HARNESSING THE POTENTIAL OF *CANARİUM STRICTUM* ROXB.: A COMPREHENSIVE ANALYSIS

Jain K. Jose¹*, Preethu P. John², Neethi Shaju³ and Dr. Santhosh M. Mathews⁴

¹M.Pharm Student, Department of Pharmacology, Pushpagiri College of Pharmacy
Thiruvalla, Kerala, India.

²Associate Professor, Department Pharmacology, Pushpagiri College of Pharmacy
Thiruvalla, Kerala, India.

³Assistant Professor, Department Pharmacology, Pushpagiri College of Pharmacy
Thiruvalla, Kerala, India.

⁴Principal, Pushpagiri, College of Pharmacy, Thiruvalla, Kerala, India.

ABSTRACT

An indigenous and endemic plant species of the Eastern and Western Ghats is *Canarium strictum* Roxb. About 100 species of tropical and subtropical trees make up the genus *Canarium*, which belongs to the family Burseraceae. It is a sizable, resinous tree species that is commercially harvested for dammar and has many useful folk medical applications. Its therapeutic qualities are caused by phytochemical components. The main components of stem and bark are procyanidins and triterpenoids. The current study lists *Canarium strictum*'s pharmacological activities, ethnobotanical relevance, and general characteristics in order to help researchers prepare for approaching the plant's utility, efficacy, and potency.

KEYWORDS: *Canarium strictum*, Larvicidal, antipyretic, antioxidant, antimicrobial activity.

INTRODUCTION

Medicinal plants are a source of bioactive lead that can produce substantial pharmacological action in the biological system. Therefore, they can play a vital role in the discovery and development of new therapeutic lead compounds for the maintenance of human health. Herbal medicine is widely practised worldwide. Natural treatments have been used for
generations to treat common maladies like colds, allergies, upset stomachs, and toothaches, and the trend is only growing. In order to prevent diseases and ailments, there has been a movement in the global trend from synthetic to herbal treatments, or what we can refer to as a "Return to Nature." Medicinal plants can be found in nature. The World Health Organization (WHO) reported that 4 billion people (80% of the world’s population) use herbal medicines for some aspect of primary healthcare.\cite{1} Herbal medicine has been recognized by WHO as essential components for primary health care and about 11\% of the 252 drugs are derived from plants.\cite{2} Plants can act as an antioxidant, antiviral, anticancer, antibacterial, antifungal, and antiparasitic, among other pharmacological functions. Plants include compounds that behave like antioxidants and scavenge free radicals, such as flavonoids, phenolic, anthocyanins, and vitamins.\cite{3}

*Canarium strictum* is a large canopy tree with bipinnate leaves that is distributed across parts of India, Myanmar, and Yunnan province, China. It can grow up to about 40 m tall and is found in moist deciduous to semi-evergreen forests at altitudes ranging from about 750 m to 1400 m. Trees are polygamous and flowers are insect pollinated. Seed predation has been reported to be very low. The fruits of *C. strictum* cannot be eaten, in contrast to many other *Canarium* species.\cite{4} The resin produced by *C. strictum* is referred to as "black dammar," and it is also known by the Indian names mand dhoop, raal dhuup, karun kungiliyam, and sambrani. In general, all forms of resins are referred to as dammar, which is a Malay term. A number of taxa have been named after dammar in taxonomy, such as the coniferous genus "Dammara," whose name refers to the plant's ability to produce resin. As a result, the common name dammar is unclear. Since *C. strictum's* resin is a dark brown colour, the term "black dammar" may have been used to describe it. The bark of the *C. strictum* tree needs to be cut in order for the resin to be produced. The resin has historically been employed as a treatment for rheumatism, asthma, venereal illness, chronic cutaneous disorders including psoriasis and pityriasis, and as a liniment for rheumatic conditions. However, topical use of *C. strictum* stem bark powder has been described as a mosquito deterrent. In addition to its medical use, *C. strictum* resins are commercially collected because of their economic value, are used to make incense, varnish, lacquers, and paints, as well as for other incense-related purposes. It is used for caulking boats.\cite{5,6}
PHYTOCHEMICAL CONSTITUENTS

Black dammer resin collected from wounded trunk of tree contains triterpenoids such as α-amyrin, β-amyrin, β-amyrin acetate, (+) junenol, canarone, epikhusinol, and Ψ-taraxasterol and epi-Ψ-taraxastane diol.[7,8] Monoterpenes, triterpenes, sesquiterpenes, cyclohexane, and sterols, tetraterpenes such as carotenoids, coumarins, and furans, lipids, and phenols (flavonoids, tannins, and phenolic acids) are some of the chemical elements present in Canarium species.[9,10]

BOTANICAL DESCRIPTION

Leaves

Compound, imparipinnate, alternate, spiral, clustered at twig ends, to 40 cm; rachis ferruginous pubescent; leaflets 3-9 pair with odd one at apex, increasing in size towards apex; petiolule 0.3-0.7 cm long; lamina 5-15 x 2.5-7 cm usually oblong, sometimes ovate, apex acuminate, base asymmetric-rounded; margin serrate or serrulate, coriaceous, rusty tomentose or pubescent beneath, glabrous above; secondary nerves strong, 11-18 pairs; tertiary nerves weakly percurrent.

Flowers

Bisexual or polygamous, in short branched axillary panicles, about 1 cm long, yellow to dull white, shortly stalked and mildly fragrant.

Fruits

Drupe, 2.5 to 5.0 cm long, pointed at ends, mesocarp fleshy, stone hard, aromatic and seeds trigonous, usually 3- celled with three seeds. The ripen fruits/drupes are collected by lopping the small branches, the fleshy mesocarp is removed with a sharp knife, and seeds are dried under proper shade.[11,12,13]

Table 1: Taxonomical classification of C. strictum.[13]

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subkingdom</td>
<td>Tracheobionta</td>
</tr>
<tr>
<td>Superdivision</td>
<td>Spermatophyta</td>
</tr>
<tr>
<td>Division</td>
<td>Magnoliophyta</td>
</tr>
<tr>
<td>Class</td>
<td>Magnoliopsida</td>
</tr>
<tr>
<td>Subclass</td>
<td>Rosidae</td>
</tr>
<tr>
<td>Order</td>
<td>Sapindales</td>
</tr>
<tr>
<td>Family</td>
<td>Burseraceae</td>
</tr>
<tr>
<td>Genus</td>
<td>Canarium</td>
</tr>
<tr>
<td>Species</td>
<td>Canarium strictum Roxb</td>
</tr>
</tbody>
</table>
Fig. 1: Fruits, leaves, flowers & resin of *Canarium strictum*. [14,15]

Table 2: Ethnobotanical uses of *Canarium strictum*. [16]

<table>
<thead>
<tr>
<th>PART USED</th>
<th>INDICATIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resin</td>
<td>Analgesic, fumigant</td>
</tr>
<tr>
<td>Bark</td>
<td>Mosquito repellent</td>
</tr>
<tr>
<td>Seed</td>
<td>Mixed with sesame oil to treat joint pain</td>
</tr>
<tr>
<td>Leaves</td>
<td>Antipyretic</td>
</tr>
</tbody>
</table>

PHARMACOLOGICAL ACTIVITIES

ANTIOXIDANT ACTIVITY (INVITRO)

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. [17] Quercetin (Sigma–Aldrich) was used as positive control.

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 [17]. Quercetin (Sigma–Aldrich) was used as positive control.
Dendritic D2SC/I cells
The murine dendritic cell line D2SC/I was cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% foetal 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% CO2.

The methanol crude extract of bark showed higher activity in the antioxidant assays 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. 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. 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 which likely explains the observed effects.[18,19]

Nitrite assay
With minimal adjustments, a nitrite test was performed as previously reported. D2SC/I cells were seeded at a density of 5 105 cells/ml in 96-well flat-bottomed plates and pre-incubated with test samples for 1 hour before being treated with 500 ng/ml LPS (Escherichia coli O55:B5, Sigma-Aldrich). DMSO (0.5% final DMSO concentration) or medium was used to dissolve the samples. The cells were cultured for 24 hours in triplicate with test solutions containing 6.25, 12.5, 25, and 50 g/ml (final concentrations). The quantity of nitrite in cell supernatants was measured colorimetrically using the Griess test to assess nitric oxide (NO). 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 NaNO2 in medium was used to construct the standard reference curve.
Percentage NO inhibition was expressed as the percentage decrease in NO production as:
\[ 100 - \frac{[\text{NO}]_a}{[\text{NO}]_b} \times 100 \]
where \([\text{NO}]_a\) represents the NO concentration for test samples and \([\text{NO}]_b\) represents the NO concentration from LPS-activated control.

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 showed low activity.\[^{[19]}\]

**Cell viability assay**

**MTT 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 labelling 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% CO2. 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) were 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.\[^{[19]}\]

**LARVICIDAL ACTIVITY**

Canarium strictum adult leaf pieces were gathered from locations in the evergreen forest of the Walayar valley, southern Western Ghats, India. The plant leaves were pulverised after being shade-dried at room temperature. One kg of dried and powdered leaf material was successively macerated in three litres of methanol for 96 hours before being filtered. On a rotating vacuum evaporator, the filtrate was then concentrated at a lower temperature. It was diluted in 100 ml of methanol to make a stock solution containing one gramme of the concentrated extract of dried leaf Canarium strictum (1g/mL). To create the necessary extract concentrations for exposure of the mosquito larvae, the stock solution was employed. An insecticide-free larvicidal bioassay was performed on vector mosquitoes that were raised in laboratories. The study used the standard WHO (2005) procedure with a few minor adjustments. Glass beakers were used for the testing. Specifically, 4th instar mosquito larvae
were collected from F1 generation laboratory colonised mosquitoes. Concentrations ranging from 50 to 550 mg/L were made from the stock solution. Each glass beaker held 50 ml of water and the test concentration, and twenty healthy larvae were introduced into each one. After the therapy, larval mortality was tracked for 24 hours. For each concentration, a total of three experiments with three replicates each were conducted. When control mortality was between 5% and 20%, mortality was noted, and it was adjusted using Abbott's formula (1925).

After 24 hours of exposure, the methanolic leaf extract of *Canarium strictum* exhibited only mild effects. The strongest toxic impact of the methanolic leaf extract had an LC50 value of 263.0 ppm against the larvae of *Culex quinquefasciatus*, and it was followed by the *Aedes aegypti* with 245.47 ppm. The evidence points to *Canarium strictum*'s methanolic leaf extract as a promising environmentally acceptable method for the management of *Aedes aegypti* and *Culex quinquefasciatus*. [20]

**ANTIPYRETIC ACTIVITY**

**Yeast induced fever**

*Canarium strictum* fresh leaves were gathered in Tamil Nadu, India's Walayar Valley, in the southern Western Ghats. Plant material was mechanically ground, sieved through 40 meshes, and allowed to dry at room temperature in the shade. In a Soxhlet device, the powdered material (100 g) was extracted with 95% methanol using the hot continuous percolation technique. Under lower pressure, the extract was condensed and dried. The 13.65 g of semi-solid, methanol-free material that was obtained for pharmacological research was suspended in 5% gum Acacia.

20% Brewer's yeast was used to produce a fever in albino mice in order to study the antipyretic efficacy. After taking the mice's rectal temperatures with a 1.5 cm digital thermometer inserted into the rectum, pyrexia was produced by subcutaneously administering a 20 ml/kg of body weight dosage of a 20% suspension of dry yeast in a 2% gum Acacia in normal saline solution. Mice that demonstrated an increase in temperature of at least 1°C after an 18-hour yeast infusion were selected for the investigation. The different groups of animals received the following care: they were separated into 5 groups of 6 rats each. The experimental protocol was as follows:

Group I: Normal saline water (control)

Group II: Paracetamol (reference standard) (150 mg/kg).
Group III: Methanolic leaf extract of *Canarium strictum* (100 mg/kg).

Group IV: Methanolic leaf extract of *C. strictum* (200 mg/kg).

Group V: Methanolic leaf extract of *C. strictum* (300 mg/kg).

Mice were given orally to each group. All of the mice in each group had their temperatures taken at 0, 1, 2, 3, and 4 hours after oral delivery. Each group's mean temperature was calculated, and it was compared to the standard medication. The methanolic leaf extract of *C. strictum* effectively lowered the yeast-induced elevation in body temperature in a dose-dependent manner, and its impact is similar to that of the common antipyretic medication, Paracetamol.[21]

**ANTI INFLAMMATORY ACTIVITY (IN-VIVO)**

Resin of the Burseraceae species *Canarium strictum* Roxb. was taken from the wounded trunks of the trees in Rayirath Gardens in Thrissur. By employing the hydro distillation process, the Clevenger type of equipment was utilised to extract the essential oil from the resin (5 kg) of *C. strictum*. The resulting yellow oil was collected, and anhydrous sodium sulphate was used to evaporate the moisture from it. EOCS was kept in glass vials with Teflon-sealed covers at a temperature of 2 ± 0.5 °C and in a dimly-lit area.

**Carrageenan-induced Paw Edema Assay (Acute Inflammatory Model)**

Using a modified version of the carrageenan-induced paw oedema technique first published by Winters CA in 1962, the anti-inflammatory effect was evaluated in mice. Three dosages of the EOCS—10, 50, and 100 mg/kg—were administered to the animals orally in addition to the vehicle (10 ml/kg), Diclofenac sodium (10 mg/kg, p.o.), and the other two medications. Carrageenan (0.02 ml of 0.1%, w/v) solution in ordinary saline was injected into the sub-plantar area of the right hind paw to cause oedema after the various test samples had been provided for 30 minutes. A calliper rule was used to measure the thickness of the paws before, 1, 2, 3, and 4 hours after carrageenan injection. In contrast to the vehicles used to dissolve EOCS, which were provided to the control group, diclofenac sodium was administered as a reference medication. All other treatments were contrasted with the vehicle-treated group, which was thought to have the most inflammation. A set of six mice (n=six) in each group.

\[
\text{Percentage inhibition} = \frac{(CT-Co)_{\text{control}} - (CT-Co)_{\text{treated}}}{(CT-Co)_{\text{control}}} \times 100
\]
Ct - paw size after a specific time interval in hours after carrageenan injection  
Co - paw size before carrageenan injection.

**Formalin Test (Chronic Inflammatory Model)**

Female Balb/C mice were separated into five groups and then subjected to five different levels of formalin-induced inhibition of chronic inflammation. Group II was given positive control Dexamethasone 10 mg/kg while Group I was retained as the control. Six days in a row, EOCS 10-100 mg/kg were given orally to Groups III, IV, and V. One hour after the test medications were given orally to all of the animals, sub plantar injection of freshly produced 0.02 ml of 2% formalin was provided to cause chronic inflammation. The formalin injection was administered 15 minutes after receiving 10 mg/kg of dexamethasone intravenously. The paw thickness was measured using the metre rule six days in a row after formalin injection. Using the supplied formula, the percentage of inhibition was computed.

\[
\text{Percentage inhibition of inflammation} = \frac{1-T}{C} \times 100
\]

T-Paw volume difference in test  
C- Paw volume difference in control

The EOCS against in vivo animal models of carrageenan and formalin produced paw edema was successfully reduced in all stages. The inflammatory episode that followed the injection of carrageenan was divided into first and second phases. Histamine, serotonin, and kinins would be released during the initial phase of the inflammation event, which lasted between 0 and 2.5 hours. Leukotrienes, prostaglandins, proteases, and superoxide radicals are released between 2.5 and 6 hours before the second phase, which is mediated by these substances. Two inflammatory events proceeded throughout the formalin test in a similar way. The release of substance P and a direct chemical stimulation in the nociceptive afferent fibres, primarily C fibres, occur during the neurogenic first phase.

At the inflamed affected region during the second phase of inflammation, inflammatory mediators such prostaglandins, histamine, bradykinin, and serotonin are released. In both experiments, the EOCS reduced the paw oedema, showing the suppression of different inflammatory mediators.\[22\]
Anti-inflammatory activity (In-vitro)

Protein denaturation inhibition assay

Using aspirin as a reference, the protein denaturation inhibition assay was used to assess the anti-inflammatory activity of the C. resiniferum extractives. As previously noted, sterile centrifuge tubes were obtained and tagged appropriately. Then, all test tubes received 2.8 mL of phosphate buffer (pH 6.4 0.2) and 1.0 mL of 5% egg albumin solution. As positive- and negative controls as well as the test group, Aspirin (0.1 mg), Tween-80, or plant sample (500 g/mL of Tween-80) were introduced. After adjusting the pH (5.6 0.2) with 1.0 N HCl, all of the reaction mixtures were heated at 57 °C for 20 min. The absorbance of each reaction mixture was measured at 660 nm using a UV-visible spectrophotometer after cooling and filtering (Whatman filter paper) (Shimadzu-1800). Three times were given the test. By measuring the absorbance of the treatment groups and turning it into inhibition of albumin protein denaturation, the anti-inflammatory activity of each test sample was calculated. Using the following formula, the percent inhibition of protein denaturation was obtained.

\[
\% \text{ inhibition of protein denaturation} = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100
\]

Here, \( A \) = Absorbance for respective groups.

During inhibition of protein denaturation assay, the chloroform fraction at the dose of 500 µg/mL showed the highest inhibitory capacity with 47.08%, followed by hexane (39.96%) and aqueous fractions (35.29%) of C. resiniferum, while the standard aspirin exhibited 77.10% inhibition of albumin denaturation.[23]

Membrane stabilisation assay

Utilising human erythrocytes, the Shinde et al. technique was employed to test the membrane stabilising action of plant extractives. Three sterile centrifuge tubes were obtained and labelled as the aspirin standard/positive control, three as the negative control, and three as the tube for each plant sample. All of the tubes were then filled with 0.5 mL of erythrocyte suspension, 2.0 mL of hyposaline, and 1.0 mL of phosphate buffer (pH 7.4 0.2). The next step involved mixing 1.0 mL of aspirin (0.1 mg) for the standard group, 1.0 mL of distilled water for the control group tubes, and 1.0 mL of plant samples (500 g/mL) for the test group as indicated. All of the reaction mixtures were centrifuged after 30 minutes of incubation at 37°C in a Bio-Oxygen Demand incubator (10min at 3000 rpm). A Shimadzu-1800 UV-visible spectrophotometer was used to measure the absorbance of the supernatants at a wavelength of 560 nm. For each plant extract, the test was conducted three times. The
suppression of hemolysis was measured in order to assess the activity of membrane stabilisation.

\[
\% \text{ inhibition of hemolysis} = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100
\]

The ability of all the solvent fractions of methanol extract of \textit{C. resiniferum} leaf to protect the RBC membrane against hypotonic solution was found to be statistically significant (P<0.01). The highest inhibition of hypotonic solution induced RBC hemolysis was displayed by the chloroform fraction (49.68%).[23]

**THROMBOLYTIC ACTIVITY (INVITRO)**

Thrombolytic effect was evaluated following a standard method in which streptokinase (SK) was used as standard. Briefly, 15 mL of venous blood was drawn from healthy volunteers without any recent history of oral contraceptive and anticoagulant therapy. The collected blood was immediately distributed into 27 previously weighed sterile alpine tubes (0.5 mL/tube) and the tubes were incubated at 37°C for 45 min to form clots. After clot formation, the serum was completely removed without disturbing the clot and each tube having clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube-weight of tube alone). Plant sample solutions (500 μg/100 μL) were added separately to each alpine tube containing pre-weighed clot. As a standard, 100 μl of streptokinase, SK (15, 00,000 I.U.) and as a control, 100 μL of methanol, hexane and chloroform were separately added to the control tubes. All the tubes were then kept at 37°C for 90 min and observed for clot lysis. After Incubation, the fluid released was removed carefully and tubes were again weighed to observe the difference in weight after clot disruption. The difference in weight taken before and after clot lysis was expressed as percentage of clot lysis.

The chloroform, hexane and aqueous soluble fractions of methanol extract of \textit{C. resiniferum} leaf at the dose of 500 μg/100 μl showed prominent thrombolytic activity by clot lysis of 57.32%, 42.85% and 34.08%, respectively.[23]

\[
\% \text{ clot lysis} = \frac{\text{weight of clot after lysis by plant sample}}{\text{weight of clot before lysis by plant sample}} \times 100
\]
ANALGESIC ACTIVITY

Central analgesic activity by tail immersion assay

Central analgesic activity of *C. resiniferum* was evaluated by tail immersion assay\[24\] in Swiss mice. Briefly, the experimental animals were randomly divided as mentioned above and received the plant extractives (500 mg/kg) by oral route. After treatment, at zero-hour, 1-2 cm of the tail of mice was immersed in warm water kept constant at 55° C. The time (in sec) taken for the mouse to flick its tail was regarded as the pain reaction time (PRT). The latency period was set as 20 s to avoid injury to mice. Following the administration of the test samples, pain reaction time was recorded at 0, 30, 60 and 90 min. Finally, the percentage of time elongation of tail immersion was estimated in respect to standard morphine (2 mg/kg) by the equation

\[
\text{% Time elongation} = \left(\frac{T_{\text{test}} - T_{\text{control}}}{T_{\text{control}}}\right) \times 100
\]

Where, \( T_{\text{test}} = \) Pain reaction time for each group.

Peripheral analgesic activity by formalin-induced pain

The peripheral analgesic activity was evaluated by formalin-induced licking and biting responses test.\[24\] Like a tail immersion test, both standard aspirin (50 mg/kg) and plant samples (500 mg/kg) were administered to the experimental animals by oral route. Pain sensation was induced by subcutaneous injection of 1% formalin into the right hind paw of the experimental animals. As long as the animals feel pain, they continue to give licking and biting responses. Each licking and biting responses are counted and taken as an indication of pain sensation. Thirty minutes after formalin injection, the number of licking and biting responses was recorded for 5 min for each mouse. The plant sample possessing analgesic activity will decrease the number of licking and biting responses in animals. The percent inhibition of formalin-induced licking in each treated group was then calculated by using the following equation:

\[
\text{% Inhibition of licking response} = \left[\frac{(N_{\text{control}} - N_{\text{test}})}{N_{\text{control}}}\right] \times 100
\]

Where, \( N = \) Mean number of licking and biting responses for each group\[23\]

In tail immersion method, the hexane fraction significantly (p<0.05) increased the pain threshold with 228.57% elongation at 90 min while the chloroform fraction showed maximum 29.82% inhibition of formalin-induced licking and biting responses in mice.
ANTIMICROBIAL ACTIVITY

Disc diffusion method
On the first day, the organisms to be examined (Gram negative bacteria, Pseudomonas aeruginosa, and Gram positive bacteria, Staphylococcus aureus) were grown on an appropriate agar medium under ideal incubation conditions to create a new overnight grown culture. 5 mL sterile suitable broth tubes were made. The second day's operations included removing a variety of unique colonies from the freshly established plate culture. They were suspended in broth-filled tubes until the turbidity (visually) equivalent to the 1.0 Mc Farland standard was obtained. Sterile cotton swabs were dipped and squeezed in the appropriate culturing medium. Mueller Hinton agar medium was used to cultivate the lawn. 15 minutes were given for the plates to dry. A pre-incubation of the cells is permitted by longer drying durations, which should be avoided. The plates were incubated shortly after the discs were applied. The discs were placed to the agar surface using sterile forceps with test compound loaded. The ideal conditions for incubation were used to incubate the plates. On the third day, using a mm ruler, the diameter of the inhibition zones was measured from the site of sudden growth inhibition to the nearest mm. The resin of *Canarium strictum* showed antibacterial activity against the organisms *Staphylococcus aureus* and *Pseudomonas aeruginosa.*\[25]\n
ANXIOLYTIC ACTIVITY

Elevated plus-maze (EPM) test
The EPM apparatus consisted of four arms, including two open (5 × 35 cm) and two closed (5 × 15 × 30 cm) arms, joined together with a central platform (5 × 5 cm). The maze was placed 60 cm above ground level. The animals from groups I-V were treated 30 min before the test was begun by placing individual animals on the central platform. The time spent in the open arms and the number of entries in the open arms were recorded over a period of 5 min.\[26]\n
Hole-board test (HBT)
The hole-board (HB) test apparatus is a wooden compartment (40×40×25 cm) with 16 holes each 3 cm in diameter. The animals from groups I-V were treated 30 min before being placed individually on the HB apparatus. The number of head-dips was counted over a 5 min period of observation.\[27]\n
**Light-dark box (LDB) test**
The LDB apparatus was a Plexiglas box with two compartments (each 25×25 cm) joined together. One of the compartments was dark and covered with a lid, the other one was brightly lit and open. The two compartments were connected by a 3 cm hole. The animals from groups I-V were treated for 60 min before being placed individually in the light compartment of the apparatus and allowed to move around. The time that the animals spent in the light and the dark compartments was recorded for a period of 5 min.\(^{[28]}\)

**ANTIDEPRESSANT ACTIVITY**

**Tail suspension test (TST)**
Animals in group IeV were treated 30 min prior to being individually hanged 50 cm above the ground using adhesive tape placed about 1 cm from the tip of their tail and for a period of 6 min. The duration of immobility (in seconds) was recorded for the suspended animals within each group.\(^{[29]}\)

**Forced swim test (FST)**
Animals in group I-V were treated 30 min prior to being placed individually for a period of 6 min inside a glass cylindrical chamber (25 cm high 10 cm diameter) filled with water (up to 19 cm) at a temperature of 23 ± 1 C. The duration of immobility (in seconds) of animals that stopped swimming was assessed during the last 4 min of the test.\(^{[30]}\)

Methanolic extract of plant, at all doses, showed dose-dependent anxiolytic activity. At 400 mg/kg, it significantly increased the time spent and number of entries in the open arms (EPM test), the number of head-dips (HBT), and the time spent into the light compartment (LDB) test compared to the control. In the TST and FST, MECR dose-dependently reduced the duration of immobility compared to untreated animals. This was significant for all doses except for 100 mg/kg in the FST model.\(^{[31]}\)

**CONCLUSION**
*Canarium strictum* is a native plant of the Eastern and Western Ghats, and this article has concluded that it is reputed to have therapeutic characteristics. Different ethnic groups use it as their traditional medicine. Additionally, it has anti-inflammatory, analgesic, antipyretic, antioxidant, and antidepressant properties. Review of the literature also states that the plant *Canarium strictum* Roxb. Has a lot of potential for future cutting-edge study and should be regarded as a beneficial herbal medicinal plant.
ACKNOWLEDGEMENT
The author is grateful to the head of department of pharmacology and other faculties, moreover to the Pushpagiri College of pharmacy (Kerala University of health sciences) Tiruvalla, India for the continuous support and encouragement.

REFERENCES


