcinogen metabolizing enzymes and their potential to modulate signal transduction pathways (Bahorun, Neergheen et al. 2005).

They may prevent tumor development by inducing tumor cell apoptosis by inhibiting DNA topoisomerase II and p53 down regulation or by causing mitochondrial toxicity, which initiates mitochondrial apoptosis (Galati et al., 2000; Birt et al., 2001; Ren et al., 2003; Galati and O'Brian, 2004).

Anti-tumor activity of *Cassia fistula* seed extract based on cytological studies reveal that a reduction in the mitotic activity can be the leading mechanism of action against tumor genesis. Indeed the appearance of membrane blebbing and intracytoplasmic vacuoles in the treated tumor cells suggest that these pathways may account for the reduction in tumor volume (Gupta et al., 2000).

3. MODULATION OF HUMORAL IMMUNITY

Synergistic effect of *Cassia fistula* in combination with antibiotic Amoxicillin was studied by (Ali Nafisa, Kazmi Shahana et al. 2008), it was found that a combination of *Cassia fistula* and amoxicillin i.e. amoxy-cassia exhibits stronger reinforcement of humoral immunity than amoxicillin alone, but the exact mechanism needs to be elucidated by further research.

This study was done on *humoral immune system of BALB/c mice*. Animals were immunized with sheep RBC and treated with *Cassia fistula* fruit, amoxy-cassia, amoxicillin and saline. Number of activated anti-SRBC producing cell in spleen was calculated by haemolytic plaque assay. Antibody titer in blood was measured by haemagglutination test. Number of plaques formed by the animal treated with Amoxy-cassia, amoxicillin, *Cassia fistula*, and normal saline were 191, 86, 53, 34 per 10(5) spleen cells respectively. Haemagglutinating Antibody (HA) titer was evaluated on post-immunized day 4, 6, 8, 10. Rising antibody titer was observed in all animals but Amoxy-cassia treated mice serum had the highest HA titer throughout the experiment suggesting its therapeutic usefulness.

4. LOWERING OF CHOLESTEROL CRYSTALS GROWTH

(Ammal, George et al. 2007) studied the effect of *cassia fistula* on inhibition of cholesterol crystals growth in an in vitro study.

The most dangerous type of cholesterol crystals present in various pathologies including gall stone and atherosclerotic patches is monohydrated type having plate like morphology.

Monohydrated cholesterol crystals could easily be identified by examining the habits of its deposits (Straffer and Bischoff, 1964).

(Ammal, George et al. 2007) studied the growth of cholesterol crystals in sodium metasilicate (SMS) gel medium. Experiments conducted using the extract of *Cassia fistula* as an additive clearly showed an inhibition on the crystal growth. The crystals formed in the control have plate like morphology. The addition of the *Cassia fistula* extract showed not only a delay in nucleation but also a change in morphology. The crystal growth was seen in control tubes within an hour of pouring the supernatant solution.

The grown crystals were clear, transparent and plate like and grown to an average size of 1.6 to 1.8 cm within 2 days. But with the addition of the additive (*Cassia fistula*) in serial dilution, the appearance of the crystals changed to needle like, and then to wool like, in the case of maximum addition of *Cassia fistula* in the observed experiments. Length of growth of the crystals is also found to decrease with the increase in concentration of the *Cassia fistula* solution. The change in morphology is a clear indication of the inhibition of cholesterol crystal growth which may be attributed to the effect of some phytoactive compound in the *Cassia fistula*. Presence of *Cassia fistula* clearly indicates control of cholesterol growth, as concentration of *Cassia fistula* increases percentage growth of cholesterol reduces – also change in morphology, crystals are soft and the reduction in growth of cholesterol is not to zero so that no damage to body, no harmful effect, but very effective in controlling the growth.

5. ANTIBACTERIAL EFFECT

Cassia fistula extract has shown antibacterial activity against a wide spectrum of bacteria namely *Escherichia Coli*, *Bacillus mycides*, *Bacillus subtilis*, *Mycobacterium smegmatis*, *Klebsiella aerogenes*, *Pseudomonas aerogenes* and *Proteus vulgaris* (Perumal et al., 1998).

6. ANT-FUNGAL ACTIVITY

(Duraipandiyan and Ignacimuthu 2007) demonstrated that *Cassia fistula* extract have both antibacterial and antifungal activity.

Antibacterial and antifungal activity of *Cassia fistula* extract was demonstrated by (Duraipandiyan and Ignacimuthu 2007); hexane, chloroform, ethyl acetate, methanol and water extracts from the flower of *Cassia fistula* (an ethnomedicinal plant) were tested against bacteria and fungi. All the extracts exhibited antibacterial activity against Gram-positive organisms with minimum inhibitory concentrations (MIC) between 0.078 and 2.5 mg/ml. Among the Gram-negative bacteria, only *Pseudomonas aeruginosa* was susceptible to the extracts. Ethyl acetate crude extract was fractionated using chromatographic techniques. A crystal was isolated, which was confirmed as 4-hydroxy benzoic acid hydrate using X-ray crystallography. It exhibited antifungal activity against *Trichophyton mentagrophytes* (MIC 0.5 mg/ml) and *Epidermophyton floccosum* (MIC 0.5 mg/ml). These facts shows that the ingredients present in *Cassia fistula* extract has anti-fungal activity.

7. LARVICIDAL AND OVICIDAL ACTIVITY

(Govindarajan, Jebanesan et al. 2008) studied ovicidal and larvicidal activity of methanolic extract of *Cassia fistula* leaves, they found that extract was more lethal to the larvae of *Anopheles stephensi* than *Culex quinquefasciatus* with LC 50 values of 17.97 and 20.57 mg/ l, respectively. Mean percent hatchability of the ovicidal activity was observed 120 h after treatment. Mean percent hatchability was inversely proportional to the concentration of extract and directly proportional to the eggs.

The egg raft of *C. quinquefasciatus* was found to be more hatchable than *A. stephensi*. The results show that the leaf extract of *Cassia fistula* is promising as a larvicidal and ovicidal agent against *C. quinquefasciatus* and *A. stephensi*.

8. ANTI-LESHMANIAL ACTIVITY

Natural products represent a rich source of new chemical entities for the development of drugs for neglected diseases. Leishmaniasis still afflicts the poorest populations in the world, with 12 million cases worldwide. (Sartorelli, Andrade Samanta et al. 2007) analyzed the crude extracts and fractions from the

fruit of *Cassia fistula* against the most dramatic and fatal disease form of Leishmaniasis, the visceral form (VL).

Hexane extract from the fruits showed significant antileishmanial activity against the promastigote form of *Leishmania L. chagasi*. The bioguided fractionation resulted in the isolation of a sterol, clerosterol, which was further analyzed in different models. Promastigotes presented an inhibitory concentration (IC₅₀) of 10.03 µg/mL and intracellular amastigotes demonstrated higher susceptibility, with an (IC₅₀) of 18.10 µg/mL. Mammalian cytotoxicity was evaluated and it was demonstrated that **clerosterol was 3.6-fold less toxic than the standard drug Pentamidine.**

µg= micro gram

9. HEPATIC EFFECTS

Scientific evidence for the usage of *Cassia fistula* against hepatic disorders is being provided by the following study done by (Pradeep, Mohan et al. 2007), the hepatoprotective and antioxidant effect of *Cassia fistula* leaf extract on liver injury induced by diethylnitrosamine (DEN) was investigated. Wistar rats weighing 200 +- 10 g were administered a single dose of DEN (200 mg/kg b.w., i.p.) and left for 30 days. For hepatoprotective studies, ethanolic leaf extract (ELE) of *Cassia fistula* (500 mg/kg b.w., p.o.) was administered daily for 30 days. AST, ALT, ALP, LDH, gamma-GT and bilirubin were estimated in serum and liver tissue. Lipid peroxidation (LPO), SOD and CAT were also estimated in liver tissue as markers of oxidative stress. DEN induced hepatotoxicity in all the treated animals were evident by elevated serum ALT, AST, ALP and bilirubin levels and a simultaneous fall in their levels in the liver tissue after 30 days. Induction of oxidative stress in the liver was evidenced by increased LPO and fall in the activities of SOD and CAT.

ELE of *Cassia fistula* administration for 30 days prevented the DEN induced hepatic injury and oxidative stress. In conclusion, it was observed that ELE of *Cassia fistula* protects the liver against DEN induced hepatic injury in rats.

TOXICOLOGY

Cassia fistula plant may contain heavy metals like Cu, Co, Ni, pb, Zn, Cr, Fe, Mn, F and K₂O, the concentration of these elements depends upon the soil mineral status as studied by (Biswas 2006).

The concentrations of these elements present in ppm (part per million except K₂O expressed in Percent), present in *Cassia fistula* leaves, in mineralized and non-mineralized areas is given below as studied by (Biswas 2006) in India:-

	Cu	Co	Ni	Pb	Zn	Cr	Fe	Mn	F	K ₂ O%
MA	1	2	13	3	19	6	107	99	1	0.71
NM	0	6	15	3	18	13	78	98	0	0.74

MN= Mineralized area, NM= Non mineralized area

The concentrations of Co(Cobalt), Ni(Nickel) and lead are higher, especially in the mineralized areas, than toxic levels according to standards given in Remington, 20th edition, 2000).The higher concentration of these heavy metals could cause serious toxicities to animals including human beings. The most toxic trace mineral is lead (Pb) and Nickel (Ni).

DISCUSSION/CONCLUSION

➤ CATHARTIC ACTIVITY

(Ahmed, Qureshi et al. 1989) Studied cathartic activity of the Sennoside glycosides from *Cassia fistula* using Lou, s method.

Cassia fistula has shown cathartic activity proving scientific evidence for ethnopharmacological use of *Cassia fistula* dried leaves as cathartic in Pakistan however the cathartic activity of cassia fistula is weaker than that of *Cassia angustifolia* , also the cathartic activity of the legumes is stronger than that of leaves depending upon the glycoside/ Sennoside content.

(Bahorun, Neergheen et al. 2005) suggested the presence of antioxidants like anthraquinone which also have laxative effect; this fact indicates that Sennoside and Anthraquinone may synergize the effect of each individual substance, on the bowl movement.

➤ ANTI-OXIDANT EFFECTS

The phytochemical studies done by (Bahorun, Neergheen et al. 2005) indicates the presence of antioxidant phenols like Anthraquinone and Flavonoids etc , which are secondary metabolite products found in *Cassia fistula* extract, the antioxidant effect of these biological molecules like free radical scavenging activity and inhibition of oxidation of biomolecules like fats etc. may have a prophylactic use against a no. of fatal diseases like cancer and heart diseases. Thus *Cassia fistula* may be used in the nutritional/food supplements this fact reinforces the traditional usage of *Cassia fistula* for various types of ailments in India and Pakistan and the other parts of the world.

Consequently, there has been a growing interest in the potential health-promoting properties of phytochemicals of plant origin. Special attention has been given to vitamin E, vitamin C and more particularly to phenolic derivatives including Anthraquinone, xanthenes, phenolic acids, phenolic diterpenes, Flavonoids, catechins, proanthocyanidine and anthocyanins.

These substances have also been reported to exhibit biological effects including antibacterial, anti-viral, anti-inflammatory, antithrombotic, antimutagenic, anticarcinogenic, antiageing and vasodilatory actions (Middleton and Kandaswami, 1994; Bravo, 1998; Carroll et al., 1998; De Bruyne et al., 1999; Di Carlo et al., 1999; Duthie et al., 2000; Middleton et al., 2000; Ferguson, 2001).

The following 2 studies further prove that *Cassia fistula* fruit has nutritional potential:- (Barthakur et al., 1995) reported the presence of 15.3%, 13% and 7.8% of aspartic acid, glutamic acid and lysine of the total amino acids respectively in the pulp. (Vasi and Kalintha, 1980) reported content of protein (19.94 %) and carbohydrate (26.3%).

➤ **MODULATION OF HUMORAL IMMUNITY**

Ali Nafisa, Kazmi Shahana et al. 2008 found that *Cassia fistula* augments humoral immune response but further detailed studies of mechanisms of immunomodulation and its probable use in immuno compromised individual are still to be investigated. These facts indicate that Amoxy-cassia may be used as an adjuvant during vaccination programs in order to reduce number of non-responder to vaccines.

➤ **ANTI-BACTERILA AND ANTI-FUNGAL EFFECTS**

(Perumal et al., 1998) studied therapeutic efficacy of *Cassia fistula* extract against a no. of bacterial species, these findings may be exploited to find alternative antibacterial medicines to curb the strains of bacterias resistant to the conventional chemotherapeutic agents.

Antibacterial and antifungal activity of *Cassia fistula* extract was demonstrated by (Duraipandiyan and Ignacimuthu 2007) during the in vitro studies done in the laboratory. The anti-fungal effects, shown by *Cassia fistula* extract may lead to a future herbal anti-fungal drug which could effectively solve the resistance to the presently used drugs keeping side effects at much lower level.

➤ ANTI-LEISHMANIAL EFFECTS

Anti-leishmanial effect of Clesterol presnt in *Cassia fistula* studied by (Sartorelli, Andrade Samanta et al. 2007) this work showing lower toxicity than traditional medicine Pentamidine and therapeutic efficacy gives a starting point to find more about it.

➤ INHIBITION OF CHOLESTEROL CRYSTALS GROWTH

The study done by (Ammal, George et al. 2007) described, efficacy of *Cassia fistula* extract on inhibition of cholesterol growth inhibition, in a growth experiment conducted using the *Cassia fistula* extract added to the superannuated solution of cholesterol. It showed that it has got an inhibitory effect on the crystallization. Crystals formed in the control have plate like morphology but the addition of the extract showed not only a delay in nucleation but also a change in morphology. The crystal turned from plate-like to needle-like and also with a wool-like appearance.

The change in morphology is a clear indication of inhibition of cholesterol crystal growth which may be attributed to some phytoactive compound in the *Cassia fistula* extract. This fact is supported by IR studies in which the hydroxyl bonds seen in control crystal were absent in the crystals grown after treatment with *Cassia fistula* extract. In oxidation of secondary alcohol to ketone, one learns to expect the disappearance of hydroxyl (O-H) stretch and appearance of carbonyl (C=O). X-ray studies proved it to be triclinic system. An addition of *Cassia fistula* extract in the growth stage can reduce the growth of cholesterol crystal i.e. additional crystallization of cholesterol can be avoided; this effect is significant in the pathologies caused by deposition of monohydrated crystals of cholesterol like atherosclerosis in the blood vessels and diseases caused by it like cerebrovascular coronary and peripheral vascular diseases.

Since the morphology of the monohydrated cholesterol crystals present in the body is same like one which is studied in the study done by (Ammal, George et al. 2007), which means that inhibition of cholesterol crystals growth and changes in morphology are significant effects. In oxidation of secondary alcohol to ketone, one learns to expect the disappearance of hydroxyl (O-H) stretch and appearance of carbonyl (C=O). X-ray studies proved it to be triclinic system. An addition of CF in the growth stage can reduce the growth of cholesterol crystal i.e. additional crystallization of cholesterol can be avoided Therefore *Cassia fistula* extract is a suitable medicine, without side effects, for the control of cholesterol crystallization.

This efficacy of *Cassia fistula* in inhibition of cholesterol crystals growth may be a milestone for the scientists trying to find substances that can replace or could be used concomitantly with synthetic substances to treat cerebrovascular coronary and peripheral vascular diseases due to deposition of cholesterol crystals in the atherosclerotic patches and gall stone diseases.

➤ **WOUND HEALING PROPERTIES**

Wound healing usage in India has been scientifically proved by the study done by (Bhakta, Mukherjee et al. 1998); *Cassia fistula* commonly known as Sundali was selected to evaluate its wound healing potentials based on traditional use and literature refs. Methanol ext. of *Cassia fistula* leaves were examined for its wound healing property in the form of an ointment in two types of wound models in rats:

(i) Excision wound model and

(ii) Incision wound model. The ointment of the leaf extract of two different concentrations. (5% and 10% wt. /wt. ointment of leaves ext. in simple ointment base) responded significantly in both models of wounds tested.

The results were also comparable to that of standard drug, nitrofurazone in terms of wound contraction ability, epithelization period, tensile strength and regeneration of tissue at wound area.

➤ **ANTI-CANCER EFFECTS**

Studies done by (Bahorun, Neergheen et al. 2005) demonstrates anticarcinogenic effect of *Cassia fistula*. Further it indicates that reduction in mitotic activity might be the main mechanism involved, thus giving starting point for further research, research also needs to

be undertaken to ascertain the mechanisms of DNA protection, hence delineating the antimutagenic and anticarcinogenic effects of *Cassia fistula* extracts.

➤ **ANTI-RHEUMATIC**

(Biswas et al.1973; Kirtikar and Basu, 1975) studied and proved the anti-rheumatic effect of *Cassia fistula* thus reinforces and scientifically prove the traditional usage of *Cassia fistula*, in Pakistan, as anti-rheumatic agent.

Another study done by (Sunil Kumar and Müller, 1998) shows inhibition of synthesis of mediators of inflammation "Leukotrienes" a mechanism involved in anti-rheumatic treatment also reinforces the traditional usage as anti-rheumatic in Pakistan.

➤ **HYPOGLYCEMIC EFFECTS**

(Esposito Avella, Diaz et al. 1991) studied the anti-diabetic effect of aqueous extract of *Cassia fistula*, the aqueous fraction produced a significant decrease in the glycemia ($p < 0.001$) at 4 and 24 hours with doses of 300 and 500 mg/kg, and at one and four hours after the dose of 1000 mg/kg ($p < 0.001$). In the glucose tolerance test, the aqueous fraction of *Cassia fistula* produced a significant decrease ($p < 0.05$) with the dose of 500 mg/kg at 0.25 and 0.5 hours. The 1000 mg/kg dose produced a significant increase ($p < 0.001$) at 0.25 and 2 hours.

Rhamnetin 3-O-beta -D-glucopyranosyl-(1->6)-beta -D-glucopyranoside is the compound responsible for hypoglycemic effect of *Cassia fistula* as studied by (Vaishnav and Jain 2004).

This work scientifically supports the traditional usage of *Cassia fistula* as hypoglycemic agent. Further research could design a safe and effective herbal hypoglycemic which either could be used as adjuvant to the synthetic drugs or as an independent drug.

REFERENCES

- Abu Sayeed M, Abbas Ali M, Astaq Mohal Khan GRM, Rahman MS (1999). Studies on the characterization and glyceride composition of *Cassia fistula* seed oil. *Bangladesh J. Sci. Indust. Res.* 34:144-148.
- Ahmed, S., S. Qureshi, et al. (1989). "Cathartic activity of Cassia species." *Pak J Pharm Sci* **2**(2): 37-45.
- Alam MM, Siddiqui MB, Hussian W (1990). Treatment of diabetes through herbal drugs in rural India. *Fitoter.* 61: 240–242.
- Ali Nafisa, H., U. Kazmi Shahana, et al. (2008). "Modulation of humoral immunity by Cassia fistula and amoxy-cassia." *Pak J Pharm Sci* **21**(1): 21-3.
- Ammal, M. S., K. V. George, et al. (2007). "Effect of phytoactive compounds on in vitro cholesterol crystal growth." *Cryst. Res. Technol.* **42**(9): 876-880.
- Asolkar LV, Kakkar KK, Chakre OJ (1992). Second supplement to glossary of Indian medicinal plant with active principles. In: Publication and Information Directorate, New Delhi. CSIR, I, . p. 177.
- Bahorun, T., V. S. Neergheen, et al. (2005). "Phytochemical constituents of Cassia fistula." *Afr. J. Biotechnol.* **4**(13): 1530-1540.
- Bhakta, T., P. K. Mukherjee, et al. (1998). "Studies on in vivo wound healing activity of Cassia fistula Linn. leaves (Leguminosae) in rats." *Nat. Prod. Sci.* **4**(2): 84-87.
- Birt DF, Hendrich S, Wang W (2001). Dietary agents in cancer prevention: flavonoids and isoflavonoids. *Pharmacol. Ther.* 90: 157-177.
- Biswas, S. N. (2006). "Comparative studies on plants as bioindicator for Cu, Co, Ni, Pb, Zn, Cr, Fe, Mn, F and K₂O in lead-zinc-fluorite mine area of Chandidongrh, Distt. Rajnandgaon (C.G.), India." *Asian J. Chem.* **18**(2): 991-996.

Biswas K, Ghosh AB (1973). In *Bharatia Banawasadhi*, Calcutta University, Advancement of learning, Calcutta., 2: 336.

Caroll KK, Guthrie N, So FV, Chambers AF (1998). Anticancer properties of flavonoids, with emphasis on citrus flavonoids. In: C.A.Rice-Evans and L. Packer (eds.) *Flavonoids in Health and disease*, Marcel Dekker Inc, New York. pp. 437-446.

De Bruyne T, Pieters L, Deelstra H, Vlietinck A (1999). Condensed vegetable tannins: biodiversity in structure and biological activities. *Biochem. Syst. Ecol.* 27: 445-459.

Di Carlo G, Mascolo N, Izzo AA, Capasso F (1999). Flavonoids: old and new aspects of a class of natural therapeutic drugs. *Life Sci.* 65: 337-353.

Duraipandiyan, V. and S. Ignacimuthu (2007). "Antibacterial and antifungal activity of *Cassia fistula* L.: an ethnomedicinal plant." *J Ethnopharmacol* 112(3): 590-4.

Duthie C, Duthie SJ, Kyle JAM (2000). Plant polyphenols in cancer and heart disease: implications as nutritional antioxidants. *Nut. Res. Rev.*13: 79-106.

El-Saadany SS, El-Massry RA, Labib SM, Sitohy MZ (1991). The biochemical role and hypocholesterolaemic potential of the legume *Cassia fistula* in hypercholesterolaemic rats. *Die Nahrung.* 35:807- 815.

Esposito Avella, M., A. Diaz, et al. (1991). "Evaluation of traditional medicine: effects of *Cajanus cajan* L. and of *Cassia fistula* L. on carbohydrate metabolism in mice." *Rev Med Panama* 16(1): 39-45.

Galati G, O'Brian PJ, (2004). Potential toxicity of flavonoids and other dietary phenolics: significance for their chemopreventive and anticancer properties. *Free Rad. Biol. Med.* 37: 287-303.

Galati G, Teng S, Moridani MY, Chan TS, O'Brien PJ (2000). Cancer chemoprevention and apoptosis mechanisms induced by dietary polyphenolics. *Drug Metab. Drug Interac.* 17: 311-349.

Govindarajan, M., A. Jebanesan, et al. (2008). "Larvicidal and ovicidal activity of *Cassia fistula* Linn. Leaf extract against filarial and malarial vector mosquitoes." *Parasitol Res* **102**(2): 289-92.

Gupta M, Mazumder UK, Rath N, Mukhopadhyay DK (2000). Antitumour activity of methanolic extract of *Cassia fistula* L. seed against Ehrlich ascites carcinoma., *J. Ethnopharmacol.* 72:151-156.

Jaipal S, Sing Z, Chauhan R (1983). Juvenile hormone like activity in extracts of some common Indian plants. *Indian J. Agric. Sci.* 53:730– 733.

Kirtikar KR, Basu BD (1975). In: B. Singh and M. Pal Singh (Eds), *Indian Medicinal Plants*, Dehradun. 2: 858.

Luximon-Ramma, A., T. Bahorun, et al. (2002). "Antioxidant activities of phenolic, proanthocyanidin, and flavonoid components in extracts of *Cassia fistula*." *J. Agric. Food Chem.* **50**(18): 5042-5047.

Middleton E, Kandaswami C (1994). The impact of plant flavonoids on mammalian biology: implication for immunity, inflammation and cancer. In: Harborne JB (ed.) *The Flavonoids: Advances in research since 1986*, Chapman and Hall, UK, pp. 619-952.

- Modi FK, Khorana ML (1952). A study of *Cassia fistula* pulp. Indian J. Pharm. 4: 61-63.
- Patel D, Karbhari D, Gulati D, Gokhale D (1965). Antipyretic and analgesic activities of *Aconatum spicatum* and *Cassia fistula*. Pharm. Biol. 157: 22–27.
- Perumal R, Samy S, Iggnacimuthu S., Sen A (1998). Screening of 34 medicinal plants antibacterial properties. J. Ethnopharm. 62: 173- 182.
- Pradeep, K., C. V. R. Mohan, et al. (2007). "Effect of *Cassia fistula* Linn. Leaf extract on diethylnitrosamine induced hepatic injury in rats." Chem.-Biol. Interact. **167**(1): 12-18.
- Raja N, Albert S, Ignacimuthu S (2000). Effect of solvent residues of *Vitex negundo* Linn. and *Cassia fistula* Linn. on pulse beetle, *Callosobruchus maculatus* Fab. and its larval parasitoid, *Dinarmus vagabundus* (Timberlake). Indian J. Exp. Bot. 38: 290-292.
- Ren W, Qiao Z, Wang H, Zhu L, Zhang L (2003). Flavonoids: promising anticancer agents. Med. Res. Rev. 23: 519-534.
- R. D. Straffer and F. Bischoff, 148th National meeting of American chemical society, Chicago, Aug – Sep. 1964.
- Sen AB, Shukia YN (1968). Chemical examination of *Cassia fistula*. J. Indian Chem. Soc. 45:744.
- Sartorelli, P., P. Andrade Samanta, et al. (2007). "Isolation of antileishmanial sterol from the fruits of *Cassia fistula* using bioguided fractionation." Phytother Res **21**(7): 644-7.
- Satyavati GV, Sharma M (1989). In Medicinal plant in India. ICMR, New Delhi.
- Sunil Kumar KC, Müller K (1998). Inhibition of leukotriene biosynthesis and lipid peroxidation in biological models by the extract of *Cassia fistula*. Phytotherapy Res. 12: 526-528.

Sharma BK, Basandrai AK (1999). Efficacy of some plant extracts for the management of Karnal bunt [*Neovossia (Tilletia) indica*] of wheat (*Triticum aestivum*). Indian J. Agric. Sci. 69: 837-839.

Vaishnav, M. M. and P. Jain (2004). "A flavonol diglycoside from *Cassia fistula*." J. Inst. Chem. (India) **76**(4): 115-117.

PHOTO REFERENCE

http://www.rain-tree.com/Plant-Images/Cassia_fistula_p2jpg.jpg.

Retrieved in December, 2008.

Trigonella foenum-graecum



BOTANICAL NAME: - *Trigonella foenum-graecum* (L)

ENGLISH NAME: - Fenugreek

URDU/ LOCAL NAME: - Methi

FAMILY: - Leguminosae

PARTS OF PLANT USED:-

Dried seeds, Leaves

DESCRIPTION

The plant is grown as green leafy vegetable and for its seeds.

The robust herb has light green leaves, is 30-60 cm tall, and produces slender beaked pods which are 10-15 cm long. Each pod contains 10-20 small hard yellowish brown seeds, which are smooth and oblong, about 3 mm long; each is grooved across one corner giving it hooked appearance (indianetzone.com).

ETHNOPHARMACOLOGY

USES IN PAKISTAN AND INDIA

USES AS FOOD

Pakistanis and Indians also like the fresh leaves, which are eaten as a very tasty vegetable and prepared like spinach, or dried and used as flavoring.

The plant is also eaten as salad and seeds are used in curry powder.

MEDICINAL USES

Both seed and plant are used medicinally.

➤ **HYPOGLYCEMIC EFFECTS**

Fenugreek has been prescribed for diabetes mellitus by the herbalists in Pakistan and India. A glass of milk or water in which a tablespoon of seeds of *Trigonella foenum-graecum* has been soaked overnight is drunk each morning.

➤ **CHOLESTEROL LOWERING EFFECT**

➤ **FOR GASTROINTESTINAL DISEASES**

● **ANTI-ULCER**

Trigonella foenum-graecum seeds extract helps against peptic ulcer by providing coating of mucilaginous matter.

● **FOR COLIC**

● **CARMINATIVE**

Fenugreek stimulates digestive process.

● **FOR LOSS OF APETITE**

● **DYSENTRY**

● **DIARRHEA**

● **DEMULCENT**

Fenugreek tea has soothing effect on inflamed stomach and intestine, further it is believed by the herbalists that it cleans the stomach, bowels and kidney (phytotherapies.org) (indianetzone.com)

➤ **ANTIPYRETIC EFFECT**

Fenugreek tea helps to perspire, dispel toxicity and shorten gestation period of fever.

➤ **EMOLIENT**

Fenugreek reduces skin irritation and inflammation on topical application (phytotherapies.org).

➤ **RESPIRATORY DISEASES**

Trigonella foenum-graecum tea has been traditionally used in the early stages of any of the respiratory infections such as bronchitis, influenza, sinusitis and catarrh, and pneumonia.

➤ **GALACTAGOGUE EFFECT** (phytotherapies.org)

The seeds of *fenugreek* have been used to make tea which is known to increase milk production in nursing women.

Dosage: - Up to four cups of tea could be taken daily.

Fenugreek seeds made in gruel and given to nursing women increases the flow of milk.

It has also been given, along with sweets, to ladies in post natal period.

Preparation of *fenugreek* tea

Soak 0.5 gram (about 1/8 teaspoonful) of crushed seed in 1 cup of cold water for 3 hours. Strain before drinking. Tea could be sweetened by honey. To improve flavor few drops of lemon also could be added.

➤ **FOR MOUTH- ULCERS**

An infusion of *Trigonella foenum-graecum* is being traditionally used as gargle for recurrent mouth ulcers.

➤ **VULNERARY EFFECT**

Fenugreek promotes healing of wounds (phytotherapies.org).

➤ **ANTI-DANDRUFF EFFECTS**

Trigonella foenum-graecum seeds are also being used to remove dandruff.

Two table spoons soaked overnight in water, softened in the morning, crushed and ground to make a paste like formulation and applied on scalp/ dandruff and left for half an hour. The hair is then thorough washed in soap-nut solution.

➤ **APHRODISIAC**

Trigonella foenum-graecum seeds extract has been used traditionally to improve sexual desire and potency by the herbalist healers.

➤ **VETERINARY USES**

Trigonella foenum-graecum seeds fine ground and mixed with *cottonseed* fed to cows is a traditional remedy being used to increase the flow of milk.

OTHER USES

➤ **IN PHARMACEUTICAL INDUSTRY**

Fenugreek also contain a sapogenin “Diosgenin”, it has steroidal structure and has been used as a starting material to produce sex hormones in the pharmaceutical industry.

➤ **AGAINST CANCER**

Fenugreek has been used in combination with a few other ingredients to formulate a preparation against cancer as described by (Ma, Zhu et al. 2006).

This combination helps to reduce side effects related to chemotherapy.

➤ **AGAINST PROSTATA**

(Guo 2006) has described that *fenugreek* can be used along with a no. of other ingredients to design a formulation which can inhibit α -receptor and delay the progression of prostatic hyperplasia, and has no obvious adverse side effects.

CHEMISTRY

SEEDS:-

The composition of an average seed is as follows, (Hemavathy and Prabhakar 1989), (indianetzone.com):-

Name of substance	Content in percent
Moisture	6.3%
Protein	9.5%
Fat	7.5.0% of dry weight (Hemavathy and Prabhakar 1989)
Fiber	18.5%
Carbohydrates	42.3%
Calcium	1.3%
Phosphorus	0.48%
Iron	0.011 %
Sodium	0.09%
Potassium	1.7%
Vitamin B1	0.41 mg/ 100g
Vitamin B2	0.36 mg/100g
Niacin	6mg/ 100 g
Vitamin C	12.0 mg/ 100g
Vitamin A	1040 I.U. / 100 g
Calorific value	370 calories / 100 g
Gums	23.06 %
Mucilage	28 %

LEAVES STEM AND PODS:-

Leaves stem and pods are also rich in Calcium, iron, vitamin A and Vitamin C. Although fresh leaves contain only 3-5 % protein, on dry basis, they are comparable to pulses (indianetzone.com).

Trigonella foenum-graecum seeds also contain both volatile oils and fixed oils.

● VOLATILE OILS

The volatile oil content of fenugreek is less than 0.02% it is not commercially very important (indianetzone.com).

● FIXED OILS

The fixed oil content of the seed is about 6% (Hardman and Jefferies 1971). The fatty acids content of the seed is largely of oleic acid, linoleic and linoleic acids. It has marked drying properties, the dried oil being golden yellow in color, and insoluble in ether. The oil has disagreeable odor and bitter taste (indianetzone.com).

CHEMICAL COMPOUNDS OF *Trigonella foenum-graecum* ISOLATED BY EXTRACTIONS AND CHROMATOGRAPHY

1. 4-Hydroxyisoleucine (Shah, Bodhankar et al. 2006).
2. Trigonellin an alkaloid (Shah, Bodhankar et al. 2006).
3. Flavonoids (Shang, Cai et al. 1998).
5 different flavonoids have been isolated from *fenugreek* extract as Vitexin, Tricin, naringenin, quercetin, and tricetin-7-O- β -D-glucopyranoside.
4. Glycosides
 - Galactomannan (Sun and Zhang 2008).
 - Furostanol (Hardman, Kosugi et al. 1980).
5. Diosgenin (Jing and Zhao 2005) (Fazli and Hardman 1968).
6. Protodioscin (Hibasami, Moteki et al. 2003).
7. Trigoneocidin VIII (Shang, Cai et al. 2001).
8. Yamogenin (sapogenin) phytotherapies.org (Hardman and Jefferies 1971).
9. Defensins (Tfgd2); 2 types of antifungal Defensins in *fenugreek* were detected by cloning and PCR (polymerase chain reaction) (Olli, Guruprasad et al. 2007).
10. Trigocumarin (Parmar, Jha et al. 1982).

BIOLOGY/ PHARMACOLOGY

1. HYPOGLYCEMIC EFFECT

- (Shah, Bodhankar et al. 2006) studied the combination therapy with 4-Hydroxyisoleucine from *fenugreek* extract and a synthetic drug *pioglitazone*, on Alloxan-induced diabetic mice, and found that combined effect of both substances is stronger than each of them individually; also combination therapy with 4-hydroxyisoleucine and *pioglitazone* is more beneficial than combination therapy with 4-hydroxyisoleucine and glyburide (Benzamide).

These findings support and provides scientific basis of traditional usage of fenugreek in Pakistan and India as hypoglycemic herb. Also combination with *fenugreek* and the synthetic drugs lowers the effective dose of the synthetic drugs thus reducing the potential side effects related to therapy with the synthetic substances alone.

These findings provide starting point for further research to minimize the potential side effects related to chemotherapy with synthetic substances. It could also be further researched to invent a co-formulation with 4-hydroxyisoleucine isolated from *Fenugreek* extract and synthetic drugs like *pioglitazone*, which may become a safer alternative as compared to synthetic drugs alone. *Fenugreek* can also be used as health food supplement.

- (Shah, Bodhankar et al. 2006) studied the pharmacological effects of *trigonelline* extracted from *fenugreek* on the *Alloxan* induced diabetic mice and found that *trigonelline* significantly reduced the blood glucose in treated groups as compared to control diabetic animals. *Trigonelline* treated group showed islet cells in the vicinity of the pancreatic duct which indicates its beneficial effects on beta cells. *Glyburide (Benzamide)* was used as a standard anti-diabetic drug and its effect on pancreatic cell was also studied. The pancreatic beta cells of *glyburide (Benzamide)* treated mice did not show any islets in the vicinity of pancreatic duct. Both *trigonelline* and *glyburide* arrested the decrease in body weight and mortality of diabetic mice. LD50 of *trigonelline* was found to be more than 5000 mg/kg.

Hypoglycemic effects of combination of two compounds isolated from *Trigonella foenum-graecum*, “4 hydroxyisoleucine” and “*trigonelline*” (4HIT) by (Shah, Bodhankar et al. 2006) in *alloxan* induced diabetic mice.

These compounds were isolated by column chromatography from *fenugreek* seeds. The combination of 4 *hydroxyisoleucine* and *trigonelline* [4HIT, 40:30, and 120 mg/kg] was administered orally in *alloxan* induced diabetic mice. The parameters studied were blood glucose, histology of pancreas, body weight, mortality and acute oral toxicity. 4HIT (120 mg/kg) showed reduction in blood glucose level within 2 hours and reduced the peak blood glucose level at 6 hours during acute study.

After 28 days treatment with 4HIT, there was significant decrease in blood glucose level. 4HIT increased the glucose threshold as compared to only *alloxan* treated group.

Histology of pancreas showed formation of new islets near the vicinity of the pancreatic duct. Decreased glycosylated Hb (hemoglobin) adds to the effect of 4HIT. *Glyburide* was used as a standard anti-diabetic drug and its effect on pancreatic cell was also studied. The pancreatic beta cells of *glyburide* treated mice did not show any islets in the vicinity of pancreatic duct. Both 4HIT and *glyburide* arrested the decrease in body weight and mortality of diabetic mice. LD50 was found to be more than 5000 mg/kg.

Result

These findings suggest that 4HIT showed hypoglycemic effect in *alloxan* induced diabetic mice. The presence of the pancreatic islets in the vicinity of duct suggested 4HIT might act by regeneration of new islets.

2. INDUCTION OF KEY LIVER ENZYMES INVOLVED IN CARBOHYDRATES AND LIPIDS METABOLISM

Fenugreek extract induces liver enzymes involved in glucose and lipid metabolism (Rashad and Moharib 2008).

The male white rats were fed with diets containing different Egyptian plant leaf fibers and the activities of some key liver enzymes of carbohydrate and lipid metabolism, as well as liver function test, were studied.

The rats were fed for 8 weeks on 5 diets: high carbohydrate basal diet 1 (fiber-free) and 4 experimental diets consisting of the first one substituted with 10% of each fiber (*turnip, sugar beet, cabbage and fenugreek green*).

The activities of *glucose 6-phosphate dehydrogenase*, *6-phosphogluconate dehydrogenase*, *6-phosphofructokinase*, *fructose-1,6-bisphosphatase*, *ATP-citrate lyase*, and *malate dehydrogenase* were significantly increased in rats fed with diets substituted with 10% of a cabbage and fenugreek green fibers (diets 4 and 5) for 8 weeks. While a significant decrease in the activities of these enzymes was observed in the liver of rats receiving turnip and sugar beet fiber-containing diets (diets 2 and 3).

The *hepatic pyruvate kinase* activity was significantly lower in rats fed on diet 5 throughout the feeding period (8 weeks).

The activities of the enzymes of liver function were lower in serum of rats fed with 4 experimental diets than the control group.

Higher reduction in serum glucose concentration was observed in rats fed on diets 3-5, compared with the control. A lower hepatic glycogen content was observed in the rats fed on diets 4 and 5, with regard to those receiving diets 1 and 2.

CONCLUSION

It can be concluded that rats fed on *cabbage* and *fenugreek* green fibers (diets 4 and 5) Showed higher effects on:-

1. Increasing the activities of some key liver enzymes which are involved in the metabolism of carbohydrates *6-phosphate dehydrogenase*, *6-phosphogluconate dehydrogenase*, *6-phosphofructokinase*, *fructose-1, 6-bisphosphatase*, *ATP-citrate lyase*, and *malate dehydrogenase*.
2. Reducing the transaminase enzymes.
3. Lowering serum glucose levels.
4. Lowering liver glycogen content than that of turnip and sugar beet fibers (diets 2 and 3) over the 8-wk feeding period.

3. CHOLESTEROL LOWERING EFFECT

(Joshi and Rajni 2007) studied the effect of *fenugreek* in lowering cholesterol. The following study demonstrated that *fenugreek* significantly lowers the total blood cholesterol.

Raw, roasted and sprouted fenugreek (methi) (*Trigonella foenum-graecum*) seeds were fed to rats for 40 days at 2.5 and 5% levels to find the effect on the concentration of blood glucose, total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) and triglyceride (TG).

A significant ($p \leq 0.05$) decrease was observed in blood glucose, TC, LDL-C and TG levels, whereas a significant ($p \leq 0.05$) increase was seen in HDL-C level as compared to control groups. Maximum rise in the HDL-C level was observed in rats fed on a diet containing 5% of sprouted *fenugreek* seeds.

4. ANTIOXIDANT EFFECT

(Annida and Stanely Mainzen Prince 2005) demonstrated antioxidant effect of *fenugreek* extract in streptozotocin-induced diabetic rat model. The antioxidant effect was evaluated by estimating *thiobarbituric acid*-reactive substances and reduced *glutathione* and measuring the activities of *catalase* and *superoxide dismutase* in liver, heart, and kidney in diabetic rats. *Fenugreek* leaf powder supplementation significantly lowered lipid peroxidation and significantly increased the antioxidant system in diabetic rats. Thus, *fenugreek* leaf powder reduces oxidative stress in experimental diabetes in rats.

5. ANTI-LEUKEMIC EFFECTS

Protodioscin (PD) induces apoptosis and inhibits growth of human leukemic cells (Hibasami, Moteki et al. 2003).

(Hibasami, Moteki et al. 2003) done a clinical trial on human beings (HL-60 cells) to study effects of Protodioscin on viability of human leukemic cells and stomach cancer cells KATO III cells (cells in the gastric lining). Protodioscin isolated from *fenugreek* extract induces apoptosis and displayed strong growth inhibitory effect against HL-60 cells, but weak growth inhibitory effect on KATO III cells. Morphological change showing apoptotic bodies was observed in the HL-60 cells treated with PD, but not in KATO III cells treated with PD.

Flow cytometric analysis showed that the hypodiploid nuclei of HL-60 cells were increased to 75.2, 96.3, and 100% after a 3-day treatment with 2.5, 5, and 10 μM PD, respectively. The fragmentation by PD of DNA to oligonucleosomal-sized fragments, that is a characteristic of apoptosis, was observed to be both concentration and time-dependent in the HL-60 cells. These findings suggest that growth inhibition by PD of HL-60 cells results from the induction of apoptosis by this compound in HL-60 cells.

PD= Protodioscin, KATO III cells = Gastric cancer cell lining

HL-60 cells= Human leukemic cells, μM = Micromole

6. GALACTAGOGUE EFFECT

(El Ridi and Shahat 1944) has discovered a lactation promoting factor, from oil extracted from *fenugreek* seeds after dietary experiments on rats.

The factor was concentrated by chromatographic adsorption of the nonsaponifiable matter. Twelve different fractions were separated; one of them was active in doses of 1 mg/ kg body weight or in the proportion of 1:75,000 of the diet consumed. Spectrographic examination of the oil, its nonsaponifiable matter and the active concentrate revealed an inflection in the region near 300 milli micro; but none in the tocopherol region (290 milli micro). The position of the band did not change after oxidation.

The nonsaponifiable matter contained 2.7 mg. % carotene, 25 mg. % sterols (of which 1.5% was ergosterol), and 3 mg. % of phospholipids.

7. COUNTERACTION OF HYPERGLYCEMIC SIDE EFFECTS ASSOCIATED WITH CORTISON TREATMENT

When an alkaloid "Trigonelline", isolated from *fenugreek*, given to rabbits Pre- injected with 0.5 mg/ kg, it countered the hyperglycemic effect (Menczel and Korine 1964).

TOXICOLOGY

No toxicological studies have been yet reported on fenugreek.

DISCUSSION/ CONCLUSION

➤ HYPOGLYCEMIC EFFECTS

(Rashad and Moharib 2008) experimentally demonstrated the induction of liver enzymes involved in carbohydrate and lipid metabolism by *fenugreek* in rats.

Fenugreek extract showed the following effects:-

- Increasing the activities of some liver key enzymes involved in carbohydrate and lipid metabolism.
- Reducing the transaminase enzymes
- Lowering serum glucose levels
- Lowering liver glycogen content

The above effects give scientific basis for traditional usage of *fenugreek* against hyperglycemia in Pakistan and India.

Hypoglycemic effect of *Trigonella foenum-graecum* on rats has been described by (Shah, Bodhankar et al. 2006) in total 3 studies done on Alloxan induced diabetic mice with 2 active ingredients 4-hydroxyisoleucine and Trigonelline, both of these substances has shown hypoglycemic effects.

When a combination of 4 hydroxyisoleucine and trigonelline was administered (4HIT, 40:30, and 120 mg/kg) this combination acts by regeneration of islets of langerhan near pancreatic duct leading to production of more insulin and reducing blood sugar level to a remarkable level.

Another study done by (Annida and Stanely Mainzen Prince 2005) demonstrated that 1g/ Kg body weight of *fenugreek* has same effect like synthetic drug *Glibenclamide*.

These studies done on rats could be either extrapolated to human beings or used to design clinical trials in human beings to check relevant effects. Because *fenugreek* has been proved safe and useful hypoglycemic agent traditionally that is why these findings may supports and provide scientific basis for traditional usage of *fenugreek* against Diabetes mellitus in Pakistan and India. Also combination with fenugreek and the synthetic drugs lowers the effective dose of the synthetic drugs thus reducing the potential side effects related to therapy with the synthetic substances alone.

These findings provide starting point for further research to minimize the potential side effects related to chemotherapy with synthetic substances. It could also be further researched to invent a co-formulation with *4-hydroxyisoleucine* and the synthetic drugs like *pioglitazone*, which may become a safer alternative as compared to synthetic drugs alone.

➤ **COUNTERACTION OF HYPERGLYCEMIA INDUCED BY CORTISON**

As described by the studies done by (Menczel and Korine 1964) on rabbits that *trigonelline* alkaloid isolated from *fenugreek* counters hyperglycemia induced by pre treatment with *cortisone* treatment. These findings could be used to design a clinical trial to check its efficacy in the patient groups (like cancer patients receiving cortisone treatment on long term basis to counteract side effects of chemotherapy/ radiation therapy like vomiting). And it may prove helpful to counteract hyperglycemia in those persons.

➤ **CHOLESTEROL LOWERING EFFECT**

(Joshi and Rajni 2007) demonstrated cholesterol lowering effect of *fenugreek* seeds on rats and this study could either be extrapolated to human beings or used to design clinical trials. Further this study gives a scientific basis for traditional usage of *fenugreek* in lowering cholesterol in Pakistan and India.

The principles derived from *fenugreek* may become a future combined treatment regime with synthetic cholesterol lowering drugs for cholesterol lowering, either being used in co-formulations or as a health food supplement (It has already being marketed as a health food supplement in the market of traditional herbal remedies in the countries like Pakistan, India and china etc).

➤ **ANTI-LEUKEMIC EFFECTS**

(Hibasami, Moteki et al. 2003) demonstrated in a human clinical trial induction of apoptosis in the Human leukemic cells by a Protodioscin isolated from *fenugreek*. This fact indicates the possibility of adjuvant treatment with the modern synthetic drugs and *fenugreek* which could further lowers side effects related to synthetic chemotherapy and it opens chances of further research.

➤ **GALACTAGOGUE EFFECT**

(El Ridi and Shahat 1944) described galactagogue principles in the *fenugreek* extract by testing in dietary experiments on rats; also fenugreek has been used both by nursing mothers and in veterinary practices to promote secretion and flow of milk, in buffalo and cows, without any side effects. It gives the scientific basis for traditional usage of *fenugreek* as galactagogue. It could be further tested by designing clinical trials in nursing human mothers and its efficacy could be compared to oxytocin.

➤ **DIOSGENIN PRODUCTION FROM FENUGREEK**

(Fazli and Hardman 1968) isolated diosgenin from *fenugreek* extract by a series of hydrolysis and extractions. Presence of a number of other associated steroids like Gitogenin, tigogenin, and 25 alpha-spirostane have been detected by thin layer chromatography. Yamogenin is not separated from diosgenin.

This study shows presence of diosgenin, a sapogenin containing steroid structure which has been used as a starting material for the synthesis of sex hormones.

1. No clinical/ animal studies are not yet available for the aphrodisiac effects, effect against gastritis, effect against gastric ulcers, mouth ulcers and effect against respiratory tract diseases of *Trigonella foenum-graecum* despite of the matter of fact that *fenugreek* has proved useful for these effects traditionally in Pakistan and India after years after years usage.
2. Toxicological studies has not yet been reported and this is a need of the hour to design toxicological studies along with clinical trials, also the studies should be extended to check effects and dose adjustments in liver and renal compromised individuals so that fenugreek could be added as adjuvant to the modern treatment to overcome the side effects related to chemotherapy and achieve design best treatment regiments.

REFERENCES

Annida, B. and P. Stanely Mainzen Prince (2005). "Supplementation of Fenugreek Leaves Reduces Oxidative Stress in Streptozotocin-Induced Diabetic Rats." J. Med. Food **8**(3): 382-385.

El Ridi, M. S. and M. Shahat (1944). "A preliminary note on a "Fat-Soluble Lactation Factor."" J. Roy. Egypt. Med. Assoc. **27**: 199-200.

Fazli, F. R. Y. and R. Hardman (1968). "The spice, fenugreek, (*Trigonella foenumgraecum*): its commercial varieties of seed as a source of diosgenin." Trop. Sci. **10**(2): 66-78.

Guo, K. (2006). Method for manufacturing pharmaceutical composition for treating benign prostatic hyperplasia. Application: CN
CN, (Peop. Rep. China). 7pp.

Hardman, R. and T. M. Jefferies (1971). "Determination of diosgenin and yamogenin in fenugreek seed by combined column chromatography and infrared spectrometry." J. Pharm., Pharmacol. **23**(Suppl.): 231S-232S.

Hardman, R., J. Kosugi, et al. (1980). "Isolation and characterization of a furostanol glycoside from fenugreek." Phytochemistry **19**(4): 698-700.

Hemavathy, J. and J. V. Prabhakar (1989). "Lipid composition of fenugreek (*Trigonella foenumgraecum* L.) seeds." Food Chem. **31**(1): 1-7.

Hibasami, H., H. Moteki, et al. (2003). "Protodioscin isolated from fenugreek (*Trigonella foenumgraecum* L.) induces cell death and morphological change indicative of apoptosis in leukemic cell line H-60, but not in gastric cancer cell line KATO III." Int. J. Mol. Med. **11**(1): 23-26.

Jing, Y. and Y.-q. Zhao (2005). "Studies on extraction technique of steroidal saponins in *Trigonella foenum-graecum* L." Zhongyao Yanjiu Yu Xinx **7**(6): 19-21.

Joshi, R. and M. Rajni (2007). "Lipid profile of rats fed on domestically processed fenugreek." J. Food Sci. Technol. **44**(5): 542-544.

Ma, J., J. Zhu, et al. (2006). Manufacture of traditional Chinese medicine for treating malignant tumors. Application: CN
CN, (Xi'an Huanghe Pharmaceutical Co., Ltd., Peop. Rep. China). 10pp.

Menczel, E. and Z. Korine (1964). "The counteraction of the hyperglycemic effect of cortisone by azacyclonol and trigonelline." Harokeach Haivri **10**: 152-153.

Olli, S., L. Guruprasad, et al. (2007). "Characterization of defensin (Tfgd2) from *Trigonella foenum-graecum*." Curr. Sci. **93**(3): 365-369.

Parmar, V. S., H. N. Jha, et al. (1982). "Trigocoumarin - a new coumarin from *Trigonella foenumgraecum*." Z. Naturforsch., B: Anorg. Chem., Org. Chem. **37B**(4): 521-3.

Rashad, M. M. and S. A. Moharib (2008). "Studies of the effect of some plant leaf fibres on the key liver enzymes intermediate carbohydrate and lipid metabolism in rats." AFS, Adv. Food Sci. **30**(1): 11-18.

Shah, S., S. Bodhankar, et al. (2006). "Combinative therapeutic approach for better blood sugar level control in alloxan diabetic mice." Int. J. Diabetes Metab. **14**(2): 104-105.

Shah, S. N., S. L. Bodhankar, et al. (2006). "Effect of trigonelline: an active compound from *Trigonella foenumgraecum* Linn. in alloxan induced diabetes in mice." J. Cell Tissue Res. **6**(1): 585-590.

Shah, S. N., S. L. Bodhankar, et al. (2006). "Hypoglycemic activity of the combination of active ingredients isolated from *Trigonella foenumgraecum* in alloxan induced diabetic mice." Pharmacologyonline(1): 65-82.

Shang, M., S. Cai, et al. (1998). "Studies on flavonoids from fenugreek (*Trigonella foenumgraecum* L.)." Zhongguo Zhongyao Zazhi **23**(10): 614-616.

Shang, M., S. Cai, et al. (2001). "Structural identification of Trigoneoside VIII from *Trigonella foenumgraecum* (fenugreek)." Yaoxue Xuebao **36**(11): 836-839.

Sun, Q. and L.-m. Zhang (2008). "Technology conditions of degradation for galactomannan from *Trigonella foenumgraecum* by cellulose." Tianjin Keji Daxue Xuebao **23**(1): 33-37.

PHOTO REFERENCE

http://www.rolv.no/images/planteleksikon/T/trigonella_foenum-graecum1.jpg

Retrieved in December, 2008.

Carica papaya



BOTANICAL NAME: - *Carica papaya* (Linn)

ENGLISH NAME: - Papaya

URDU/ LOCAL NAME: - Papeeta

FAMILY: - Caricaceae

PARTS OF PLANT USED:-

Fruit pulp, seeds, roots, leaves.

ETHNOPHARMACOLOGY

USES IN PAKISTAN

1. Contraception (Unripe fruit)
2. Anthelmintic
3. Nutritional food supplement

OTHER USES

Fruit

- Ring worm, the mature ripe fruit is used.
- High blood pressure, seeds and green fruit is used.
- Aphrodisiac

Seeds

- Anti-inflammatory
- Analgesic
- Anthelmintic
- Anti-fungal

Leaves

- As heart tonic
- Analgesic
- Stomachic

Roots

- Analgesic

CHEMISTRY

1. FRUIT (Pendzhiev 2002):-

The milky juice (latex) of papaya fruits is a source of proteolytic enzymes (Papain, chymopapain, peptidases A and B, and lysozyme), these enzymes possess Anti-inflammatory, anticoagulant, analgesic, bactericidal, anthelmintic and hemolytic properties. Capable of decomposing the proteins to polypeptides and amino acids and dissolving necrotized cells (while not affecting intact ones); these enzymes are widely used in medicine.

- Papain, a cystein protease enzyme which is used for digestive problems as tablets.

(Azadbakht, et al. 2006) described a method for isolation and study papain enzymes from *Carica papaya* as follows:-

MATERIALS AND METHOD

The full-grown but unripe fruit was subjected to shallow incisions on several sides (depth of incision was important). After collection of 100 gram latex, the coagulated lumps were shredded and dried by the use of heat (30°C).

Extraction and sedimentation procedures were used for extraction of papain. In this method, cysteine hydrochloride at adjusted pH was used for extraction and sodium chloride for sedimentation. 1.2 gram of Papain was isolated from 100 gram latex after extraction and sedimentation.

The extracted papain was identified by the use of an electrophoresis method and also by comparing its IR (infrared spectrum) with the standard papain. The activity of extracted papain was detected by USP (United States Pharmacopoeia) procedure. Electrophoresis pattern and IR spectrum of extracted papain were identical with the standard papain.

- (Chen, Hsu et al. 2007) described the presence of chitinase a glycosyl hydrolase.

2. SEEDS

Following fatty acids have been found in seeds (Pendzhiev 2002):-

- (i) Oleic acid (75.5%)
- (ii) Palmitic acid (11.38%)
- (iii) Stearic acid (5.25%)
- (iv) Linolic acid (2.13%), and
- (v) Arachidonic (0.31%) acids

3. LEAVES (Pendzhiev 2002):-

The leaves of papaya contain free and bound phenolic compounds, tannins, organic acids, steroidal and triterpene saponins, flavonoids, lipids, coumarins, glycosides, and alkaloids.

The crust of the tree contains a substance effectively suppressing the growth of some malignant tumors.

PHARMACOLOGY/ PHARMACOLOGY

1. CONTRACEPTIVE AND ABORTIFACIENT PROPERTIES

➤ ABORTIFACIENT AND CONTRACEPTIVE EFFECTS IN WOMEN

The scientific research work has been done by the Britain's university of Sussex, which proves the contraceptive qualities of *Carica papaya* fruit (Asia week, 1994).

Mechanism of Action

The *Carica papaya* contain an enzyme called Papain which suppresses Progesterone, a hormone which is needed to maintain conception and maintain pregnancy.

Another possibility may be papain which can be used to tenderize meat may break down a membrane vital to the development of the fetus.

Recommended dose

• CONTRACEPTIVE EFFECTS

In Sri-lanka, India and Pakistan, the traditional healers recommend eating unripe papaya fruit daily to avoid pregnancy in young women.

When pregnancy is required it could be attained by just stopping usage of papaya.

• ABORTIVE EFFECTS

(Tharmalingam Senthilomohan, rep. by Asia week 1994) recommends that abortion could be induced by consumption of unripe fruit of *Carica papaya* for 3 consecutive days.

- (Oderinde, Noronha et al. 2002) studied the abortifacient potential of aqueous extract of *Carica papaya* (Linn) seeds in female Sprague-Dawley rats in the following study:-

AIM OF THE STUDY

Experiments were conducted to investigate the abortifacient potential of aqueous extract of *Carica papaya* (Linn) seeds in female Sprague-Dawley rats. Oral doses of 100 and 800 mg/kg body weight were administered once a day from days 1 to 10 post-coitum. No significant differences in total body weight were found in fetuses exposed to these regimes. However, in the group treated with 100 mg/kg body weight, there was a significant increase ($p < 0.05$) in the implantation sites and fetal weight was significantly decreased ($p < 0.05$) compared to the controls. No dead or malformed fetuses were found. However, in the group treated with 800 mg/kg body weight, there was obvious vaginal bleeding but no treatment related increase in implantation sites compared with control. There was however, complete resorption of about 30% of the fetuses. The surviving fetuses were stunted when compared with the control but were without any external malformations. The results of the present investigations lead to the clear conclusion that low dose aqueous crude extract of *Carica papaya* (Linn) seeds do not adversely affect prenatal development. The altered toxicological profile indicates that the abortifacient property is a high dose side effect. The results indicate that *Carica papaya* toxicity can adversely affect the fetus.

➤ **CONTRACEPTIVE EFFECTS IN MALE**

(Lohiya Nirmal, Manivannan et al. 2006) demonstrated a significant male anti-fertility effects of *Carica papaya* seeds extract in the following pharmacological study on albino rats:-

In this study there were studied semen profile, fertility, body and organ weight response, and toxicology in male albino rats. The *Carica papaya* seed extract was administered at the dose regimens of 10 and 50 mg/animal/day orally for 30, 60, and 90 days and 0.1 and 1.0 mg/animal/day intramuscularly for 15 and 30 days.

Caudal epididymal sperm motility and count was reduced significantly at low and high dose regimens both in the oral as well as the intramuscular groups. The reduced sperm motility was associated with morphological defects. Testicular sperm counts were also reduced in all the treatment groups except the low dose intramuscular group. Fertility tests showed dose- and duration-dependent reduction and zero fertility was observed at high dose regimens of the oral and intramuscular groups following 60 and 30 days of treatment, respectively.

Testicular weight was reduced in all the treatment groups, whereas accessory sex organs showed a variable response. Body weight and toxicological observations did not show any untoward response. Fertility and all other associated changes returned to normal within 45 and 30 days of treatment cessation in the oral and intramuscular groups, respectively.

RESULT

The data revealed that reversible sterility could be induced in male rats by papaya seeds aqueous extract treatment without adverse effects on libido and toxicological profile.

2. ANTI-PARASITAL EFFECTS

●ANTHELMINTIC ACTIVITY

(Stepek, Lowe et al. 2007) examined the mechanism of action and compared the anthelmintic efficacy of cystein proteinases from papaya, pineapple, fig, kiwi fruit and Egyptian milkweed in vitro using the rodent gastrointestinal nematode *Heligmosomoides polygyrus*. Within a 2 hour incubation period, all the cystein proteinases, with the exception of the kiwi fruit extract, caused marked damage to the cuticle of *H. polygyrus* adult male and female worms, reflected in the loss of surface cuticular layers. Efficacy was comparable for both sexes of worms, was dependent on the presence of cystein and was completely inhibited by the cystein proteinase inhibitor, E-64. LD50 values indicated that the purified proteinases were more efficacious than the proteinases in the crude latex, with purified ficin, papain, chymopapain, Egyptian milkweed latex extract and pineapple fruit extract, containing fruit bromelain, having the most potent effect.

RESULT

Carica papaya along with the other plants under study (except kiwi) exhibited a potent anthelmintic effect.

The mechanism of action of these plant enzymes (i.e. an attack on the protective cuticle of the worm) suggests that resistance would be slow to develop in the field.

●ANTI-*Trichomonas vaginalis* EFFECT

(Calzada, Yopez-Mulia et al. 2007) performed the invitro susceptibility assay on crude methanolic extracts from 22 Mexican medicinal plants. All these plants under study were

screened for anti-trichomonal activity against *Trichomonas vaginalis*, which is the etiological agent of trichomoniasis. Among the plants tested *Carica papaya* and *Cocos nucifera* showed the best anti-trichomonal activity with IC₅₀ values of 5.6 and 5.8 µg/ml, respectively.

The extracts of *Bocconia frutescens*, *Geranium mexicanum*, and *Lygodium venustum* showed moderate activity with IC₅₀ values ranging from 30.9 to 60.9 µg/ml. All the other plant extracts were inactive (IC₅₀ > 100 µg/ml). All extracts tested were less active than metronidazol (IC₅₀ 0.037 µg/ml); an antiprotozoal drug used as positive control.

RESULT

Carica papaya (and *Bocconia frutescens*) has shown best anti-trichomonal activity.

IC 50= Inhibitory concentration required to kill 50 % of the organisms under test.

●ANTI-FUNGAL PROPERTIES

(Chen, Hsu et al. 2007) described the presence of Chitinase enzyme in *Carica papaya* fruit in the following study:-

Chitinase cDNA clone (CpCHI, 1002 bp) was isolated from papaya fruit, which encoded a 275 amino acid protein containing a 28 amino acid signal peptide in the N-terminal end.

The predicted molecular mass of the mature protein was 26.2 kDa (Kilo Dalton), and its pI value was 6.32. On the basis of its amino acid sequence homology with other plant chitinases, it was classified as a class IV chitinase. An active recombinant CpCHI enzyme was over expressed in *Escherichia coli*. The purified recombinant papaya chitinase showed an optimal reaction temperature at 30°C and a broad optimal pH ranging from 5.0 to 9.0. The recombinant enzyme was quite stable, retaining >64% activity for 3 weeks at 30 °C. The spore germination of *Alternaria brassicicola* could be completely inhibited by a 76 nM level of recombinant CpCHI.

Recombinant CpCHI also showed antibacterial activity in which 50% of *E. coli* was inhibited by a 2.5 micro Molar concentration of the enzyme.

cDNA= complimentary DNA produced by reverse transcriptase

N- terminal= Refers to the end of protein or polypeptide terminated by an amino acid with a free amino group. When protein is translated from mRNA, it is created from N-terminal to C-terminal.

PI value= means percent inhibition value

nM= nanomolar concentration

TREATMENT OF DIGESTIVE INSUFFICIENCY

(Bykov, Demina et al. 2000) described the usage of papain enzyme, isolated from *Carica papaya* extract, in the co-formulations along with other enzymes needed to cure digestive insufficiency.

Commercial products containing Papain for digestive insufficiency:-

1. WOBENZYM
2. UNIENZYM

3. ANTI-OXIDANT PROPERTIES

(Mehdipour, Yasa et al. 2006) studied the anti-oxidant properties of *Carica papaya* juice both in laboratory and pharmacological studies in rats and found the anti-oxidant properties are comparable to α -tocopherol. The study done by (Mehdipour, Yasa et al. 2006) is as follows:-

AIM OF THE STUDY

The present study was designed to explore the toxicological and antioxidant potential of dried *Carica papaya* juice *in vitro* and *in vivo*.

The oral LD50 of the juice of *Carica papaya* was determined, and the antioxidant potentials determined by DPPH and FRAP tests.

In vivo examination was performed after oral administration of dried papaya juice to rats for 2 weeks at doses of 100, 200 and 400 mg/kg. Blood TBARS and FRAP assays were used to determine the potential of the juice to act against oxidative stress. The acute toxicity test (LD50) demonstrated that papaya juice is not lethal up to a dose of 1500 mg/kg after oral

administration and thus is considered nontoxic. In treated groups, no sign of toxicity was observed.

In vitro evaluation of the antioxidant effects of papaya showed that the highest antioxidant activity (80%) was observed with a concentration of 17.6 mg/ml. Blood lipid peroxidation levels decreased significantly after administration of all doses of papaya juice (100, 200, 400 mg/kg/day) to 35.5%, 39.5% and 40.86% of the control, respectively, compared with a value of 28.8% for vitamin E. The blood total antioxidant power was increased significantly by all doses of papaya juice (100, 200, 400 mg/kg/day) to 11.11%, 23.58% and 23.14% of the control, respectively. The value for vitamin E was 18.44%. This preliminary study indicates the safety and antioxidative stress potential of the juice of *C. papaya*, which was found to be comparable to the standard antioxidant compound α -tocopherol.

DPPH= Diphenyl picryl hydrazyl

FRAP= Ferric reducing ability of plasma

TBARS= Thio barbituric acid

LD 50= Lethal dose required to kill 50 % of the organisms under study

4. MEDICINAL USES OF PAPYA ENZYMES

(Pendzhiev 2002) isolated different enzymes present in *Carica papaya* extract including proteolytic enzymes (papain, chymopapain, peptidases A and B, and lysozyme) having anti-inflammatory, anticoagulant, analgesic, bactericidal, Anthelmintic and hemolytic properties. These enzymes are capable of decomposing the proteins of polypeptides and amino acids and dissolving necrotized cells (while not affecting intact ones); these enzymes are widely used in medicine.

USE OF ENZYMES IN SURGERY

- The proteolytic and anti-inflammatory properties of papain allow this enzyme to be applied in surgery for the treatment of various fistulas, cleaning wounds from necrotized tissues, and preparing trophic ulcers for skin grafting epidermatoplasty). Good cleaning of wounds and the improved condition of soft tissues allowed the duration of using metal structures to be prolonged, thus increasing the consolidation of broken bones.

- The ability of papain to soften and partly dissipate newly formed pathological connecting tissues and hematomas is employed in ophthalmology. The proteolytic enzyme preparations are introduced by the Burgignon technique with the aid of an eye-bath, by endonasal electrophoresis, or by phonophoresis.

- Papain is also used in neurosurgery for facilitating cicatricial adhesions in the shells of brain and spinal cord, peripheral nerves, and spinal roots, which are typical complications of many neurosurgical disorders. The enzyme preparations are also used for the treatment of open and closed craniocerebral injuries and spinal-cord traumas, inflammatory and degenerative processes in the spinal column and ligamentous apparatus, and various formations in the peripheral nervous system. The use of proteolytic enzymes significantly improves the total effect of the active surgical invasion.

- In orthopedics, papain is used in vertebral surgery for the treatment of lumbar osteochondrosis (intravertebral introduction).

- Papain can be also employed for the treatment of intravertebral disk herniation and connective tissue disorders, such as the initial stage of Dupuytren's contracture. In the stage of pronounced digital contracture, papain can be used for partly decreasing the degree of contracture, preparing soft tissues for operation, and eliminating post-operation recidivations.

- In urology, papain can be used for the treatment of prostatitis by electrophoretic methods.

Due to presence of phenolic compounds, tannins, organic acids, glucose, flavonoids, lipids, coumarins, steroidal and triterpene saponins, and alkaloids, the leaves of papaya possess biligenic(bile like) properties. Papain was used in phlebology for the treatment of chronic venous insufficiency of lower extremities, which is typically caused by the primary varix dilatation or deep venous thrombosis. In surgical cosmetology, papain is used for accelerating post-operation scar tissue resorption and debriding small residual traces (hypertrophic and keloid scars) after plastic operations.

In gastroenterology, papain preparations are used for the treatment of gastritis, gastric ulcers, and constriction of Schatzki's ring (esophageal ring), [(Abdullaev, et al. 1998), (Pendzhiev, et al, 2000), (Starkov, et al. 1975), (Zaslavskii, et al, 1975), (Zaslavskii, et al, 1971)].

TOXICOLOGY

- (Wilson, Kwan et al. 2002) performed the following pharmacological study on animals (dog carotid artery) to study the effects of *Carica papaya* extract on mammalian vascular smooth muscles.

AIM OF THE STUDY

To investigate their potentially toxic effects on mammalian vascular smooth muscle.

MATERIALS AND METHODS

Pentane extracts of papaya seeds and the chief active ingredient in the extracts, benzyl isothiocyanate (BITC), were tested for their effects on the contraction of strips of dog carotid artery. BITC and the papaya seed extract caused relaxation when added to tissue strips that had been pre-contracted with phenylephrine (PE). Incubation of the tissue with papaya seed extract or BITC caused inhibition of contraction when the strips were subsequently contracted with KCl (Potassium chloride) or PE.

This relaxation and inhibition of contraction did not appear to be endothelium-dependent, as endothelium-denuded rings showed the same degree of relaxation or inhibition of contraction in response to the preparations/drugs as those with the endothelium intact. Inhibition of relaxatory effects of carbachol (CCh) further supports this relaxatory effect independent of endothelial derived relaxatory factors.

The effects of both BITC and the extract were irreversible, i.e., the tissue did not recover to normal contractile ability after extensive washing. Exposure of the tissue to the papaya seed extract caused slower relaxation of the tissue, compared to controls, both after contraction with PE and subsequent addition of carbachol (CCh), and after contraction with KCl and then washing. Calcium imaging studies using cultured endothelial cells showed strong influxes of

Ca²⁺ into the cells, in a tissue specific manner, in response to addition of the papaya seed extract.

RESULT

It was concluded that these extracts induced vasorelaxatory effects on vascular smooth muscles in a dose dependent manner; when present in high concentration, are cytotoxic by increasing the membrane permeability to Ca²⁺ it would interfere with the ability of vascular smooth muscle to maintain vascular tone, and lead to toxic effects in the animal causing paralysis of cells as is evident by the morphological changes in cells. The vascular effects of papaya seed extracts are consistent with the notion that BITC is the chief bio-active ingredient.

RESULT/CONCLUSION

Carica papaya extracts contains a bio-active ingredient benzyl isothiocyanate (BITC) which is responsible for the relaxatory/ vasodilatory effect of its extract. But increased influx of Ca²⁺ causes cytotoxicity.

- (Mehdipour et al, 2006) determined in studies in rats that up to 1500mg/ Kg body weight is not toxic.
- *Carica papaya* unripe fruit has also been traditionally used as Abortifacient that is why it should not be given to pregnant women and the women trying to become pregnant.

DISCUSSION/ CONCLUSION

➤ CONTRACEPTIVE AND ABORTIFICIENT EFFECTS

The studies done by Britain's University of Sussex (Asia week, 1994) have proven the abortifacient and contraceptive effects of *Carica papaya* in women. The proteolytic enzyme Papain present in unripe fruit of papaya has been suggested to be the ingredient responsible for this effect. It suppresses the progesterone and can also break down a membrane vital for the development of fetus.

(Oderinde 2002) demonstrated dose dependent abortifacient effects of unripe *Carica papaya* fruit extract in animal studies done in rats. Further it was observed that abortifacient potential is related to higher doses greater than 800mg/kg.

These facts give scientific basis for abortifacient and contraceptive usage of unripe fruit of *Carica papaya* in Asia Minor.

➤ TREATMENT OF DIGESTIVE INSUFFICIENCY

(Bykov et al, 2000) The persons having abnormal enzyme production due to disease conditions like Chronic pancreatitis with excretory insufficiency, pancreatectomy and pancreatic cell carcinoma, liver and bile tract disorders upon stomach and/or small intestine resection, chronic enteritis, gastritis with secretory insufficiency etc, needs treatment with digestive enzymes like, pepsin, lipase and papain. The extract obtained from *Carica papaya* is rich in Papain proteolytic enzyme along with other enzymes, is a part of commercial preparations to cure digestive insufficiency. These facts support the traditional usage of papaya for digestive problems.

> ANTI-PARASITIAL EFFECTS

●ANTHELMINTIC EFFECTS

The scientific work done by (Stepak et al, 2007) proves the traditional anthelmintic usage of *Carica papaya* in humans and livestock.

In addition the mechanism of action of these plant enzymes (i.e. an attack on the protective cuticle of the worm) suggests that resistance would be slow to develop in the field.

The efficacy and mode of action make plant cysteine proteinases potential candidates for a novel class of anthelmintic urgently required for the treatment of humans and domestic livestock. However the toxicity and the potential side effects should be further researched to find the safe and effective dosages.

●ANTI-TRICHOMONAL EFFECTS

(Calzada 2007) concluded, in the in vitro scientific work done on the *Trichomonas vaginalis*; vaginitis causing parasite, that *Carica papaya extract* shows the best inhibitory concentration with IC 50 5.6 µg/ ml .The results of the antiprotozoal screening support the traditional usage of *Carica papaya* for urogenital disorders. However, seeds of *Carica papaya* and aerial parts of *Bocconia frutescens* should be used in herbal medicine with care to avoid toxicity.

There is also need for the clinical studies and the studies to find out the active ingredients responsible for this effect.

●ANTI-FUNGAL ACTIVITY

(Chen, Hsu et al. 2007) described the presence of Chitinase enzyme (glycosyl hydrolase) in the *Carica papaya* fruit; this enzyme contributes to plant defense due to their ability to degrade the cell wall and inhibit the growth of fungal pathogens. These facts support the traditional usage of *Carica papaya* as anti-fungal. Further pharmacological research could lead to a novel pharmaceutical topical anti-fungal ointments or cream, which could treat effectively the problem of various types of fungus infections in humans.

It could also be employed as a natural defense against pathogens to economically important plant crops.

➤ ANTI-OXIDANT EFFECTS

(Mehdipour et al, 2006) demonstrated by both in vivo and in vitro studies that the anti-oxidant potential of *Carica papaya* is comparable to the α -tocopherol supports the traditional usage of *Carica papaya* for various types of ailments.

The anti-oxidant activity may be due to presence of flavonoids and vitamin C.

The inhibition of lipid peroxidases in the studies done by (Mehdipour et al, 2006) may support the usage of *Carica papaya* as analgesic and anti-pyretic in the traditional health care settings in Pakistan and India.

➤ VASODILATORY EFFECTS

(Wilson, Kwan et al. 2002) studied the relaxatory effects caused by the *Carica papaya* extract on intact dog artery. The papaya extract contains benzyl isothiocyanate (BITC) which is responsible for vasodilatory effects, which is independent of endothelial derived relaxatory factors i.e. Nitric oxide (NO) etc.

The increased influx of Ca^{2+} damages the cells as is observed by the morphological studies of dog carotid artery after exposure to *Carica papaya* extract (BITC) in a dose dependent manner.

The calculated lethal dose for *Caenorhabditis elegans* [a free living nematode (round worm)] is 0.04-0.1 μ L in a 0, 5 ml in vitro assay (1:1000).

When *Carica papaya* containing BITC is taken orally it result in a final dilution of 1:40,000 which is equivalent to a BITC dilution of about 1:4,000,000 or 0.19 μ M, assuming that it is widely distributed in the body tissues(there is about 40 Liter water in the human body). After dilution BITC reaches up to a level which does not cause irreversible relaxation/ damage to tissues.

These facts may support the traditional usage for high blood pressure. However further detailed clinical research is needed before *Carica papaya* extract could be recommended to safe usage for high blood pressure for human beings.

➤ **MEDICINAL USES OF PAPA ENZYMES**

(Pendzhiev 2002) described various applications in surgery, of enzymes isolated from milky juice obtained from *Carica papaya* fruit. This scientific work gives scientific basis for traditional usage of *Carica papaya* for various diseases.

These facts indicate that the *Carica papaya* contains proteolytic enzymes which offer the same chondrolytic properties close to those of the analogous imported preparations. Using this domestic preparation, it is possible to ensure the effective therapy of patients with various disorders and restrict the use of deficient and expensive preparations. This could be a good alternative for a developing country like Pakistan etc.

Chondrolysis= the degeneration of cartilage cells that occurs in the process of intracartilaginous ossification (formation of cartilages).

➤ **COSMETIC INDUSTRY**

Carica papaya enzymes has also been used by the cosmetic industry in the production of exfoliating creams, skin shining creams, anti-wrinkle and skin protecting formulations (Shetgiri, Shetgiri et al. 2008), (Cho, Kim et al. 1999; Ye 2004), (Ye 2004; Adachi, Tada et al. 2006).

REFERENCES

A. K. Abdullaev and A. M. Pendzhiev. *Zdrav. Turkmen.*, No. 4, 20 – 22 (1998).

A. M. Pendzhiev, *Author's Abstract of Doctoral Thesis* [in Russian], Moscow (2000).

Adachi, K., T. Tada, et al. (2006). Skin-lightening cosmetics containing *Carica papaya* extracts. Application: JP

JP, (Mikimoto Pharmaceutical Co., Ltd., Japan). 12 pp.

Bykov, V., N. Demina, et al. (2000). "Enzyme preparations used for the treatment of digestion insufficiency (a review)." Pharmaceutical Chemistry Journal **34**(3): 105-109.

Calzada, F., L. Yepez-Mulia, et al. (2007). "Effect of Mexican medicinal plant used to treat trichomoniasis on *Trichomonas vaginalis* trophozoites." J Ethnopharmacol **113**(2): 248-51.

Chen, Y.-T., L.-H. Hsu, et al. (2007). "Gene Cloning and Characterization of a Novel Recombinant Antifungal Chitinase from Papaya (*Carica papaya*)." J. Agric. Food Chem. **55**(3): 714-722.

Cho, B. K., Y. J. Kim, et al. (1999). Basic cosmetic compositions having wrinkle-suppressing effect without skin irritation. Application: KR
KR, (Pacific Co., Ltd., S. Korea). No pp given.

Lohiya Nirmal, K., B. Manivannan, et al. (2006). "Toxicological investigations on the methanol sub-fraction of the seeds of *Carica papaya* as a male contraceptive in albino rats." Reprod Toxicol **22**(3): 461-8.

Mehdipour, S., N. Yasa, et al. (2006). "Antioxidant potentials of Iranian *Carica papaya* juice in vitro and in vivo are comparable to alpha-tocopherol." Phytother Res **20**(7): 591-4.

Oderinde, O., C. Noronha, et al. (2002). "Abortifacient properties of aqueous extract of *Carica papaya* (Linn) seeds on female Sprague-Dawley rats." Niger Postgrad Med J **9**(2): 95-8.

Pendzhiev, A. M. (2002). "Proteolytic Enzymes of Papaya: Medicinal Applications." Pharmaceutical Chemistry Journal **36**(6): 315-317.

Shetgiri, N. P., P. P. Shetgiri, et al. (2008). Novel composition of papain as desloughing skin care agents and method thereof. Application: IN IN, (India). 27pp.

Steppek, G., A. E. Lowe, et al. (2007). "The anthelmintic efficacy of plant-derived cysteine proteinases against the rodent gastrointestinal nematode, *Heligmosomoides polygyrus*, in vivo." Parasitology **134**(10): 1409-1419.

Wilson, R. K., T. K. Kwan, et al. (2002). "Effects of papaya seed extract and benzyl isothiocyanate on vascular contraction." Life Sciences **71**(5): 497-507.

Ye, G. (2004). Skin protecting preparations containing papaya ingredients. Application: CN CN, (Ye Yongxin, Peop. Rep. China). No pp given.

PHOTO REFERENCE

http://www.vuatkerala.org/static/mal/advisory/agri/papaya/image/Carica_papaya_-_papaya_-_var-tropical_dwarf_papaya_-_desc-fruit%5B1%5D.jpg

Retrieved in December, 2008.

Dioscorea floribunda



BOTANICAL NAME: - *Dioscorea floribunda* M.Martens & Galeotti

URDU/ LOCAL NAME: - Ratalo

FAMILY: - Dioscoreaceae

PARTS OF PLANT USED:-

Dried root tuber.

DESCRIPTION

Dioscorea floribunda is cultivated in tropical and subtropical regions of the world as a source of natural diosgenin. Various steroidal drugs like corticosteroids, sex hormones and anabolic steroids are derived from diosgenin by artificial synthesis.

Dioscorea floribunda like other yams is a tropical plant and requires a long frost-free growing season. The palm like pyramidal tuber is divisible into three distinct parts -crown (stem end with preformed buds), median (middle) and tip (distal end). All the three parts are used for vegetative propagation (Bammi, RK et al. 1982). A monsoon climate followed by a dry season with shorter days is ideal for the cultivation of this crop (Martin, FR. 1972). Both day length and temperature influence the developmental cycle. In the spring, when the days lengthen and temperature increases, the plants develop vigorous vines, and with the change in season the rate of tuber development increases as the days become shorter. Finally, a time is reached, apparently controlled by day length but also influenced by the availability of water and by low temperature, when the vines die back and the tuber enters a period of dormancy. The tubers are able to withstand low temperature and sprout again in spring to follow a normal life cycle. It has been suggested that ABA (Abscisic acid) may be involved in the dormancy mechanisms of tubers of other vegetable species (Coleman, WK. et al. 1984) and this may well be the case in *Dioscorea* (Gupta, S et al. 1979).

ETHNOPHARMACOLOGY

USES IN PAKISTAN

- ANTITUSSIVE

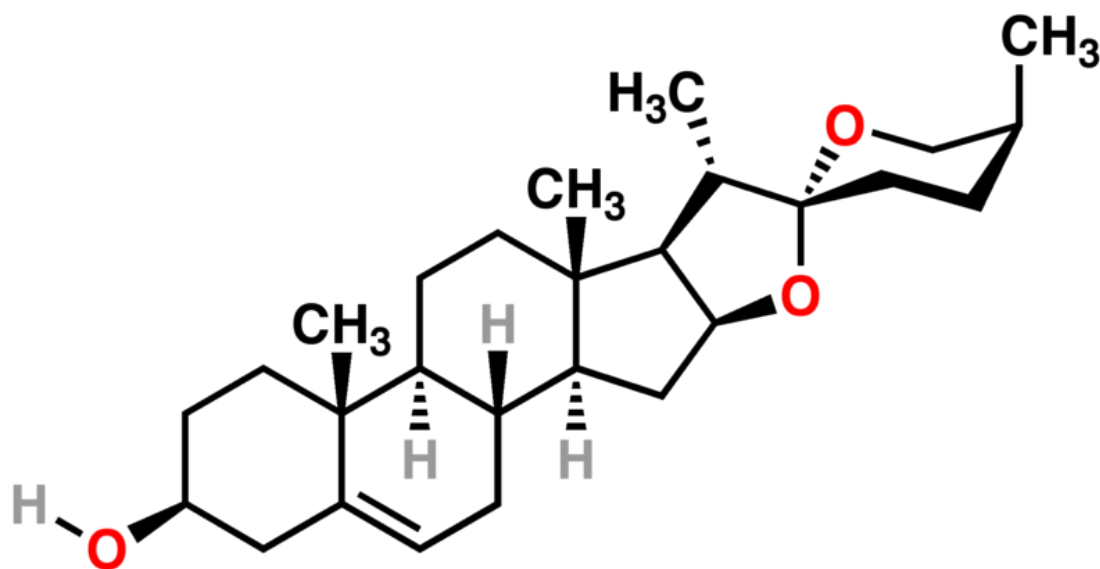
USES IN ENGLAND

- Rheumatoid arthritis to relive pain and inflammation
(British herbal pharmacopoeia)

CHEMISTRY

- The principle ingredients present are saponin glycosides of which sapogenin (non glycoside portion “aglycon”) “DIOSGENIN” is therapeutically important.

(De and De 2005)



Structure of Diosgenin as proposed by (Anthony, C. et al. 2002)

- Genetic improvement (cloning) gave increased Diosgenin production as studied by (Dixit, Banerji et al. 2000).
- Abscisic acid and phthalic acid and Batatasin-1 are present. Concentrations of these growth inhibitors vary during dormancy and active growth periods as studied by (Farooqi, Shukla et al. 1989).

- The root also contains phytosterols, alkaloids, tannin and a high level of starch. Other materials present include Aluminum, Ascorbic-acid, Ash, Beta-carotene, Calcium, Chromium, Cobalt, Iron, Magnesium, Manganese, Niacin, Phosphorus, Potassium, Protein, Riboflavin, Selenium, Silicon, Sodium, Thiamin, Tin, Zinc (Anthony C. et. al, 2002).

PHARMACOLOGY

1. ANTIPHLOGISTIC EFFECTS

Saponins and sapogenins were tested for anti-inflammatory effects in rats and mice at a rate of 10 mg/kg using exudative and proliferative models. The strongest antiphlogistic effect was seen amongst the furostane group of saponins (which yield diosgenin) as studied by (Syroc et. al, 1992).

2. OESTROGENIC EFFECTS

Diosgenin was given to ovariectomised mice at 20 & 40 mg/kg daily for 15 days. The diosgenin stimulated the growth of mammary epithelium as shown by an increase in DNA content, number of ducts and the appearance of terminal end buds. Concomitant treatment with oestrogen and diosgenin showed augmentation of the oestrogenic effect of diosgenin especially at the higher dose. Diosgenin showed a lack of progesterogenic activity as shown by the absence of alveolar development even in the presence of exogenous oestrogen (Rao, et al. 1992).

3. TREATMENT OF BREAST CANCER

The role of Diosgenin in the treatment of breast cancer was studied by (Chiang, Way et al. 2007) in the following study:-

Fatty acid synthases (FAS) expression is markedly elevated in HER2-overexpressing breast cancer cells. In this study, diosgenin, a plant-derived steroid, was found to be effective in suppressing FAS expression in HER2-overexpressing breast cancer cells. Diosgenin preferentially inhibited proliferation and induced apoptosis in HER2-overexpressing cancer cells. Furthermore, diosgenin inhibited the phosphorylation of Akt and mTOR, and enhanced phosphorylation of JNK. The use of pharmacological inhibitors revealed that the modulation of Akt, mTOR and JNK phosphorylation was required for diosgenin-induced FAS suppression. Finally it was proved that diosgenin could enhance paclitaxel-induced cytotoxicity in HER2-overexpressing cancer cells. These results suggested that diosgenin has the potential to advance as chemo preventive or chemotherapeutic agent for cancers that over express HER2.

4. ANTIAGING EFFECT

Diosgenin has shown anti aging effect owing to stimulation of estrogenic receptors in the skin, anti wrinkles effect and anticollagenase activity as shown by the following studies:-

➤ ANTI WRINKLES EFFECT

Sapogenin (non glycoside portion of a saponin glycoside) is a part of external formulation to treat wrinkles (Besne 2003).

➤ ANTICOLLAGENASE ACTIVITY

Sapogenin portion of *Dioscorea floribunda* (Diosgenin) extract exhibits anticollagenase activity thus contribute to keep firmness of skin and protect against/ slowdown aging as studied by (Liviero and Allec 2002).

➤ FACE LIFT THROUGH MUSCULO-APONEUROTIC SYSTEM

Diosgenin by interacting with musculo-aponeurotic system or the receptors of estrogen or progesterone present in human thus skin plays a role in face-lift products (Eymard 2004).

5. VASODILATORY EFFECT

Vasodilatory/ vasorelaxant effect of Dioscorea extract was studied by (Dias, Correia et al. 2007) in the following study:-

MATERIALS AND METHODS

The aim of this study was to investigate the vasorelaxant effect induced by diosgenin in superior mesenteric rings in rat superior mesenteric artery. In rings pre-contracted with phenylephrine (10 μ M), diosgenin caused concentration-dependent relaxations [$EC_{50} = (3.3 \pm 1.2) \times 10^{-4}$ M, $E_{max} = 94.2 \pm 2.6$ %]. Vascular relaxation induced by diosgenin was significantly inhibited after removal of the endothelium ($E_{max} = 46 \pm 8.8\%$, $p < 0.001$) or after pre-treatment of the rings with *N*-nitro-*l*-arginine methyl ester (l-NAME) 100 or 300 μ M ($E_{max} = 35.3 \pm 4\%$; $28.1 \pm 3.3\%$, respectively, $p < 0.001$), atropine 1 μ M ($E_{max} = 24.6 \pm 3.4\%$, $p < 0.001$), hydroxocobalamin 30 μ M ($E_{max} = 54.0 \pm 9.6\%$, $p < 0.001$), 1H-[1,2,4]oxadiazolo-[4,3-*a*]quinoxalin-1-one (ODQ) 10 μ M ($E_{max} = 46.0 \pm 8.0\%$, $p < 0.001$) or indomethacin 1 μ M ($E_{max} = 22.6 \pm 11.8\%$, $p < 0.001$). Vasorelaxation evoked by diosgenin was significantly inhibited after pre-treatment of preparations with both selective and non-selective inhibitors of large conductance Ca^{2+} -activated K^+ (BK_{Ca}) channels, iberiotoxin 100nM or tetraethyl ammonium (TEA) 1mM, respectively ($E_{max} = 62.5 \pm 9.1\%$; $65.7 \pm 1.1\%$, $p < 0.001$).

Conversely, in endothelium-denuded vessels, none of BK_{Ca} channel blockers modified the relaxant effect induced by diosgenin. In mesenteric endothelial cells loaded with FURA-2 diosgenin was able to increase intracellular calcium concentrations, which were significantly decreased by atropine 1 μ M. In addition, in isolated mesenteric rings, diosgenin induced marked increase in nitric oxide (NO) levels, which was completely abolished after functional endothelium removal. The results obtained here demonstrated that diosgenin-induced relaxation appears to involve endothelial muscarinic receptor activation with increase in intracellular calcium concentrations and consequent release of endothelium-derived relaxing factors (EDRFs), mainly NO and cyclo-oxygenase derivatives, which activate BK_{Ca} channels. Nevertheless, further studies are necessary to clearly elucidate residual endothelium-independent relaxation induced by diosgenin.

EC 50= Half maximal effective concentration; Concentration of a drug which induces a response halfway between baseline and maximum.

Emax= The concentration required to produce maximum effect.

mM= Millimolar concentration

μ M= Micromolar concentration

nM= Nanomolar concentration

FURA-2= This is a ratiometric fluorescent dye which binds to free intracellular calcium.

TOXICOLOGY

1. Upon intravenous injection saponin glycosides causes hemolysis(phytotherapies.org).
2. Weak diarrhoea is being reported in some cases that is why the person having weak digestion should use it carefully(phytotherapies.org).
3. Dioscorea is generally considered safe in all trimesters of pregnancy and is traditionally indicated for nausea of pregnancy (McIntyre, A. 1995), and for prevention of early miscarriage.

DISCUSSEION/CONCLUSION

The activity of *Dioscorea floribunda* has been attributed to the action of various steroidal saponins (diosgenin an aglycon).

➤ **ANTITUSSIVE EFFECTS**

Dioscorea floribunda extract suppresses cough reflexes (Phytotherapies.org) this theory reinforces traditional usage of *Dioscorea floribunda* as antitussive in Pakistan.

➤ **VASORELAXATORY EFFECTS**

Diosgenin has shown vasorelaxant effect in the studies done, on endothelial cells isolated from rat superior mesenteric artery, by (Dias, Correia et al. 2007). It was demonstrated that diosgenin induces vasorelaxation in an endothelium dependent manner. Endothelium dependent relaxatory effects is mediated through (BK_{Ca}) channels, also involves NO/ CGMP pathway, and is significantly inhibited by pre-treatment with (BK_{Ca}) channels inhibitors. This effect was completely abolished by removal of endothelium.

Increase in the intracellular calcium levels regulates the activity of endothelial NOS and neuronal NOS isozymes that produce NO (nitric oxide) in vessels and neurons, respectively (Lopez-Ramos et al. 2005). In primary culture of endothelia mesenteric cells, diosgenin and acetylcholine induced an increase in the intracellular calcium levels and this effect was significantly abolished after atropine (1μM) administration, suggesting that the increase in the intracellular calcium levels are probably due to muscarinic receptor activation present in endothelial cells.

In conclusion it has been demonstrated in the work done by the studies named above that EDRFs (mainly NO and COX derivatives) and activation of (BK_{Ca}) channels play a major role in the vasorelaxation induced by diosgenin in rat superior mesenteric artery. Nevertheless, further studies are necessary to clearly elucidate the residual endothelium- independent vasorelaxatory effect induced by diosgenin in mesenteric arteries.

➤ **ANTI-INFLAMMATORY EFFECTS**

There is no doubt that the phytosterols (of which diosgenin is a member) will give soothing and anti-inflammatory effect to the extract and so be a very useful topical additive to a skin cream for mature and dry skin types. This is being borne out in some of the latest studies being published on Wild Yam extracts targeted at the skin care formulator (Anthony C. et. al, 2002).

➤ **ANTI-RHEUMATIC EFFECTS**

Steroidal nature of diosgenin justifies the use of *Dioscorea floribunda* along with other *Dioscorea* species in England against rheumatism related inflammation and pain (Anthony C. et al 2002).

➤ **ANTI-CANCER EFFECTS**

(Chiang, Way et al. 2007) demonstrated the effects of *Dioscorea floribunda* extract on HER2 expressing tumors but further studies should be done to find out either this activity could be used as adjuvant to modern treatment methods.

➤ **COMMERCIAL SYNTHESIS OF STEROIDS**

Sugar free aglycon; Diosgenin derived from *Dioscorea floribunda* and other members in the *Dioscoreaceae* family can be used as a starting material in the production of steroids(like cortisone, pregnenolone and progesterone as studied by (Marker and Krueger 1940).

➤ **COSMETIC USES**

Anticollagenase effects induced by diosgenin as studied by (Liviero and Allec 2002) and usage in facial and neck cosmetics for anti aging as described by (Liviero and Allec 2002) and anti-wrinkle treatment(Besne 2003) and face lift(Eymard M, 2004) clearly gives scientific basis for anti-aging effects and traditional usage of *Dioscorea floribunda* in topical anti-aging formulations.

REFERENCES

Anthony C. Dweck FLS FRSH FRSC The Wild Yam – review of *Dioscorea* species, Personal Care Magazine **3**, 3, p.7-9. (2002).

Besne, I. (2003). Use of a sapogenin or a sapogenin-containing plant extract for smoothing skin lines and wrinkles. Application: EP, (L'Oreal, Fr.). 10 pp.

Bammi RK and Gangadhara Rao G (1982) Commercial cultivation of *Dioscorea floribunda* ,
In: Atal CK and Kapur BM, eds . Cultivation and Utilization of Medicinal Plants, pp
.77-86 . Regional Research Laboratory, Jammu-Tawi (India).

British Herbal Pharmacopoeia, The: 1983. ISBN 0-903032-07-4. British Herbal
Manufacturers Association (B.H.M.A).

Chiang, C. T., T. D. Way, et al. (2007). "Diosgenin, a naturally occurring steroid, suppresses fatty acid syntheses expression in HER2-overexpressing breast cancer cells through modulating Akt, mTOR and JNK phosphorylation." Journal.

Coleman WK and King RR (1984) Changes in endogenous abscisic acid, soluble sugars and proline levels during tuber dormancy in *Solanum tuberosum* L. Am Potato J 61:437-449.

De, D. and B. De (2005). "Elicitation of diosgenin production in *Dioscorea floribunda* by ethylene-generating agent." Fitoterapia **76**(2): 153-156.

Dias, K. L. G., N. d. A. Correia, et al. (2007). "Mechanisms involved in the vasodilator effect induced by diosgenin in rat superior mesenteric artery." European Journal of Pharmacology **574**(2-3): 172-178.

Dixit, B. S., R. Banerji, et al. (2000). "High diosgenin content in some selected clones of *Dioscorea floribunda*." Indian J. Pharm. Sci. **62**(1): 53-54.

Eymard, M. (2004). Topical diosgenin-based compositions. Application: FR
FR, (Laboratoire de Dermo-Cosmologie, Fr.). 17 pp.

Farooqi, A. H. A., Y. N. Shukla, et al. (1989). "Endogenous inhibitors and seasonal changes in abscisic acid in *Dioscorea floribunda* Mart. & Gal." Plant Growth Regul. **8**(3): 225-32.

Gupta S, Sobti SN and Atal CK (1979) Tuber dormancy in *Dioscorea composita* Hemsil: Effect of growth regulators. *Indian J Exp Biol* 17: 612-614.

Krochmal, Arnold & Connie: A guide to the Medicinal plants of the United States. Quadrangle, the New York Times Book Co. 1973. ISBN No. 0-8129-0261-0.

Liviero, C. and J. Allec (2002). Use of at least a sapogenin for preventing skin ageing symptoms. Application: WO
WO, (L'oreal, Fr.). 21 pp.

López-Ramos, J.C., Martínez-Romero, R., Molina, F., Cañuelo, A., Martínez-Lara, E., Siles, E., Peinado, M.A., 2005. Evidence of a decrease in nitric-oxide-storage molecules following acute hypoxia and/or hypobaria, by means of chemiluminescence analysis. *Nitric Oxide* 13, 62–67.

McIntyre, A, 1995, *The Complete Woman's Herbal*, Hodder Headline Pty Ltd, Australia, p 29.

Marker, R. E. and J. Krueger (1940). "Sterols. CXII. Sapogenins. XLI. The Preparation of Trillin and its Conversion to Progesterone." J. Am. Chem. Soc. **62**(12): 3349-3350.

Martin FR (1972) Yam production methods: Production research report no. 147. Agriculture Research Service USA: P.1-17.

Rao, A, Rao, AR, Kale, RK, Diosgenin - A growth stimulator of mammary gland of ovariectomised mouse, *Indian J Exp Biol.* 30(5): pp 367-70, 1992.

Syroc, VN, Kravets, KD, Khushbaktova, ZA, et al, Steroid genins and glycosides as anti-inflammatory agents, *Khim-Farm Zh.* 26(5): pp 71-6, 1992.

REFERENCES FROM INTERNETT

www.phytotherapy.org (Tricky R)

PHOTO REFERENCE

<http://www.bicco.com/imaze/image012.jpg>

Retrieved in December, 2008.

Citrullus colocynthis



BOTANICAL NAME:-*Citrullus colocynthis* (L.) schrad.

ENGLISH NAME: - COLOCYNTH

URDU/ LOCAL NAME: - Korhtumma

FAMILY: - Cucurbitaceae

PARTS OF PLANT USED:-

Dried fruit pulp, leaves.

ETHNOPHARMACOLOGY

USES IN PAKISTAN

- CATHARTIC
- TREATMENT OF PARASITIC DISEASES IN LIVSTOCK

(Farooq, Iqbal et al. 2008), (Jabbar, Raza Muhammad et al. 2006)

USES IN UAE(United Arab Emirates)

- Diabetes (Al-Ghaithi, El-Ridi et al. 2004)

USES IN AFRICA (Nigeria)

For the treatment of sick camels(Antoine-Moussiaux, Faye et al. 2007).

USES IN USA

Citrullus colocynthis extract has been used in a topical preparation for the treatment of dermal abrasions in animals to avoid licking of wound.

(Berdami 2001) described usage of *Citrullus colocynthis* in a topical preparation, being used in the United States of America for epidermal abrasions, as bitter principle to avoid pets from licking wounds. This topical preparation contains *Citrullus colocynthis* powder 2-5 gram

along with other ingredients including bacitracin zinc 300 neomycin 35, polymyxin-B sulfate 10,000 mg, and pramoxine-HCl 10 mg. White petroleum jelly is used as a solvent and carrier for the active ingredients.

USES IN IRAN

- Abortifacient
- Treatment of constipation
- Edema
- Bacterial infections
- Cancer
- Diabetes

(Delazar, Gibbons et al. 2006)

CHEMISTRY

CUCURBITACIN GLYCOSIDES

(Tannin-Spitz, Grossman et al. 2007).(Tannin-Spitz, Bergman et al. 2007),

(S, E. Adam, et al. 2001)

Citrullus colocynthis extract contain:-

- Cucurbitacin A
- Cucurbitacin B
- Cucurbitacin C
- Cucurbitacin D
- Cucurbitacin E(α -elaterin)

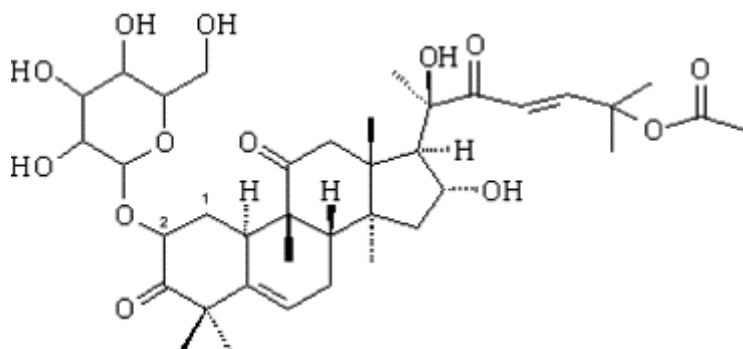


Fig. Chemical structure of Cucurbitacin B glycoside (MW = 739). Structure of Cucurbitacin E glycoside (MW = 737) is the same as Cucurbitacin B glycoside except for a double bond between carbons 1 and 2 (MW= Molecular weight).

(Delazar, Gibbons et al. 2006) isolated following two Cucurbitacin glycosides from the *Citrullus colocynthis* extract:-

4. 2-O-beta -D-glucopyranosylcucurbitacin I
5. 2-O-beta -D-glucopyranosylcucurbitacin L

FLAVONOIDS

(Delazar, Gibbons et al. 2006) isolated 3 flavonoids and two Cucurbitacin glycosides (given above) by extractions and reverse phase preparative HPLC (High performance liquid chromatography).

1. Isosaponarin
2. Isovitexin
3. Isoorientin 3`-O-Methyl ether

1-3 have shown significant antioxidant properties.

MATERIALS AND METHODS

The reversed-phase preparative HPLC was employed to isolate compounds from the butanol fraction of the hydro-methanolic (70%) extract of the fruits of the locally grown *Citrullus colocynthis*. Structures of the isolated compounds [1-5] were elucidated by spectroscopic means. Three flavones glycosides, Isosaponarin [1], Isovitexin [2] and Isoorientin 3'-O-Me ether [3] and two Cucurbitacin glycosides, 2-O-beta -D-glucopyranosylcucurbitacin I [4] and 2-O-beta -D-glucopyranosylcucurbitacin L [5] were isolated and identified.

ASCORBIC ACID CONTENT

(Hussain, Khan et al. 2008) determined Vitamin C content of *Citrullus colocynthis* to be 161.42 mg/ 100g.

PHARMACOLOGY

1. ANTI-DIABETIC EFFECTS

(Sangameswaran, Balakrishnan et al. 2008) proved the hypoglycemic effects of *Citrullus colocynthis* on alloxan induced diabetic rats in the following study:-

AIM OF THE STUDY

This study was designed to investigate the anti-hyperglycemic effect of leaves of *Citrullus colocynthis* (Cucurbitaceae) in normal and alloxan induced diabetic rats.

Diabetes was induced in adult Male rats of Wistar strain, weighing 150-200g by administration of alloxan (150 mg/kg body wt.) i.p (Intraperitoneally). Diabetic rats showed an increase in levels of blood glucose.

The methanolic and aqueous extracts of *Citrullus colocynthis* were given to the experimental group for a period of 12 days. *Citrullus colocynthis* extracts showed significant hypoglycemic activity in experimental rats as compared with the diabetic control group in a 12 days study. The anti-diabetic activity was also compared with the standard anti-diabetic agent, glibenclamide (500micro g/kg). The result indicates that methanol and aqueous extracts showed significant ($P < 0.01$) anti-diabetic activity when compared with standard and diabetic control.

2. ANTIOXIDANT EFFECT

(Delazar, Gibbons et al. 2006) isolated 3 different flavonoids from the *Citrullus colocynthis* extract i-Isosaponarin, ii- Isovitexin and iii- Isoorientin 3`-O-Methyl ether. All of them exhibited significant anti-oxidant effect in 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay.

3. ANTI-CANCER(CYTOTOXIC) EFFECTS

(Tannin-Spitz, Grossman et al. 2007) studied the effect of *Citrullus colocynthis* plant on human breast cell cancer in the laboratory studies as follows:-

AIM OF THE STUDY

Aim of the study was to study the effects of Cucurbitacin glycosides extracted from *Citrullus colocynthis* leaves on human breast cancer cell growth.

Leaves were extracted, resulting in the identification of Cucurbitacin B/E glycosides.

The Cucurbitacin glycoside combination (1:1) inhibited growth of ER+ MCF-7 and ER_ MDA-MB-231 human breast cancer cell lines.

Cell-cycle analysis showed that treatment with isolated Cucurbitacin glycoside combination resulted in accumulation of cells at the G2/M phase of the cell cycle. Treated cells showed rapid reduction in the level of the key protein complex necessary to the regulation of G2 exit and initiation of mitosis, namely the p34CDC2/cyclin B1 complex.

Cucurbitacin glycoside treatment also caused changes in the overall cell morphology from an elongated form to a round-shaped cell, which indicates that Cucurbitacin treatment caused impairment of actin filament organization. This profound morphological change might also influence intracellular signaling by molecules such as PKB, resulting in inhibition in the transmission of survival signals.

Reduction in PKB phosphorylation and inhibition of survivin, an antiapoptosis family member, was observed.

The treatment caused elevation in p-STAT3 and in p21WAF, proven to be a STAT3 positive target in absence of survival signals. Cucurbitacin glycoside treatment also induced apoptosis, as measured by Annexin V/propidium iodide staining and by changes in mitochondrial membrane potential (DC) using a fluorescent dye, JC-1.

These facts indicates that Cucurbitacin glycosides exhibit pleiotropic effects on cells, causing both cell cycle arrest and apoptosis. These results suggest that Cucurbitacin glycosides might have therapeutic value against breast cancer cells.

PKB= Protein kinase B

Pleiotropiy= The quality of a gene to manifest itself in more than one way i.e. to produce more than one phenotypic expression.

4. ANTI-PARASITAL EFFECTS

(Farooq, Iqbal et al. 2008) described anti-parasital effects of *Citrullus colocynthis* in the following study on livestock in Pakistan.

AIM OF THE STUDY

This study was conducted to document the ethno veterinary medicinal (EVM) practices for the treatment of different parasitic diseases of livestock in Cholistan desert, Pakistan.

MATERIALS AND METHODS

An initial reconnaissance survey (rapid rural appraisal) among the local shepherds was conducted to identify the traditional healers. Information was collected from the traditional healers using a well-structured questionnaire through open-ended interviews and guided dialogue technique.

RESULTS

The parasitic diseases reported in livestock were:

- (i)- Tick and lice infestation
- (ii) Mange
- (iii) Myiasis; a condition caused by infestation of the body by fly maggots.
- (iv) Helminthiasis.

A total of 77 ethno veterinary practices comprising of 49 based on plant usage and 28 based on dairy products, chemicals and other organic matter were documented. A total of 18 plant species representing 14 families were documented to treat the parasitic diseases.

The plants included: *Citrullus colocynthis* (Cucurbitaceae), *Aerva javanica* (Amaranthaceae), *Aizoon carariense* (Aizoaceae), *Azadirachta indica* (Meliaceae), *Brassica campestris* (Cruciferae), *Capparis decidua* (Capparaceae), *Capsicum annuum* (Solanaceae), *Cyperus rotundus* (Cyperaceae), *Calligonum polygonoides* (Polygonaceae), *Eruca sativa* (Cruciferae), *Ferula assafoetida* (Umbelliferae), *Haloxylon salicornicum* (Chenopodiaceae), *Mallotus philippinensis* (Euphorbiaceae), *Nicotiana tabacum* (Solanaceae), *Pinus roxburghii* (Pinaceae), *Salsola baryosma* (Chenopodiaceae), *Solanum surratens* (Solanaceae) and *Zingiber officinale* (Zingiberaceae).

(Jabbar, Raza Muhammad et al. 2006) surveyed in southern Punjab, Pakistan, in order to document existing ethnobotanical knowledge by the herdsmen/key respondents about anthelmintic in ruminants. A 3 stage process was used to document the plants being used to treat and/or control helminthes. This study describes 29 plants to treat helminthiasis in ruminants. The main plants used were *Citrullus colocynthis* (L.), *Lamium amplexicaule* L., *Mallotus philippinensis* Muell, *Withania somnifera* (L.) Dunal, *Azadirachta indica*.

A few of these plants have been scientifically validated for their claim by herdsmen on modern lines while majority of them still needs to be evaluated by scientific work.

TOXICOLOGY

(Adam, Al-Farhan et al. 2000) described the toxicological properties of *Citrullus colocynthis* and a few other plants in the following study:-

MATERIALS AND METHOD

The effect of oral administration of 0.25 g/kg/day of *Citrullus colocynthis* fruits, 0.25 g/kg/day of *Rhazya stricta* leaves or mixture of the two plants at 0.25 g/kg/day of *Citrullus colocynthis* fruits plus 0.25 g/kg/day of *Rhazya stricta* leaves in Najdi sheep was examined. Oral administration of 0.25 g/kg/day of *Citrullus colocynthis* fruits or 0.25 g/kg/day of *Rhazya stricta* leaves for 42 days proved not fatal but that of the mixture of the two plants (0.25 g + 0.25 g/kg/day) proved fatal within 26 days with profuse diarrhea, dehydration, loss in condition, ataxia and recumbency, prior to death. These manifestations accompanied by enterohepatonephrotoxicity, gelatinization of the renal and epicardial fat and transudate in serous cavities were correlated with alterations in serum LDH and AST activities and concentrations of total protein, albumin, globulin, bilirubin, cholesterol and urea and hematology.

These results shows cathartic potential of *Citrullus colocynthis* and the other herb under study *Rhazya stricta* but the individual effect of both herbs become stronger in combination treatment causing diarrhea.

DISCUSSION/ CONCLUSION

➤ CATHARTIC EFFECTS

(Adam, Al-Farhan et al. 2000) demonstrated the cathartic potential of *Citrullus colocynthis* by studies done in sheeps. But the effect on motility of intestine becomes even stronger in the presence of *Rhazya stricta*.

These findings should be further investigated to find the active principles responsible for cathartic effect. However these facts support the traditional usage of *Citrullus colocynthis* as a cathartic herb in Pakistan.

➤ ANTI-DIABETIC EFFECTS

The studies done by (Sangameswaran, Balakrishnan et al. 2008) on rats proved Anti-hyperglycemic effects of *Citrullus colocynthis* extract; which supports the traditional usage of *Citrullus colocynthis* as anti-diabetic agent. But there is need for further research to find the active ingredients responsible for this effect.

A combination of *Citrullus colocynthis* and the synthetic drugs could also be researched to reduce the effective dose of the synthetic drugs hence reducing the side effects and getting effective and safe management of diabetes.

➤ ANTI-OXIDANT PROPERTIES

(Delazar, Gibbons et al. 2006) isolated 3 flavonoids from the *Citrullus colocynthis* extract. Since reactive oxygen species are important contributors to tissue injury, inflammation, cancer and many other diseases, the antioxidant properties of 3 isolated flavonoids probably contribute, at least to some extent, to the pharmacological and traditional uses of *Citrullus colocynthis*. It has been widely accepted that beneficial effects, shown by flavonoids, against cancer and heart diseases, is not only due to their antioxidant properties but also by other mechanisms.

➤ TREATMENT OF BREAST CANCER

The laboratory study done by (Tannin-Spitz, Grossman et al. 2007) demonstrates the growth inhibitory effects of Cucurbitacin B and E on ER+ MCF-7 and ER₋ MDA-MB-231 human breast cancer cell lines. These results could be used to design a clinical trial and scientifically support the affectivity of this traditional remedy against one of the challenging diseases to modern clinical sciences. *Citrullus colocynthis* extract could lead to production of a significant, safe and effective remedy after application of toxicological and clinical parameters. It could also become adjuvant to the anti-breast cancer regimens made up of synthetic drugs which could helps to lower the side effects.

➤ TREATMENT OF PARASITIAL DISEASES IN LIVSTOCK

(Farooq, Iqbal et al. 2008) and (Jabbar, Raza Muhammad et al. 2006) studied the traditional usage of *Citrullus colocynthis* and the other herbs as anti-parasital in livestock, among the traditional herbalists in the Pakistan. The traditional usage shows the significance of safe and effective usage of *Citrullus colocynthis* and other plant remedies in the parasital diseases but it is needed to design further pharmacological and clinical work, to find the active ingredients

responsible for this effect, and determine safe and toxic doses using standard parasitological procedures.

Further the outcomes from the animal studies could be extrapolated to human beings to find herbal anti-parasital remedies.

REFERENCES

• Adam, S. E. I., A. H. Al-Farhan, et al. (2000). "Effect of combined *Citrullus colocynthis* and *Rhazya stricta* use in Najdi sheep." Am J Chin Med 28(3-4): 385-90.

Al-Ghaithi, F., M. R. El-Ridi, et al. (2004). "Biochemical effects of *Citrullus colocynthis* in normal and diabetic rats." Mol. Cell. Biochem. 261(1&2): 143-149.

Antoine-Moussiaux, N., B. Faye, et al. (2007). "Tuareg ethnoveterinary treatments of camel diseases in Agadez area (Niger)." Trop Anim Health Prod 39(2): 83-9.

Berdami, L. A. (2001). Topical composition for treating epidermal abrasions. Application: US US, (USA). 3 pp.

Delazar, A., S. Gibbons, et al. (2006). "Flavone C-glycosides and cucurbitacin glycosides from *Citrullus colocynthis*." Daru, J. Fac. Pharm., Tehran Univ. Med. Sci. 14(3): 109-114.

Farooq, Z., Z. Iqbal, et al. (2008). "Ethnoveterinary practices for the treatment of parasitic diseases in livestock in Cholistan desert (Pakistan)." J Ethnopharmacol 118(2): 213-9.

Hussain, I., L. Khan, et al. (2008). "Comparative study of vitamin C contents in fruits and medicinal plants." J. Chem. Soc. Pak. 30(3): 406-409.

Jabbar, A., A. Raza Muhammad, et al. (2006). "An inventory of the ethnobotanicals used as anthelmintics in the southern Punjab (Pakistan)." J Ethnopharmacol 108(1): 152-4.

Sangameswaran, B., B. R. Balakrishnan, et al. (2008). "Oral hypoglycaemic activity of extracts of leaves of *Citrullus colocynthis* (Schrad)." J. Pharm. Res. 7(3): 138-140.

Tannin-Spitz, T., M. Bergman, et al. (2007). "Cucurbitacin glucosides: Antioxidant and free-radical scavenging activities." Biochemical and Biophysical Research Communications 364(1): 181-186.

Tannin-Spitz, T., S. Grossman, et al. (2007). "Growth inhibitory activity of cucurbitacin glucosides isolated from *Citrullus colocynthis* on human breast cancer cells." Biochem Pharmacol 73(1): 56-67.

PHOTO REFERENCE

http://www.botanik.uni-karlsruhe.de/garten/fotos-carolus/Citrullus_colocynthis_HC.jpg

Retrieved in December, 2008.

Ferula asafoetida



BOTANICAL NAME: - *Ferula asafoetida* (H.Karst.)

Family: - Umbelliferae

English name: - Asafetida

Urdu name: - Hing

Local name in Pakistan: - Rawe (chitral) Suff (Gilgit)

Location in Pakistan: - Harchonullah, Bateashi, kamari, mauskhi, Zarghum.

DISTRIBUTION:-

The plant grows abundantly on dry exposed steep hill slopes of Harchonullah, near the village Batwashi along the road side and near Kamari village 1515 m to 2727 m asl.

(www.parc.gov.pk/data/medicinal/medsearch.asp/)

Asafoetida is the oleogum resin obtained by incision of the roots of various plants from the genus *Ferula* also *Ferula asafoetida* (family Umbelliferae) (Takeoka 2001).

PARTS USED

1. **OLEORESIN EXUDATE**, Asafoetida or Asafetida is the dried latex, Oleo- gum or oleo- resin exuded from the taproots of perennial herbs belonging to many species of the genus *Ferula*(George 2006).
2. Dried roots, Crushed roots powder.

DESCRIPTION

Asafoetida is the milky juice (latex), which forms a resin, from the root of *Ferula asafoetida*. Asafoetida contains 400-640 g/kg resin, 250 g/kg gum, 100-170 g/kg volatile oil and 15-100 g/kg ash (7). The chief constituent of oil is secondary butyl prophenyl disulphide(Thyagaraja and Hosono 1996) .

It has an unpleasant smell, is herbaceous and perennial and grows up to 2 m high.

ETHNOPHARMACOLOGY

USES IN PAKISTAN

1. Asthma(Damotahi)
2. Common cold
3. Coughs
4. Kudakan (child disease)
5. Toothache
6. Gastric problems like ulcer.
7. Constipation(Qabzi)
8. Arthritis
9. To prepare cows for mating.

(www.parc.gov.pk/data/medicinal/medsearch.asp/)

USES IN IRAN

1. Abdominal pain
2. Constipation
3. Diarrhea
4. Anthelmentic

USES IN NEPAL

- Emmenagogue
- Anthelmentic
- Aphrodisiac (Eigner et al, 1990)

USES IN UNITED STATES OF AMERICA

1. Emmenagogue
2. Expectorant
3. Anthelmentic
4. Aphrodisiac
5. Tonic, stimulant to the brain and nerves.
6. Antispasmodic

OTHER USES

➤ **ANTIFUNGAL (Takeoka 2001)**

Field of usage: - preservation of food.

USE AS FOOD SPICE

Asafoetida has a strong, tenacious, sulfurous odor and is an important spice used in Indian, European and some Middle Eastern foods(Takeoka 2001).

CHEMISTRY

➤ **GUM FRACTION (Kajimoto, 1989)**

Asafoetida contain 25% gum fraction including glucose, galactose, l-arabinose, rhamnose and glucuronic acid.

➤ **RESIN FRACTION**

Resin fraction comprises 40–64 % (Kajimoto, 1989), which contains ferulic acid esters (60%), free ferulic acid(1.3%), coumarin derivatives(e.g. Umbeliferone) and volatile oils (3-17%), and monoterpenes.

➤ **Volatile oil**

Ferula asafoetida yields 3-17% volatile oils on extraction (Kajimoto 1989) (Mahran, El Alfy et al. 1975).

Volatile oil contains following ingredients:-

1. Alpha- and beta- pinene
2. Diallyl sulfate
3. Diallyl sulfide
4. Secondary butyl propenyl disulfide
5. Secondary butyl propenyl disulfide
6. Alpha-phellandrene
7. Geranyl acetate

➤ **MINERAL COMPOSITION**

Mineral composition is as followed as described by(Uma Pradeep, Geervani et al. 1993) p.141.

mg/100g asafoetida

- Calcium 1007.4 mg/100 g
- Phosphorus 299.5 mg/ 100 g
- Iron 59.90 mg/100 g
- Manganese 1.63 mg/ 100g
- Magnesium 108.9 mg/ 100g
- Zinc 0.62 mg/ 100g

➤ Starch 19.89 %

➤ Proximate composition and energy content of *Ferula asafoetida*

(g/100 g edible portion)

Energy (Kilo calorie)	Dry matter	Ash	Nitrogen	Protein	Fat	CHO*
90.76	4.97	0.68	0.68	4.25	2.65	78.89

*CHO = Carbohydrate composition by different mean triplicate analysis

- Dietary fiber, uronic acid, tannins and phytic acid in spices:-

Dietary fiber (%DM)			Total Fiber (g/100g)	Uronic acid (% DM)	Tannins (g/100 g)	Phytic acid (%DM)
IDF	SDF	TF				
1.9	56.42	52.9		11.22	0.80	---
58.3						

%DM= Percent dry mass

IDF= Insoluble dietary fiber

TF = Total Fiber

BIOLOGY/ PHARMACOLOGY

1. ANTI-FUNGAL EFFECTS

(Thyagaraja and Hosono 1996) demonstrated that *Ferula asafoetida* non-aqueous extract inhibits significantly the fungi responsible for food spoilage.

Materials and Methods

1. Spices

Spices procured from India, namely *chilli, coriander, cumin, pepper, turmeric and asafoetida* were ground to fine powder in a Warring blender and extracted in 950 g/L ethanol. The extract containing 10 mg/ml equivalent of spices was used in an inhibition assay.

2. Moulds and incubation

The organisms *Rhizopus azygosporus* (IFO 31989), *Mucor dimorphosporous* (IFO 4555), *Penicillium commune* (IFO 5763) and *Fusarium solani* (IFO 9425), were used as indicator organisms. These were selected to represent moulds commonly encountered in food spoilage. These fungi were maintained in potat-dextros agar medium following recommendations from IFO.

3. Inhibition assay

The assay was carried out by filter disk method (4). The inhibition assay was carried out in potato dextrose agar (Oxoid) by the paper disc method. The inoculum (0.1 ml) was transferred into a well-dried PDA plate and spread on the surface to make a lawn. After allowing sufficient time for absorption of the moisture, a paper disc and 40 ml of spice extract was placed in the centre of the agar. The plate was incubated at 25°C and inhibition observed by measuring the clear zone around the paper disc. All the assays were carried out in triplicate and repeated at least twice (means of two experiments are reported).

In the above study (Thyagaraja and Hosono 1996) demonstrated that it was only *Ferula asafoetida* which inhibited mould growth up to a significant level.

Further it was found that the active ingredient responsible for mould inhibition is present in diethyl-ether extract and aqueous extract obtained by hot water does not have any inhibitory activity.

IFO= (Institute of Fermentation, Osaka, Japan)

2. ANTI-SPASMODIC EFFECTS

(Fatehi, Farifteh et al. 2004) demonstrated anti-spasmodic effects of *Ferula asafoetida* gum extract on Guinea-pig ileum and blood pressure on anesthetized rats in the following experiment:-

EFFECT ON PRE-CONTRACTED GUINEA PIG ILEUM

The effects of *Ferula asafoetida* gum extract on the contractile responses of the isolated guinea-pig ileum induced by acetylcholine, histamine and KCl and on the mean arterial blood pressure of rat were investigated. In the presence of *Ferula asafetida* extract (3 mg/ml), the average amplitude of spontaneous contractions of the isolated guinea-pig ileum was decreased to 54 +/- 7% of control.

Exposure of the pre-contracted ileum by acetylcholine (10 µM) to *Ferula asafoetida* gum extract caused relaxation in a concentration-dependent manner.

Similar relaxatory effect of the extract was observed on the pre-contracted ileum by histamine (10 µM) and KCl (28 mM). However, when the preparations were pre-incubated with indomethacin (100 nM) and different antagonists, such as propranolol (1 µM),

atropine (100 nM), and chlorpheniramine (25 nM) then were contracted with KCl, exposure to the extract (3 mg/ml) did not cause any relaxation.

μM= micro molar concentration.

EFFECT ON BLOOD PRESSURE OF ANESTHESIZED RATS

Furthermore, *Ferula asafoetida* gum extract (0.3-2.2 mg/100g body weight) significantly reduced the mean arterial blood pressure in anaesthetized rats. It might be concluded that the relaxant compounds in *Ferula asafoetida* gum extract interfere with a variety of muscarinic, adrenergic and histaminic receptor activities or with the mobilization of calcium ions required for smooth muscle contraction non-specifically.

3. ANTIOXIDANT ACTIVITY

(Sujatha and Srinivas 1995) Studied the antioxidant effects of a number of Indian plants including *Ferula asafoetida* and demonstrated that *ferula asafoetida* has a significant antioxidant effect.

MATERIALS AND METHODS

The following materials were used in this research:-

1. Bovine serum albumin
2. Nitroblue tetrazolium (NBT)
3. Superoxide dismutase (SOD)
4. 12-O-tetradecanoyl phorbol-13-acetate (TPA)
5. Tert-butyl hydroperoxide (tBHP)
6. Butylated hydroxyanisole (BHA)
7. Curcumin was purchased from Sigma Chemicals (St Louis, MO, USA).
8. Ferrous sulfate
9. Ascorbic acid
10. Hanks' balanced salt solution was purchased from Himedia Private Ltd (India).
11. Thiobarbituric acid was purchased from Loba Chemie (India).

All other chemicals and reagents were of Analar grade and were purchased from Glindia Laboratories (India). Solvents were distilled before use and were peroxide free.

Erythrocyte ghosts were prepared by hypotonic lyses of human blood according to the procedure of Dodge et al. (1963). The SOD was removed by washing the pellets thoroughly with isotonic saline. Protein was estimated according to the method of Spector (1978). The ghost suspension, containing 300 μ g protein, was subjected to peroxidation induced by exposure to ferrous sulfate-ascorbate (10:100 μ mol) for 20min at 37°C according to the Procedure of Shimasaki *et al.* (1984).

Lipid peroxidation was estimated in terms of thiobarbituric acid reactive substances (TBARs) at 535 nm using a Shimadzu Spectrophotometer.

Conjugated dienes were also estimated as a sensitive index of lipid peroxidation, according to the method of Stoffel and Ahrens (1958). The membrane lipids, after peroxidation were extracted with chloroform- methanol (2: 1, v/v), evaporated under nitrogen, and redissolved in hexane. The absorbance spectra were recorded at 200-320 nm.

Studies on the prevention of peroxidation were carried out using antioxidants such as BHA, Curcumin and fresh aqueous extracts of spices and vegetables. Appropriate blanks and controls were included along with the test samples to take into account any non-specific contribution to the final chromophore or interference with the assay. The concentration of standard antioxidants used was based on the literature.

Aqueous extracts of spices were prepared by adding 50 mg of the powdered spices to 5 ml boiling distilled water. The mixture was vortex mixed and centrifuged (1 500 rpm, 15 min, 25°C). Known volumes were taken from the clear supernatant for the assay.

To prepare the vegetable extracts, 1 g of vegetable was finely chopped and crushed in a glass pestle and mortar, and then 5 ml boiling distilled water was added and the fresh extracts containing known amounts of solubles were used for the inhibition assays. Boiling water was used to destroy vitamin C; the known antioxidant in vegetables to improve the solubility and to simulate the culinary (something related to kitchen) practices that are usually followed.

The extracts alone were used as blanks, and any interference in the chromophore formation was taken into account. The residue after extraction was weighed to calculate the solubility (Salimath et al., 1986).

Lipid peroxides were prepared by subjecting the human erythrocyte membrane (300 µg protein) to peroxidation by exposure to ferrous sulfateascorbate (10: 100 µmol) for 20 min at 37°C. The peroxidized lipid was extracted with 5 ml ethanol-diethyl ether (3: 1, v/v), evaporated under nitrogen and suspended in 2 ml 0.9% saline (Frankel et al., 1987).

50µl of the extract was equivalent to 1.75 nM malondialdehyde (MDA)/mg protein.

Lipid peroxide-induced membrane lysis was studied using human erythrocytes as the model system by monitoring the release of haemoglobin due to haemolysis, according to the procedure of Mack *et al.* (1972). 1 ml freshly drawn human blood was diluted immediately with 9 ml 0.9% saline and centrifuged at 1500 rpm for 10 min at 25°C. The pellets were washed twice with 0.9% saline and resuspended in 7.5 ml 0.9% saline. 1 ml of the erythrocyte suspension was incubated with lipid peroxides in the presence and absence of aqueous extracts of spices in a total volume of 5 ml 0.54% saline. Incubation was carried out for 20 min at 37°C, at the end of which the solution was centrifuged at 1500 rpm and the optical densities of the supernatants at 540nm were measured.

Human PMNLs were used as another model system to investigate lipid peroxide-mediated membrane events. The possible prevention of lipid peroxidation by aqueous extracts of spices was also carried out.

RESULTS

Lipid peroxidation of erythrocyte membrane: effect of aqueous extract of spices

Aqueous extracts of spices were tested for their antioxidant activity and compared with the known antioxidants BHA and Curcumin. In the presence of BHA (400 µM) and Curcumin (400µ M) there was 77 and 68% inhibition, respectively.

Aqueous solution of *Ferula asafoetida* inhibited peroxidation at concentration 300 µg/ ml up to 85%.

Abbreviations:-

BHA = butylated hydroxyanisole;

BHT = butylated hydroxytoluene;

fMLP = formyl methionyl _ leucinyl -phenylalanine;

MDA = malondialdehyde;

NBT = nitroblue tetrazolium;

PMNL = polymorphonuclear leucocyte;

SOD = superoxide dismutase;

TBars = thiobarbituric acid reactive substances.

nM= nano molar concentration.

tBHP = tertiary-butylhydroperoxide.

TPA = 12-O-tetradecanoyl phorbol-13-acetate.

4. ANTI-COAGULENT

(Leung et al, 1980) *Ferula asafoetida* gum has anti-coagulant activity.

5. MOLLUSCICIDAL ACTIVITY

(Kumar and Singh 2006) demonstrated effect of dried root latex powder of *Ferula asafetida* in fascioliasis caused by a snail *Lymnaea acuminata*; the intermediate host of which causes endemic fascioliasis in the cattle population of northern India.

The molluscicidal activity of dried root latex powder of *Ferula asafoetida*, flower-bud powder of *Syzygium aromaticum*, and seed powder of *Carum carvi* against the snail *Lymnaea acuminata* was studied. The molluscicidal activity of all these

3 plant products were both time and concentration dependent. The toxicity of *Syzygium aromaticum* flower-bud powder (96 h LC50:51.98 mg/L) was more pronounced than that of root latex powder of *Ferula asafoetida* (96 h LC50:82.71 mg/L) and seed powder of *Carum carvi* (96 h LC50:140.58 mg/L). Ethanol extract was more toxic than other organic extracts. The ethanol extract of *Syzygium aromaticum* (24 h LC50:83.53 mg/L) was more effective than that of *Ferula*

asafoetida (24 h LC50:132.31 mg/L) and *Carum carvi* (24 h LC50:130.61 mg/L) in killing the test animals. The 96 h LC50 of column purified fraction of seed powder of *Carum carvi* was 5.40 mg/L whereas those of flower-bud powder of *Syzygium aromaticum* and dried root latex powder of *Ferula asafoetida* were 7.87 and 9.67 mg/L, resp. The product of *Ferula asafoetida*, *Syzygium aromaticum*, and *Carum carvi* may be used as potent molluscicides.

6. ANTI-ULCER EFFECTS

(Rao, Kumar et al. 2007) described a novel herbal remedy for the treatment of Gastric ulcer; this contains *Ferula asafoetida* and a number of other herbal ingredients. These findings in this study support traditional usage as an anti-ulcer herb.

7. ANTI-TRICHOMONAS VAGINALIS EFFECT

(Ramadan Nashwa and Al Khadrawy Faisal 2003) studied the effect of oleo-gum resin from *Ferula asafoetida* on growth of *Trichomonas vaginalis* in vitro. *Ferula asafoetida* gum exhibited inhibitory effect on *Trichomonas vaginalis*. In this study a comparison was done to compare the effectiveness of gum obtained from *Ferula asafoetida* with standard synthetic compound *Metronidazol* as a reference drug. Gum *Ferula asafoetida* showed potent anti-parasitic effect on *Trichomonas vaginalis* as compared to *Metronidazol*.

This is a significant finding.

TOXICOLOGY

Higher doses taken orally cause diarrhea, meteorism, headaches, dizziness, and enhanced libido (Kapoor, 1990).

DISCUSSION/ CONCLUSION

➤ ANTI-SPASMODIC AND BLOOD PRESSURE LOWERING EFFECT

(Fatehi, Farifteh et al. 2004) experimentally determined that *Ferula asafoetida* has relaxant effect on contracted (contraction induced by Acetylcholine, histamine and KCl) gastric smooth muscles, in dose dependent manner, in Guinea pig ileum and vascular smooth muscles in anesthetized rats.

The exact mechanism of action is not known but a few hypotheses could be derived following the observations done in this study:-

- Contraction of gastric muscles involves cycles of depolarization and repolarization involving influx of calcium Ca⁺ ions through voltage activated channels (Walsh and Singer, 1980; Branding, 1981). Therefore extract of gum *Ferula asafoetida*, may contain a few substances, which interfere with contractions induced by calcium channels.
 - The contractions produced by acetylcholine are mediated through muscarinic receptor sub-type M3 and the guinea pig ileum may have a preponderance of M2 sub-type muscarinic binding sites, these facts indicate that *Ferula asafoetida* may interfere with muscarinic receptors to counteract contractions produced by acetylcholine.
 - The relaxatory/ reparatory effect produced in the histamine pretreated ileum is greater than that with Acetylcholine pre treated ileum.
- The contraction induced by KCl is due to an increase in K⁺ and depolarization of Smooth muscle fibers, leading to increased influx of calcium through L-type voltage-operated channels (Gilani et al., 1994).

However, the spasmolytic activity of the extract could not be attributed solely to any pure antagonistic effect, since the tissue contracted by KCl (Potassium chloride) was also relaxed after exposure to the extract.

Considering lack of the relaxatory effect of the extract in the presence of atropine, chlorpheniramine and propranolol, one might suggest that these antagonists competed with the relaxant compounds of the extract for binding to their acceptors. Therefore, there is no good reason to exclude any interaction between some compounds of the extract and cholinergic, histaminergic and adrenergic receptors. On the other hand, smooth muscle contractile tone can be relaxed by increased levels of 3, 5-cyclic adenosine monophosphate (cAMP) (Berridge, 1975). Therefore, the extract may have its relaxatory effect through an

increase in cAMP independent of any specific receptor activity, then a reduction in Ca²⁺ levels. The relaxatory effects of the *Ferula asafoetida* gum extract on vascular smooth muscle as well as on ileum smooth muscle may suggest that, this natural product reduce the cytosolic Ca²⁺ in a non-specific manner.

There need for further research to find out the exact mechanism, however these findings scientifically supports the traditional usage of *Ferula asafoetida* gum for abdominal pain and blood pressure.

➤ **ANTIOXIDANT ACTIVITY**

Study done by (Sujatha and Srinivas 1995)for antioxidant effect of

Ferula asafoetida gum extract in vitro studies using human erythrocytes, demonstrated clearly a significant anti-oxidant activity induced by *Ferula asafoetida*. Further *Ferula asafoetida* inhibited the effect of peroxidases at a significant level as compared to the other herbs under study.

Inhibition of conjugated dienes (a sensitive indicator of peroxidation) by *Ferula asafoetida* and other herbs under study demonstrated that diene conjugation inhibition was more effectively done by these herbs under study as compared to α -tocopherol chain breaker in free radical reaction. This indicates that anti-oxidant effect exhibited by these herbs under study is not only done by chain breaking but also effectively done by either quenching the free radicals, scavenging the radicals, or reacting with radicals and thus sparing the targets, or by transforming them into non-reactive products or by chelating the metal ions that catalyze the free-radical reaction (Halliwell and Gutteridge, 1990).

In the light of said study it is needed to do further research to find out the active ingredients responsible for this effect.

However these findings may provide scientific support for the traditional usage of *Ferula asafoetida* as an anti-oxidant and preventive substance in many diseases including cancer etc.

➤ ANTI-PARASITAL EFFECTS

(Ramadan Nashwa and Al Khadrawy Faisal 2003) demonstrated that ole-gum resin obtained from *Ferula asafoetida* has potent inhibitory effect on the growth of an anaerobic parasite *Trichomonas vaginalis*, causing vaginitis in women, in invitro study. Further it was found that the effect presented by *Ferula asafoetida* gum is stronger than the reference drug *Metronidazol*. This finding could be researched further by designing clinical trials and may provide safe and effective herbal remedy against vaginitis, which could also help in the parasites resistant to the effect of Metronidazol.

Further the anti-parasital effect could be tested on other types of parasites.

➤ ANTI-FUNGAL EFFECT

The study done by (Thyagaraja and Hosono 1996) clearly demonstrated the anti-fungal effect of *Ferula asafoetida*, but it is necessary to research further to isolate the active ingredient responsible for this effect. The anti-fungal effect could also be tested in the fungi causing diseases in human beings like athlete's foot and vaginal candidiasis.

➤ MOLLUSCICIDAL EFFECT

(Kumar and Singh 2006) demonstrated the molluscicidal effect, of *Ferula asafetida* and two other plant species, by doing laboratory tests on *Lymnae acuminata*. This work supports the traditional usage of *Ferula asafetida* and the other plant species under test. It is more environment friendly and cost effective to use herbs than the synthetic drugs in order to avoid the side effects related to synthetic therapy. It also a matter of facet that the farmers living in the rural areas can't afford the costs associated with synthetic therapy.

➤ GASTRIC ULCER

(Rao, Kumar et al. 2007) described a novel herbal remedy for the treatment of Gastric ulcer containing *Ferula asafoetida* and a number of other plants are used like , *Speragus racemosus*, *Glycyrrhiza glabra*, *Seaamum indicum*, *Musa sapientum* and *Trachyaparmum roxburghicinum* and optionally, powdered plant parts of *Cyclea peltate*, *Embelia ribes*, *Coriandrum sativum* *Ferula asafoetida*, *Aloe barbadensis* and

Evolvulus aisinodes. These ingredients used in powder form synergize the effects of each other. This study supports the traditional usage of *ferula asafoetida* as anti-ulcer herb.

(Uma pardeep et al, 1993p.141) described that *Ferula asafoetida* contain about 10 mg/g herb; It is known that calcium has an acid neutralizing ability that may further explain anti-ulcer effect.

➤ **ANTI- ASTMA, COUGH SUPPRESSENT**

There is not found any scientific study which could support the traditional usage of *Ferula asafoetida* against asthma and as expectorant in Pakistan and other countries but the study done by (Fatehi, Farifteh et al. 2004) for relaxant effects of ferula asafoetida on intestinal smooth muscles opens the possibility of relaxant effects on bronchial smooth muscles, there is need for a research trial on the bronchial smooth muscles find the scientific basis for the traditional usage of *Ferula asafoetida* as anti-asthmatic and cough suppressant.

➤ **ANTI-CONSTIPATION EFFECT**

There are not found any study which could give scientific support to the traditional usage of ferula asafoetida against constipation but the study done by (Uma pardeep et al 1993p.141) describes that *Ferula asafoetida* contain 52.09% fibers; fibers has a known stimulatory effect on the intestines this could explain anti-constipation effect of *Ferula asafoetida* but it could be further researched in a separate trial.

REFERENCES

- Berridge, M.J., 1975. The interaction of cyclic nucleotides and calcium in control of cellular activity. *Advanced Cyclic Nucleotides Researches* 6, 1–98.
- Brading, A.R., 1981. How do drugs initiate contraction in smooth muscle? *Trends in Pharmacological Sciences* 2, 262–265.
- Dodge J. T., Mitchell C. and Hanahan D. J. (1963) the preparation and chemical characteristics of hemoglobin free ghosts of human erythrocytes. *Archives of Biochemistry and Biophysics* 100, 119-130.
- Eigner, D., Scholz, D., 1990. Das Zauberkraut chlein der Gyani Dolma. *Pharmazie in unserer Zeit* 19, 141–152.
- Fatehi, M., F. Farifteh, et al. (2004). "Antispasmodic and hypertensive effects of *Ferula asafoetida* gum extract." J Ethnopharmacol FIELD Full Journal Title: Journal of ethnopharmacology 91(2-3): 321-4.
- Frankel E. N., Neff W. E., Brooks D. D. and Fujimoto K. (1987) Fluorescence formation from the interaction of DNA with lipid oxidation degradation products. *Biochemical et Biophysics Acta* 919, 239-244.
- George, C. K. (2006). "Asafetida." Handb. Herbs Spices FIELD Full Journal Title: Handbook of Herbs and Spices 3: 221-229.
- Gilani, A.H., Janbaz, K.H., Zaman, M., Lateff, A., Tariq, S.R., Ahmad, H.R., 1994. Hypotensive and spasmolytic activities of crude extract of *Cyperus scariosus*. *Archives of Pharmacological Research* 17 (3), 145–149.
- Halliwell B. and Gutteridge J. M. C. (1990), the antioxidants of human extracellular fluids. *Archives of Biochemistry and Biophysics* 280, I-10.

Kajimoto, T., 1989. Sesquiterpenoid and disulphide derivatives from *Ferula asafoetida*. *Phytochemistry* 28, 1761–1763.

Kapoor, L.D., 1990. Handbook of Ayurvedic Medicinal Plants. CRC Press, Boca Raton, FL, p. 185.

Kumar, P. and D. K. Singh (2006). "Molluscicidal activity of *Ferula asafoetida*, *Syzygium aromaticum* and *Carum carvi* and their active components against the snail *Lymnaea acuminata*."

Chemosphere FIELD Full Journal Title: Chemosphere 63(9): 1568-1574.

Leung, A.Y., 1980. Encyclopedia of Common Natural Ingredients used in Food, Drugs and Cosmetics, Wiley, New York.

Mack J. P., Lui N. S. T., Roels D. A. and Anderson O. K. (1972), the occurrence of vitamin A in biological membranes. *Biochimica et Biophysica Acta* 288, 203.

Mahran, G. H., T. S. M. A. El Alfy, et al. (1975). "A phytochemical study of volatile oil of Afghanian Asafoetida." Bull. Fac. Pharm., Cairo Univ. FIELD Full Journal Title: Bulletin of the Faculty of Pharmacy (Cairo University) 12(2): 101-17.

Markert N., Andrews P. C. and Babior B. M. (1984), Measurement of O₂ - production by human neutrophils. The preparation and assay of NADPH oxidase containing particles from human neutrophils. *Methods in Enzymology* 105, 358.

Rao, J. M., U. S. Kumar, et al. (2007). Novel herbal composition for the treatment of gastric ulcer. Application: IN
IN, (Council of Scientific and Industrial Research, India).

Ramadan Nashwa, I. and M. Al Khadrawy Faisal (2003). "The in vitro effect of Asafoetida on *Trichomonas vaginalis*." J Egypt Soc Parasitol FIELD Full Journal Title: Journal of the Egyptian Society of Parasitology 33(2): 615-30.

Salimath B. P., Sundaresh C. S. and Leela Srinivas (1986) Dietary factors inhibit erythrocyte membrane lipid peroxidation. *Nutrition Research* **6**, 1 I7 I-I 178.

Shimasaki H., Ueta N., Mowri H. O. and Inoue K. (1984), Formation of age pigment like fluorescent substances during peroxidation of lipids in model membranes, *Biochimica et Biophysica Acta* **792**, 123-128.

Spector T. (I 978) Refinement of the Coomassie blue method of protein quantitation. *Analytical Biochemistry* **86**, 142-145.

Stoffel W. and Ahrens E. H. (1958) Isolation and structure of Cl6 unsaturated fatty acids in menhaden body oil. *Journal of the American Chemical Society* **80**, 6604-6608

Sujatha, R. and L. Srinivas (1995). "Modulation of lipid peroxidation by dietary components." *Toxicol. in Vitro* **9**(3): 231-6.

Takeoka, G. (2001). "Volatile constituents of asafoetida." ACS Symp. Ser. FIELD Full Journal Title:ACS Symposium Series **794**(Aroma Active Compounds in Foods): 33-44.

Thyagaraja, N. and A. Hosono (1996). "Effect of Spice Extract on Fungal Inhibition." Lebensmittel-Wissenschaft und-Technologie **29**(3): 286-288.

Uma Pradeep, K., P. Geervani, et al. (1993). "Common Indian spices: nutrient composition, consumption and contribution to dietary value." Plant Foods Hum Nutr FIELD Full Journal Title:Plant foods for human nutrition (Dordrecht, Netherlands) **44**(2): 137-48.

Walsh Jr., J.V., Singer, J.J., 1980. Calcium action potentials in single freshly isolated smooth muscle cells. *American Journal of Physiology* **239**, C162–C174.

PHOTO REFERENCE

<http://karawan.ir/upimages/asafoetida1.jpg>

Retrieved in December, 2008.

Caesalpinia Crista



BOTANICAL NAME: - *Caesalpinia crista* (Linn)

LOCAL NAME: - *Ka-lain* in Myanmar, *Bagore* in Indonesia, *Taepee* in Thailand, *nantenkadsura* in Japanese and *Letskanta* in Bangladesh.

SYNONYMS: - *Caesalpinia nuga* (L).

(Kinoshita, Haga et al. 2005)

FAMILY: - Caesalpinaceae

PARTS OF PLANT USED:-

Leaves, flowers, fruit, root, bark, seeds.

DESCRIPTION (Indian medicinal plants volume II p. 842)

Caesalpinia crista plant is an extensive climber; branches finely grey-downy, armed with hooked and straight hard yellow prickles.

Leaves 36-60 cm long; petioles prickly; stipules a pair of reduced pinnae at the base of leaf each furnished with a long mucronate point; pinnae 6-8 pairs, 5-7.5 cm. long, with a pair of hook stipulary spines at the base.

Leaflets 6-9 pairs, 2-3.8 by 1.3-2.2 cm., membranous, elliptic-oblong, obtuse, strongly mucronate, glabrous above, more or less puberulous beneath; petiolules very short; stipels of short hooked spines.

Flowers in dense (usually spicate) long-peduncled terminal and supraaxillary racemes dense at the top, lax downwards, 15-25 cm. long; pedicles very short in bud, elongating to 5 mm. in flower and 8 mm. in fruit, brown-downy; bracts squarrose, linear, acute, reaching 1 cm. long, fulvous-hairy. Calyx 6-8 mm. long, fulvous-hairy; lobes obviate-oblong, obtuse.

Petals oblanceolate, yellow. Filaments declinate, flattened at the base, clothed with long white silky hairs. Pods shortly stalked oblong, 5-7.5 by 4.5 cm, densely armed on the faces with wiry prickles. Seeds 1-2, oblong, lead-colored, 1.3 cm. long.

DISTRIBUTION

Caesalpinia crista is distributed throughout India and Pakistan, generally in the tropical areas.

ETHNOPHARMACOLOGY

USES IN PAKISTAN AND INDIA

ROOT

1. ANTI-TUMOR

The root bark is good for tumors and for removing the placenta. The sprouts are useful in the treatment of tumors.

2. ANTHELMINTIC

3. ELEPHANTIASIS: - An infectious disease marked by inflammation and obstruction of lymphatic's and hypertrophy of the skin and subcutaneous tissues, chiefly affecting the legs and genitalia. or

Hypertrophy and thickening of the tissues from any cause. Elephantiasis is usually seen in tropics.

4. SMALL POX

FLOWER

Flower is bitter and heating to body.

5. DEODORANT; destroys the bad odor due to perspiration.

6. KAPHA AND VATA;

7. ASCITES ; Flower ash is used

FRUIT

The fruit is acrid (Scarp, bitter), heating to the body.

8. ASTRINGENT TO THE BOWELS

9. APHRODISIAC

10. ANTHELMINTIC, in the countries including Myanmar.

11. CURES URINARY DISCHARGES

12. LEUCORRHOEA

13. PILES

14. WOUNDS

15. ULCERS; the oil from the fruit is good for the indolent ulcers (Ayurvedic).

SEED

The seed is hot and dry

16. STYPTIC

17. ANTIPERIODIC

18. ANTHELMINTIC, also in Myanmar.

19. PREVENT CONTAGIOUS DISEASES

20. ANTI-INFLAMMATORY

21. ANTI-COLIC

22. MALARIA

23. HYDROCELE; In Madras India, Pakistan and Madagascar an ointment is being made from the powdered seeds with castor oil and applied externally in hydrocele and orchitis.

24. SKIN DISEASES

25. LEPROSY (In the unani system)

26. ANTI-PERIODIC

27. ANTI-PYRETIC; Valuable in all ordinary cases of simple, continued and intermittent fevers. (Also in Myanmar)

28. TONIC; in Madagascar and Indonesia

29. FEBRIFUGE; in Madagascar

30. BLENNORRHAGIA; in Madagascar

31. ASTMA; Have been found useful in a few cases of asthma.

32. VESICANT; in Guinea pounded seeds are used

33. MALARIA; were administered with equal part of pepper powder. But didn't proved good in Malignant malaria.(Also in Myanmar)

34. ANTI-BLENNORRHAGIC ; in Madagascar

35. TOPICAL FORMULATION FOR INFLAMMATION, INFLAMED PILES AND ORCHITIS

The leaves and seeds are roasted with castor oil and are applied externally to inflammatory swellings especially to inflamed piles, hydrocele, and orchitis (Koman)

LEAVES

36. LIVER DISORDERS; tender leaves are considered very efficacious.

37. DEOBSTRUENT; In China

38. EMMENGOUGE: in China and Madagascar
39. ANTI-CONVULSENT; an oil expressed from it in China is given in convulsions, palsy and similar complaints.
40. INTERMITTENT FEVERS
In Malaya the young leaves are used in intermittent fevers, and for expelling intestinal worms, also in Ceylon.
41. TOOTHACHE; In Ceylon
42. SORE THROAT; the boiled leaves are used as a gargle for sore throat.

ROOTS

43. FEBRIFUGE (an herbal remedy which can reduce fever); in Madagascar and Guinea a decoction of root is used to reduce the fever.
44. ANTHELMINTIC; in Madagascar
45. LEUCORRHEA; in Madagascar
46. BLENNORRHAGIA; in Madagascar
47. ANTI-PYRETIC; in Guinea a decoction of roots is used in fever
48. ANTI-RHEUMATIC; in Indonesia a root decoction is used as ant-rheumatic.

CHEMISTRY

➤ **DITERPENES**

Caesalpinia crista contains two major types of Diterpenes as follows:-

1. Cassane type Diterpenes(Caesalpins).
2. Norcassane type Diterpenes(Nor-caesalpins).

(Awale, Linn et al. 2006) isolated *10 types of Furano-cassane type diterpenes from the CH₂Cl₂ (Dichloromethane) extract of *Caesalpinia crista* (from *Indonesia*) seed kernels in the following study.

MATERIALS AND METHOD

Air-dried seed kernels of *Caesalpinia crista* Linn, were extracted with CH₂Cl₂ by overnight percolation at room temperature. The CH₂Cl₂ extract was then fractionated by silica gel column chromatography with a benzene/EtOAc gradient system into nine fractions.

The fractions 6—8 were further subjected to repeated silica gel column chromatography, followed by normal- and reversed-phase preparative TLC, to afforded Caesalpinins H—P (**1—9**) and norcaesalpinin F (**10**) together with 13 known diterpenes, Caesalpinin C, caesalpinins D, norcaesalpinin E, 2-acetoxyl-3-deacetoxycaesaldekarin e, caesaldekarin e, caesalmin E, 1-deacetoxy-1-oxocaesalmin C, 2-acetoxycasaldekarin e, 3-deacetoxy-6-acetoxycasaldekarin e, α -caesalpin, and bonducellpins A-C.

1. CASSANE DITERPENES (CAESALPINS)

They are commonly called Furano-cassane-diterpenes but are phytochemically classified as Cassane diterpenes. Another synonym name is Caesalpins.

(C-G) determined by (Linn, Awale et al. 2005)

(H-P) determined by (Awale, Linn et al. 2006)

- **Caesalpinin C:** colorless amorphous solid;

HRFABMS m/z 417.2314 (M + H) + determines the molecular formula to be C₂₄H₃₃O₆.

- **Caesalpinin D:** colorless amorphous solid;

HRFABMS m/z 447.2025 (M +H) + determines the molecular formula to be C₂₄H₃₁O₈.

- **Caesalpinin E:** colorless amorphous solid; HRFABMS m/z 463.2317

(M + H) determines the molecular formula to be C₂₅H₃₅O₈.

- **Caesalpinin F:** colorless amorphous solid; HRFABMS m/z 419.2051

(M + H)+ determines the molecular formula to be C₂₃H₃₁O₇.

- **Caesalpinin G:** colorless amorphous solid; HRFABMS m/z 447.2009

(M + H)+ determines the molecular formula to be C₂₄H₃₁O₈.

- **CAESALPININ H**

This compound have a molecular formula C₂₂H₂₈O₇, can also be chemically described as 1-o-deacetyl-caesalpinin D.

It was isolated as a colorless, amorphous solid. It showed quasimolecular ion at m/z 405.1915 (M+H) + in HR-FAB-MS.

- **CAESALPININ I**

It was isolated as a colorless amorphous solid and its molecular formula C₂₂H₂₆O₇ was determined by HR-FAB-MS.

- **CAESALPININ J**

It was isolated as colorless amorphous solid with a molecular formula C₂₅H₃₃O₉ by HR-FAB-MS.

- **CAESALPININ K**

It was isolated as colorless amorphous solid. The molecular formula was C₂₂H₃₂O₅ determined by HR-FAB-MS.

- **CAESALPININ L**

HR-FAB-MS of Caesalpinin L (5) showed the Quasimolecular ion at m/z 435.2336(M+H) ⁺, consistent with the molecular formula C₂₂H₃₄O₇.

- **CAESALPININ M**

It was isolated as a colorless amorphous solid. The molecular formula determined by HR-FAB-MS was C₂₅H₃₄O₉.

- **CAESALPININ N**

The ¹³C NMR spectra resembled with Caesalpinin K (4) but were characterized by disappearance of signals due to tertiary methyls with the appearance of signals due to an aldehyde group.

- **CAESALPININ O**

It was separated as colorless amorphous solid.

Its molecular formula was determined to be C₂₂H₂₈O₇ by HR-FAB-MS.

- **CAESALPININ P**

It was separated as colorless amorphous solid.

It showed quasi- molecular ion at m/z 39.12096(M+H) ⁺ consistent with the molecular formula C₂₂H₃₀O₆.

2. NOR-CASSANE DITERPENES (NOR-CAESALPINS)

D and E determined by (Linn, Awale et al. 2005)

F determined by (Awale, Linn et al. 2006)

Norcassane-type diterpenes, named nortaepeenin A–B (Cheenpracha, Srisuwan et al. 2005)

- **NORCAESALPININ A-C** (Linn, Awale et al. 2005)
- **Norcaesalpinin D:** colorless amorphous solid; HRFABMS m/z 477.2106 (M + H)
+ determines the molecular formula to be C₂₅H₃₃O₉.

- **Norcaesalpinin E:** colorless amorphous solid; HRFABMS m/z 377.1946 (M + H)
+ determines the molecular formula to be C₂₁H₂₉O₆.

- **NOR-CAESALPININ F**

It was isolated as colorless amorphous solid with molecular formula C₂₁ H₂₆ O₇ as determined by HR-FAB-MS.

- NORTAEPEENIN a (C₂₀H₂₆O₄) White solid, mp 157–158 °C.
- NORTAEPEENIN B (C₂₀H₂₆O₅) White solid, mp 145–146 °C.

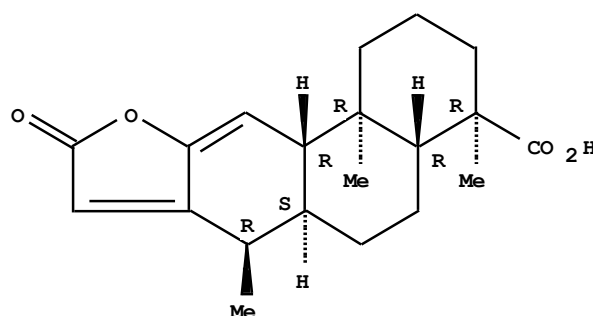
*= There was found 20 new Diterpenes from *Caesalpinia crista* from Myanmar (Burma)

CH₂Cl₂= Dichloromethane, used as a solvent.

EtOAc= CH₃COOCH₂CH₃= Ethyl acetate

Quasimolecular ion= A term used to represent a prorogated molecule.

HR-FAB-MS = High Resolution Fast Atom Bombardment Mass Spectrogram.



Norcaesalpinin I Molecular formula C₂₀ H₂₆ O₄

NEO-CASSANE DITERPENES

(Kinoshita, Haga et al. 2005) isolated a new class of chemical compounds from *Caesalpinia crista* extract known as Neocassane diterpenes.

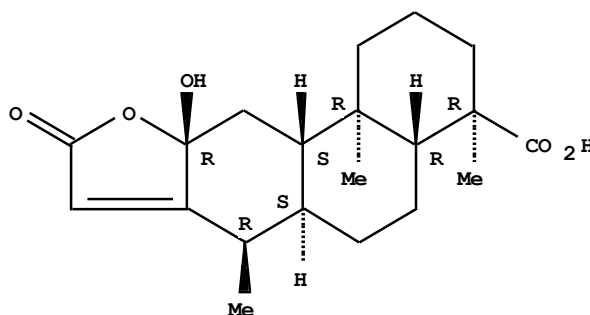
- i) Neocaesalpins H
- ii) Neocaesalpins I

These are characterized by α , β -butenolide hemiacetal ring that is rare in nature. They lack **5-hydroxy group** which distinguishes them from cassane diterpenes (caesalpins).

(i) Neocaesalpins H

It was isolated as optically active colorless needles, mp 255—256 °C. The molecular formula was deduced as C₂₀H₂₈O₅ from the high-resolution mass spectrometry.

The UV absorption maximum at 213 nm along with the IR absorption band at 1738 cm⁻¹ attested to the presence of an α , β -butenolide ring.



Diptyxyic acid; Neocaesalpin H, Molecular formula C₂₀ H₂₈ O₅

(ii) Neocaesalpins I

Neocaesalpin I (**2**) was obtained in optically active colorless fine needles, Mp >260 °C. It had the molecular formula of C₂₀H₂₆O₄ according to the High-resolution mass spectrometry. The acid dehydration of Neocaesalpin H led to production of a compound identical in all respects to Neocaesalpin I.

The UV absorption maximum at 277 nm indicated that it had α , β -butenolide ring conjugated with one extra double bond. This type of a conjugate α , β -butenolide ring was found only in Neocaesalpin D.

OTHER CASSANE DITERPENES

(Cheenpracha, Srisuwan et al. 2005) isolated nine new Cassane-type diterpenes as following:-

- TAEPEENIN A: - White solid, mp 156–157 °C, C₂₁H₂₆O₃.
- TAEPEENIN B: - White solid, mp 221–222 °C, C₂₀H₂₄O₃.
- TAEPEENIN C: - White solid, mp 154–155 °C, C₂₁H₂₆O₄.
- TAEPEENIN D: - White solid, mp 118.5–119 °C, C₂₃H₂₈O₅.
- TAEPEENIN E: - White solid, mp 57–58 °C, C₂₁H₂₄O₄.
- TAEPEENIN F: - White solid, mp 194–195 °C, C₂₁H₂₆O₄.
- TAEPEENIN G: - Viscous oil, C₂₀H₃₂O.
- TAEPEENIN H: - White solid, mp 164.5–165 °C.
- TAEPEENIN I: - White solid, mp 83–84 °C, C₂₁H₃₀O₄.

were isolated, by various steps process involving extractions and chromatography, from the stems and roots of *Caesalpinia crista* along with three known diterpenes: vinhaticoic acid, methyl vinhaticoate and ent-11b-hydroxy-rosa-5, 15-diene. Their structures were elucidated on the basis of spectroscopic analysis. In addition, the structure of Taepeenin A was confirmed by X-ray diffraction analysis.

➤ **PHYTOCHEMICAL ANALYSIS OF FIXED OIL FROM**

Caesalpinia crista

(Njoku, Okeke et al. 1999)isolated a fixed oil from *Caesalpinia crista* in the following study:-

MATERIALS AND METHOD

Fixed oil were extracted by soxlet from dried seeds, moisture content (5 %), with petroleum ether (40-60%) and analyzed immediately for iodine value, saponification value, refractive index, unsaponifiable matter, acid number and peroxide value by AOCS (American Oil Chemists Society) methods. Vitamin A and E were quantitatively examined by the method of Bassiar, sterol by the method of Stadam, glycolipid was assayed by the method of plumber, and phospholipids by the method of Totani et al, Gossypol and Mycostoxins were assayed by the method of Leutor et al and the official method of AOCS.

The gum content of the pulverized seeds was determined according to the method reported by whistler. The oil was tested for the presence of unconventional fatty acids-hydroxy acid using the turbidity test of Lakshaninarayana, epoxy and cyclopropane fatty acids by the picric acid method of [Fioriti et al and Halphen test method of Mailey and Mandal].

Table 1. Proximate analysis of seeds of *Caesalpinia crista*

DETERMINATION	% YEILD
Moisture content	14
Protein	5
Ash	2
Fibre	22
Fat	7
Carbohydrate	49

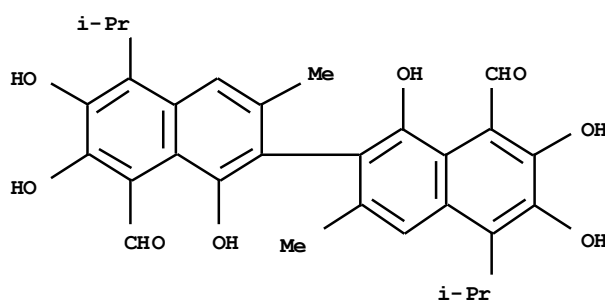
Table II. Physicochemical properties of the oil of *Caesalpinia crista* seed

DETERMINATION	
COLOR	Brown
% YEILD	10
STATE AT ROOM TEMPERATURE	LIQUID
SPECIFIC GRAVITY	0.9200
REFRACTIVE INDEX AT 30°C	1.4632
VISCOSITY	11.05
ACID VALUE(mg KOH g-1)	3.92
PEROXIDE VALUE (meq/l)	2.0
SAPONIFICATION NUMBER (mg KOH g-1)	164
IODINE VALUE	124
UNSAPONIFIABLE MATTER	0.7
GOSSYPOL	TRACE
GLYCOLIPID	PRESENT
TOTAL GUM	23 %
STEROL	PRESENT
AFLATOXIN B	PRESENT
OCHRATOXIN A	PRESENT
PHSOPHOLIPID mg 100 m-1	3.8

VITAMIN A	PRESENT
VITAMIN E	PRESENT
CYCLOPROPANE FATTY ACIDS	PRESENT
HYDROXY FATTY ACIDS	PRESENT
EPOXY FATTY ACIDS	PRESENT

TABLE III. PHYTOCHEMICAL ANALYSIS

CHARACTERISTICS	
FLAVONOIDS	ABSENT
SAPONINS	PRESENT
ALKALOIDS	ABSENT
CYNOGENIC GLYCOSIDES	PRESNET
TANNINS	PRESENT
ANTHRACENE GLYCOSIDES	PRESENT
CARDIAC GLYCOSIDES	PRESENT
STEROIDAL GLYCOSIDES	PRESENT
REDUCING SUGAR	PRESENT



Structural formula of Gossypol , molecular formula C₃₀ H₃₀ O₈ (Liang and Wang 2007).

PHARMACOLOGY

1. ANTI-MALARIAL ACTIVITY

- (Awale, Linn et al. 2006) demonstrated the anti-malarial activities of the substances isolated from *Caesalpinia crista*; Caesalpinins H-P(1-9) and Nor-Caesalpinins (10) except for **5** were tested for their inhibitory activities against growth of *Plasmodium falciparum* FCR-3/A2 *in vitro*. All of them displayed different potency activity in a dose dependent manner. Among the newly isolated compounds, **Caesalpinin K** and **norcaesalpinin F** showed the most potent inhibitory activity with an IC₅₀ value of 120 and 140 nM., respectively, and were less than that reported for a well known antimalarial drug, chloroquine (IC₅₀, 283—291 nM) [(Ringwald et al, 1999) (Pradines et al, 1999)].

The IC₅₀ value is defined as that concentration of compound producing 50% growth inhibition relative to untreated control.

nM= nano molar concentration= (10⁻⁶ mol/ Liter)

- (Linn, Awale et al. 2005)
The *in vitro* studies done by them on *Plasmodium falciparum* indicates that all cassane and Norcassane type diterpenes isolated from the *Caesalpinia crista* plant extract exhibit dose dependent anti-malarial effect but the most potent effect was observed in **Norcaesalpinin E** having IC₅₀ value 90nM (0.090μM), which is significantly stronger than the standard clinical drug chloroquine having IC₅₀ value 0.29μM.
- (Kalauni, Awale et al. 2006) studied the anti-malarial effects, against the malaria parasite *Plasmodium falciparum* FCR-3/A2 clone *in vitro*, of 44 cassane and Norcassane type diterpenes isolated from *Caesalpinia crista* of

Myanmar and Indonesia; they found that all the isolated diterpenes exhibited anti-malarial activity in a dose dependent manner but the most stronger antimalarial effect was seen in Norcaesalpinin E with IC50 value 0.090 μ M, stronger than clinical drug Chloroquine with IC 50 value 0.29 μ M.

μ M= Micro molar concentration

2. TREATMENT OF LEKORRHAGIA(LEUCORRHEA)

(Duan 2006) described an herbal preparation, for the treatment of Leukorrhagia, including *Caesalpinia crista* 30-60 g and a few other herbs including *Punica granatum* 80-110 g, *Cinnamomum cassia* bark 30-60 g, *Myristica fragrans* seed 30-60 g, *Piper nigrum* 30-60 g, *Carthamus tinctorius* flower 30-60 g and sucrose 800-900 g, by the steps of extracting volatile oil from *Carthamus tinctorius* flower, decocting the residue of *Carthamus tinctorius* flower and the rest ingredients in water, filtering, concentrating the filtrate to obtain an extract adding sucrose, granulating, drying, and adding the above volatile oil. The inventive granule can be used for the treatment of Leukorrhagia, with the advantages of high efficiency, rapid and long-lasting action, less toxic and adverse side effects, small volume and convenience for administration, storage and transport.

3. ACCELERATION OF FIBULIN-5 PRODUCTION/ ACTIVITY

In a scientific study done by (Ogura, Amano et al. 2006) *Caesalpinia crista* as a part of composition accelerates fibulin-5 production and/or enhances fibulin-5 activity, the other herbs present includes *Psophocarpus tetragonolobus* extract, phytic acid and physiologically acceptable salts thereof, and L-hydroxyproline and physiologically acceptable salts.

4. ANTI-PARASITIC EFFECTS

➤ TREATMENT OF FASCIOSIS IN BUFFALOS

Fasciolosis is an important helminth disease caused by two trematodes *Fasciola hepatica* (the common liver fluke) and *Fasciola gigantica*.

(Maqbool et al, 2004) studied the anti-parasitic effects of *Caesalpinia crista* extract in the following study done on buffalos:-

MATERIALS AND METHOD

One hundred and eighty buffalo were used in 18 controlled experiments to compare the efficacy of *Caesalpinia crista* seeds and certain other indigenous drugs, including *Nigella sativa* seeds, *Fumaria parviflora* aerial parts, and *Saussurea lappa* roots with triclabendazole against fasciolosis. Efficacy was quantified by determining the difference of eggs per g feces (EPG) pre- and post-treatment. *Nigella saliva* seeds, after a single dose of 30, 40 and 50 mg/kg body mass, reduced EPG by 54.16, 57.4 and 58.33 per cent, respectively. After the second dose the respective reduction in EPG was 79.16, 80.85 and 81.25 per cent. *Fumaria parviflora* aerial parts at a rate of 40, 50 and 60 mg/kg body mass were 50.0, 57.44 and 78.72 per cent, resp., whereas efficacy at two dose levels with the same dose rate was 82.6, 89.36 and 95.74 per cent, resp. *Caesalpinia crista* seeds at 30, 40 and 50 mg/kg body mass were 48.9, 50.0 and 57.7 per cent effective, resp., whereas efficacy at two dose levels was 80.0, 84.09 and 86.6 per cent, resp. *Saussurea lappa* at a rate of 50, 100 and 200 mg/kg body mass was 46.6, 57.4 and 61.7 per cent effective, resp., at one dose level and was 62.2, 72.3 and 78.7 per cent effective at two dose levels. Triclabendazole at one dose level at a rate of 10 mg/kg body mass was 82.6 per cent effective and at two dose levels it was 95.7 per cent effective. The efficacy order was triclabendazole, *Fumaria parviflora*, *Caesalpinia crista*, *Nigella saliva*, and *Saussurea lappa*. No side effects were noted due to the use of any of these plant-origin drugs.

This study demonstrates the Anthelmintic effect of *Caesalpinia crista* but the strongest effect, among the herbal drugs under study, has been seen with *Fumaria parviflora*.

➤ **ANTHELMINTIC EFFECT IN LIVESTOCK IN PAKISTAN**

(Javed, Akhtar et al. 1994) studied the Anthelmintic effects of *Caesalpinia crista* in the following comparative study with another Anthelmintic herb *Chenopodium album*.

The aim of the study was to determine the Anthelmintic activity of *Caesalpinia crista* (L.) seed kernel and *Chenopodium album* (L.) (Chenopodiaceae) whole plant in order to justify their traditional use in veterinary medicine.

Materials and methods

In vitro Anthelmintic activity of crude aqueous methanolic extract (AME) of both the plants was determined using mature *Haemonchus contortus* and their eggs in adult motility assay and egg hatch test, respectively. In vivo Anthelmintic activity was evaluated in sheep naturally infected with mixed species of gastrointestinal nematodes by administering crude powder (CP) and AME in increasing doses (1.0–3.0 g/kg).

Results

Both plants exhibited dose- and time-dependent Anthelmintic effects by causing mortality of worms and inhibition of egg hatching.

Caesalpinia crista (LC50 = 0.134 mg/ml) was found to be more potent than *Chenopodium album* (LC50 = 0.449 mg/ml) in egg hatch test.

In vivo, maximum reduction in eggs per gram (EPG) of faeces was recorded as 93.9 and 82.2% with *Caesalpinia crista* and *Chenopodium album* AME at 3.0 g/kg on day 13 and 5 post-treatment, respectively. Levamisole (7.5 mg/kg), a standard Anthelmintic agent, showed 95.1–95.6% reduction in EPG.

Conclusions

These data show that both *Caesalpinia crista* and *Chenopodium album* possess Anthelmintic activity in vitro and in vivo, thus, justifying their use in the traditional medicine system of Pakistan.

TOXICOLOGY

The phytochemical analysis on the fixed oil extract from the seeds of *Caesalpinia crista* done by (Njoku, Okeke et al. 1999) revealed the presence of

ANTI- NUTRITIONAL TOXINS

- GOSSYPOL
- CYANOGENIC GLYCOSIDES
- CYCLOPROPANE
- HYDORXY AND EPOXY FATTY ACIDS

➤ **DECREASE IN EGG PRODUCTION AND REDUCED EGG AND CHICKEN SIZE IN CHICKEN**

In the studies done on chicken; the Gossypol and Cyclopropane cause decrease in egg production, size and chicken weight (Bhide, et al.1976).

➤ **ANTIFERTILITY IN MICE AND RAT**

The mice and rat fed on the *Caesalpinia crista* meal showed antifertility effect. This antifertility effect could be attributed to the compounds like Gossypol and especially Cyclopropane fatty acids; which has been implicated as an antifertility factor (Egbo E.A, 1984).

➤ **Anti-fertility effects of Gossypol has been demonstrated by (Brocas, Rivera et al. 1997)during pharmacological study done on bulls.**

Gossypol; a polyphenolic yellow pigment is also present in cotton plants (*Gossypium specie*) have been implicated as a reproductive toxicant.

In this study it was demonstrated that Gossypol (50 and 100 µg/ml) decreased the percentage of sperm, in bulls, that completed the swim-up procedure. This effect was not blocked by glutathione monoethyl ester. Cleavage rates were not different between oocytes inseminated with gossypol-treated spermatozoa (10 or 50 µg/ml) and oocytes inseminated with control

spermatozoa. Development to the blastocyst stage at Day 7 after insemination was reduced when spermatozoa treated with 50 µg/ml gossypol were used for fertilization. Gossypol toxicity was evident in cows fed cottonseed meal because erythrocyte fragility was greater than for control cows. However, there were no differences between cottonseed meal and control groups in number of oocytes collected per cow, cleavage rate after in vitro maturation and fertilization, or the proportion of oocytes or embryos that developed to blastocyst. Similarly, exposure of oocytes to 2.5-10 µg/ml gossypol during in vitro maturation did not affect cleavage rates or subsequent development. In contrast, addition of 10 µg/ml gossypol to embryos reduced cleavage rate. Moreover, development of cleaved embryos was reduced by culture with 5 or 10 µg/ml gossypol and tended to be reduced by 2.5 µg/ml gossypol.

In conclusion, bovine gametes are resistant to gossypol at concentrations similar to those in blood of cows fed cottonseed meal. In contrast, the developing embryo is sensitive to gossypol.

DISCUSSEION/ CONCLUSION

➤ ANTI-MALARIAL ACTIVITY

Malaria is one of the most life-threatening infectious diseases worldwide and claims the millions of people's life each year. The appearance of drug-resistance *Plasmodium falciparum* has made the treatment of malaria increasingly problematic, and thus, it is a dire need to search the new alternatives of current drugs.

Studies done by (Awale, Linn et al. 2006), (Kalauni, Awale et al. 2006), (Linn, Awale et al. 2005), on 44 cassane and Norcassane diterpenes shows the dose dependent anti-malarial effect, in invitro studies on *plasmodium falciparum* FCR-3/A2 clone, of the substances isolated from *Caesalpinia crista* extract including Caesalpinin K and norcaesalpinin F showed IC 50 values 120 nM and 140 nM respectively.

But that most potent effect was seen with Norcaesalpinin E with IC50 value 0.090µM is stronger than the clinical drug chloroquine having IC50 value 0.29 µM.

These in vitro studies give scientific basis for the usage of; these guide lines could be used to design clinical trials in humans and efficacy could be tested for sub-types of malaria. And this herbal remedy which has been effectively used in Asia and Africa as a traditional antimalarial remedy could become a significant anti-malarial drug in the future and could effectively solve the resistance problems associated with the marketed drugs.

➤ **ACCELERATION OF FIBULIN-5 PRODUCTION/ ACTIVITY**

The work done by (Ogura, Amano et al. 2006) found the modulation of Fibulin-5 by a co-formulation including *Caesalpinia crista* and a few other plants; Fibulin-5 is a calcium dependent elastin-binding glycoprotein which participate in calcium signaling pathways (by binding to calcium) with their role in signal transduction. Calcium-binding proteins contribute to all aspects of the cell's functioning from homeostasis to memory.

Further Fibulin- 5 is and integrin-binding extracellular protein that mediates endothelial cell adhesion; also being a calcium dependent elastin-binding protein it scaffolds cells to elastic fibers, thereby preventing elastinopathy in the skin, lung and vasculature (Schiemann, Blobe et al. 2002).

These facts could provide the scientific basis for usage of *Caesalpinia crista* as tonic herb in the traditional healthcare in Asia and Africa, and other effects like protection against elastinopathy, through modulation of fibulin-5.

But the effect of *Caesalpinia crista* in this composition should be further studied as individual drug to further verify this effect and find the ingredients responsible for this effect.

➤ **TREATMENT OF LEKORRHAGIA**

The scientific work done by (Duan 2006) described an effective and long acting anti-leukorrhagic herbal remedy including *Caesalpinia crista* a number of other ingredients. This may scientifically support the traditional anti-leukorrhagic usage but it is further needed to search the active ingredient responsible for this effect.

➤ **ANTI-PARASITAL EFFECTS**

The study done by (Maqbool, Hayat et al. 2004) and (Javed, Akhtar et al. 1994) gives scientific basis for the traditional usage of *Caesalpinia crista* as Anthelmintic drug in Asia and Africa. However the effect is not stronger than that of *Chenopodium album* but weaker than the synthetic drug Triclabendazole and Levamisole.

Research could be done to work on a combination formulation including triclabendazole or Levamisole and *Caesalpinia crista* and/ or other herbs proved useful Anthelmintic to design a formulation which could reduce side effects related to monotherapy alone with the synthetic substances.

By using these guidelines after successful usage in animals *Caesalpinia crista* could also be clinically tested on humans and may provide a significant effective and cost effective alternative to the presently marketed anti-parasital drugs and could meet the challenges of resistance to the clinical drugs in use.

➤ **CHEMICAL STUDIES ON FIXED OIL FROM *Caesalpinia crista***

The chemical studies done on the fixed oil obtained from *Caesalpinia crista* showed little nutritional value but it could be employed as a local cheap source of industrial oil and could substitute many industrial processes, could be employed in the cosmetics industry, considering some components of the oil especially the vitamins and lecithin present.

➤ **GOSSYPOL A MALE CONTRACEPTIVE AGENT**

The study done by (Brocas, Rivera et al. 1997) demonstrates that at concentrations above 10µg/ ml gossypol caused a concentration-dependent decrease in the percentage of sperm successfully completing the swim-up procedure as compared to sperm without gossypol. Both 50 µg/ml and 100µg/ ml reduced the proportion of sperm

motility hence reduced the proportion completing the swim-up procedure indicating gossypol induced failure to fertilize.

Further addition of 10µg/ ml gossypol after fertilization caused a reduction in cleavage rate leading to reduced development of embryo.

(Ji 2008) has invented a method of production of gossypol acetate; a mile stone towards production of herbal contraceptives for males.

These facts give scientific basis for traditional usage of *Caesalpinia crista* as male contraceptive and Abortifacient. It is a significant finding since there is not available any synthetic male contraceptive in the market. Further research could be done to design a safe and effective herbal male contraceptive.

Further (Wang, Li et al. 2006) demonstrated in a pharmacological in vitro study that gossypol could induce differentiation in the leukemia HL-60 cells, and it may be a potential therapeutic agent, chemoprevention or chemotherapeutic adjuvant especially in combination drug therapy for leukemia.

A lot of other traditional usages in Asia and Africa need to be proven by scientific and clinical work. Further the efficacy during pregnancy looks risky due presence of anti growth substances like gossypol and cyclopropane compounds.

REFERENCES

AOAC (1975), Official methods of analysis of the Association of Official analytical chemists, 12th edition, AOAC Washington D.C.

Awale, S., T. Z. Linn, et al. (2006). "Constituents of *Caesalpinia crista* from Indonesia." Chem. Pharm. Bull. **54**(2): 213-218.

Bassiar O. (1978), Handbook of practical Biochemistry, University press Ibadan, Nigeria pp.38.

Bhide M.B., Nikam, S.T. and Chavann S.R. Indian Council of Medicinal Research pp. 228(1976).

Brocas, C., R. M. Rivera, et al. (1997). "Deleterious actions of gossypol on bovine spermatozoa, oocytes, and embryos." Biol Reprod **57**(4): 901-907.

Cheenpracha, S., R. Srisuwan, et al. (2005). "New diterpenoids from stems and roots of *Caesalpinia crista*." Tetrahedron **61**(36): 8656-8662.

Duan, Z. (2006). Manufacture of traditional Chinese medicine granule for treating Leukorrhagia. Application: CN

CN, (Peop. Rep. China). 4pp.

Egbo E.A.(1984) B.Sc Thesis University of Nigeria, Nsukka.

Javed, I., M. S. Akhtar, et al. (1994). "Comparative anthelmintic efficacy and safety of *Caesalpinia crista* seed and piperazine adipate in chickens with artificially induced *Ascaridia galli* infection." Acta Vet Hung **42**(1): 103-9.

Ji, P. (2008). Method for producing gossypol acetate. Application: CN

CN, (Peop. Rep. China). 8pp.

Kalauni, S. K., S. Awale, et al. (2006). "Antimalarial activity of cassane- and Norcassane-type diterpenes from *Caesalpinia crista* and their structure-activity relationship." Biol. Pharm. Bull. **29**(5): 1050-1052.

Kinoshita, T., Y. Haga, et al. (2005). "The isolation and structure elucidation of new cassane diterpene-acids from *Caesalpinia crista* L. (Fabaceae), and review on the nomenclature of some *Caesalpinia* species." Chem. Pharm. Bull. **53**(6): 717-720.

Letutour B. Totaoni Elaraki A. and Ihal I. JAOCs 60(4) 835 *(1938).

Liang, L. and S.-m. Wang (2007). "Research advances of antitumor effect of gossypol." Guangdong Yixue **28**(9): 1535-1537.

Linn, T. Z., S. Awale, et al. (2005). "Cassane- and Norcassane-Type Diterpenes from *Caesalpinia crista* of Indonesia and Their Antimalarial Activity against the Growth of *Plasmodium falciparum*." Journal of Natural Products **68**(5): 706-710.

Maqbool, A., C. S. Hayat, et al. (2004). "Comparative efficacy of various indigenous and allopathic drugs against fasciolosis in buffalo." Vet. Arh. **74**(2): 107-114.

Njoku, O. U., U. Okeke, et al. (1999). "Preliminary investigation on some nutritional and toxicological properties of *Caesalpinia crista* seed and oil." Boll. Chim. Farm. **138**(10): 552-554.

Pradines, B.; Mamfoumbi, M.; Parzy, D.; Medang, O.; Lebeau, C.; Mbina, J. R. M.; Doury, J. C.; Kombila M. Am. J. Trop. Med. Hyg. **1999**, 60, 105-108.

Ogura, Y., S. Amano, et al. (2006). Composition and method for accelerating fibulin-5 production and/or enhancing fibulin-5 activity. Application: WO, (Shiseido Company, Ltd., Japan). 25 pp.

Ringwald, P.; Eboumbou, E. C. M.; Bickii, J.; Basco, L. K. Antimicrob. Agents Chemother. **1999**, 43, 1525-1527.

Schiemann, W. P., G. C. Blobe, et al. (2002). "Context-specific Effects of Fibulin-5 (DANCE/EVEC) on Cell Proliferation, Motility, and Invasion. FIBULIN-5 IS INDUCED BY TRANSFORMING GROWTH FACTOR-beta AND AFFECTS PROTEIN KINASE CASCADES." J. Biol. Chem. **277**(30): 27367-27377.

Stadam T.C. (1957) preparation and assay of cholesterol and ergosterol, In: Colowick S.P. and Kaplan N. (eds.), *Methods in Enzymology*, vol 3, Academic Press New York, pp 392.

Wang, W.-Q., R. Li, et al. (2006). "Gossypol-induced differentiation in human leukemia HL-60 cells." *Int. J. Biomed. Sci. (Monterey Park, CA, U. S.)* 2(4): 395-400.

Whistler R.L. (1984) starch, *Chemistry and Technology*, 2nd edition, Academic Press London, pp.337.

PHOTO REFERENCE

<http://homepage3.nifty.com/inagiyasou/photo/iriomote06/jmk2/nantenkazra.jpg>

Retrieved in December, 2008.

Smilax ornata



BOTANICAL NAME: - *smilax ornata* (Lem)

ENGLISH NAME: - *sarsaparilla*

URDU/ LOCAL NAME: - Ashba

FAMILY: - Smilacaceae

INTRODUCTION

A number of plants in genus *smilax* are called *sarsaparilla* including *smilax ornata*, *smilax regelii*, *smilax sarsaparilla* etc; in this literature search emphasis is given to *smilax ornata* but a few other species known as *sarsaparilla*, having significant traditional medicinal usage, have also been described.

PARTS OF PLANT USED: - Dried root and rhizome.

ETHNOPHARMACOLOGY

USES IN PAKISTAN

1. Rheumatism
2. Gout
3. Skin disorders
 - Eczema
 - Psoriasis

USES IN MORROCO

Treatment of Leprosy.

HEALTH SUPPLIMENT USED IN USA

(Hastings, Barnes et al. 2001) describes a health supplement, in which *sarsaparilla* root is present as 10.75%, along with other herbs, for healthy joints.

CHEMISTRY

(Power and Salway 1914), (Van Der Haar, 1929), (British Herbal compendium, 1992)

ISOLATION OF ESSENTIAL OIL (Power and Salway 1914)

For the purpose of complete chemical examination 22.1 Kilograms of the ground material were extracted by continuous percolation with hot alcohol. After the removal of greater portion of the alcohol, a viscid, dark-colored extract was obtained, amounting to 2.95 kilogram.

The whole of above extract was mixed with water, and a vigorous current of steam passed through the mixture for several hours. The distillate, on extraction with ether, yielded 2.6 gram of an essential oil. Being thus equivalent to about 0.01 percent of the weight of root employed. This essential oil, when distilled under diminished pressure, passed over between 70 and 200°/15 mm. as a pale yellow liquid, which possessed a pleasant, somewhat aromatic odor, a density of 0.977 at 15°/15°, and was not completely soluble in 70 percent alcohol. It was found to contain furanaldehyde, and also gave a bluish-black color with ferric chloride, thus indicating the presence of a phenolic substance.

ISOLATION OF RESINS

After above operation the steam distillation flask contained a considerable quantity of a brown resin, which formed with the aqueous liquid a viscous emulsion.

Since the resin did not separate on keeping, the mixture was agitated with hot amyl alcohol. By this means a very dark-colored aqueous liquid (A) was obtained, whilst the amyl-alcoholic extract contained the resin (B), partly in solution and partly in suspension. The extract was filtered, the filtrate well washed with water, the solvent then removed, and the residual semi-solid resin, together with that portion which was insoluble in amyl alcohol was separated.

Resin separated is composed of following:-

- Sitosterol(C₂₇ H₄₆O)
- Sitosterol-d-glucoside(Phytosterolin), C₃₃H₅₆O₆

The following are main substances obtained from *Sarsaparilla* root extract.

1. Parillin (C₂₆H₄₄O₁₀, 2 ½ H₂O) Crystalline and almost insoluble in cold water.
2. Sarsaponin (C₄₅H₇₄O₁₇), a crystalline solid, isolated from the alcoholic solution of resin.

Readily soluble in water, it has melting point 230-240 degree centigrade (Van Der Haar, 1929). And formula, on hydrolysis it yields one molecule of Sarsapogenin (Parigenin), two molecules of glucose and one molecule of rhamnose.

Sarsapogenin is chemically classified as “Digitalis glycosides”, should not be confused with cardiac glycosides which is a different chemical group having different chemical functions. In addition Sarsapogenin has no pharmacological action on cardiac muscles. A still further differentiation lies ability of digitalis saponins to hemolyze erythrocytes. Furthermore the standardization of this Sarsaponin compound by the hemolytic index serves completely to control optimal quality, uniformity and composition.

3. Sarsapogenin (C₂₆ H₄₂ O₃), obtained by hydrolysis of Sarsaponin.
4. Sarsapic acid (C₆H₄O₆), it is isolated from the aqueous liquid (A). It is soluble in alcohol, sparingly soluble in cold water and ether.
5. Smilasaponin (C₂₀H₃₂O₁₀)₅,12H₂O, Amorphous and soluble in water.
6. Smilacin(Smilasaponin)
7. Smilagenin
8. Steroidal saponins
9. Fatty acids
10. Phytosterol(isolated from resinous residue)
11. Starch
12. Minerals(Potassium) (British Herbal Compendium, 1992).

ENZYMES (Power and Salway 1914)

In the following test described in “The British pharmacopoeia” it was confirmed that sarsaparilla extract contain enzymes.

500 g of powdered sarsaparilla root was mixed with water and kept for two days at ordinary temperature. The mixture was then filtered under pressure, and alcohol added to the filtrate. A flocculent precipitate was thus produced. Which when dried in vacuum over sulphuric acid, amounted 2.6 gram or 0.52% of the weight of root employed. This substance slowly hydrolyzed *amygdaline*, thus indicating its enzymic activity.

Sarsaparilla has been erroneously touted to contain testosterone and/or other anabolic steroids. While it is a rich source of steroidal saponins, it has never been proven to have any anabolic effects, nor is testosterone found in sarsaparilla or any other plant souce.

BIOLOGY/ PHARMACOLOGY

1. ANTI-INFLAMMATORY

(Ageel et al, 1989) researched on *sarsaparilla* and a six other plants, being used in Saudi Arabia for their anti-inflammatory effects in *Carrageenan*-induced acute inflammation in rats. The plant materials were extracted with 96% ethanol. The dried extract was dissolved in water for pharmacological testing. The rats were administered an oral dose of 500mg/kg body weight of each extract 1 hour before production of inflammation by *Carrageenan* injection.

The paw volume was measured at 0, 2, 3 and 4 hours the injection.

Sarsaparilla (25 %) and three other plants out of seven plants inhibited Carrageenan induced acute inflammation. *sarsaparilla* also inhibited cotton pellet induced exudation. According to this study *sarsaparilla* has 25 % anti-inflammatory activity which is a significant effect.

2. TONIC EFFECTS

Sarsaparilla has been traditionally used as tonic; it improves tone, vigor and function of a particular body organ or body system. It has also been used as male tonic and it is believed that *sarsaparilla* tonifies the male reproductive system.

CLINICAL STUDIES

1. TRETMENT OF SKIN DIORDERS/PSORIASIS

BAKGROUND

(Ransom. 1901), in an out –moded way, demonstrated a chemical affinity between the saponins and serum cholesterin by which the saponins acted on tissues without causing haemolysis.

A few researchers believed that psoriasis is related to higher cholesterol/ lipid in the blood and described that disturbance in the metabolism of lipids to be the cause but these arguments were countered by opposite observations relating lower cholesterol to be the cause of psoriasis.

(Madden et al, 1939) using cholesterol-tolerance tests, observed that hypercholesteremia tests, observed that hypercholesteremia did not generally exist but a lower fat diet was of definite value in 68 % cases tested for psoriasis. Madden, s patients were hospitalized. He inclined to believe that favorable effect of diet might be explained on the basis of general alignment of metabolism, rather than correction of disturbed fat metabolism and *sarsaparilla* because of its saponins contents has especial affinity for glucose.

Despite these conflicting findings Wise and Sulzberger, 1940 state that there is no doubt that certain cases of psoriasis are much improved by a strict “fatless” diet.

USAGE OF SARSAPARILLA FOR PSORIASIS

(Philippsohn 13) studied effectiveness of sarsaparilla extract leading to gradual relief in psoriasis. A no. of patients, under this study, took 1000cc of taped water, took 15 gram sarsaparilla in it and allowed the mixture to stand overnight, and then boiled for twenty minutes next morning. While the mixture was still hot they drunk half of that liquid that morning, the remainder was consumed afternoon same day.

(Ritter 14) studied a clinical trial in which 19 private patients were given 10 tablets each daily. Nine patients became completely or nearly completely free from psoriasis. The shortest required duration of treatment was two weeks.

CLINICAL TRIAL DONE BY (THURMON, 1942)

In these clinical trials, covering two years, 92 patients were studied. Of these 75 patients of psoriasis were treated with *sarsaparilla* tablets and 17 served as controls (only given topical treatment with salicylate ointments, sunlight etc.).

TREATMENT

Oral treatment

Adult dosage:-

1 tablet twice daily, preferably taken with warm liquid, when empty stomach.

A larger dose comprising of two tablets twice daily or even two tablets morning and evening and one at noon.

Children:-

Children aged 5 years or under, half a scored tablet given twice daily is sufficient.

Local treatment

The 75 treated patients did not get any local treatment in the first 12 months. After 12 months it was determined that *sarsaparilla* has given relief. Afterwards local treatment with irradiation in the form of sunshine or ultraviolet lamp, boric acid ointment, lanolin petroleum, olive oil, mild precipitate ointment, salicylate and sulphur ointment, and modified coal tars.

RESULTS

62 percent of the treated patients gave good results to treatment. 14 percent became clearly well from psoriasis including a patient who has been sick of psoriasis for the last 18 years.

2. TREATMENT OF LEPROSY

(Leprosy)

The Norwegian doctor Gerhard Armauer Hansen identified the etiologic agent, *Mycobacterium leprae*, which causes the infectious disease leprosy or Hansen's disease that affect mainly the peripheral nerves of the human being skin.

(Rollier 1959) clinically demonstrated effect of leprosy, by a clinical study in combination with a synthetic anti-leprotic drug called DDS (4, 4-diaminodiphenylsulfone); pathologies of nasal mucosa and lepromas were measured to find out the clinical effect.

Material and methods

1. Weak aqueous extracts of *Smilax ornata*
2. Tablets containing 240 mg of the extracts.

The leprosy effected patients were divided to two major groups; the first one receiving a combination treatment with *Smilax ornata* and DDS (4, 4-diaminodiphenylsulfone) {DDS-smilax} and the second one only DDS as a monotherapy to serve as control.

It is notable that all cases had positive skin lesions, but that several in each group gave negative nasal smears. It was impossible to make the groups strictly comparable, and the DDS-smilax group had slightly greater degree of positivity, which makes the findings more significant.

The first group comprised 111 cases-33 L1 (29%), 43 L2 (39 %), 30 L3 (27%), and 5 regressive (5%).

The DDS was given in progressive doses, starting at 25 mg and increasing by 25 mg every 3 weeks, never exceeding a daily dose of 150 mg for adults weighing 70 kg.

The sarsaparilla dosage was increased from 4 to 10 tablets in the first week, remaining at the daily dose of 10 tablets for a minimum period of 6 months and a maximum of 1 year. Subsequently, the treatment was continued with DDS alone in the classical dose of 150 mg per day for adults.

The bacteriologic, clinical and biological results, supported to some extent by pathologic findings, have been compared with those obtained with DDS alone in a comparable lot of 140 patients, distributed as follows: 42 L1 (30%), 56 L2(40%), 38 L3(27%), and 4 regressive (3%). The patients of this second group were given the same doses of DDS as those in the first group. With both groups the treatment was given 6 days a week.

BACTERIOLOGIC CHANGES IN NASAL MUCOSA

The degree of bacteriologic passivity in the two groups, group 1 on the combination treatment and group 2 on DDS alone, in the nasal mucosa are given below in the table.

Degree of bacteriological positivity in percentages, before, during and after treatment, of Group 1 (DDS-smilax, 111 cases) and Group 2 (DDS control, 140 cases), in the nasal mucosa.

GROUP 1 (DDS-*smilax*, 111 cases)

Period of treatment	Negative	1+	2+	3+	4+
Before	0a	14	34	42	10
After 6 months	54	29	13	4	0
After 1 year	83	9	8	0	0
After 1 ½ year	93	7	0	0	0
After 2 year	100	0	0	0	0
After 2 ½ year	100	0	0	0	0

a of the 111 cases in Group 1 , only 100 were positive in the nasal mucosa before treatment, and they are taken as 100 percent in this table.

GROUP II (DDS control, 140 cases)

Period of treatment	Negative	1+	2+	3+	4+
Before	0b	22	33	35	10
After 6 months	27	34	29	10	0
After 1 year	52	37	8	3	0
After 1 ½ year	74	21	4	1	0
After 2 year	86	12	2	0	0
After 2 ½ year	98	2	0	0	0

b of the 140 cases in Group II only, 120 were positive in the nasal mucosa and are taken as 100 percent.

With respect to the changes under treatment, considering first the cases positive in the nose (100 of the 111 in the combined-treatment group and 120 of the 140 in the DDS control group), 54 percent of the first group became negative within 6 months, and 83 percent within a year; whereas of the second group only 25 % had become negative after 6 months and 52 percent in a year. The nasal smears of all the patients in the experimental group were negative by the end of 2 years and those of control group in 3 years.

BACTERIOLOGIC CHANGES IN LEPROMAS

Degree of bacteriological positivity in percentages, before, during and after treatment of Group 1 (DDS-smilax, 111cases) and Group 2 (DDS control, 140 cases), in the lepromas.

Group 1 (DDS-smilax group, 111 cases)

Period of treatment	Negative	1+	2+	3+	4+
Before	0 ^c	6	16	50	28
After 6 months	13	23	30	32	2
After 1 year	24	22	32	22	0
After 1 ½ year	37	27	29	7	0
After 2 year	51	41	10	0	0
After 2 ½ year	57	32	11	0	0
After 3 years	61	33	6	0	0

^c All the cases were positive in lepromas.

Group II (DDS control group, 140 cases)

Period of treatment	Negative	1+	2+	3+	4+
Before	0 ^c	16	31	32	22
After 6 months	4	30	32	28	6
After 1 year	8	48	29	11	4
After 1 ½ year	22	51	21	5	1
After 2 year	41	42	13	4	0
After 2 ½ year	57	31	11	1	0
After 3 years	61	27	12	0	0

c All the cases were positive in lepromas.

All the patients were positive to lepromas and were biopsied routinely before treatment and simultaneously several punctures were made. These punctures and biopsies were repeated every month for 6 months and every 6 months thereafter. The same person took the biopsy specimens and made the smears and the same physician interpreted the microscopic findings, so that co-efficient of error is the same in the two groups.

RESULTS

The above tables show significance of combined treatment with (DDS-smilax); having greater effect as all the patients getting rid of nasal smears within 2 years as compared to control group.

After 6 months 13 percent of Group 1 (*DDS-smilax* group) became leproma fri as compared to only 4 percent in Group II (DDS alone, control group). Also no patient showed highest positivity 4+ within a year from Group 1 but there was still 4 percent in the Group II having highest bacteriologicity at the end of one year.

These findings prove that the effect of *sarsaparilla* is unquestionable. Its effects persisted even after the cessation of its administration; since it was not until the end of the third year of treatment that a lot of patients who received DDS alone come up to the same level of negativity as those who received the combined treatment in the first year with both *DDS* and *smilax ornata*.

TOXICOLOGY

There are not reported toxicological studies done on *sarsaparilla* in the authentic scientific literature.

Two women used sarsaparilla tablets during pregnancy got significant effect for psoriasis without any harmful effects on fetus (Thurmon, 1942).

(Philipsohn¹³) did not notice untoward effects from *sarsaparilla*; one patient who has used sarsaparilla extract in twenty years did not experience a single relapse of psoriasis.

DISCUSSION/ CONCLUSION

➤ ANTI-INFLAMMATORY ACTIVITY

(Ageel et al, 1989) demonstrated anti-inflammatory effect of *sarsaparilla* in pharmacological studies in rats; these findings could be used to design clinical trials in humans. The traditional usage for years has already proved it safe and effective. This study gives scientific basis, for traditional usage of *sarsaparilla* as

Anti-inflammatory herb, in the treatment of rheumatism and gout, in Pakistan and use in USA as a part of health food supplement (Hasting et al, 2001).

These findings open for further research to find out the active ingredients having anti-inflammatory effect and may lead to good herbal alternative, which could be used either alone or in combination with synthetic substances, to reduce a lot of serious side effects related with synthetic NSAIDs.

➤ ANTI-LEPROTIC TREATMENT

With the advent of modern medication with mullet drug therapy for leprosy; this disease is vanishing slowly vanishing. But we can meet it principally in the tropical zone countries. Brazil has the second greatest number of leprosy cases around the world with almost 30,000 new cases diagnosed in 2005(Jose M et al, 2007).

The studies done by (Rollier 1959) gave scientific/ epidemiologic prove for the efficacy of *Smilax ornata* being used by the Moroccans in the traditional settings. It gives considerable interest in the mixed treatment, for endemic areas where it is technically impossible to hospitalize systematically all open cases.

A combination of *Smilax ornata* and synthetic drugs could be even more effective treatment which helps to overcome the problem of drug resistance.

➤ TREATMENT OF PSORIASIS

A new drug, the water soluble saponins, called “Sarsaponin, obtained from Honduras *Sarsaparilla* is described in the study done by (Thurmon et al, 1942). It shows 62 percent got beneficial effect of oral treatment with sarsaparilla. 14 percent gave complete relief from psoriasis. These facts support the traditional usage of *sarsaparilla* for the treatment of psoriasis, however the exact mechanism of actions has not yet been determined and there is need for further research to find out the active ingredient. Also combined therapeutic approaches could be employed including local treatments to get the best results. This study gives scientific basis for the traditional usage of *Sarsaparilla* against skin disorders like psoriasis etc.

REFERENCES

- Ageel AM, Mossa JS, Al-Yahya MA, et al. Experimental studies on antirheumatic crude drugs used in Saudi traditional medicine. *Drugs Exp Clin Res* 1989;15:369–72.
- British Herbal Medicine Association, *British Herbal Compendium, A handbook of scientific information on widely used plant drugs*, Volume 1, BHMA, pp 443-444, 1992.
- Hastings, C. W., D. J. Barnes, et al. (2001). Dietary supplement for nutritionally promoting healthy joint function. Application: US, (Reliv International, Inc., USA). 4 pp.
- Jose M et al, *Revista Brasileira de Farmacognosia Brazilian Journal of Pharmacognosy* 17(1): 141-148, Jan./Mar. 2007.
- Madden, J. F. Cholesterol balance and low fat diet in psoriasis. *Arch. Dermat. & Syph.* 39:268-277,1939.
- Philippsohn, A. Ekzem-und Psoriasisbehandlung nach eigener Methode. *Dermat. Wchnschr.* 93:1220-1223,1931.
- Power, F. B. and A. H. Salway (1914). "Chemical examination of sarsaparilla root." *J. Chem. Soc., Trans.* **105**: 201-19.
- Ransom, F. Saponin und sein Gengengift. *Deutsche med. Wchnschr.* 27:194-199,1901.
- Ritter, H. Über dei Behandlung der Psoriasis mit Sarsaparilla. *Deutsche med. Wchnschr.* 62:1629-1631,1936.
- Rollier, R. (1959). "Treatment of lepromatous leprosy by a combination of DDS and sarsaparilla (*Smilax ornata*)." *Int J Lepr* **27**: 328-40.
- Schulz, w.v. Constituents of Sarsaparilla. *Pharm. Journal* 52:6, 1892.
- Thurmon FM, "The treatment of psoriasis with sarsaparilla compound", *New England Journal of Medicine*, 337, pp 128-133, 1942.
- Van der Haar, A. Untersuchungen über die Saponine und verwandte köper. *Rec. trav. Chim. D. pays-bas.* 48:726-742,1929.
- Wise, F., and Sulzberger, M. B. *The 1940 Year Book of Dermatology and Syphilology*. PP. 782, Chicago: The year book publishers, inc., 1940, p. 4.

PHOTO REFERENCE

http://www.globalherbalsupplies.com/herb_information/images/sarsaparilla.jpg

Retrieved in December, 2008.

Styrax benzoin



BOTANICAL NAME: - *Styrax benzoin* (Dryand)

ENGLISH NAME: - Benzoin (USP 2008 p.1506)

URDU/ LOCAL NAME: - LOBAN

FAMILY: - Styracea

PARTS OF PLANT USED:-

Incised stem exudates.

DESCRIPTION

Benzoin obtained from *Styrax benzoin* is composed of blocks or lumps of varying size, made up of tears, compacted together, with a reddish brown , reddish gray , or grayish brown resinous mass. The tears are externally yellowish or rusty brown, milky white on fresh fracture; hard and brittle at ordinary temperature but softened by heat and becoming plastic on chewing (USP 2008 p. 1506-07).

➤ **PRODUCTION OF BENZOIN**

(Reinitzer 1926) described the production of *Styrax benzoin*.

Stems of the vigorous and healthy trees are incised and hammered at cambium level to obtain better flow of resin. The resin that first appears is amorphous, unsightly and therefore rejected of no value. Later a crystalline milk-white resin exudes which is collected. Collection is effected every 3rd month, first by removal of a bark-free layer, later a darker layer containing some bark and finally one dark and grossly contaminated layer. These 3 sorts are brought to the native towns in Sumatra and are further classified and packed in cases and distributed for sale worldwide.

ETHNOPHARMACOLOGY

USES IN PAKISTAN

1. Expectorant, Respiratory catarrh.
2. Antiseptic.

OTHER USES

➤ **USES IN THE PHARMACEUTICAL INDUSTRY**

Resinous material obtained from *Styrax benzoin* can be used to formulate incompatible vitamins, minerals and dietary supplements in the same tablets by coating around individual components. The coating can be done by dissolving the resinous material in an organic solvent and thoroughly mixing the concerned material in it followed by evaporation (Anderson 1946).

➤ **Adhesive** to surgical instruments

➤ **USED AS FIXATIVE IN PERFUMARY**

Gum benzoin can be used as fixative in perfumery, slows down dispersion of perfume particles (Boelens, De Rijke et al. 1982).

➤ **Used in cosmetics** and pharmaceutical colorless nail lacquer, nail lacquer bases and nail hardener compositions containing alcohol, shellac and benzoin oil Wahnhas (2002)

➤ **USAGE AGAINST TINNITUS**

(Joan scurlock et al p.7) described the usage of *Styrax benzoin* against tinnitus by the ancient Babylonian physicians, as described in the old manuscripts.

➤ **CO-INGREDIENT IN A FORMULATION AGAINST ENVIRONMENTAL POLLUTION CAUSED DISEASES**

Styrax benzoin has been used in an Ayurvedic preparation along with other ingredients against a no. of diseases caused by environmental pollution (Nityanand 1998).

CHEMISTRY

• Identification test:-

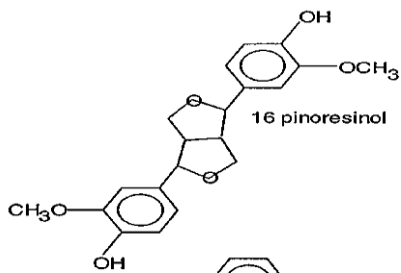
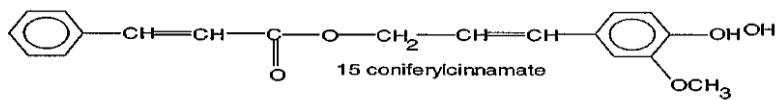
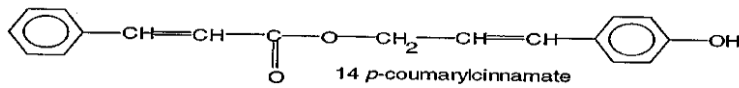
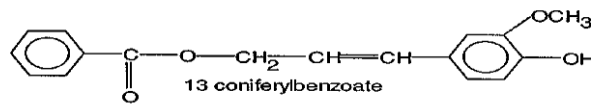
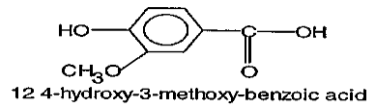
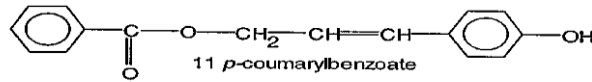
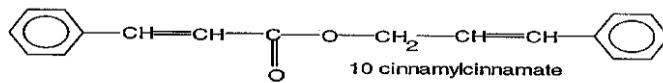
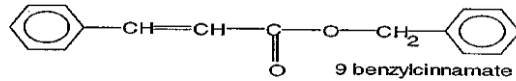
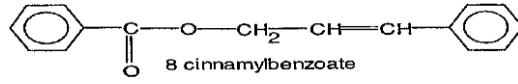
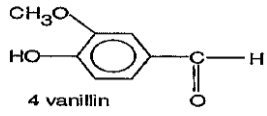
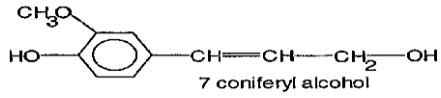
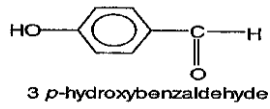
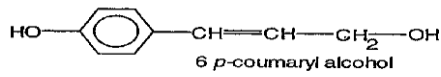
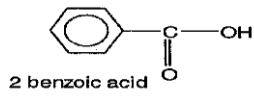
A solution in alcohol becomes milky upon the addition of water, and the mixture is acid to litmus paper (USP 2008 Vol II P.1507).

• Chemical composition:-

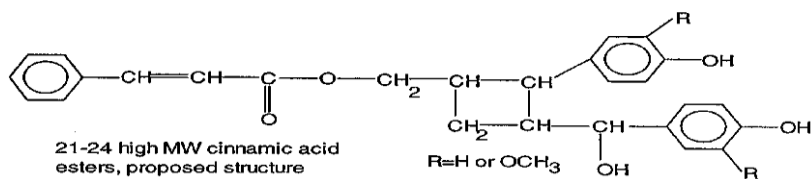
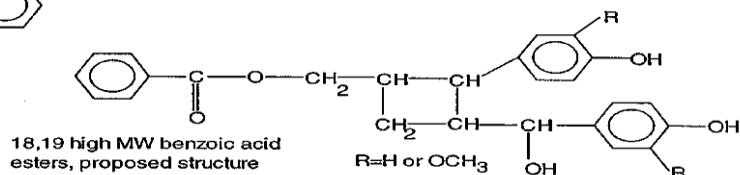
- Benzoin resin (a water insoluble resin) also commonly known as Sumatra benzoin or gum benzoin or gum Benjamin, this is mainly composed of balsamic acids i.e Benzoic acid.
- Free benzoic acids and cinnamic acids
- Esters of benzoic and cinnamic acids with coniferyl and p-coumaryl alcohols f.eks. Coniferyl benzoate.
- Pinoresinol

Different qualities of gum resin obtained from *Styrax benzoin* can be classified into six different classes based on color and particle sizes and method of harvesting. The first two classes are harvested from the stem under bark and are lighter in color and the remaining classes are harvested from the stem outside the bark and are darker in color; class sixth is the darkest one. Dark color is due to reaction with light for several days (I. Pastorova 1997).

Figure on next page:-Components identified in the gum benzoin resins from *Styrax paralleloneurum* Perk and from *Styrax benzoin* Dryand (Pastorova, de Koster et al. 1997).



17,20 unknown, pentacyclic triterpenes, $C_{30}H_{48}O_4$



• **Compounds identified by GC-MS and quantified by GC-FID in gum benzoin resins from *Styrax benzoin* percentage composition** ^a(Pastorova, de Koster et al. 1997).

Name compound <i>b</i>	Class <i>c</i>	Rn <i>d</i>	Ist <i>e</i>	3 rd <i>e</i>	Mix <i>e</i>
Benzoic acid	A	1	19.2	28.2	46.9
Cinnamic acid	B	0.94	1.8	7.5	40.4
Cinnamyl benzoate	C	0.74	0	0.5	0.5
Benzyl cinnamate	D	0.72	0	0	0
Cinnamyl cinnamate	D	0.72	0	0	0
<i>p</i> -coumaryl benzoate	C	0.86	52.5	30.5	0
Coniferyl benzoate	C	1.8	18.2	15.6	0
<i>p</i> -coumaryl cinnamate	D	0.83	1	1.1	0
Coniferyl cinnamate	D	0.9	0	0	0
Pinoresinol	F	0.86	4.1	10.8	6.1
Benzoic acid ester	F	0.88	1.1	1.4	0.6
Benzoic acid ester	F	0.88	1.3	2	0.6
Triterpene	E	0.78	1.3	3.1	2.3
Cinnamic acid ester	F	0.87	0	0	0

^aOnly compounds with a relative amount of more than 0.5% (normalized to the total composition) are listed; amounts less than 0.5% have been set to

Zero.

cA—free benzoic acid; **B**—free cinnamic acid; **C**—benzoic acid esters;
D—cinnamic acid esters; **E**—Triterpene; **F**—Pinoresinol and high molecular
Weight aromatic esters.

Rn *d*—relative molar response factor.

eQuality of resin tested: 1st—first quality; 3rd—third quality; Mix—a mixture
of third, fourth and fifth qualities.

BIOLOGY/ PHARMACOLOGY

1. STYRAX BENZOIN AGAINST OSTEOPOROSIS

Styrax benzoin extract is a calcium absorption acceleration activator and can be used both to prevent and cure bone diseases due to deficiency of Calcium like osteoporosis (Nomura, Murase et al. 2008).

Preparation of a gum benzoin extract involves:- Resin 10g of gum benzoin , 50% ethanol 100mL was added, left for seven days, after settlement extraction, it was filtered and extract, 92mL, was obtained at room temperature (Nomura, Murase et al. 2008).

2. ANTIALLERGIC ACTIVITY

(Suzuki, Shimada et al. 2006) studied and described that *Styrax benzoin* lowers production of IL 4(Interleukin 4) by 58%, when used in a concentration of 33% microgram/ ml, by T cells in the presence of antigen presenting cells (like allergens).

3. MELANIN FORMATION PROMOTER ACTIVITY IN A

CO-FORMULATION

(Suzuki, Umishio et al. 2002) invented a formulation comprising of various extracts of plants including *Styrax benzoin* to prevent gray hair. The mechanism of action is to improve cAMP (Cyclic Adenosine monophosphate) of the melanocytes which leads to increased melanin production, a decrease in white melanism and tanning effect. As a result prevention of grey hairs is achieved.

4. SKIN WHITENING EFFECT

According to (Tanaka 2006) *Styrax benzoin* along with other ingredients inhibits phagocytosis of melanosomes by keratinocytes in the skin epidermis thus inhibiting the transport of melanin, produced by melanosomes, to skin epidermis thus giving the skin a brighter color.

The substituting method which induces phagocytosis using false melanosomes, such as a latex bead, is reported (Klaus Wolff, et.al., J.Ultrastructure research, 39:262-280-1972).

TOXICOLOGY

1. Benzoin can cause skin sensitization (Martindale 33rd edition page 1668).
2. Allergic reactions have been reported to gum benzoin including eczematous reactions etc.

DISCUSSION/ CONCLUSION

> ANTISEPTIC USAGE

- The overuse, misuse and prophylaxis to the microbes making resistance being the major problem in the health care setting of antiseptics are gaining renewed interest due to having lower propensity for resistance instead of the matter of fact that antiseptics could be more lethal to mammalian cells as compared to the traditional antibiotics. Molecular iodine and silver are broad-spectrum antimicrobial agents that encourage healing in the microbially compromised wounds. New compounds inhibiting microorganisms like benzoin (*Styrax benzoin*) and emetine has been isolated from plants

(Muthusamy S K et al p. 94-95).

- Sumatra benzoin is used in topical preparations for its antiseptic and protective properties (Martindale 33rd edition p. 1668)

The following formulations for topical usage are available:-

--- (BP 2001) Compound Benzoin tincture (Frisar, s Balsam)

--- (BPC 1954) Compound Benzoin Tincture

---U8SP 2008 vole II) Compound Benzoin tincture

- Facts about antimicrobial activity of benzoin described by (Muthusamy et al, 2008)

The antiseptic usage of Benzoin obtained from *Styrax benzoin* as described above in the authentic scientific literature gives scientific evidence for antiseptic usage in wound healing/ disinfection *in* Pakistan in the traditional health care settings.

> RESPIRATORY CATARRH (EXPECTORANT)

- Sumatra benzoin obtained from *Styrax benzoin* is an ingredient of inhalations which are used in the treatment of the upper respiratory tract catarrh (Martindale 33rd edition)

Preparations available in (BP 1998), Benzoin inhalation.

Usage of *Styrax benzoin* against respiratory catarrh as described above in (BP 1998) and (Martindale 33rd edition) scientifically attests the traditional usage of *Styrax benzoin* for Respiratory Catarrh in Pakistan.

➤ USAGE AS CALCIUM ABSORPTION ACCELERATOR

Styrax benzoin extract acts as a calcium absorption accelerator by active transport process in the small intestine, it increases the gene expression of calcium- transporter protein Ca T1 (by manifesting mRNA) (Nomura, Murase et al. 2008).

This discovery could be used to develop oral drinks, tablets or other types of formulations by using suitable ingredients.

Besides, gum benzoin or its extract, calcium preparations, such as calcium carbonate and a calcium chloride, DFAIII (twin TOSU), FOS (fructo oligosaccharide), CPP (casein phosphopeptide), Citrate malic acid calcium, vitamin D, a vitamin K, soy isoflavone, collagen, etc. can be blended suitably if needed, and improvement in calcium ingestion and bone strength can be aimed at (Nomura, Murase et al. 2008).

The above findings could be a milestone to find alternatives to the modern treatment of bone diseases like osteoporosis, it could also be researched to combine both modern treatments available and benzoin formulation to get best effect and reduce frequency of side effects.

➤ **USAGE FOR TINNITUS**

Scurlock et al, year, described the anti Tinnitus usage of *Styrax benzoin* by the ancient Babylonian physicians (Babylonians was an ancient civilization many thousands of years ago).

The Babylonian physicians advised the persons sick of Tinnitus to Wrap Resin (Benzoin resin) in *Arantu* grass and *kukru* in a tuft of wool and put it down in water and boil over fire. Then to drop the extract in ears cavity

.

This description in the old manuscripts could be a starting point for the scientists looking for herbal solutions for Tinnitus.

REFERENCES

Anderson, C. N. (1946). Vitamin and mineral dietary supplement. US, (Lever Brothers Co.).

Boelens, H. M., D. De Rijke, et al. (1982). "Studies of some balsamic in perfumery." Perfum. Flavor. **6(6)**: 7-8, 10-14.

Joan Scurlock et al, A ringing endorsement for Assyro-babylonian medicine: The diagnosis and treatment of tinnitus in 1st Millenium BCE Mesopotamia; *Audiological medicine* 13.12.2007.

JP, (Ichimaru Pharcos Inc., Japan). 67 pp.

Martindale "The complete drug reference" 33rd edition 2002, p. 1634.

Muthusamy SK et al, *Journal of Surgical research* 144, 94-101(2008).

Nityanand, K. (1998). Process for the preparation of a novel ayurvedic anti-pollution medicine. Application: IN
IN, (Mantra Health & Herbais Private Limited, India). 7 pp.

Nomura, T., H. Murase, et al. (2008). Calcium absorption accelerators comprising Styrox benzoin. Application:JP, (Kao Corp., Japan). 8pp.

Pastorova, I., C. G. de Koster, et al. (1997). "Analytical study of free and ester bound benzoic and cinnamic acids of gum benzoin resins by GC-MS and HPLC-frit FAB-MS." Phytochem. Anal. **8(2)**: 63-73.

Reinitzer, F. (1926). "Production of benzoin." Arch. Pharm. Ber. Dtsch. Pharm. Ges. **264**: 368-82.

Suzuki, R., K. Umishio, et al. (2002). Gray hair-preventing agents, melanin formation accelerators, and cAMP formation accelerators contain plant-derived substances. Application: JP, (Shiseido Co., Ltd., Japan). 14 pp.

Suzuki, T., S. Shimada, et al. (2006). Interleukin 4 and 5 produced by T cells in the presence of antigen-presenting cells, Staphylococcus enterotoxin B and test substance for screening antiallergic agent and preventive. Application: JP, (Yakult Honsha Co., Ltd., Japan). 12pp.

Tanaka, K. (2006). Phagocytosis inhibitors containing specified plant extracts, and cosmetics containing the same. Application: JP, (Ichimaru Pharcos Inc., Japan). 67 pp.

USP 2008” The official compendia of standards volume II

Wohnhas, J. (2002). Cosmetic and pharmaceutical colorless nail lacquer, nail lacquer bases and nail hardener compositions containing alcohol, shellac and benzoin oil. Application: DE DE, (Wohnhas K.-G., Germany). 4 pp.

PHOTO REFERENCE

<http://astroromp.com/skyla/Styrax%20officinalis.jpg>

Retrieved in December, 2008.

Crocus sativus



BOTANICAL NAME: - *Crocus sativus* (L)

FAMILY: - Iridaceae

ENGLISH NAME: - Saffron

URDU NAME: - Zafran, Kesar

INTRODUCTION

The origin of saffron is obscure, but the plant is believed to have originated in the eastern Mediterranean, probably Asia Minor and Persia. The name “saffron” is derived from Arabic *zaʿ-*faran** “be yellow.” The spice was known to the Greeks as *Krokos* (as mentioned by Homer in the Iliad), but the name is pre-Greek and possibly of Babylonian-Assyrian origin (Schormüller 1970).

The chemical composition of saffron has been thoroughly studied and considerable knowledge about volatile as well as non-volatile saffron constituents, their analysis, stability and generation has been accumulated within the last decades. Whereas initially most of the research activities were directed to the analysis and structural elucidation of saffron pigments, the focus in recent studies has shifted towards the characterization of the complex saffron flavor as well as toward questions regarding the generation of some of the key flavor compounds. In the course of the still ongoing search for phytochemicals with antitumor activity, saffron carotenoids were also tested for possible activity. In different in vitro studies, saffron carotenoids were found to be highly effective. They inhibited, for example, proliferation of a leukemic cell line. Such findings stimulated renewed interest in the possible biological properties of a spice that has been in the pharmacopoeias of many countries for centuries.

PARTS OF THE PLANTS USED

Dried flowers.

Saffron is a spice derived from a flower of *Crocus sativus*, a species of family Iridaceae. The flower has three stigmas, which are the distal ends of the plant's carpels. Together with its style, the stalk connecting the stigmas to the rest of the plant, these components are often dried and used in cooking as a seasoning and coloring agent. For decades, saffron has been the world's most expensive spice by weight.

EHTNOPHARMACOLOGY

USES IN PAKISTAN:-

1. FOOD: - (i) Saffron is a flavoring agent in sweets and other types of dishes.

Saffron has aromatic odor and has been used in food making since the ancient times
(www.parc.gov.pk/data/medicinal/medsearch.asp).

(ii) Saffron has been used as food color
(www.parc.gov.pk/data/medicinal/medsearch.asp).

2. MEDICINAL:-

Saffron has following applications in the medical science in Pakistan[⊕]

- APHRODISIAC; In improving Sexual desire /erectile function e.g. HAMADOGEN[®] hamdard laboratories Pakistan/India/Bangladesh)
- ANTI-DEPRESSIVE
- EXPECORANT
- SEDATIVE
- STIMULANT; stimulates central nervous system
(www.parc.gov.pk/data/medicinal/medsearch.asp).

3. CHEMICAL USES

Saffron has been used chemically as:-

- a. Coloring agent
- b. Flavoring agent
- c. Astringent
- d. Resolving
- e. Detersive

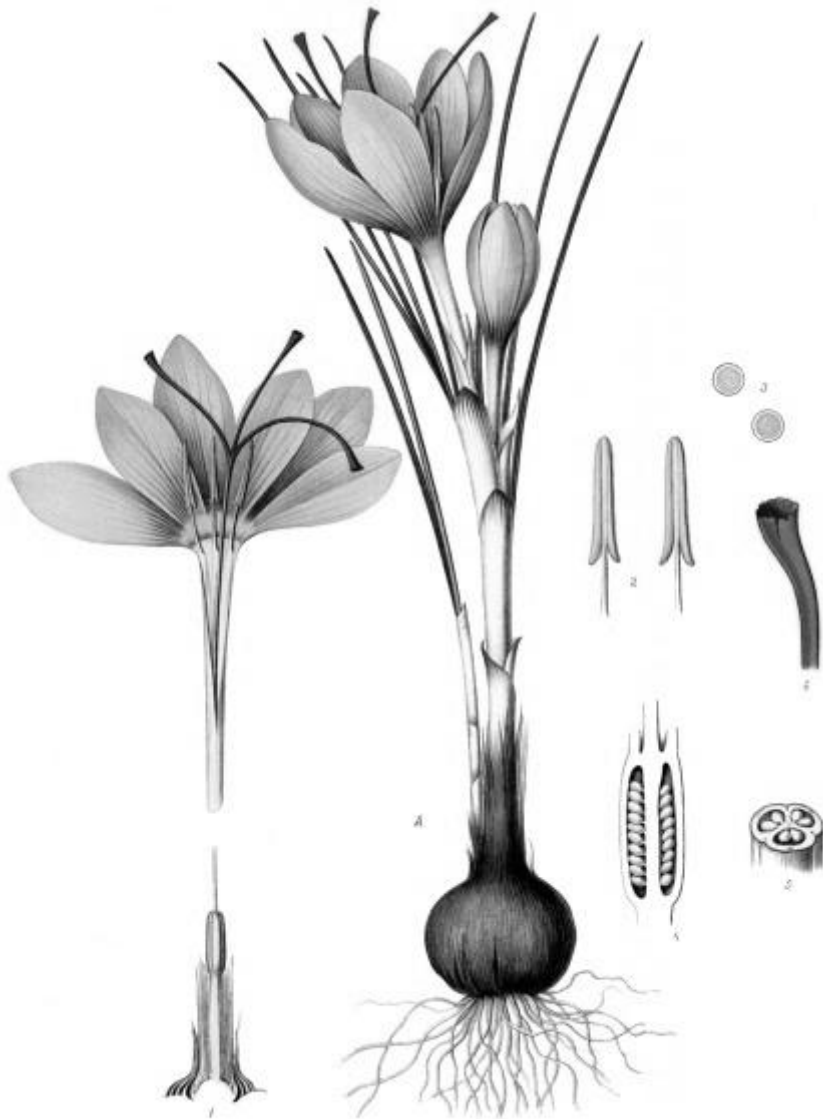
(www.parc.gov.pk/data/medicinal/medsearch.asp).

USES IN EUROPE

Saffron has a long history in European cuisine and today the spice is still used for Traditional fish and seafood dishes (*Risotto alla Milanese* in Italy, *Bouillabaisse* in France or *Paella Valenciana* in Spain). Saffron is a traditional ingredient in a few cakes, e.g., the German saffron cake ‘‘Gugelhupf.’’ Also the medicinal uses of saffron are diverse. Ancient Romans had hoped to benefit from its reputed ability to prevent hangovers by steeping the spice in their wine. Saffron has formerly being used as abortifacient(Straubinger 2000).

BIOLOGY OF SAFFRON

Petals of *Crocus sativus* flower are lilac to mauve and the outstanding feature of the flower is its three bright red stigmas which can reach a length of 25–30 cm(Straubinger 2000).



Saffron flowers are sterile, therefore the plant is not able to set viable seed and must be propagated vegetatively by the root tuber (G.A.Burdock 1995) (H.E.Laux 1996) (W.Franke 1989).

Plant is dormant in summer and gives flowers in autumn (Straubinger 2000).

CHEMISTRY

Saffron is characterized by a bitter taste and an Iodoform or hay-like fragrance

Caused by:-

- (i) Picrocrocin (Straubinger 2000).

And aroma is due to:-

- (ii) Safranal and 4-hydroxy-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde (HO-safranal) (Straubinger 2000).

It also contains a carotenoids dye:-

- (iii) Crocin which gives food a rich golden yellow hue (color) (Straubinger 2000).

VOLATILE COMPONENTS

Saffron contains more than 150 volatile and aroma-yielding compounds including Safranal. For a long time, safranal (2, 6, 6-trimethyl-1, 3-cyclohexadiene-1-carboxaldehyde) was considered to be the character impact compound of saffron's aroma. Only recently have sensory studies revealed that additional trace constituents in the volatile fraction are equally important for the typical aroma of the spice (W.Rödel 1991) (K R. Cadwallader 1997).

Quantitatively, safranal and its hydroxyl-derivative predominate in the aroma extract (47% and 13% of the total amount of volatiles, respectively). For both aldehydes (Zarghami and Heinz, 1971) assumed secondary formation via picrocrocin cleavage.

Next in concentration were compounds a (6%), b (6%), and c (3%). Each of the remaining volatiles was only present in a low concentration range of 0.3–2% of the total aroma fraction.

a=4-hydroxy-3,5,5-trimethyl-2-cyclohexene-1-one.

b=4-hydroxy-3-oxo-2,6,6-trimethyl-1,4-cyclohexadiene-1-carboxaldehyde.

c=3-oxo-2, 6, 6-trimethyl-1, 4-cyclohexadiene-1-carboxaldehyde.

NON-VOLATILE COMPOUNDS

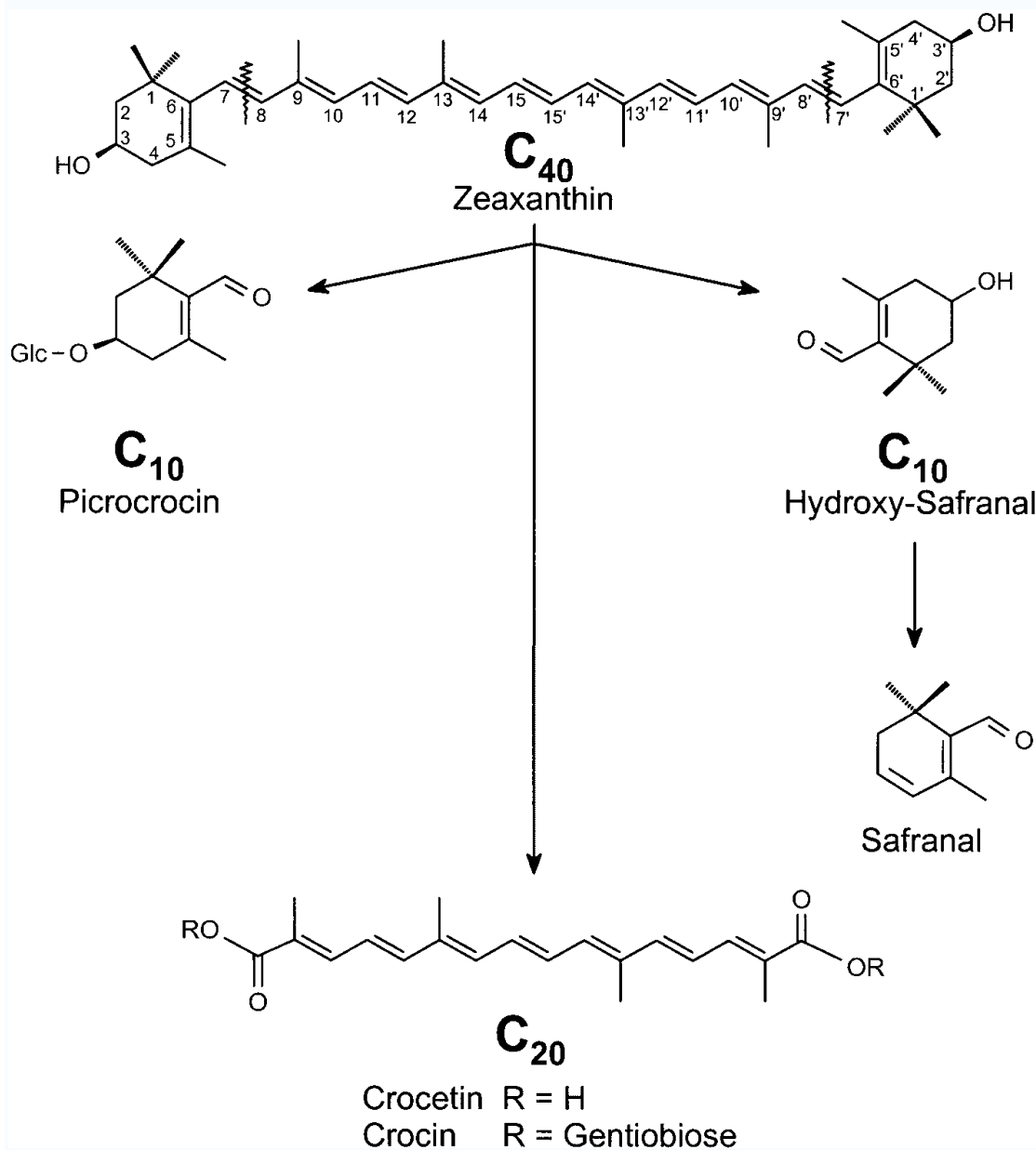
1. CROCIN

Crocus sativus extract contain many nonvolatile active components (Abdullaev, 2002), many of which are carotenoids, including zeaxanthin, lycopene, and various α - and β -carotenes. However, saffron's golden yellow-orange color is primarily the result of α -crocin. This crocin is trans-crocetin di-(β -D-gentiobiosyl) ester (systematic (IUPAC) name: 8, 8-diapo-8, 8-carotenoic acid). This means that the crocin underlying saffron's aroma is a digentiobiose ester of the carotenoid crocetin (Abdullaev, 2002). Crocins themselves are a series of hydrophilic carotenoids that are either monoglycosyl or diglycosyl polyene esters of crocetin (Abdullaev, 2002). Meanwhile, crocetin is a conjugated polyene dicarboxylic acid that is hydrophobic, and thus oil-soluble. When crocetin is esterified with two water-soluble gentiobioses (which are sugars), a product results that is itself water-soluble. The resultant α -crocin is a carotenoid pigment that may comprise more than 10% of dry saffron's mass. The two esterified gentiobioses make α -crocin ideal for coloring water-based (non-fatty) foods such as rice dishes (McGee, 2004).

2. PICROCROCIN

The bitter glycoside picrocrocin is responsible for saffron's flavor.

(chemical formula: $C_{16}H_{26}O_7$; systematic name: 4-(β -D-glucopyranosyloxy)-2, 6, 6-trimethylcyclohex-1-ene-1-carboxaldehyde) is a union of an aldehyde sub-element known as safranal (systematic name: 2, 6, 6-trimethylcyclohexa-1, 3-diene-1- carboxaldehyde) and a carbohydrate. It has insecticidal and pesticidal properties, and may comprise up to 4% of dry saffron. Significantly, picrocrocin is a truncated version (produced via oxidative cleavage) of the carotenoid zeaxanthin and is the glycoside of the terpene aldehyde safranal. The reddish-colored (Leffingwell, 2002) zeaxanthin is, incidentally, one of the carotenoids naturally present within the retina of the human eye.



(Postulated cleavage of zeaxanthin giving rise to saffron secondary metabolites)
(Straubinger 2000).

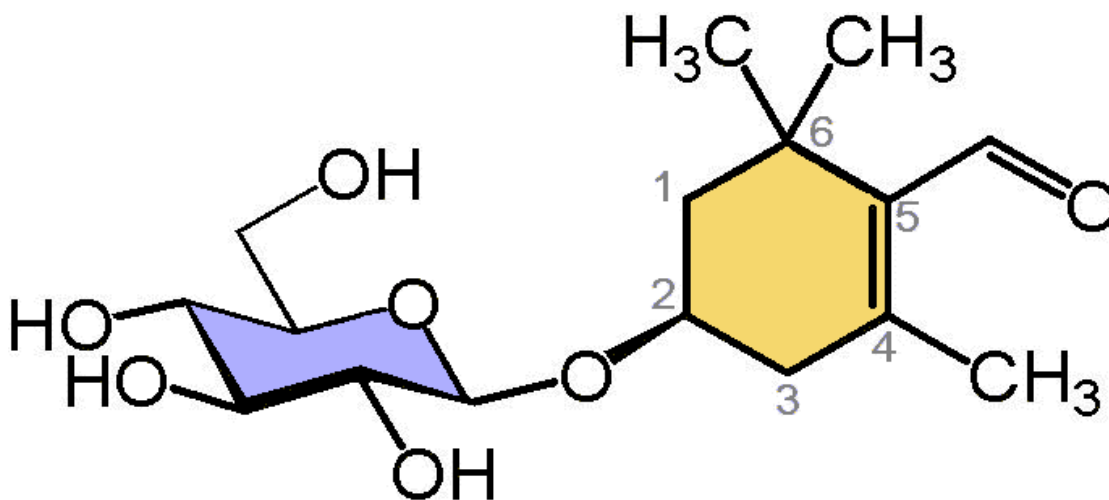
When saffron is dried after its harvest, the heat, combined with enzymatic action, splits picrocrocin to yield D-glucose and a free safranal molecule (Abdullaev, 2002). Safranal, a volatile oil, gives saffron much of its distinctive aroma (McGee, 2004, p.423), (Dharmananda,

s. 2005). Safranal is less bitter than picrocrocin and may comprise up to 70% of dry saffron's volatile fraction in some samples (Leffingwell, 2002).

A second element underlying saffron's aroma is 2-hydroxy-4, 4, 6-trimethyl-2, 5-cyclohexadien-1-one, the scent of which has been described as "saffron, dried hay like"(Leffingwell, 2002, p.3). Chemists found this to be the most powerful contributor to saffron's fragrance despite its being present in a lesser quantity than safranal (Leffingwell, 2002, p.3). Dry saffron is highly sensitive to fluctuating pH levels, and rapidly breaks down chemically in the presence of light and oxidizing agents. It must therefore be stored away in air-tight containers in order to minimize contact with atmospheric oxygen. Saffron is somewhat more resistant to heat.

β-D-glucopyranose derivative.

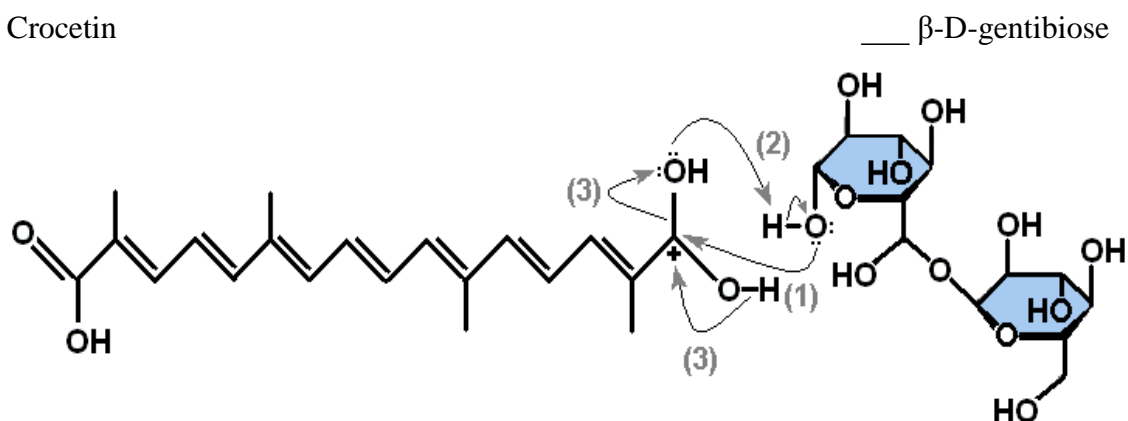
Safranal moiety



Chemical structure of PICROCROCIN

3. THE FORMATION OF CROCIN

Crocetin



___ β -D-gentiobiose

(THE ESTERIFICATION REACTION BETWEEN CROCETIN AND 2 GENIBIOSE SUGAR MOLEDULES)

4. Proximate nalysis of saffron

Component	Mass %
Water-soluble components	53.0
→ Gums	10.0
→ Pentosans	8.0
→ Pectins	6.0
→ Starch	6.0
→ α -Crocin	2.0
→ Other carotenoids	1.0
Lipids	12.0
→ Non-volatile oils	6.0
→ Volatile oils	1.0
Protein	12.0
Inorganic matter ("ash")	6.0
→ HCl-soluble ash	0.5
Water	10.0

Fiber (crude) 5.0

**5. Chemical composition
of saffron**

Component	Mass %
	12.0–
Carbohydrates	15.0
	9.0–
Water	14.0
	11.0–
Polypeptides	13.0
Cellulose	4.0–7.0
Lipids	3.0–8.0
Minerals	1.0–1.5
Miscellaneous	40.0
non-nitrogenous	

(Source: Dharmananda 2005)

BIOLOGY & PHARMACOLOGY

1. ANTI-SPASMODIC AND ANTI-DEPRESSIVE EFFECTS

(Akhondzadeh, Tahmacebi-Pour et al. 2005) studied the anti-spasmodic effects of *Crocus sativus* in the following clinical study:-

AIM OF THE STUDY

This clinical study was done to assess the efficacy of the stigmas of *Crocus sativus* (saffron) in the treatment of mild to moderate depression in a 6-week double-blind, placebo-controlled and randomized trial.

MATERIALS AND METHODS

Forty adult outpatients who met the Diagnostic and Statistical Manual of Mental Disorders, 4th edition for major depression based on the structured clinical interview for DSM IV participated in the trial. Patients had a baseline Hamilton rating scale for depression score of at least 18. In this double-blind, placebo-controlled, single-centre and randomized trial.

DOSAGE

The patients were randomly assigned to receive a capsule of saffron 30 mg/ day (BD) (Group 1) or a capsule of placebo (BD) (Group 2) for a 6-week study. At 6 weeks, *Crocus sativus* produced a significantly better outcome on the Hamilton depression rating scale than the placebo (d.f. = 1, $F = 18.89$, $p < 0.001$). There were no significant differences in the two groups in terms of the observed side effects.

RESULTS

The results of this study indicate the efficacy of *Crocus sativus* in the treatment of mild to moderate depression. A large-scale trial is justified.

2. PAIN RELIEVING(ANTI-NOCICEPTIVE) EFFECTS

Nociceptive receptors are the receptors which send signals which cause pain perception, in response to pain stimuli.

(Hosseinzadeh and Shariaty 2007) studied the pain relieving effects of a principle “safranal” present in *Crocus sativus* extract in the following study done in mice:-

MATERIALS AND METHODS

Safranal is one of the main constituents of saffron. In view of previous reports of anti-nociceptive activity of saffron there was examined the anti-nociceptive property of safranal. Antinociceptive activity was determined using hot-plate, writhing and formalin tests in mice. Safranal at doses 0.1, 0.3 and 0.5 ml/kg/i.p. inhibited the abdominal constrictions induced by acetic acid and also at 0.5 mL/kg/i.p. increased the pain threshold of mice against the thermal source only at 30 min after treatment.

In formalin test, safranal at doses 0.05 mL/kg/i.p. significantly decreased pain-related behaviors in phase I and with lower dose (0.05 and 0.025 mL/kg/i.p.) phase II. Generally, naloxone (2 mg/kg, s.c.) did not abolished the anti-nociceptive effects of safranal completely.

RESULTS

The results showed that safranal have anti-nociceptive activity in chem. (formalin and acid acetic tests) methods and this effect may be medicated more peripherally.

Naloxone=an opioid agonist.

s.c= subcutaneous, ml= milliliter

i.p=intraperitoneally, kg= Kilogram.

3. APHRODISIAC EFFECTS

(Hosseinzadeh, Ziaee et al. 2008) demonstrated the aphrodisiac properties of saffron in the studies done on rats.

MATERIALS AND METHODS

In this study, the aphrodisiac activities of two major ingredients crocin and safranal present in *Crocus sativus* stigma aqueous extract and its constituents. The aqueous extract (80, 160 and 320mg/kg body weight), crocin (100, 200 and 400mg/kg body wt.), safranal (0.1, 0.2 and 0.4ml/kg), sildenafil (60mg/kg body wt., as a positive control) and saline were administered intraperitoneally to male rats. Mounting frequency (MF), intromission frequency (IF), erection frequency (EF), mount latency (ML), intromission latency (IL) and ejaculation latency (EL) were the factors evaluated during the sexual behavior study.

RESULTS

Crocin, at all doses, and the extract, especially at doses 160 and 320mg/kg body weight, increased MF, IF and EF behaviors and reduced EL, IL and ML parameters. Safranal did not show aphrodisiac effects. The present study reveals an aphrodisiac activity of saffron aqueous extract and its constituent crocin.

4. ANTI-LEUKEMIC EFFECTS

(Straubinger 2000) described the anti-leukemic effects of crocetin derivatives based on various anti-cancer studies.

Carotenoid metabolites, especially the retinoids (i.e., vitamin A and derivatives), have attracted attention because of their possible anticarcinogenic properties. Although inhibition of growth of certain types of tumor cell lines was observed for retinoids, their level of toxicity precludes a general therapeutical application in humans. Contrary to retinoids and their parent compounds the carotenoids, saffron pigments exhibit a diapocarotenoid structure and, thus, crocetin derivatives do not act as vitamin A precursor. According to the working hypothesis in studies on antitumor activity of crocetin derivatives reduced toxicity at higher doses in cancer chemotherapy was expected. Several in vitro studies with saffron pigments

have been carried out (42– 50). It is important to note that the mixture of the crocetin derivatives as well as the dicarboxylic acid crocetin and its methyl ester dimethyl crocetin were found to be highly effective in inhibiting the proliferation of a leukemic cell line. Concentrations that induced 50% inhibitions of cell growth were only slightly higher as observed for all-*trans* retinoid acid. Thus, saffron carotenoids are suggested as alternative antitumor agents, which alone or in combination with other chemical substances may have potential for the treatment of certain forms of cancer in the future.

5. INSECICIDAL AND PESTICIDAL EFFECTS

(Leffingwell, 2002) demonstrated that safranal; a principle isolated from *Crocus sativus* extract exhibits insecticidal and pesticidal effect. This fact could present Saffron as safe and effective herbal insecticide and pesticide which is more environment friendly than other synthetic insecticides and pesticides and may have potential to substitute the synthetic substances.

TOXICOLOGY

Saffron is toxic if taken in high doses. It has been used as abortifacient by the traditional healers (Straubinger 2000).

Due to dose dependent abortifacient effect it should not be used by pregnant women and those who are planning to become pregnant.

DISCUSSION/ CONCLUSION

➤ ANTI-SPASMODIC EFFECTS

(Akhondzadeh, Tahmacebi-Pour et al. 2005) demonstrated the anti-spasmodic effects of saffron in a double blind clinical trial by using 40 patients and proved efficacy of saffron as an herbal drug to treat mild to moderate depression.

Depression is a serious disorder in today's society, with estimates of lifetime prevalence as high as 21% of the general population in some developed countries. Further research involving much larger patient population could uncover more details about the anti-depressive effects of saffron. However this clinical study gives scientific basis for safe and effective traditional usage of saffron for the treatment of depression.

➤ PAIN RELIEVING EFFECT

The pharmacological studies done by (Hosseinzadeh and Shariaty 2007) proves therapeutic efficacy of safranal isolated from *Crocus sativus* as a pain killer through inhibition of pain signaling through nociceptive receptors. The opioid agonist Naloxone did not inhibit the anti-nociceptive effect that is why this pain relieving effect is not mediated through opioid receptors but by inhibition of synthesis or action of prostaglandins.

This scientific work scientifically supports the traditional usage of *Crocus sativus* as abdominal pain killer in Pakistan.

➤ ANTI-LEUKEMIC EFFECTS

The anti-leukemic effects described by (Straubinger 2000) gives anti-leukemic potential of saffron. Thus, saffron carotenoids are suggested to be alternative anti-leukemic agents. Saffron could also be used as adjuvant to synthetic drugs to lower the side effects related to anti-leukemic therapy.

However a number of other traditional uses including Emmenagogue and expectorant need to be verified by further scientific work.

α -crocin is responsible for yellowish orange colour saffron gives during food preparation (Zarghami and Heinz, 1971).

REFERENCES

Akhondzadeh, S., N. Tahmacebi-Pour, et al. (2005). "Crocus sativus L. in the treatment of mild to moderate depression: a double-blind, randomized and placebo-controlled trial." Phytother Res **19**(2): 148-51.

Abdullaev, FI (2002), "Cancer chemopreventive and tumoricidal properties of saffron (*Crocus sativus*L.)", *Experimental Biology and Medicine* **227** (1), <<http://www.ebmonline.org/cgi/content/full/227/1/20>>. Retrieved on January 10, 2006. PMID 11788779.

Dharmananda, S (2005), "Saffron: An Anti-Depressant Herb", *Institute for Traditional Medicine*, <<http://www.itmonline.org/arts/saffron.htm>>. Retrieved on January 10, 2006.

G.A.Burdock, F. (1995). Handbook of Flavor Ingredients, CRC Press: Boca Raton. op. Rep. China). 7pp.

H.E.Laux, H. L., A.Tode, *Gewürzpflanzen*, Franckh-kosmos, Stuttgart, 1996 (1996).

Hosseinzadeh, H. and V. M. Shariaty (2007). "Anti-nociceptive effect of safranal, a constituent of *Crocus sativus* (saffron), in mice." Pharmacologyonline(2): 498-503.

Hosseinzadeh, H., T. Ziaee, et al. (2008). "The effect of saffron, *Crocus sativus* stigma, extract and its constituents, safranal and crocin on sexual behaviors in normal male rats." Phytomedicine **15**(6-7): 491-495.

K R. Cadwallader, H. H. B., And M. Cai (1997). Spices-flavor Chemistry and antioxidant Properties, " ACS symp. ser. 660, (S.J. Risch and C.T. Ho, eds.): 66-79.

Leffingwell, JC (2002), "Saffron", *Leffingwell Reports* **2** (5) p.1-3, <<http://www.leffingwell.com/download/saffron.pdf>>. Retrieved on January 10, 2006. 209

McGee, H (2004), *On Food and Cooking: The Science and Lore of the Kitchen* p. 422-423, Scribner, ISBN 0-684-80001-2, <<http://books.google.com/books?ie=UTF-8&hl=en&id=iX05JaZXRz0C>>. Retrieved on January 10, 2006.

Schormüller, J. (1970). "Handbuch der Lebensmittelchemie." **Vol. VI**.

Straubinger, P. W. h. M. (2000). "Saffron-Renewed interest in an ancient spice, *Food Reviews International*." Technische Universität Braunschweig, Braunschweig, Germany **16:1**: 39-59.

W.Franke, N., Thieme: Stuttgart, New York (1989).

W.Rödel, M. P. a. (1991). High Res. Chromatography **14**: 771-774.

N. S. Zarghami and D. E. Heinz, *Lebensm.-Wiss. u. Technol.*, **4**, 43-45 (1971).

PHOTO REFERENCE

http://boga.unibe.ch/data/public/Website_ab_2005/Bilder_gross/Schauhaeuser-und-Vitrinen-Steppenhaus/Crocus-sativus-380.JPG

Retrieved in December, 2008.

CONCLUSION

Since a lot of synthetic drugs are designed by using plants products as a mother molecule in one or other way. Also plants still contains a lot of valuable entities which could help to solve the mysteries of the modern therapeutics. That is why it is need of the hour to pay valuable attention to scientific research keeping traditional remedies in mind to get the best results of therapeutics.

In this work it could be concluded that a lot of scientific work is required to find the scientific basis of the traditional usages of plant products which has been safely used for hundreds of years.

It could be concluded from this scientific work that the following plant remedies may change the future of modern therapeutics.

1. *Trigonella foenum-graecum*

(Shah, Bodhankar et al. 2006) studied that the active principles from the fenugreek leads to production of fresh β - cells of islets of langerhans and induction of hepatic enzymes involving carbohydrate metabolism in rat studies, the former effect is the unique one and could provide a better combination with hypoglycemic agents like Metformin. A lot of scientists working on fenugreek could give hope about a combination which could change the future of treatment of Diabetes type II.

2. *Carica papaya*

A principle obtained from *Carica papaya* “papain” has been proved to be a marvelous anthelmintic agent, papain attacks the cuticle of worms and the generation of resistance to this effect is very slow (Stepak, Lowe et al. 2007) this effect make papain a very attractive in a lot of successful veterinary remedies. Further research is needed to be done.

Carica papaya has been used both as a male and female contraceptive and abortifacient agent in females, in the traditional healthcare settings in Asia and Africa.

Since we have not any male contraceptive available in the market, further work on positive findings in male rat studies done by (Lohiya Nirmal, Manivannan et al. 2006) could lead to synthesis of a safe and effective male contraceptive.

3. *Cassia fistula*

(Ammal, George et al. 2007) found, in the laboratory tests, that *Cassia fistula* extract changes the morphology of the cholesterol crystals, from plate like to needle like, deposited the atherosclerotic patches. It could be an adjuvant to the treatment of coronary diseases. Further work should be done to prove it clinically.

A lot of other effects done by these useful traditional remedies like the effect of *Citrullus colocynthis* as anthelmintic in veterinary medicines, estrogenic effects of *Dioscorea floribunda*, anti spasmodic and anti-hypertensive effects of *Ferula asafetida*, anti- parasitic/ anti-malarial effects done by *Caesalpinia crista*, and anti-psoriatic effects done by *Smilax*

ornata, and acceleration of uptake of calcium in the osteoporotic patients by *Styrax benzoin* extract should be further clinically studied to find safe effective and much better therapeutic agents and lower the frequency of potential side effects.