Investigations On Anti-Termite Activity Of Eucalyptus Globulus Leaf Extract

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Abstract
This study aims to investigate the anti-termite activity of eucalyptus globulus leaf extract. Termites are destructive pests that cause significant damage to wooden materials that ultimately leads to financial losses as well as structural instability. Natural remedies for termite control are gaining attention due to concerns over the environmental and health impacts of synthetic pesticides. The Ethanolic extract of Eucalyptus globulus leaves show 90% mortality rate at 2mg/ml concentration whereas essential oil shows 80% mortality rate at same concentration. This shows that, the Ethanolic extract of Eucalyptus globulus leaves were more effective as compared to the essential oil. So, this conveys that with increase in concentration, the mortality rate of termites also increases. This research show that both Ethanolic extract of Eucalyptus globulus leaves and essential oil shows excellent inhibitory activity against termites. These results will be helpful for future researchers for the development of potent, safe and cost effective pesticides.

Keywords: Eucalyptus globulus, Essential oils, ethanolic extract, phytochemicals, anti-termite activity, natural pesticides.

Introduction
Eucalyptus globulus is also named as Blue gum eucalyptus, Tasmanian blue gum, and southern blue gum belonging to family Myrtaceae. In India, it is commonly known as Nilgiri and in Sanskrit Tailpama. Tipu Sultan first brought Eucalyptus tree in India in 1785. It is widely grown in Nilgiri hills of South India, Andhra Pradesh, Telangana, Tamil Nadu, Punjab, Haryana and Karnataha (Skolmen et al., 1985; Chen et al., 1987). Numerous therapeutic activities of Eucalyptus globulus were reported including antioxidant (Pan et al., 2020; Gonzalez et al., 2004; Neiva et al., 2018), antimicrobial (Sharma et al., 2021; Aita , 2021), antibacterial (Brezáni et al., 2018; Ahmad et al., 2021), antifungal (Ajilore et al., 2021), antiviral (Hayat et al., 2015), anti-diabetic (Saka et al., 2017), anthelmintic (Kesharwani et al., 2018; Taur et al., 2010), antihistaminic (Kesharwani et al., 2018), anti-inflammatory (Kesharwani et al., 2018), antitermite (Emamjomeh et al., 2021), antiplaque (Agarwal et al., 2013; Shah et al., 2012; Osawa et al., 1996), and anticancer (Abiri et al., 2021). Different chemical constituents had been identified and isolated from Eucalyptus globulus like Proteocatechuic acid (anti-oxidant), 1, 8-cineole (anti-microbial and Anti-inflammatory properties), Monoterpenes (anti-bacterial), Alkaloids and essential oil (antifungal), borneol, cineol, linalool, gernayl acetate, safrol, antheol (anti-anthelmintic), oxygenated and non-oxygenated terpenes in essential oil (Antitermites), and Citral monoterpenoid (anticancer activity). This plant is also enriched with nutritional components including high level of manganese. Eucalyptus globulus is well known plant for insecticidal activity of in natura and maltodextrin/Áng gum nano-encapsulated essential oils of Eucalyptus globulus Labill and Zataria multiflora Bioss was evaluated against the third instar larvae of Ephhestia kuehiella (Lepidoptera: Pyralidae) (Emamjomeh et al., 2021). Myzus persicae causes damage to its host by direct feeding, the transmission of plant viruses and the production of honeydew. The herb Eucalyptus globulus extract was used for insecticidal property for pest control (Khoshraftar et al., 2019). But there is still need to explore the anti-termite activity of Eucalyptus globulus. Therefore, the present study is designed to evaluate the anti-termite activity of Ethanolic extract of Eucalyptus globulus leaves.

Methodology:
Collection of leaves: Fresh leaves of Eucalyptus globulus were collected from the nearby villages of Nadaun, Himachal Pradesh on 5th March, 2023 at latitude: 31.78 and longitude: 76.35.

Organoleptic Evaluation: Evaluation was done by examining the leaves of Eucalyptus globulus with naked eyes under natural light.
Microscopic and histological study of leaves

**Study of transverse section (T.S.) of leaves:** Fresh leaves of *Eucalyptus globulus* were boiled in water and section was taken by free hand cutting of leaf from the lower epidermal layer. The section was transferred on the slide and treated with few drops of HCl followed by phloroglucinol. T.S. was observed under microscope.

**Quantitative microscopy:** Quantitative microscopy was performed to determine the stomatal Number and stomatal index.

**Essential oil extraction:** The process of extracting essential oil involved the utilization of fresh *Eucalyptus globulus* leaves. These leaves, sourced from the neighboring village of our educational institution in Bela, Nadaun, were carefully collected. Hydrodistillation using the Clevenger steam distillation apparatus was employed to extract the essential oil from the aforementioned leaves, weighing 50 grams. Before the extraction, the fresh *Eucalyptus* leaves underwent a thorough washing and cutting process. Heat was then applied at a temperature of 60°C for duration of 6 hours. The extraction of essential oil persisted for a total of 6 days, culminating in the collection of the valuable oil within a sealed vessel.

**Preparation of ethanolic extract:** Fresh and fully grown leaves were gathered from the neighboring villages near our college in Bela, Nadaun. These leaves were carefully dried in a shaded environment. We then took 30 grams of dried *Eucalyptus globulus* leaves and coarsely ground them using a grinder. A thimble was prepared to facilitate the extraction process. Next, we removed the fats and oils from the plant material by using n-hexane (275 ml) as a solvent at a temperature of 70°C for a period of 3 days. Following the defatting procedure, only 27 grams of the desired substance remained. Defatted material (27g) was extracted with 350 ml of ethanol at 30°C for 5 days using Soxhlet apparatus, concentrated on rotary evaporator and dried in desicator using silica gel. The dried extract was kept at 4 ℃ till further use.

**Phytochemicals screening:** Phytochemical screening was performed to ascertain the presence of various phytoconstituents.

A. **Test for Carbohydrate:**
   1. **Molish test (general test):** To 1ml of Ethanolic extract added few drops of α- naphthol, shake it well and added few drops of concentrated H₂SO₄ from the sides of test tube. At the junction, purple to violet color ring appears.
   2. **Fehling’s test:** Mix 1ml Fehling’s A to 1ml Fehling’s B solution and boil for 1 min. Added equal volume of Ethanolic extract. Heat the test solution in the hot water bath for 5-10 min. Firstly, yellow color then brick red precipitate is observed.
   3. **Barfoed’s test:** Mix equal volume of Barfoed’s reagent and test solution. In boiling water bath heat the test solution for 1min and cool it. Red precipitates were formed.
   4. **Benedict’s test:** To 1ml test solution added 4% NaOH and few drop of 1% CuSO₄ solution. Violet or pink color appeared.
   5. **Selwinoff’s test:** Heat 1ml of test solution and 3ml of Selwinoff’s reagent in boiling water bath for 1min. Red color was appeared.
   6. **Tollen’s test:** Mix 2.5 ml concentrated HCl and 4ml phloroglucinol. Added 1ml test solution. Heat the mixture. Yellow to red color appeared.

B. **Tests for Proteins:**
   1. **Xanthoprotein test:** Mix 1ml test solution with 1ml of concentrated H₂SO₄ White precipitate is formed. Boil it. Precipitate turn into yellow. Added NH₄OH precipitate turns orange.
   2. **Biuret’s test (general test):** To 1ml test solution added 4% NaOH and few drop of 1% CuSO₄ solution. Violet or pink color appeared.
   3. **Millon’s test:** Mix 1ml test solution with 1ml Millon’s reagent. White precipitates were formed. Warm precipitate turns brick red color precipitate.

C. **Test for Amino acid:**
   1. **Ninhydrin test:** 1ml test solution and 3 drops of 5% Ninhydrin solution were heated in boiling water bath for 10 minutes. Formation of purple or bluish color is appeared.
   2. **Test for tyrosine:** heat 1ml test solution and added few drops Millon’s reagent. Solution shows dark red color.

D. **Test for Steroids:**
   1. **Salkowski reaction:** to 1ml Ethanolic extract, added 1ml chloroform and 1ml concentrated H₂SO₄ and shake it well. Chloroform layer appear red and acid layer shows greenish yellow fluorescence.

E. **Test for Glycosides:**
   • **Test for cardiac glycosides:**
     1. **Baljet’s test:** A thick section shows yellow to orange color with sodium picrate.
2. **Legal's test:** To Ethanolic extract, added 1ml pyridine and 1ml sodium nitroprusside. Pink to red color appeared.

3. **Tests for deoxysugar (Keller–killiani test):** to 1ml extract, added glacial acetic acid, one drop 5% FeCl₃ and concentrated H₂SO₄. Reddish brown color appears at junction of the two liquid layers and upper layer appears bluish green.

4. **Kedde's test:** To 1ml Ethanolic extract, added 1drop 85% alcohol, 2 drops of 2% nasty sqn. Purple color appears.

- **Test for Saponins:**
  - Foam test: Shake the drug extract with water. Persistent foam was observed.

**F. Test for Flavonoids:**

1. **Shinoda Test:** To dry powder or extract, added 5 ml 90% ethanol/t-butyl alcohol, few drops conc. HCl and 0.5 g magnesium turnings. Orange, pink, red to purple color appeared (flavonols, dihydro derivatives and xanthenes).

**G. Tests for alkaloids:**

1. **Dragendorff's test:** To 1 ml ethanolic extract, added few drops Dragendorff’s reagent. Orange brown precipitates were formed.

2. **Mayer's test:** To 1 ml ethanolic extract, added few drops of Mayer's reagent it gives precipitates.

3. **Hager's test:** 1 ml ethanolic extract with Hager's reagent gives yellow precipitates.

4. **Wagner's test:** 1ml filtrate with few drops Wagner's reagent gives reddish brown precipitates.

**H. Test for Tannins:**

1. **Lead acetate solution test:** 1ml of extract was put in beaker and then few drops of lead acetate solution were added in it after that white precipitate was obtained.

2. **Gelatin solution:** 1ml of extract was put in beaker and then few drops of gelatin solution were added in it after that white precipitated was obtained.

3. **Acetic acid solution:** 1ml of extract was put in beaker and the few drops of acetic acid solution were added in it after that red coloured solution was obtained.

4. **Potassium dichromate:** few drops of potassium dichromate were added in 1 ml of extract. Red coloured precipitate was obtained.

5. **Dilute iodine solution:** Few drops of dil. Iodine solution was added in 1 ml of extract. Transient red coloured solution was observed.

6. **Dilute HNO₃:** Few drops of Dil. HNO₃ were added in 1 ml of extract. Reddish to yellow coloured solution was observed.

7. **Dilute HN₄OH and potassium ferricyanide solution:** Few drops of dilute HN₄OH and potassium ferricyanide solution were added in 1ml of extract. Red coloured solution was observed.

8. **Dilute Potassium Permanganate solution:** few drops of dilute potassium permanganate solution were added in 1ml of extract. Decolouration was observed.

**Thin layer chromatographic profile:**

In order to develop TLC plates, silica gel was used. Glass plates were activated at 120°C for 30 minutes. TLC profile of ethanolic extract was developed using [chloroform: methanol (7:3)] as mobile phase. TLC plates were seen under iodine chamber as well in UV cabinet at a wavelength of 254 nm and 365 nm.

**Anti-termite activity**

The “no-choice” bioassay method adopted by Kang et al. (1985) was followed to evaluate the antitermite’s activity of the plant essential oil and Ethanolic extract. Samples of different concentration (0.5, 1, 1.5, 2 mg/ml) of *Eucalyptus globulus* essential oil and extract were prepared in acetone and applied to filter paper discs (Whatman no. 1, 85 mm in diameter and thickness 1.5 mm) separately. Discs of filter paper treated with acetone alone were used as a control.

Solvent was removed from the treated filter papers and air-dried at room temperature. Active termites (20) were selected randomly and introduced on each filter paper impregnated with the test material in an empty Petri dish (85 mm in diameter 12 mm in height). The Petri dishes with the test samples were then placed in an incubator and maintained at a temperature of 25± 2 °C for 08 days. After 08 days, live termites were counted to determine the mortality rates. Only those termites were considered to be dead if appendages did not move when prodded with a forcep. It was previously determined that the filter paper treated with acetone solvent individually followed by air-drying at room temperature had no effect on termite mortality.

The mortality rate (%) of essential oil and Ethanolic extract were calculated by using formula:

\[
\text{Mortality rate} \% = \frac{\text{Number of dead termites after 8 days of the test}}{\text{Number of initial termites in the test}} \times 100
\]
Results:

Organoleptic evaluation:

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Criterion</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Color</td>
<td>Dark green</td>
</tr>
<tr>
<td>2.</td>
<td>Odor</td>
<td>Characteristics</td>
</tr>
<tr>
<td>3.</td>
<td>Taste</td>
<td>Bitter, pungent</td>
</tr>
<tr>
<td>4.</td>
<td>Leaf base</td>
<td>Symmetrical</td>
</tr>
<tr>
<td>5.</td>
<td>Shape</td>
<td>Juvenile</td>
</tr>
</tbody>
</table>

Table 1: Organoleptic evaluation of *Eucalyptus globulus* leaves.

Microscopic evaluation:

Study of transverse sections:

Fig1: T.S. of *Eucalyptus globulus* leaves [ST- Stomata, EC- Epidermal cells, VI- Vein islets, VT- Vein termination].

Quantitative microscopy:

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Parameter</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Stomatal Number</td>
<td>10.47</td>
</tr>
<tr>
<td>2.</td>
<td>Stomatal Index</td>
<td>2100</td>
</tr>
</tbody>
</table>

Table 2: Quantitative microscopy of leaves of *Eucalyptus globulus*

Phytochemicals screening:

The percentage yield of ethanolic extract was found to be 17.6%. The following tests were conducted to analyze different chemical constituents present in extract as shown in table 3.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Test Name</th>
<th>Observation</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.</td>
<td>Test for carbohydrates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td>Molish’s test</td>
<td>Violet color ring form at the junction</td>
<td>Positive</td>
</tr>
<tr>
<td>b.</td>
<td>Fehling’s test</td>
<td>Brick color precipitate formed</td>
<td>Positive</td>
</tr>
<tr>
<td>c.</td>
<td>Benedict’s test</td>
<td>Formation of red color solution</td>
<td>Positive</td>
</tr>
<tr>
<td>d.</td>
<td>Barfoed’s test</td>
<td>Red color precipitate formed</td>
<td>Positive</td>
</tr>
<tr>
<td>e.</td>
<td>Tollen’s test (galactose)</td>
<td>No red color appeared</td>
<td>Negative</td>
</tr>
<tr>
<td>f.</td>
<td>Selwinoff’s test</td>
<td>No red color solution formed</td>
<td>Negative</td>
</tr>
<tr>
<td>B.</td>
<td>Test for proteins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td>Xanthoprotein test</td>
<td>Yellow color precipitate formed</td>
<td>Positive</td>
</tr>
<tr>
<td>b.</td>
<td>Biuret’s test</td>
<td>No violet or pink color appear</td>
<td>Negative</td>
</tr>
<tr>
<td>c.</td>
<td>Million’s test</td>
<td>Red precipitate formed</td>
<td>Positive</td>
</tr>
<tr>
<td>C.</td>
<td>Test for Amino acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td>Ninhydrin test</td>
<td>No bluish color appear</td>
<td>Negative</td>
</tr>
<tr>
<td>b.</td>
<td>Test for tyrosine</td>
<td>No dark red color appear (orange color appear)</td>
<td>Negative</td>
</tr>
<tr>
<td>D.</td>
<td>Test for steroids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td>Salkowski reaction</td>
<td>Red color layer appear on top and greenish yellow color appear</td>
<td>Positive</td>
</tr>
</tbody>
</table>
E. **Test for cardiac glycosides**

<table>
<thead>
<tr>
<th>Test</th>
<th>Appearance</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Baljet’s test (cardenoloids)</td>
<td>Yellow to orange color</td>
<td>Positive</td>
</tr>
<tr>
<td>b. Legal’s test</td>
<td>Orange-reddish color</td>
<td>Positive</td>
</tr>
<tr>
<td>c. Keller-killani test</td>
<td>Reddish-brown color</td>
<td>Positive</td>
</tr>
<tr>
<td>d. Kedde’s test</td>
<td>Purplish color</td>
<td>Positive</td>
</tr>
</tbody>
</table>

F. **Test for Flavonoids**

<table>
<thead>
<tr>
<th>Test</th>
<th>Appearance</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shinoda test</td>
<td>Orange color</td>
<td>Positive</td>
</tr>
</tbody>
</table>

G. **Test for alkaloids**

<table>
<thead>
<tr>
<th>Test</th>
<th>Appearance</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Dragendorff’s test</td>
<td>Orange-brown precipitate formed</td>
<td>Positive</td>
</tr>
<tr>
<td>b. Mayer’s test</td>
<td>Precipitate present</td>
<td>Positive</td>
</tr>
<tr>
<td>c. Hager’s test</td>
<td>Precipitate present</td>
<td>Positive</td>
</tr>
<tr>
<td>d. Wagner’s test</td>
<td>Reddish brown color</td>
<td>Positive</td>
</tr>
</tbody>
</table>

H. **Test for saponins**

<table>
<thead>
<tr>
<th>Test</th>
<th>Appearance</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foam formation</td>
<td>Formation of Foam</td>
<td>Positive</td>
</tr>
</tbody>
</table>

I. **Test for tannins**

<table>
<thead>
<tr>
<th>Test</th>
<th>Appearance</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Dilute iodine test</td>
<td>Red color is appear</td>
<td>Positive</td>
</tr>
<tr>
<td>b. Dilute nitric test</td>
<td>Red to yellow color</td>
<td>Positive</td>
</tr>
<tr>
<td>c. Dilute potassium per magnet solution</td>
<td>Discoloration of KMnO₄</td>
<td>Positive</td>
</tr>
<tr>
<td>d. Dilute ammonium hydroxide and potassium ferricyanide solution</td>
<td>Red color is appear</td>
<td>Positive</td>
</tr>
<tr>
<td>e. Potassium dichromate</td>
<td>Red precipitate appear</td>
<td>Positive</td>
</tr>
<tr>
<td>f. Acetic acid solution</td>
<td>Red color appear</td>
<td>Positive</td>
</tr>
<tr>
<td>g. Gelatin solution</td>
<td>White precipitate appear</td>
<td>Positive</td>
</tr>
<tr>
<td>h. Lead acetate</td>
<td>White precipitate formed</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Table 3: Phytochemicals screening of *Eucalyptus globulus* leaves extract.

Thin layer chromatographic profile:

![Thin layer chromatographic profile](image)

**Fig2: TLC Profile of Eucalyptus globulus leaf extract.**

**Anti-termite activity:**

In present study, *Eucalyptus globulus* plant was evaluated for their anti-termite potency. Tested leaves extract exert significant anti-termite activity at 2 mg/ml concentration against termites as shown in table 4.

Termite mortality was highest in leaf ethanol extracts. *Eucalyptus globulus* essential oil exerts less significant effects than leaves Ethanol extract.
Anti-termite response of essential oil and ethanolic extract of *Eucalyptus globulus* of different concentration

<table>
<thead>
<tr>
<th>Tested components</th>
<th>Concentration (mg/ml)</th>
<th>Mortality rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol extract</td>
<td>0.5</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>90</td>
</tr>
<tr>
<td>Essential oil</td>
<td>0.5</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>80</td>
</tr>
</tbody>
</table>

Table 4: Effect of the *Eucalyptus globulus* leaves extracts and essential oil on termite mortality.

**Conclusion**

The result indicated that essential oil and ethanolic extract of *Eucalyptus globulus* leaves have tendency to control termite’s activity. Phytochemical screening of ethanolic extract help us to determine the presence of alkaloids, carbohydrates, cardiac glycosides, proteins, saponins, tannins, sterides and flavonoids. Macroscopic evaluation showed shape, size, color and other morphological character of different parts of *Eucalyptus globulus*. Microscopic evaluation involved quantitative microscopy and study of T.S. of *Eucalyptus globulus* leaves. The Ethanolic extract of *Eucalyptus globulus* leaves show 90% mortality rate at 2mg/ml concentration whereas essential oil shows 80% mortality rate at same concentration. This shows that, the Ethanolic extract of *Eucalyptus globulus* leaves were more effective as compared to the essential oil for anti-termite activity. These results may be helpful for future researchers to develop cost effective and environmental safe pesticids.
References: