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 Karnataka (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 Protocatechuic 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, saffrol, 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/Angum gum nano-encapsulated essential oils of *Eucalyptus globulus* Labill and Zataria multiflora Bioss was evaluated against the third instar larvae of Ephestia kuehniella (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 leave 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 H_2SO_4 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:** Mix equal volume of test solution and Benedict's reagent and heat in boiling water bath for 5min. On the basis of reducing sugar, the green, yellow or red solution was 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 H_2SO_4. White precipitate is formed. Boil it. Precipitate turn into yellow. Added NH_4OH precipitate turns orange.
2. **Biuret's test (general test):** To 1ml test solution added 4% NaOH and few drop of 1% $CuSO_4$ 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 H_2SO_4 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 –killani test):** to 1ml extract, added glacial acetic acid, one drop 5% $FeCl_3$ and concentrated H_2SO_4. 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. HCI 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 HNO_3:** Few drops of Dil. HNO_3 were added in 1 ml of extract. Reddish to yellow coloured solution was observed.
7. **Dilute HN_4OH and potassium ferricyanide solution:** Few drops of dilute HN_4OH 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℃ 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 ℃ 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:

S.No.	Criterion	Characteristics
1.	Color	Dark green
2.	Odor	Characteristics
3.	Taste	Bitter, pungent
4.	Leaf base	Symmetrical
5.	Shape	Juvenile

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

Microscopic evaluation:

Study of transverse sections:

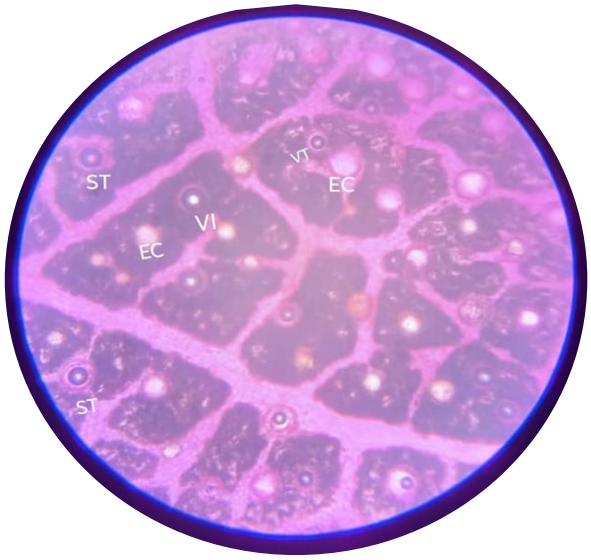

Fig1: T.S. of *Eucalyptus globulus* leaves [ST- Stomata, EC- Epidermal cells, VI- Vein islets, VT- Vein termination**]**.

Quantitative microscopy:

Sr. No.	Parameter	Number
1.	**Stomatal Number**	10.47
2.	**Stomatal Index**	2100

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.

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

E.	**Test for cardiac glycosides**		
	a. Baljet's test	Yellow to orange color appear	Positive
	b. Legal's test (cardenoloids)	Orange-reddish color appear	Positive
	c. Keller-killani test (deoxysugar)	Reddish-brown color appear	Positive
	d. Kedde's test	Purplish color appear	Positive
F.	**Test for Flavonoids**		
	Shinoda test	Orange color appear	Positive
G.	**Test for alkaloids**		
	a. Dragendorff's test	Orange-brown precipitate formed	Positive
	b. Mayer's test	Precipitate present	Positive
	c. Hager's test	Precipitate present	Positive
	d. Wagner's test	Reddish brown color appear	Positive
H.	**Test for saponins**		
	Foam formation	Formation of Foam	Positive
I.	**Test for tannins**		
	a. Dilute iodine test	Red color is appear	Positive
	b. Dilute nitric test	Red to yellow color appear	Positive
	c. Dilute potassium per magnet solution	Discoloration of $KMnO_4$	Positive
	d. Dilute ammonium hydroxide and potassium ferricyanide solution	Red color is appear	Positive
	e. Potassium dichromate	Red precipitate appear	Positive
	f. Acetic acid solution	Red color appear	Positive
	g. Gelatin solution	White precipitate appear	Positive
	h. Lead acetate	White precipitate formed	Positive

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

Thin layer chromatographic profile:

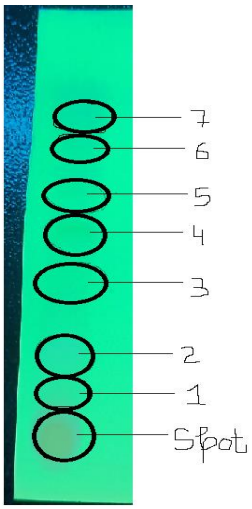

Fig2: TLC Profile of *Eucalyptus globulus* leaf extract.

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 Ethanolic extract.

Anti-termite response of essential oil and Ethanolic extract of *Eucalyptus globulus* of different concentration

Tested components	Concentration (mg/ml)	Mortality rate (%)
Ethanolic extract	0.5	25
	1	45
	1.5	70
	2	90
Essential oil	0.5	20
	1	45
	1.5	60
	2	80

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

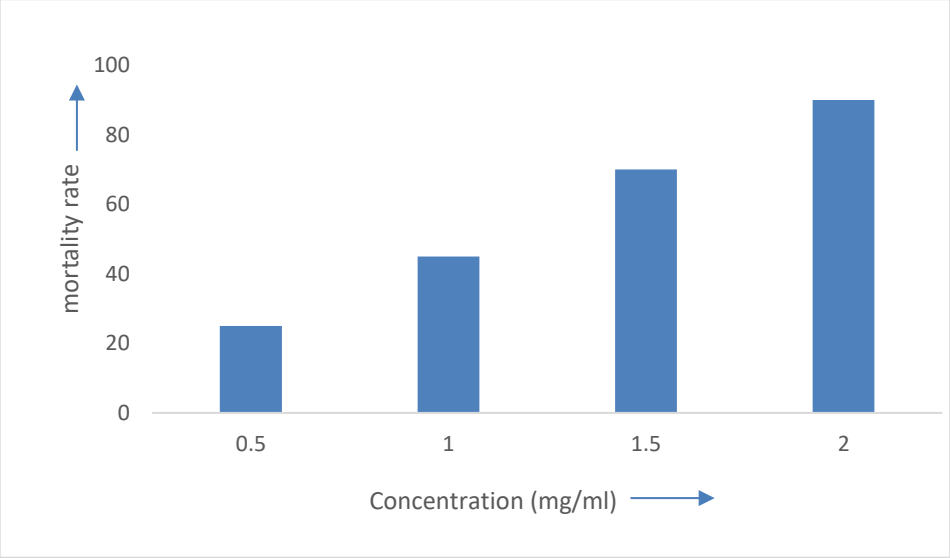

Fig3: Anti-termite activity of ethanolic extract of *Eucalyptus globulus* leaves.

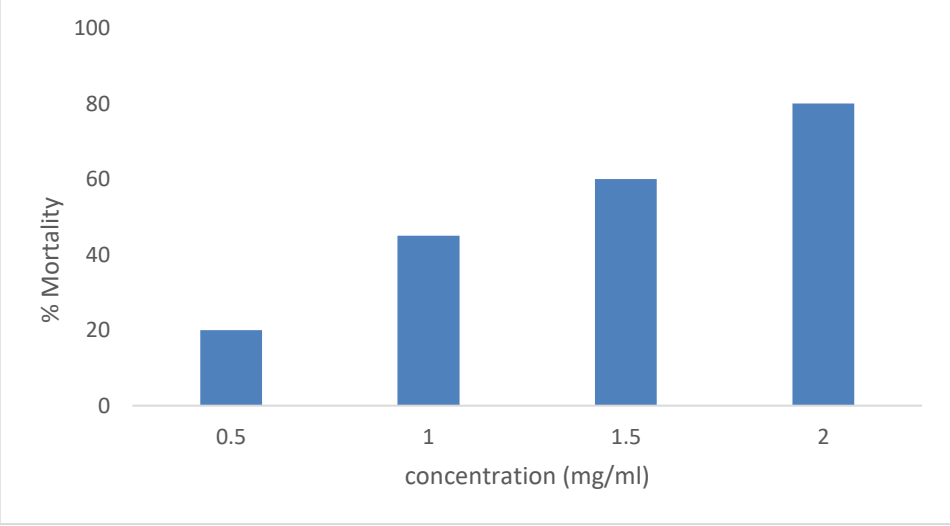

Fig4: Anti-termite activity of essential oil of *Eucalyptus globulus* leaves.

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, steroids and flavanoids. 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.

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