Ethnopharmacology, biological activities and chemical compounds of *Canarium strictum*: An important resin-yielding medicinal tree in India

Gopalakrishnan Saroja Seethapathy [a,b,c], Christian Winther Wold [a], Kaliamoorthy Ravikumar [c], Hugo J. de Boer [b], Helle Wangensteen [a,*]

[a] *Section for Pharmaceutical Chemistry, Department of Pharmacy, University of Oslo, P.O. Box 1068, 0316 Oslo, Norway*
[b] *Natural History Museum, University of Oslo, P.O. Box 1172, 0318 Oslo, Norway*
[c] *The University of Trans-Disciplinary Health Sciences and Technology, Foundation for Revitalisation of Local Health Traditions (FRLHT), 74/2 Jarakabande Kaval, Post Attur via Yelahanka, Bangalore 560064, India*

ARTICLE INFO

ABSTRACT

The resin of *Canarium strictum* Roxb. is used for rheumatism and asthma; the bark is used as a mosquito repellent. The major compounds in the resin are triterpenoids, but as no studies have been performed on the bark, this study investigated this economically important resource. Ten folk healers were interviewed about their medicinal uses of *C. strictum*. Resin and bark were extracted with dichloromethane followed by methanol using accelerated solvent extraction. The extracts were fractionated using different chromatographic methods, and isolated compounds were identified by NMR spectroscopy and GC–MS. Resin and bark extracts were investigated for DPPH radical scavenging, 15-lipoxygenase inhibition, effects on nitric oxide (NO) production in LPS-activated dendritic D2SC/I cells and toxicity against *Artemia salina* nauplii. Traditional healers used resin to treat colds, airway afflictions and rheumatoid arthritis. α-Amyrin and β-amyrin were identified as the major constituents in the dichloromethane resin extract. From the stem bark, procyanidins, gallic acid, methyl gallate, scopoletin, 3,3′-di-*O*-methylellagic acid 4-*O*-α-arabinofuranoside and elephantorrhizol (3,3′,4′,5,6,7,8-heptahydroxyflavan) were isolated and identified. By GC–MS, α-amyrin and β-amyrin and their acetates, lupeol, and taraxasterol were identified. Radical scavenging, 15-lipoxygenase inhibitory activity and inhibition of NO production was observed from resin and bark extracts, and no toxicity towards *Artemia salina* nauplii was found. Triterpenoids and procyanidins are the major compounds in *C. strictum* resin and stem bark, respectively. The high content of triterpenoids might contribute to anti-inflammatory effects and give a rationale for the widespread usage of the resin in India.

1. Introduction

Canarium strictum Roxb. (Burseraceae) is a polygamodioecious tree distributed across parts of India, Myanmar and China. Flowers are insect pollinated, and canopy trees can grow up to about 40 m tall and are found in moist deciduous to semi-evergreen forests at altitudes ranging from about 750 m to 1400 m [45]. Unlike many other *Canarium* species, the fruits of *C. strictum* are not edible [45]. *C. strictum* yields the resin known as 'black dammar', and the local names in India include *mand dhuup*, *raal dhuup*, *karunkungiliyam* and *sambrani* [22]. *Dammar* is a Malay word and in general it refers to all types of resins. In taxonomy, a number of taxa were named after *dammar*, for example a coniferous genus '*Dammara*' and the name obviously represents the plant's producing resins. In several taxonomic revisions, species in the genus *Dammara* were assigned to the *Protium* Burm.f. (Burseraceae), *Shorea* Roxb. ex C.F.Gaertn. (Dipterocarpaceae), and *Agathis* Salisb (Araucariaceae; *Agathis dammara* (Lamb.) Rich. resin yielding tree). Thus, the vernacular name *dammar* has confusions. The resin of *C. strictum* is dark brown, therefore it might have been called black *dammar* [26] The resin from *C. strictum* is not secreted from the bark itself; the tree needs to be incised for the secretion of resin. Traditionally the resin is used to treat rheumatism, asthma, venereal disease, and for chronic cutaneous diseases such as psoriasis and pityriasis; and as a liniment in rheumatic affections [22]. On the other hand, topical application of stem bark powder of *C. strictum* is reported as a mosquito repellent [47]. Apart from medicinal uses, resins of *C. strictum* are commercially harvested for

---

their economic importance to local communities, used as incense, and for the manufacture of varnish, lacquers, and paints [45].

Despite the widespread use of this tree in southern Asia, relatively little is known about its secondary metabolites. Triterpenes such as α-amyrin, β-amyrin, β-amyrin acetate, (+) junenol, canarone, epi-khusinol, and -Ψ-taraxasterol and epi-Ψ-taraxastane diol are reported to be the major phytochemical compounds present in the resin of *C. strictum* [15,16]. Chemical compounds reported in *Canarium* species are monoterpenes [13], triterpenes [4], tetraterpenes like carotenoids, sesquiterpenes, cyclohexane and sterols [30], carboxylic acids, coumarins, furans, lipids and phenols (flavonoids, tannins, phenolic acids) [8,24,30]. *C. strictum* leaf extracts have shown antibacterial and antifungal effects, however the isolated compounds from the extracts were not studied [46]. In another study, antioxidant activity using trolox equivalent antioxidant capacity and ferric reducing antioxidant power assays of resin methanol extract was screened, and but the results showed relatively low activity [41].

Notwithstanding the widespread use of this plant in traditional system of Indian medicines, no systematic surveys of its use among healers have been undertaken, and phytochemical studies on the stem bark have not hitherto been performed. Therefore, the aim of this paper was to document the medicinal uses of *C. strictum* by Indian folk healers; to investigate the constituents of the resin and stem bark, and test the radical scavenging, antioxidant and anti-inflammatory properties of the resin and stem bark extracts.

2. Material and methods

2.1. Ethnopharmacological data collection

Ten folk healers aged 45–67 (7 males and 3 females) were interviewed in 2018 and their knowledge on *C. strictum* was documented using a semi-structured questionnaire. The present survey was conducted in the village of Kotagiri in the Nilgiri Biosphere Reserve in the Western Ghats (11.43° N, 76.88° E), India. The major ethnic group interviewed were *Kurumba, Baduga* and *Erula* in Western Ghats. Prior to the ethnobotanical survey, the purpose of the study was explained to the informants and the consent to conduct the study was requested and agreed. The interviews were conducted in the informants' native language *Tamil*. Prior to ethnobotanical data collection, ethical approval for this study was obtained from the National Biodiversity Authority, Government of India.

2.2. Plant material

The resin and stem bark of *C. strictum* was collected from a polygamodioecious tree in Kolli Hills (11.105°N, 78.150°E) in July 2016. The tree was tapped for the oozing of resin 3 days prior to collection. Plant nomenclature follows The Plant List (http://www.theplantlist.org), and vouchers were identified by Professor Kaliamoorthy Ravikumar at the University of Trans-Disciplinary Health Sciences and Technology, Bangalore, India. The voucher specimen (resin: Cl 781R; and stem bark: Cl 781B) was deposited in the FRLH-Herbarium and Raw Drug Repository of University of Trans-Disciplinary Health Sciences and Technology, India.

2.3. General methods

1D and 2D NMR spectroscopy was conducted on a Bruker AVII400 or a Bruker AVI600 instrument (Bruker, Rheinstetten, Germany). CD_3OD (deuterated methanol) or $CDCl_3$ (deuterated chloroform) was used as solvent with tetramethylsilane (TMS) as reference. Mass Spectrometry data was obtained in positive mode on a Waters QTOF MS instrument coupled to a Waters 2695 HPLC (Waters Corporation, Milford, MA, USA). GC–MS analysis was performed on a GCMS-QP2010 (Shimadzu Corporation, Kyoto, Japan) using electron impact ionization (70 eV),

with a Restek Rxi-5MS silica column (30 m, i.d. 0.25 mm, 0.25 μm film thickness), split injection and set at a constant pressure mode. Initial flow was 1 ml/min. The injector and interface temperatures were 280 °C. At the time of injection, the column temperature was 65 °C, then, after 2 min, the temperature was increased with 10 °C/min up to 310 °C at which it was kept for 20 min. Ion source temperature was 280 °C. Helium was used as the carrier gas. Spectra were analyzed using GC–MS solution software, Version 2.10 (Shimadzu Corporation). Various methods for column chromatography (CC) were used: VersaPak silica gel columns (8 cm × 30 cm, and 4 cm × 15 cm) and C18 column (2.3 cm × 11 cm) (Sigma–Aldrich, St. Louis, MO, USA) on a Versa Flash chromatography system (Supelco, Bellefonte, PA, USA) or laboratory packed columns with Sephadex LH-20 gel (Pharmacia, Uppsala, Sweden). Fractions from CC were combined as indicated by TLC. Silica gel either 60F$_{254}$ or 60RP-18F$_{254}$S, 0.2 mm thickness foils (Merck, Darmstadt, Germany) were used for analytical TLC, and spots were visualized by UV irradiation (254 and 366 nm), by spraying with $Ce(SO_4)_2$ (1% in 10% aqueous H_2SO_4) followed by heating (105 °C, 5 min), or by spraying with diphenylpicrylhydrazyl (DPPH) (0.04% (*w/v*) solution in MeOH). Analytical HPLC was performed on a LaChrom Elite HPLC system (VWR-Hitachi) equipped with an L-2455 diode array detector. A Kinetex C18 100A (150 × 4.6 mm) (Phenomenex, Torrance, CA, USA) was used for separation. Preparative HPLC was carried out on a ProStar Polaris system (Varian, Palo Alto, CA, USA) equipped with a Kinetex C18 100A (150 mm × 21.2 mm, 5 μm) column (Phenomenex, Torrance, CA, USA). The flow rate was 20 ml/min, 1.0 ml was injected and 280 nm was used for UV detection. UV absorbance was measured on a Biochrom Libra S32PC instrument (Biochrom, Cambridge, UK).

2.4. Extraction and isolation

The stem bark (185 g) and resin (85 g) were air dried and powdered, followed by extraction using an Accelerated Solvent Extraction instrument, ASE350 Solvent Extractor (Dionex, Sunnyvale, CA, USA). The plant material was extracted with dichloromethane (40 °C), followed by methanol (60 °C). After drying in a rotary evaporator, the extraction yields were for stem bark: 3 g dichloromethane extract (CS_B_DCM) (1.7% yield) and 12.5 g methanol extract (CS_B_MeOH) (6.8% yield), and for resin: 46 g dichloromethane extract (CS_R_DCM) (54.1% yield) and 12.1 g methanol extract (CS_R_MeOH) (14.2% yield), respectively.

CS_R_DCM extract (20 g) was applied to a VersaPak silica gel column (8 cm × 30 cm) and chromatographed with a gradient of ethyl acetate/dichloromethane (10–100% EtOAc) to yield 12 fractions (N1–N12). Fraction N11 (1.2 g) was applied to VersaPak silica gel column (4 cm × 15 cm) with a gradient of ethyl acetate/dichloromethane (10–100% EtOAc) to yield 5 fractions (N11-F1 to F5). Fraction N11-F4 (200 mg) was applied to VersaPak silica gel column (4 cm × 15 cm) to yield 7 fractions (N11-F4-FF1to FF7).

Stem bark methanol (CS_B_MeOH) extract (2 g) was suspended in 8 ml H_2O/methanol (3:5), applied on a Sephadex LH-20 column (2.5 cm × 20 cm) conditioned with 35% MeOH and fractionated with a stepwise gradient of H_2O/MeOH (35%, 50%, 75% and 100% MeOH) and H_2O/Acetone (3:7) yielding 8 fractions (BF1-BF8). Fraction BF4, BF5 and BF6, each 80 mg, were separately applied to a VersaPak C18 column (2.3 cm × 11 cm) with a gradient of H_2O/acetonitrile (5–100% acetonitrile) followed by H_2O/acetone (3:7) to yield 12 fractions (BF4-R1 to R12), 9 fractions (BF5-R1 to R9) and 6 fractions (BF6-R1-R6), respectively. Guided by NMR and HPLC-DAD, selected fractions were purified by preparative HPLC (gradient of 0.1% TFA in water and acetonitrile).

For proanthocyanidin analysis, phloroglucinol degradation of CS_B_MeOH_BF6_R2 was carried out as described by Foo et al. [11], but with some modifications [49]. Proanthocyanidin samples (25 mg) were dissolved in 0.6 ml EtOH, and 40 mg phloroglucinol was dissolved in 2 ml of 1% HCl in EtOH. The solutions were combined followed by continuous shaking until complete dissolution (ca. 15 min), then reduced to a volume of 2.5 ml in a stream of N_2 gas. The resulting

solution was applied on a Sephadex LH 20 column (25 × 150 mm) conditioned in 30% EtOH and eluted with 30%, 75% and 96% aqueous EtOH. Column fractions (á 30 ml) were combined according to TLC pattern (cellulose TLC-plates, mobile phase: acetic acid-water (3:47) and analyzed by ^{1}H NMR [23].

2.5. DPPH scavenging

DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging was tested using test substances dissolved in DMSO or MeOH, and the assay was carried out as previously described, measuring decrease in absorbance at 517 nm [50]. Quercetin (Sigma–Aldrich) was used as positive control.

2.6. Inhibition of 15-lipoxygenase (15-LO)

Test substances were dissolved in DMSO, and the assay was carried out as previously described, measuring formation of conjugated dienes as increase in absorbance at 234 nm [50]. Quercetin (Sigma–Aldrich) was used as positive control.

2.7. Dendritic D2SC/I cells

The murine dendritic cell line D2SC/I was cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum, 1% 100 mM sodium pyruvate, 1% Pen Strep (10,000 U/ml penicillin and 10,000 μg/ml streptomycin), and maintained in a 37 °C humidified incubator containing 5% CO_2.

2.8. Nitrite assay

A nitrite assay was carried out as previously described [18] with minor modifications. D2SC/1 cells at a density of 5×10^5 cells/ml were seeded into 96-well flat-bottomed plates and pre-incubated with test samples for 1 h before the addition of 500 ng/ml LPS (Escherichia coli O55:B5, Sigma-Aldrich). Samples were dissolved in DMSO (0.5% final DMSO concentration) or medium. The cells were incubated for 24 h (37 °C, 5% CO_2) in duplicates containing 6.25, 12.5, 25, and 50 μg/ml of test samples (final concentrations). Nitric oxide (NO) in cell supernatants was determined by measuring the amount of nitrite colorimetrically using the Griess assay. Briefly, the cell supernatants (50 μl) were mixed with 50 μl of Griess reagent A (1% sulfanilamide in 5% phosphoric acid) and incubated at room temperature in the dark for 10 min. Then 50 μl Griess reagent B (0.1% N-(1-naphthyl) ethylenediamine dihydrochloride in water) was added, and the absorbance was measured at 540 nm using a BioRad microplate reader (Hercules, CA, USA). Quercetin was used as a positive control. A serial dilution of $NaNO_2$ in medium was used to construct the standard reference curve. Percentage NO inhibition was expressed as the percentage decrease in NO production as: 100 - $[NO]^a/[NO]^b \times 100$, where $[NO]^a$ represents the NO concentration for test samples and $[NO]^b$ represents the NO concentration from LPS-activated control.

2.9. Cell viability assay

Cell viability was determined by the MTT assay subsequent to the nitrite assay. The assay is based on the cleavage of the yellow tetrazolium salt MTT to purple formazan crystals by metabolic active cells. The method was done according to the manufacturer's procedure (Roche, Cell Proliferation Kit I (MTT)). Briefly, cell supernatants from the nitrite assay were removed, and 100 μl fresh cell medium was added to each well. Next, 10 μl of MTT labeling reagent was added to each well. After 4 h, 100 μl of MTT solubilization reagent was added, and the samples were incubated overnight at 37 °C, 5% CO_2. The quantity of formazan (presumed to be directly proportional to the number of metabolically active cells) was measured by absorbance at 595 nm using a BioRad microplate reader (Hercules, CA, USA). Cells treated with 500 ng/ml LPS in 0.5% DMSO (final concentrations) was used as negative control and 20% DMSO as positive control for dead cells. The results were expressed as percentage viability compared to the negative control.

2.10. Assay for toxicity against Artemia salina nauplii

The toxicity against Artemia salina nauplii was evaluated as previously described [48]. Podophyllotoxin (Sigma-Aldrich) was used as positive control.

2.11. Statistics

All samples were analyzed in triplicate and results are given as averages ± SD, calculated using GraphPad Prism 7.0 for Windows (GraphPad Software, San Diego, CA, USA).

3. Results and discussion

3.1. Ethnopharmacology

The present ethnopharmacological study revealed that the folk healers residing in the Nilgiri Biosphere Reserve of Western Ghats, India, mostly utilized the resins of C. strictum for various medicinal usages. Table 1 shows the detailed information about the medicinal usages provided by the individual healers. In the Nilgiri Biosphere Reserve region, a total of ten healers were interviewed, and the use of C. strictum resin for four different indications were documented. Among these, common cold, runny noses and sneezing were the most commonly mentioned indications. All the healers in the region used C. strictum resin, but usage of seeds was also described. Youmsi et al. [52] reported that the resin of Canarium schweinfurtii Engl. is used as an insect repellent, and either dry resin is burned to produce smokes in the house, or fresh sap is mixed with ash and applied topically to repel insects. Similarly, in our study it was found that C. strictum resin is burned, which in turn acts as an insect repellent. On the other hand, stem bark powder of C. strictum is also reported as a mosquito repellent [47]. Previously, Silambarasan and Ayyanar [40] reported that the resin is used to treat rheumatism, and similarly in our study it was observed that

Table 1
Collective information from healers in Kotagiri, Nilgiri Biosphere Reserve about the medicinal uses of Canarium strictum.

Age/Sex	Indication/Reason for use	Plant part, preparation and administration
67/M 64/M 62/M 60/F	Common cold, runny nose and sneezing	Resin. Burned with 2–5 garlic pulps or a few leaves of tulsi (Ocimum tenuiflorum L.), and the smoke is inhaled at the time of common cold, runny nose and sneezing. For the children up to 5 years of age, the smoke is used to dry the hair after head bath which helps to prevent common cold.
46/M 45/F	Incense and fumigation	Resin. Burned for the fragrance during rituals. Also burned with the bark of Azadirachta indica A.Juss. to avoid insects like house flies and mosquitoes in households as well as in cattle farms.
55/M 50/M	Bronchial diseases	Resin. Powdered resin is burned and the smoke is inhaled at the time of breathing diseases. This practice eases the breathing process when it is administrated two times a day for 3–4 days.
55/M 56/F	Rheumatoid arthritis	Resin and seed. Either powdered resin or seed is mixed with sesame oil (Sesamum indicum L.) and applied topically to relieve joint pains.

the resin is used to treat joint pains. A different use was reported by Namsa et al. [32] who documents that fresh resins are molted and applied to the skin to sooth rash from poisonous caterpillar hairs.

Varghese and Ticktin [45] reported that harvesters in the Nilgiri Biosphere Reserve were aware of male and female trees of *C. strictum*, and that resin-yielding trees were female trees. Our study ascertains the same, and the healers interviewed in our study also were aware of male and female plants. Seethapathy et al. [38] reported a similar case among healers in parts of the Eastern Ghats who were aware that male trees produced less resin than the female trees. In that study, it was also noticed that the intensity of resin fragrance varied based on the dryness of the resin [38]. Topical application of *C. strictum* stem bark powder is reported to act as mosquito repellent [47], whereas stem bark *C. bengalense* Roxb. is used as to treat tumours and liver damage in Vietnam [27].

3.2. Phytochemistry

α-Amyrin (**1**) [36] and β-amyrin (**2**) [36] were isolated from the DCM crude extracts of *C. strictum* resin and identified by ^{13}C NMR spectroscopy and corroborated by comparison with published values. Signals from these two compounds gave rise to the major signals in the spectrum of the DCM resin crude extract and are previously reported from *C. strictum*. GC–MS analysis confirmed the presence of α- and β-amyrin [10] as the major constituents in the resin. In addition, taraxasterol (**3**) and lupeol (**4**) were tentatively identified by GC–MS by comparing their fragments and relative intensities with literature values (Table 2) [2,3,10,42].

^{13}C NMR of the DCM bark extract showed signals typical for α-amyrin and β-amyrin, as well [36], but with two additional minor signals from carbonyl groups at δ 171.08 and 171.09 ppm and a signal at 81.0 ppm which can be ascribed to the acetate group and a slightly deshifted position of C-3 in α- and β-amyrin acetate [7]. GC–MS analysis confirmed the presence of α-amyrin acetate (**5**) and β-amyrin acetate (**6**) [10]. Taraxasterol (**3**) and lupeol (**4**) were also for the stem bark tentatively identified by GC–MS (Table 2) ([2,3]; [10]; [42]).

From the MeOH crude extract of *C. strictum* bark, the 1D and 2D NMR spectra of isolated compounds obtained from preparative HPLC resulted in the identification of gallic acid (**7**) [21], methyl gallate (**8**) [17], scopoletin (**9**) [19], 3,3′-di-*O*-methylellagic acid 4-*O*-α-arabinofuranoside (**10**) [43] and elephantorrhizol (3,3′,4′,5,6,7,8-heptahydroxyflavan) (**11**) [1,20,31,44] (Fig. 1). The identification of compounds **7**, **8** and **9** were confirmed by comparison with authentic standards (obtained from Sigma-Aldrich). The structure of 3,3′-di-*O*-methylellagic acid 4-*O*-α-arabinofuranoside was confirmed by HMBC spectral data and ESI-MS (positive mode) *m/z* 485.07 [M + Na]$^+$, 463.09 [M + H]$^+$. It is noteworthy that the finding of elephantorrhizol in *C. strictum* is of chemotaxonomic interest as it is the first report of this compound in the Burseraceae family (Fig. S1). 3,3'-Di-*O*-methylellagic acid 4-*O*-α-arabinofuranoside is also a rare natural product, only reported in four plant

species previously [37]. However, Wu et al. 2017 [51] found 3,3-di-*O*-methylellagic acid-4-*O*-rhamnopyranoside from the leaves of *Canarium pimela* K.D.Koenig.

From ^{1}H and ^{13}C NMR of the CS_B_MeOH extract and the fractions BF6–8 obtained from this crude extract, it appeared that the major constituents of these fractions were proanthocyanidins. ^{13}C NMR is well suited for determination of the average degree of polymerization of proanthocyanidins, since the terminal unit of the proanthocyanidin molecule gives a C-3 signal at ~68 ppm, while C-3 in the extender units gives signals at ~72 ppm [9]. Information of the monomeric units can also be obtained since catechin type monomers (2,3-*trans*) give a C-2 signal at ~82 ppm, while epicatechin type monomers (2,3-cis) has this signal at ~87 ppm. Under acidic conditions proanthocyanidins are depolymerized and release the terminal units. Terminal units can be trapped by nucleophilic reagents, such as phloroglucinol, to generate analyzable adducts. Thus, ^{13}C NMR spectra were integrated for the region 81.5–83.5 ppm (catechin type) versus 76.5–78.5 ppm (epicatechin type) and indicated the presence of catechin:epicatechin stereochemistry in the ratio of 1:9 for CS_B_MeOH_BF6 and 1:26 for CS_B_MeOH_BF7. Furthermore, the integration of the region 71.5–73.5 ppm (extender units) versus 68–69.5 ppm (terminal unit) indicated a ratio of 1:5.5 for CS_B_MeOH_BF6 and 1:14 for CS_B_MeOH_BF7 [9,28]. Phloroglucinol degradation of CS-B-MeOH-BF6-R2 resulted in the cleavage of the terminal unit, identified by ^{1}H NMR as catechin by comparison with authentic standard (catechin, Koch-Light), and epicatechin trapped by phloroglucinol. From ^{13}C NMR and phloroglucinol results, fraction CS_B_MeOH_BF6_R2 was found to contain catechin as the starter unit and epicatechin as the extender units with an average degree of polymerization of ca 6.5. CS-B-MeOH-BF7 seems to contain mostly epicatechin as monomeric units, with an average chain length of ca. 15 monomeric units.

3.3. Antioxidant effects

Investigation of the crude extracts for their antioxidant potential using DPPH scavenging and 15-lipoxygenase (15-LO) inhibition showed noteworthy activity.

The methanol crude extract of bark showed higher activity in the antioxidant assays (Table 3) than the methanol and DCM extracts of the resin. This finding could be explained by the presence of high content of polyphenols in the bark. Procyanidins are known to have high DPPH radical scavenging properties due to the high content of catechol groups in these molecules [33]. The resin, containing mostly lipophilic compounds, have no hydrogen donating capacity and explain the inactivity of the resin extracts. On the other hand, enzyme inhibition of 15-LO of methanol and DCM crude extract of bark showed relatively high activity compared with the positive control quercetin. The 15-LO inhibitory activity of the methanol bark extracts is probably due to the procyanidins [6]. The DCM extracts have a high content of α-amyrin and β-amyrin. α-Amyrin has shown strong inhibition of 15-LO with an IC 50 value of 15 ± 3 μM [14] which likely explain the observed effects.

3.4. Anti-inflammatory effects

Compounds that inhibit NO production can be inflammatory modulators. Dendritic cells play a major role in the initiation and modulation of immune responses [12]. An induction of iNOS in dendritic cells via stimulation with LPS has been described in the literature [5]. The dendritic cells may function as the most potent antigen-presenting cells for T cell activation, and increased levels of NO may inhibit T cell proliferation and apoptosis in the dendritic cells [12]. The inhibitory effect of resin and bark extracts on NO production by dendritic D2SC/I cells exposed to LPS are shown in Fig. 2. Methanol and DCM extracts of resin showed a concentration dependent anti-inflammatory effect by reducing levels of NO, without being toxic to the cells. DCM bark extract also showed significant inhibition of NO, whereas the MeOH bark extract
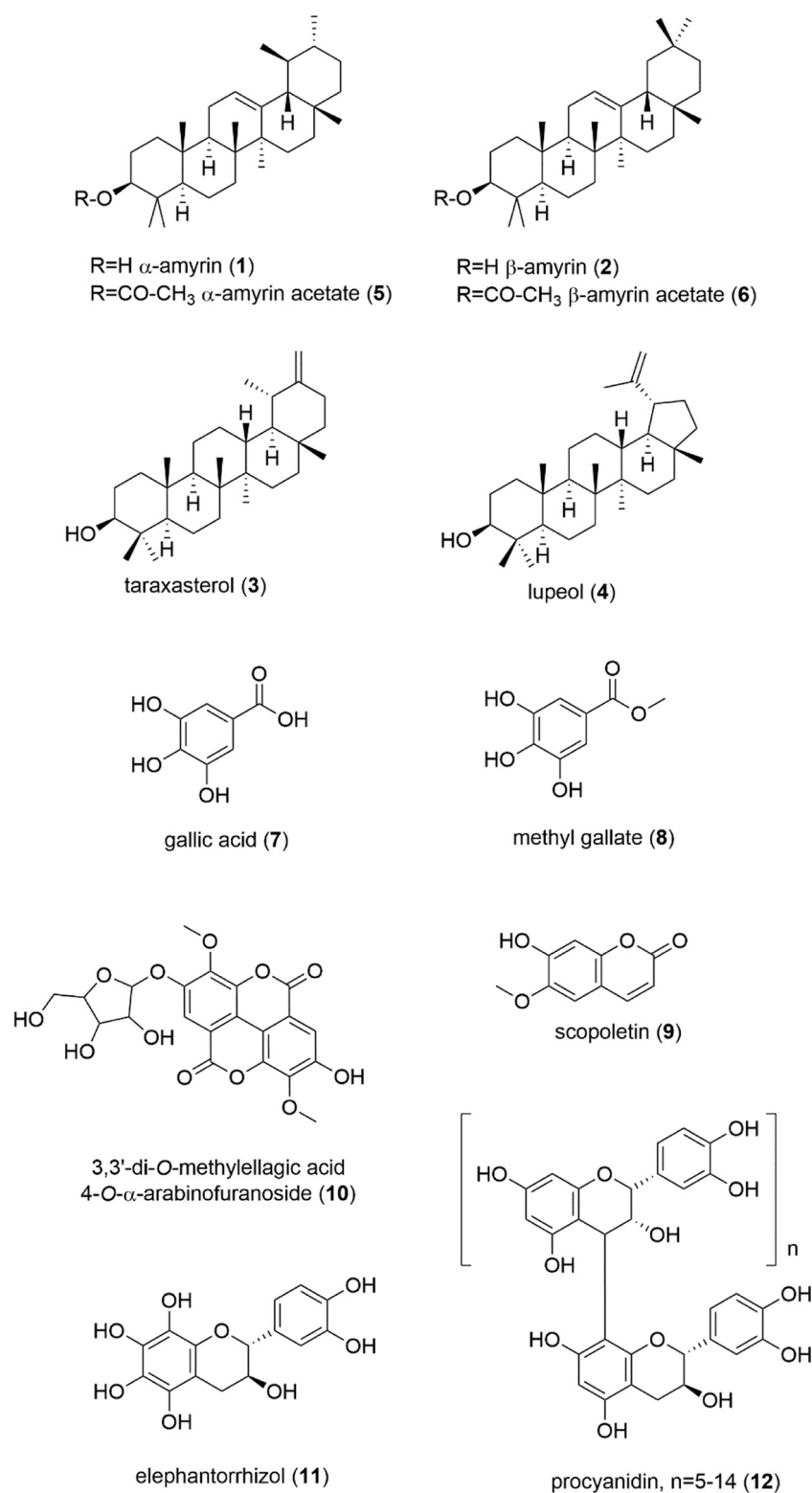
Table 2
GC–MS data of triterpenoids identified in *Canarium strictum*.

Compound	Mass (M+)	Fragments, m/z (decreasing intensity)	Retention time (min)	Plant part
α-Amyrin (**1**)	426	218, 189, 203	30.002	Bark, resin
β-Amyrin (**2**)	426	218, 281, 203, 189, 105, 253, 393	30.042	Bark, resin
Taraxasterol (**3**)	426	207, 189, 109, 121, 107	30.133	Bark, resin
Lupeol (**4**)	426	218, 203, 135, 119, 189	30.092	Bark, resin
α-Amyrin acetate (**5**)	468	218, 203, 189, 408	31.142	Bark
β-Amyrin acetate (**6**)	468	218, 203, 189, 408	31.725	Bark

Fig. 1. Chemical structures of compounds (1−12) isolated from *Canarium strictum* resin and stem bark.

showed low activity. However, the MTT assay revealed reduced cell viability for the DCM bark extract, and this was likely the main reason for the observed reduction in NO, rather than a direct anti-inflammatory effect of the extract (Fig. 3). It can therefore be concluded that the resin extracts, but not the bark extracts, showed potent anti-inflammatory effects in this particular experimental setup, and the potency of the extracts was comparable to the positive control quercetin. Reduction in

NO concentration was mainly observed in the extracts rich in triterpenes. Triterpenes are well known for anti-inflammatory effects in in vitro assays, reviewed by Ríos [34], and studies have documented inhibitory effects of α- and β-amyrin on NO production in LPS-activated macrophages [35,39]. Similarly, several studies have reported anti-inflammatory properties of other *Canarium* species [25,29], and thus the results presented here are in accordance with previous findings.

Table 3
DPPH radical scavenging, 15-lipoxygenase (15-LO) inhibition of crude extracts and fractionated compounds from *Canarium strictum*. IC_{50} values $\pm$ SD are shown.

Test sample	DPPH (µg/ml)	15-LO (µg/ml)
CS_B_DCM	> 167	32.6 ± 1.1 µg/ml
CS_B_MeOH	17.0 ± 3 µg/ml	28.5 ± 1.4 µg/ml
CS_R_DCM	> 167	> 167ml
CS_R_MeOH	> 167	19.3 ± 7.7 µg/ml
Quercetin (positive control)	9.0 ± 1.2 µg/ml	28.3 ± 1.8 µg/ml
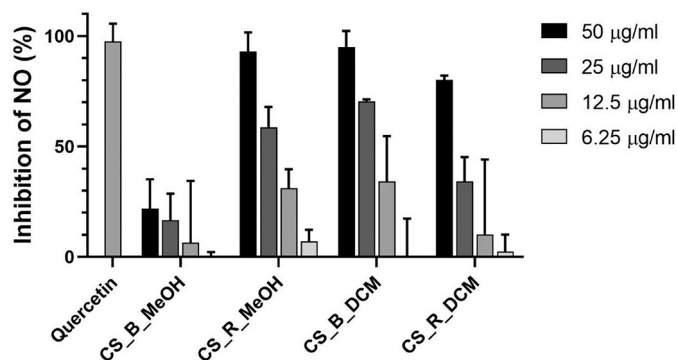
Fig. 2. NO inhibitory effects of *C. strictum* resin and stem bark crude extracts using LPS stimulated dendritic D2SC/I cells.
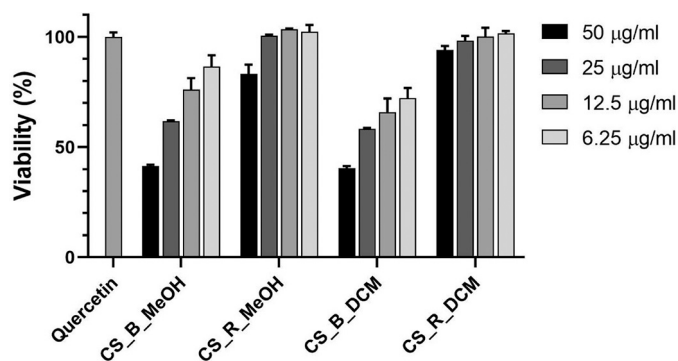
Fig. 3. Viability of D2SC/1 cells after treatment with LPS and *C. strictum* extracts, using the MTT assay. Viability is given as percent compared to cells treated with LPS alone.

The methanol and DCM extracts of bark and resin were not toxic against *Artemia salina* nauplii, and LD_{50} values for all the extracts obtained from *C. strictum* were ≥ 100 µg/ml.

4. Conclusion

The present ethnopharmacology study indicates the various uses *Canarium strictum* by healers in Nilgiri Biosphere Reserve region and that a preference for the use of resin is common among the healers. Triterpenoids and procyanidins are the major compounds in *C. strictum* resin and stem bark, respectively. The high content of triterpenoids might contribute to anti-inflammatory effects and give a rationale for the wide spread usage of the resin in India. Furthermore, the identified compounds can be utilized to comparatively study the phytochemical profile of male and female plants of *Canarium strictum*.

Funding

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fitote.2021.104920.

References

[1] J.H. Ahn, E.S. Kim, C. Lee, S. Kim, S.-H. Cho, B.Y. Hwang, M.K. Lee, Chemical constituents from *Nelumbo nucifera* leaves and their anti-obesity effects, Bioorg. Med. Chem. Lett. 23 (12) (2013) 3604–3608.
[2] A.N. Assimopoulou, V.P. Papageorgiou, GC-MS analysis of penta- and tetra-cyclic triterpenes from resins of *Pistacia* species. Part I. *Pistacia lentiscus* var. Chia, Biomed. Chromatogr. 19 (4) (2005) 285–311.
[3] A.N. Assimopoulou, V.P. Papageorgiou, GC-MS analysis of penta- and tetra-cyclic triterpenes from resins of *Pistacia* species. Part II. *Pistacia terebinthus* var.. Chia, Biomed. Chromatogr. 19 (8) (2005) 586–605.
[4] W.M. Bandaranayake, Terpenoids of *Canarium zeylanicum*, Phytochemistry 19 (2) (1980) 255–257.
[5] C. Bogdan, Nitric oxide and the immune response, Nat. Immunol. 2 (2001) 907.
[6] M. Bräunlich, R. Slimestad, H. Wangensteen, C. Brede, K.E. Malterud, H. Barsett, Extracts, anthocyanins and procyanidins from *Aronia melanocarpa* as radical scavengers and enzyme inhibitors, Nutrients 5 (3) (2013) 663–678.
[7] U.P. da Silva, G.M. Furlani, A.J. Demuner, O.L.M. da Silva, E.V.V. Varejao, Allelopathic activity and chemical constituents of extracts from roots of *Euphorbia heterophylla* L, Nat. Prod. Res. 33 (18) (2019) 2681–2684.
[8] B.C. Davidson, B.N. Nkeh, The lipid and fatty acid profiles of the fruit of *Canarium schweinfurthii*, S. Afr. J. Sci. 99 (7–8) (2003) 319–320.
[9] T.L. Eberhardt, R.A. Young, Conifer seed cone proanthocyanidin polymers: characterization by ^{13}C NMR spectroscopy and determination of antifungal activities, J. Agric. Food Chem. 42 (8) (1994) 1704–1708.
[10] C.E. Fingolo, T.D.S. Santos, M.D.M.V. Filho, M.A.C. Kaplan, Triterpene esters: natural products from *Dorstenia arifolia* (Moraceae), Molecules 18 (4) (2013) 4247–4256.
[11] L.Y. Foo, R. Newman, G. Waghorn, W.C. McNabb, M.J. Ulyatt, Proanthocyanidins from *Lotus corniculatus*, Phytochemistry 41 (2) (1996) 617–624.
[12] F. Fossiez, O. Djossou, P. Chomarat, L. Flores-Romo, S. Ait-Yahia, C. Maat, J.J. Pin, P. Garrone, E. Garcia, S. Saeland, D. Blanchard, C. Gaillard, B. Das Mahapatra, E. Rouvier, P. Golstein, J. Banchereau, S. Lebecque, T cell interleukin-17 induces stromal cells to produce proinflammatory and hematopoietic cytokines, J. Exp. Med. 183 (6) (1996) 2593–2603.
[13] P.M. Giang, W.A. Konig, P.T. Son, Chemical composition of the resin essential oil of *Canarium album* from Vietnam, Chem. Nat. Compd. 42 (5) (2006) 523–524.
[14] M.-T. Gutierrez-Lugo, J.D. Deschamps, T.R. Holman, E. Suarez, B.N. Timmermann, Lipoxygenase inhibition by anadanthoflavone, a new flavonoid from the aerial parts of *Anadenanthera colubrina*, Planta Med. 70 (03) (2004) 263–265.
[15] V.K. Hinge, S.K. Paknikar, K.G. Das, A.K. Bose, S.C. Bhattacharyya, Terpenoids-LXXXVI: structure of epi-ψ-taraxastanonol and epi-ψ-taraxastanediol, Tetrahedron 22 (8) (1966) 2861–2868.
[16] V.K. Hinge, A.D. Wagh, S.K. Paknikar, S.C. Bhattacharyya, Terpenoids-LXXI: constituents of Indian black dammar resin, Tetrahedron 21 (11) (1965) 3197–3203.
[17] D.M.N. Hisham, J.M. Lip, J.M. Noh, A. Normah, M.N. Nabilah, Identification and isolation of methyl gallate as a polar chemical marker for *Labisia pumila* Benth, J. Trop. Agric. Food Sci. 39 (2011) 279–284.

[18] G.T.T. Ho, M. Bräunlich, I. Austarheim, H. Wangensteen, K.E. Malterud, R. Slimestad, H. Barsett, Immunomodulating activity of *Aronia melanocarpa* polyphenols, Int. J. Mol. Sci. 15 (7) (2014) 11626–11636.

[19] F. Imai, K. Itoh, N. Kishibuchi, T. Kinoshita, U. Sankawa, Constituents of the root bark of *Murraya paniculata* collected in Indonesia, Chem. Pharm. Bull. 37 (1) (1989) 119–123.

[20] J.Y. Jeong, E.J. Mo, B.Y. Hwang, M.K. Lee, Phenolic constituents of nelumbinis semen and their tyrosinase inhibitory activity, Korean J. Pharmacog. 46 (2015) 1–5.

[21] S. Kamatham, N. Kumar, P. Gudipalli, Isolation and characterization of gallic acid and methyl gallate from the seed coats of *Givotia rottleriform*is Griff. and their anti-proliferative effect on human epidermoid carcinoma A431 cells, Toxicol. Rep. 2 (2015) 520–529.

[22] C.P. Khare, *Canarium strictum* Roxb, Indian Medicinal Plants: An Illustrated Dictionary, Springer New York, New York, NY, 2007, pp. 1.

[23] N. Köhler, P. Winterhalter, Large-scale isolation of flavan-3-ol phloroglucinol adducts by high-speed counter-current chromatography, J. Chromatogr. 1072 (2) (2005) 217–222.

[24] J. Koudou, A.A. Abena, P. Ngaissona, J.M. Bessière, Chemical composition and pharmacological activity of essential oil of *Canarium schweinfurthii*, Fitoterapia 76 (7) (2005) 700–703.

[25] C.-T. Kuo, T.-H. Liu, T.-H. Hsu, F.-Y. Lin, H.-Y. Chen, Protection of Chinese olive fruit extract and its fractions against advanced glycation endproduct-induced oxidative stress and pro-inflammatory factors in cultured vascular endothelial and human monocytic cells, J. Funct. Foods 27 (2016) 526–536.

[26] J.H. Langenheim, Biology of amber-producing trees: focus on case studies of *hymenaea* and *Agathis*, amber, resinite, and fossil resins, Am. Chem. Soc. (1996) 1–31.

[27] H.T. Le, D.T. Ha, C.T.A. Minh, T.H. Kim, P. Van Kiem, N.D. Thuan, M. Na, Constituents from the stem barks of *Canarium bengalense* with cytoprotective activity against hydrogen peroxide-induced hepatotoxicity, Arch. Pharm. Res. 35 (1) (2012) 87–92.

[28] Q. Lv, F. Luo, X. Zhao, Y. Liu, G. Hu, C. Sun, X. Li, K. Chen, Identification of proanthocyanidins from litchi (*Litchi chinensis* Sonn.) pulp by LC-ESI-Q-TOF-MS and their antioxidant activity, PLoS One 10 (3) (2015), e0120480.

[29] R. Mogana, K. Teng-Jin, C. Wiart, The medicinal timber *Canarium patentinervium* Miq. (Burseraceae Kunth.) is an anti-inflammatory bioresource of dual inhibitors of Cyclooxygenase (COX) and 5-Lipoxygenase (5-LOX), ISRN Biotechnol. 2013 (2013) 986361.

[30] R. Mogana, C. Wiart, *Canarium* L.: a phytochemical and pharmacological review, J. Pharm. Res. 4 (8) (2011) 2482–2489.

[31] F. Moyo, B.A. Gashe, R.R.T. Majinda, A new flavan from *Elephantorrhiza goetzei*, Fitoterapia 70 (4) (1999) 412–416.

[32] N.D. Namsa, H. Tag, M. Mandal, P. Kalita, A.K. Das, An ethnobotanical study of traditional anti-inflammatory plants used by the Lohit community of Arunachal Pradesh, India, J. Ethnopharmacol. 125 (2) (2009) 234–245.

[33] M. Plaza, T. Pozzo, J. Liu, K.Z. Gulshan Ara, C. Turner, E. Nordberg Karlsson, Substituent effects on in vitro antioxidizing properties, stability, and solubility in flavonoids, J. Agric. Food Chem. 62 (15) (2014) 3321–3333.

[34] J.-L. Ríos, Effects of triterpenes on the immune system, J. Ethnopharmacol. 128 (1) (2010) 1–14.

[35] A. Romero-Estrada, A. Maldonado-Magaña, J. González-Christen, S.M. Bahena, M. L. Garduño-Ramírez, V. Rodríguez-López, L. Alvarez, Anti-inflammatory and antioxidative effects of six pentacyclic triterpenes isolated from the Mexican copal resin of *Bursera copallifera*, BMC Complement. Altern. Med. 16 (1) (2016) 422.

[36] M.A. Saeed, A.W. Sabir, Irritant potential of some constituents from the seeds of *Caesalpinia bonducella* (L.) Fleming, J. Asian Nat. Prod. Res. 5 (1) (2003) 35–41.

[37] SciFinder, Chemical Abstracts Service, Columbus, OH, 2021. https://scifinder.cas. org (accessed January 18, 2021).

[38] G.S. Seethapathy, K. Ravikumar, B.S. Paulsen, H.J. de Boer, H. Wangensteen, Ethnobotany of dioecious species: traditional knowledge on dioecious plants in India, J. Ethnopharmacol. 221 (2018) 56–64.

[39] M.-F. Shih, Y.-D. Cheng, C.-R. Shen, J.-Y. Cherng, A molecular pharmacology study into the anti-inflammatory actions of *Euphorbia hirta* L. on the LPS-induced RAW 264.7 cells through selective iNOS protein inhibition, J. Nat. Med. 64 (3) (2010) 330–335.

[40] R. Silambarasan, M. Ayyanar, An ethnobotanical study of medicinal plants in Palamalai region of Eastern Ghats, India, J. Ethnopharmacol. 172 (2015) 162–178.

[41] S. Surveswaran, Y.-Z. Cai, H. Corke, M. Sun, Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants, Food Chem. 102 (3) (2007) 938–953.

[42] S. Szakiel, B. Niżyński, C. Pączkowski, Triterpenoid profile of flower and leaf cuticular waxes of heather *Calluna vulgaris*, Nat. Prod. Res. 27 (15) (2013) 1404–1407.

[43] N. Tanaka, T. Tanaka, T. Fujioka, H. Fujii, K. Mihashi, K. Shimomura, K. Ishimaru, An ellagic compound and iridoids from *Cornus capitata* root cultures, Phytochemistry 57 (8) (2001) 1287–1291.

[44] R.-N. Tang, X.-B. Qu, S.-H. Guan, P.-P. Xu, Y.-Y. Shi, D.-A. Guo, Chemical constituents of *Spatholobus suberectus*, Chin. J. Nat. Med. 10 (1) (2012) 32–35.

[45] A. Varghese, T. Ticktin, Regional variation in non-timber forest product harvest strategies, trade, and ecological impacts: the case of black dammar (*Canarium strictum* Roxb.) use and conservation in the Nilgiri Biosphere Reserve, India, Ecol. Soc. 13 (2) (2008).

[46] A. Venkatachalapathi, S. Paulsamy, J. Thambiraj, Antimicrobial efficacy of the ethnomedicinal plant species, *Canarium strictum* roxb, Int. J. Pharm. Pharm. Sci. 8 (2) (2016) 339–341.

[47] A. Venkatachalapathi, T. Sangeeth, M.A. Ali, S.S. Tamilselvi, S. Paulsamy, F.M. A. Al-Hemaidc, Ethnomedicinal assessment of Irula tribes of Walayar valley of Southern Western Ghats, India, Saudi J. Biol. Sci. 25 (4) (2018) 760–775.

[48] H. Wangensteen, M. Alamgir, S. Rajia, T.J. Meza, A.B. Samuelsen, K.E. Malterud, Cytotoxicity and brine shrimp lethality of rotenoids and extracts from *Sarcolobus globosus*, Nat. Prod. Commun. 2 (8) (2007) 841–844.

[49] H.M. Wangensteen, G. Duong, M. Alamgir, M.B. Sarder, A.B. Samuelsen, K. Malterud, Biological activities of limonoids, catechins, procyanidins and extracts from *Xylocarpus granatum*, Nat. Prod. Commun. 1 (2006) 985–990.

[50] H. Wangensteen, A.B. Samuelsen, K.E. Malterud, Antioxidant activity in extracts from coriander, Food Chem. 88 (2) (2004) 293–297.

[51] J. Wu, X.a. Fang, Y. Yuan, Y. Dong, Y. Liang, Q. Xie, J. Ban, Y. Chen, Z. Lv, UPLC/ Q-TOF-MS profiling of phenolics from *Canarium pimela* leaves and its vasorelaxant and antioxidant activities, Rev. Bras 27 (6) (2017) 716–723.

[52] R.D.F. Youmsi, P.V.T. Fokou, E.Z.o Menkem, I. Bakarnga-Via, R. Keumoe, V. Nana, F.F. Boyom, Ethnobotanical survey of medicinal plants used as insects repellents in six malaria endemic localities of Cameroon, J. Ethnobiol. Ethnomed. 13 (1) (2017) 33.