

Research Article

In vitro antitermite activity of alkaloids from *Epipremnum aureum* (Linden and Andre) Bunting (Araceae) against Indian white termite *Odontotermes obesus*

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ABSTRACT

Plants of Araceae family are well known sources of secondary metabolites with a wide range of biological activities, including antibacterial, antifungal, anticancerous, antioxidative, analgesic, and antipyretic properties, among others. In the present study laboratory experiments were conducted to evaluate antitermite response of alkaloids isolated *Epipremnum aureum* (Linden and Andre) Bunting leaf, stem and root against Indian white termite *Odontotermes obesus*. Purified fractions of alkaloid showed remarkable toxicity against *O. obesus* at forty times dilution with respect to control. Soil sample with termites were collected from field and healthy termites were sorted out. Thirty active workers were released on each treated Whatman filter paper (90mm diameter) fitted inside glass petridishes. Mortality of termites was observed after fifteen minutes and compared with untreated filter papers. Highest mortality rate has been recorded with alkaloids isolated from leaves of *E. aureum*. Results of present study may be useful for development of herbal formulation for spot treatment in termite infested buildings, agriculture, furniture and to overcome synthetic termiticides borne problems.

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Introduction

Epipremnum aureum (Linden and Andre) Bunting is a popular ornamental foliage belonging to the botanical family Araceae. It is a common house plant with several cultivars and capable of removing indoor air pollutants such as xylene, formaldehyde and benzene¹. Aerial roots and leaves of *E. aureum* show great potential for antimicrobial activity².

Alkaloids function as a very important class of constitutive defense compounds among the various secondary metabolites produced by plants. Alkaloids are known for their toxicity to vertebrates at very low concentration. It possess wide range of insecticidal and antimicrobial properties, thus having the prospective for commercial development as wood management agents³⁻⁵.

Termites are soft bodied arthropods that utilize cellulose with mouth parts. They are extremely destructive and largely damage plants and agricultural crops⁶, house hold equipments and finished goods. They pose international problem all around the world, particularly in tropical areas having high relative humidity. The antitermitic activity is related to the type of plant and products or part that is being utilized like leaf, root, seed, fruit, essential oil, wood, bark and resins⁷.

The purpose of the present work is to isolate and purify alkaloids using the various explants of *E. aureum* and test the efficacy of different parts for the mortality of termites.

Materials and methods

Chemicals

In this study, ethyl acetate, petroleum ether, diethyl ether, chloroform (analytical grade) and methanol (HPLC grade) were purchased from Merck chemicals, Germany. Ammonium hydroxide, sulphuric acid and ammonium sulfate from Sigma-aldrich were used.

Plant material and termites

Plant material was collected in July 2014 from Jaipur city of Rajasthan, India. The plant species was identified and authenticated by the Botanical Survey of India, Jodhpur, India, as *Epipremnum aureum* (Linden and Andre) Bunting. Collected plant material was washed, air dried and used for further studies. Collection of termites was done from the fields of Banasthali University and identified by the Department of Entomology, Indian Agricultural Research Institute, New Delhi as *Odontotermes obesus*.

Isolation of alkaloid

A powdered plant material (50 g) was treated with 15 mL of 25% NH₄OH at room temperature and solvent extraction was performed with 300 mL of ethyl acetate for 72 h. The extract was filtered and air dried at 40°C. The residue was dissolved in distilled water and acidified with H₂SO₄ to pH 3-4. It was extracted with petroleum ether and diethyl ether and adjusted the pH of aqueous phase to 9-10 with 25% NH₄OH. It was extracted with chloroform and washed with distilled water. Crude alkaloids was concentrated to dryness under reduced pressure and dried over sodium sulfate⁸.

Purification of alkaloid

Purification of the crude alkaloids extract was done using silica gel column chromatography. Silica gel (mesh size 60-120) was used for column packing and mixtures of chloroform and methanol were used as mobile phase. Fractions of 2%, 5%, 7%, 10%, 40%, 60% and 100% of methanol in chloroform were collected and checked for their antitermite activity. Bioactive fractions were combined and concentrated. It was again purified by silica gel column chromatography and determined their antitermite activity.

Antitermitic assay

Direct choice assay was used to determine the antitermite activity of alkaloids extract. In brief, circular Whatman filter papers were placed in the bottom of petridish and treated with 1 ml of 40 times diluted alkaloid fractions. After proper absorption of alkaloid fraction, population of 20 termite workers (*Odontotermes obesus*) were introduced in each petridish and covered with black paper. Mortality of termites was observed after 15 minutes and dead termites in each petriplates were sorted out using forcep. Solvent in the same dilution was used as control^{2, 9}.

Statistical analysis

All the experiments were repeated twice in triplicates and data is presented as mean \pm standard error.

Results and discussion

In the present study, isolation and purification of alkaloids from various parts of *Epipremnum aureum* was carried out and tested for their antitermite activity against *Odontotermes obesus*. Purified fractions of alkaloids isolated from *E. aureum* possess high antitermite potential. Orientation and movement of termites were found to be significantly suppressed in presence of forty times dilution of purified alkaloids fraction, with high mortality rate in 15 minutes. The leaf and aerial roots of *E. aureum* possess insecticidal and insect repellent components and these protect plants from insect damage. Leaf fractions showed 40% to 50% mortality of termites with 5% fraction showing the highest mortality of termites. Root fractions showed average 35% to 45% termite mortality rate. Stem fractions were less active and showed 25% to 35% termite mortality rate (Fig. 1). Purified fractions of alkaloids of different explants of *E. aureum* obtained through second silica gel column chromatography had higher percentage of termite mortality rate. Leaf fractions showed 50% to 70 % termite mortality rate and root fractions had 45% to 55% mortality of termites. Stem fractions were least active with an average 25% mortality of termites (Fig. 2).

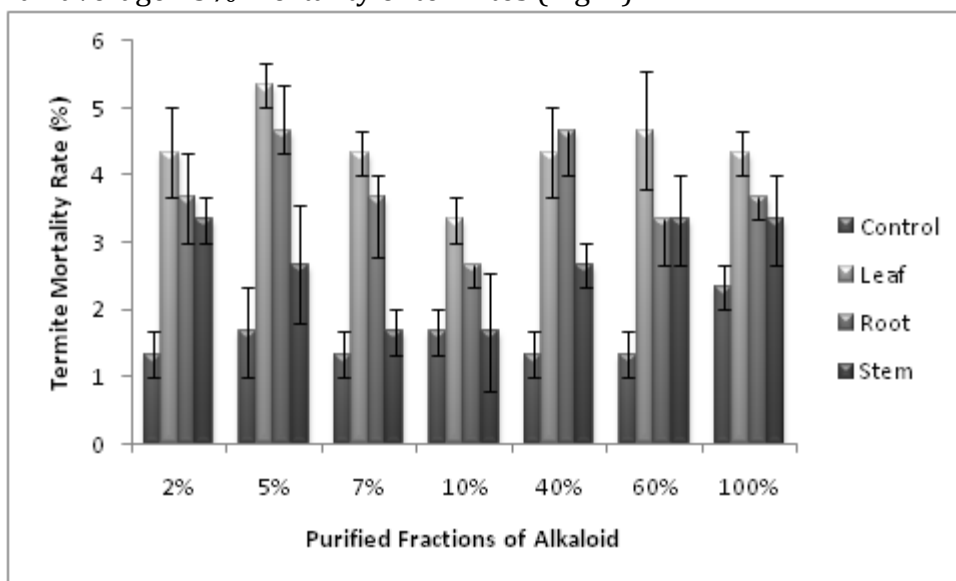


Fig. 1 Percentage termite mortality rate (Mean \pm SE) of purified alkaloid fractions of *Epipremnum aureum* explants obtained through first silica gel column chromatography

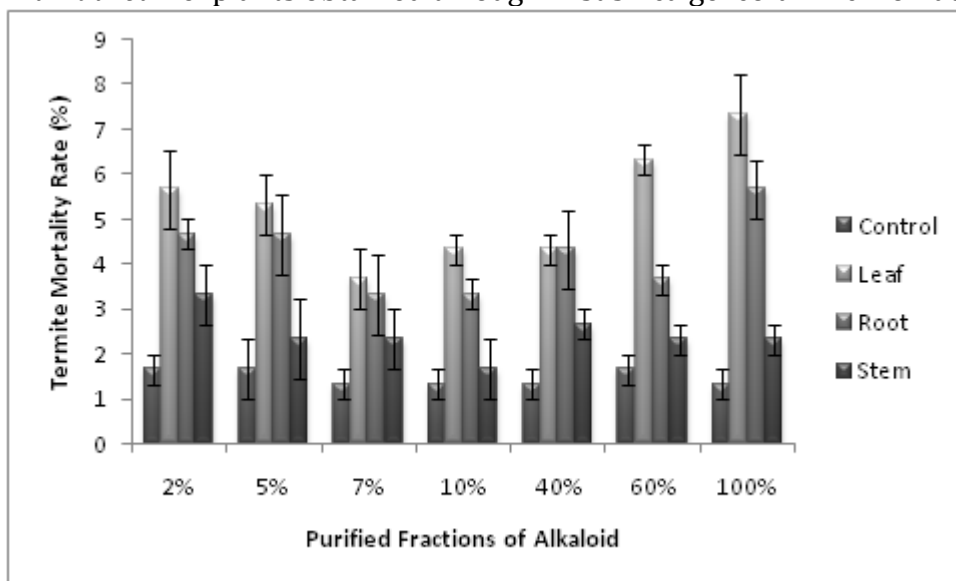


Fig. 2 Percentage termite mortality rate (Mean \pm SE) of purified alkaloid fractions of *Epipremnum aureum* explants obtained through second silica gel column chromatography

The alkaloids extract when applied to the insects bring about disturbance in the nervous system and cause death¹⁰. Earlier studies have shown termites mortality from ninety six to hundred percent using aerial root extract in ethanol after 5 minutes². Similar toxic and repellent activity of plant products have been reported¹¹. In a filter paper-based bioassay for termiticide, guineesine, a minor constituent isolated from *Piper nigrum*, has shown >90% mortality in termites at 1% wt/wt application. Moreover, root extracts of *Diospyros sylvatica* impose significant repellent activity and cause high mortality in subterranean termite, *Odontotermes obesus* in filter paper disc bioassay due to presence of plumbagin, isodiospyrin, and microphyllone or quinones¹². No doubt, *E. aureum* possesses enough antitermite potential to control Indian white termite, *O. obesus* population. In another study, Ahmad et al. (2007) showed that the seed extracts of *Withania somnifera*, *C. tiglium* and *Hygrophila auriculata* has potential as antitermitic agent. However, it can be concluded leaf extracts of *E. aureum* offer a source of naturally occurring chemicals that could be used as termite controlling agents and this activity is accredited with the presence of phytochemicals of diverse structure that had repellent or toxic effects on termite¹³. *E. aureum* active components can be used for controlling the damage and termite infestation if used as spray, fumigant or in form of poison baits. Hence, strong recommendations are being made to develop eco-friendly antitermite formulation from *E. aureum* plant for effective control of field termites.

Conclusion

In the current time, termite's threat is a serious problem in various regions of Rajasthan district. Indian white termite is an outrageous insect pest which seriously infests forest products and agricultural crops. Present study revealed the presence high alkaloids in *Epipremnum aureum* with remarkable antitermitic activity. The plant leaf extracts have the potential to develop new and eco-friendly products against *Odontotermes obesus*, a naturally occurring termite especially in the campus of Banasthali University, Rajasthan. These plant derived natural products could serve as a valuable alternative for synthetic chemicals. *E. aureum* is easy to grow and maintain as it is hydroponic, easily available, accessible and affordable. Therefore, the usage of *E. aureum* should be promoted. Screening results suggest that aerial roots and stem have promising potential in termites control with highest activity in leaves. This plant may serve as sustainable antitermite agent and its extract could be exploited to develop new wood preservatives to protect wooden structures, agricultural crops, plants and trees. The result of the present study supports the usage of *E. aureum* compounds with toxic activity against termites. Broad study need to be carried out to further explore the economic importance of pothos for mankind and the environment.

Conflict of interests

The authors declare that they have no conflict of interests.

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