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A Comparative Study of Insecticidal Activity of Leaf Extract of *Zanthoxylum armatum* DC (Rutaceae) Prepared in Polar and Non-Polar Solvents against Termites

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ABSTRACT

Plants extracts are rich in phytochemicals and have diverse properties. *Odontotermes obesus* (Isoptera: Odontotermitidae), the white Indian termite, is a highly destructive polyphagous insect pest cause a great threat to plants, agricultural fields, and structures of economic importance At present time, most of the control measures to control termites rely mainly on synthetic chemicals which are harmful to both the environment and human health. Plants extracts have gained significant attention for their insecticidal properties, offering an eco-friendly alternative to synthetic chemicals. Thus, present research aims to study the insecticidal activity of leaf extracts of *Zanthoxylum armatum* DC. A comparative study has been done on the efficacy of leaf extracts prepared in polar and non-polar solvents by contact toxicity test. Solvents used in the preparation of extracts play a crucial role in the extraction of bioactive compounds and also effect the yield, efficiency of plants extracts. In line with this, present work depicts the comparative account of solvents used in preparation of extracts. Results of the present work indicated that leaf extract of *Zanthoxylum armatum* DC prepared in the polar solvent is most effective as it shows the highest mortality and lowest LC_{50} value in comparison to non-polar solvent.

Keywords: *Zanthoxylum armatum*; Insecticidal; Termites; Mortality; Dose treatment

NOMENCLATURE

LC50	: Lethal concentration 50
SPSS	: Statistical package for social sciences
R^2	: Coefficient of determination
SEM	: Standard error of mean

1. INTRODUCTION

Zanthoxylum armatum DC, commonly known as Timur in Nepal and winged prickly ash in English, belongs to the Rutaceae family. Other vernacular names include Timar, Timbar, and Tenjmal. This small tree is characterised by a strong pungent and aromatic smell and is widely distributed in the hot valleys of the sub-tropical Himalayas. Traditionally, various parts of this plant, including leaves, seeds, thorns, and stems, have been used in ethnobotanical practices for their medicinal properties. It has been employed as an appetizer, carminative, antipyretic, and for the treatment of dyspepsia, among other ailments[1-2]. Additionally, its branches are commonly used as a remedy for toothache, leading to its nickname, the toothache plant.

Termites are among the most destructive insect pests, causing significant damage to agriculture, forestry, wooden structures, and commercial products containing cellulose, such as paper and fibers. The annual economic loss due to termite infestation is estimated at approximately $30 billion[3]. These social insects live in colonies comprising a queen, king, soldiers, and workers, exhibiting highly coordinated behavior. In tropical regions, termites constitute around 10 % of total biomass. There are four primary types of termites worldwide: drywood termites, subterranean termites (most destructive, responsible for 95 % of global damage), dampwood termites, powderpost termites. Subterranean termites are particularly damaging as they build underground tunnels and mounds that can reach up to five meters in height. High relative humidity accelerates termite population growth, posing a significant threat to crops, households, and forests alike.

Currently, termite management relies primarily on synthetic pesticides, including organochlorines and organophosphates, which exhibit high mortality rates and distinct modes of action[4]. However, their prolonged use has several drawbacks such as environmental toxicity, human health risk, resistance to synthetic chemicals. These chemicals infiltrate soil up to 20-30 cm deep, harming soil microbiota[5]. Pesticide residues pose significant health hazards. Over time, termites develop resistance to synthetic chemicals, reducing efficacy of insecticides. Botanical insecticides, such as plant extracts, present an eco-friendly alternative as they are biodegradable and generally non-toxic to warm-blooded animals. *Zanthoxylum*

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armatum exhibits notable insecticidal properties, including repellency and larvicidal effects. Studies have reported its effectiveness against pests such as *Spodoptera litura* and *Plutella xylostella*[6]. The plant's phytochemical analysis has revealed the presence of secondary metabolites like alkaloids, lignans, flavonoids, terpenoids, and phenols, which contribute to its various bioactive properties, including antiviral, hepatoprotective, and insecticidal effects[7]. Specifically, the leaf extract of *Zanthoxylum armatum* prepared in n-hexane has demonstrated larvicidal activity against *Plutella xylostella*[8]. In another study, ethanolic, chloroform, and n-hexane extracts of *Zanthoxylum armatum* were effective against grain-feeding pests such as those found in rice, wheat, and pulses[9]. Additionally, extracts and essential oils from other *Zanthoxylum* species have shown significant bioactivity against *Pieris brassicae* (cabbage butterfly), *Plutella xylostella*, and mosquitoes[10-16]. Several natural compounds, such as linalool and furanoid linalool oxide found in *Lindera umbellata var. membranacea*, have shown promising antitermitic activity[17]. Similarly, *Mentha spicata* extract has exhibited termite repellency when used as a wood coating[18]. Furthermore, extracts from Gabonese forest species have demonstrated strong antitermitic properties when prepared using ethanol, dichloromethane, and acetone[19].

The search for natural, eco-friendly alternatives to synthetic pesticides is crucial to sustainable pest management. *Zanthoxylum armatum* possesses significant insecticidal properties, but its potential as an antitermitic agent remains underexplored. Conducting detailed research on its bioactive compounds and efficacy against termites could pave the way for developing natural, environmentally safe termite control solutions. Despite the well-documented insecticidal properties of *Zanthoxylum armatum*, studies on its antitermitic potential are limited. Due to environmental and health concerns associated with synthetic pesticides, investigating the efficacy of *Zanthoxylum armatum* extracts prepared in polar and non-polar solvents against termites is of significant importance.

Thus present study aims to compare the insecticidal activities of *Zanthoxylum armatum* leaf extracts obtained using different solvents as solvent type significantly influences the extraction efficiency and the profile of secondary metabolites.

2. MATERIALS AND METHODS

2.1 Preparation of Plant Extracts

The collection of plants was done from the district Solan of Himachal Pradesh. The plant was identified by comparing it with a specimen present in Panjab University Chandigarh, herbarium (PAN). Voucher no. of the plant is 22849. Plants were chopped into smaller parts, washed thoroughly, and shade-dried for almost four weeks. These were ground into a grinder and were sieved through a sieve of 0.25 mm-sized pores to obtain refined dust particles. Plants powder was stored in separate bottles with screw caps at room temperature. Plant extracts were prepared in methanol and n-hexane by maceration method. 20g of plant powder was dissolved in 50 ml of each solvent. (% yield was 30 % in methanolic extract while 14 % in hexane extract).

2.2 Collection of Test Organisms

The population of termites was collected from the termitarium in the field. A mound of termites was dug up using a shovel. Soil containing termites was put on plastic sheets. After that by using a camel brush termites were collected and placed in plastic containers. Dry wood and smaller parts of leaves were put in containers to provide them with food. Muslin cloth was used to cover the container to allow free flow of air.

2.3 Bioassay Procedure

1 mg/ml stock of each solvent was prepared. From the stock solution different conc. such as 200, 400 600, 800, and 1000 ppm were prepared using distilled water. At conc. of 0.05 % polysorbate 80 was used as an emulsifier in the final test solution[20] (with slight modification).

2.4 Testing Procedure

Toxicity was checked by a contact toxicity test. The experiment was repeated in triplicates each conc. treatment contains 20 termites**.** The procedure of which is given below:

2.4.1 Flowchart Depicting Methodology of the Experiment

Preparation of plant extracts in methanol and hexane solvents

↓

Loading of different serial concentrations of plant extracts on Whatmann filter paper

↓

Air drying of filter papers to remove the solvent and placing them in different Petri dishes

↓

Mortality check at 24hr and 48hr interval of time

2.4.2 Mortality %: Formula to Calculate Mortality % are Given Below

MORTALITY% = (NO. OF DEAD TERMITES ÷ TOTAL NO. OF TERMITES) × 100

2.5 Statistical Analysis

The average termite mortality data were calculated by using probit analysis for finding LC_{50} by Microsoft Office Excel and One Way ANOVA and Duncan's Multiple Range test were done by SPSS 2017 software. Results that have $p < 0.05$ were given due consideration as significant statistically.

3. RESULTS

In this study, *Zanthoxylum armatum* was evaluated for its activity against termites. *Zanthoxylum armatum* extracts in both polar and non-polar were tested against termites. The polar solvent taken for testing is methanol and the non-polar is hexane. A comparative analysis of both solvents is done. Significant differences were observed between methanolic and hexane extracts. All test materials were toxic to termites. The highest mortality values of methanolic extracts were 60±10 % and 68±10 % for 1000 ppm conc. after 24 hrs and 48 hrs respectively (Table 1). In the case of n-hexane extracts, these were 40±10 % and 45±10 % for 1000 ppm after 24 hrs and 48 hrs respectively (Table 1). The lowest LC50 value for methanolic extract was 73.451 for 24 hrs of dose treatment and hexane was 139.315 for 48 hrs of dose treatment (Table 2). Graphs (Graph 1,2,3,4) are regression curves. Graphs depicting R^2 values measure goodness of fit of the experiment. Goodness of fit indicates how well the experiment's predictions match the actual data points. Values closer to one marks the better goodness of fit.

Table 1. Table showing different parameters of *Zanthoxylum armatum* DC methanol and hexane leaf extracts

	Methanol Extract				Hexane Extract			
Dosage (ppm)	Mortality% 24 hrs	Probit analysis	Morality% 48 hrs	Probit analysis	Morality% 24 hrs	Probit analysis	Morality% 48 hrs	Probit analysis
200	28±6.00[b]	4.42	29±5.29[b]	4.45	0	0	15±3.46[a]	3.96
400	34±10.14[b]	4.59	37±5.29[bc]	4.67	17±2.64[b]	4.05	18±2.00[a]	4.08
600	54±13.52[c]	4.80	45±7.00[cd]	4.87	25±4.00[c]	4.33	28±3.60[b]	4.42
800	52±6.55[c]	5.05	54.900[d]	5.10	30±7.00[c]	4.48	31±2.64[b]	4.50
1000	60±5.56[c]	5.25	68±10.00[e]	5.47	40.5.56[d]	4.75	45±2.64[c]	4.87

Values are expressed as mean±SEM ; values having superscripts within columns are significantly different ($p < 0.05$) by Duncan's multiple range test

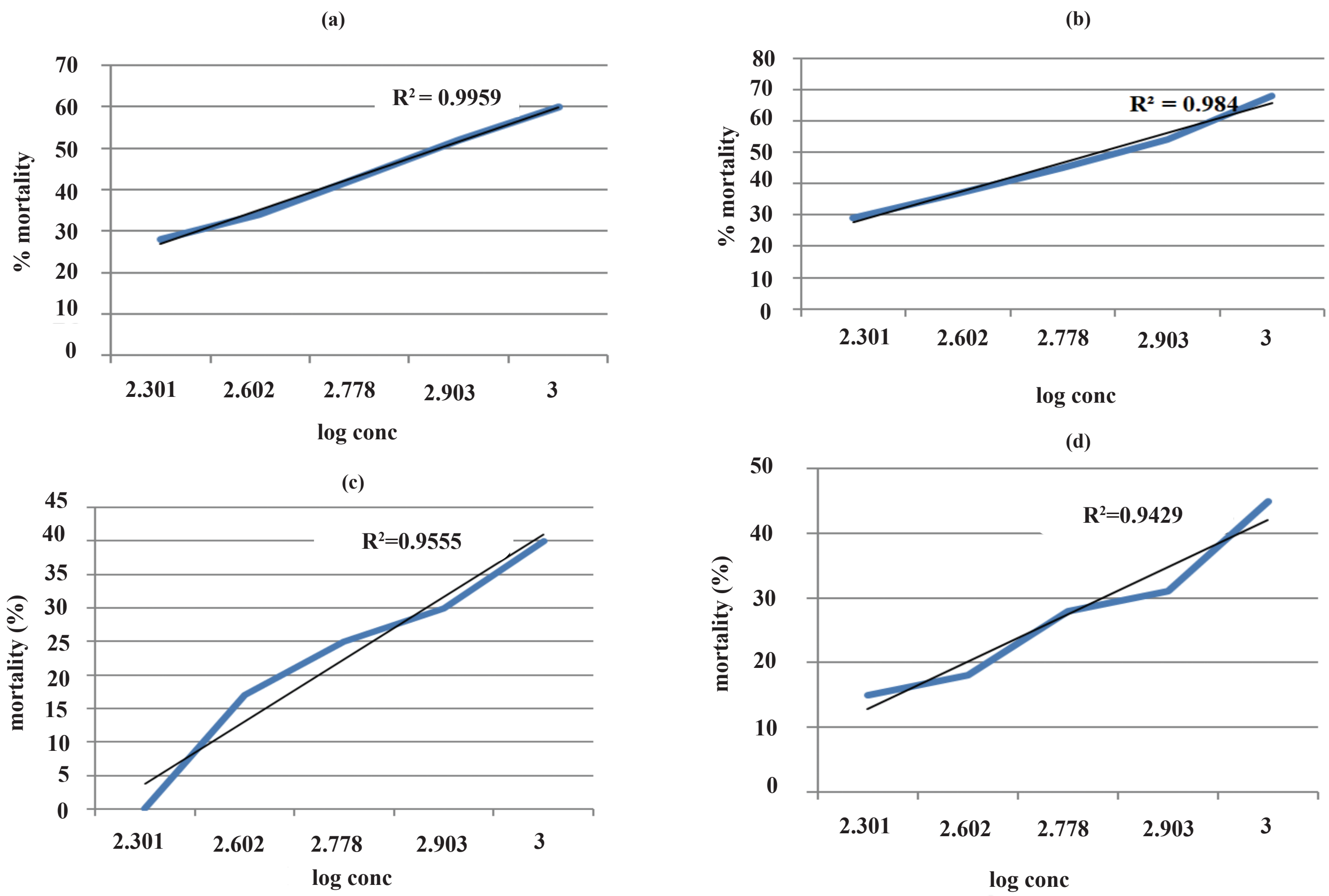

Figure 1. (a) Graphs showing regression curve for methanolic extract of *zanthoxylum armatum* (24hrs) (b) Graph showing regression curve for methanolic extract of *zanthoxylum armatum* (48 hrs (c) Graph showing regression curve for hexane extract of *zanthoxylum armatum* (24hrs) (d) Graph showing regression curve for hexane extract of *zanthoxylum armatum* (48 hrs).

Table 2. Table showing normal slope and LC_{50} values of extracts.

Plant extracts	Assay time (hr)	Slope	LC_{50}
Methanolic extract	24	1.866	73.451
Methanolic extract	48	1.912	81.658
Hexane extract	24	2.395	248.313
Hexane extract	48	2.145	139.315

4. DISCUSSION

The detrimental impact of synthetic insecticides on agroecological systems has driven extensive research into botanical alternatives. Plant-derived secondary metabolites have shown significant potential for insect pest control, particularly in mitigating termite damage, which results in an estimated 15-25 % loss in maize yield and financial losses of approximately 1478 million rupees in India[21]. Various studies highlight the efficacy of plant essential oils and extracts against termites. For instance, vetiver grass oil contains nootkatone, a potent termiticide and repellent that disrupts termite feeding for up to 12 months[22-23]. Potting media, including wood, soil, and mulch, can be infused with nootkatone and vetiver oil to curb Formosan termite populations[24].

Several plant compounds, including alkaloids, flavonoids, and monoterpenoids, have demonstrated strong antifeedant activity[25-26]. Additionally, members of the Rutaceae family, such as *Zizyphus jujuba*, show notable antitermitic activity, with crude methanolic extracts effectively targeting *Heterotermes indicola*[27]. Species of *Flourensia* possess multiple bioactive compounds including chromenes, coumarins, benzofurans, and flavonoids exhibiting insecticidal, antifeedant, and antibacterial properties[28]. Essential oils from *Origanum vulgare*, *Lavandula latifolia*, and *Syzygium aromaticum* also show promising anti-termite activity, comparable to bio-insecticides such as *Bacillus thuringiensis*[29]. Extracts of *Zanthoxylum armatum*, particularly 2-undecanone and 2-tridecanone, demonstrate strong insecticidal activity against diamondback moths[8] and significant termiticidal effects against *Globitermes sulphureus* and *Coptotermes gestroi*[30]. Similarly, its chloroform and n-hexane extracts cause approximately 90% mortality in *Rhyzopertha dominicia* within 36 hours[9]. Anti-termite efficacy of various plants' essential oils of the family Rutaceae was tested and the essential oil of *Zanthoxylum armatum* was found to have good insecticidal and toxicological properties[3].

Present comparative studies on extraction solvents reveal that methanol extracts of *Zanthoxylum armatum* exhibit higher toxicity than hexane extracts, with mortality rates of 60±10 % and 68±10 % at 1000 ppm concentrations after 24 and 48 hours, respectively. Lower LC50 values for methanol extracts (73.451 for 24-hour exposure) indicate greater toxicity than hexane extracts (139.315 for 48-hour exposure), reinforcing the efficiency of polar solvents in extracting bioactive compounds. Similar findings were observed in *Decalepis hamiltonii*, where methanolic root extracts displayed high toxicity against *Sitophilus oryzae*[31]. Research suggests that polar solvents enhance the extraction of diverse secondary metabolites such as flavonoids, alkaloids, saponins, tannins, and terpenoids, leading to improved insecticidal efficacy[32]. The crude organic extracts of *Flourensia cernua* in polar and non-polar solvents show potential antitermitic activities[33]. These findings emphasize the potential of botanical insecticides as sustainable alternatives to chemical pesticides in termite management.

5. CONCLUSION

The present comparative experimental studies on extraction solvents indicate that methanolic extracts exhibit higher toxicity against termites than hexane extracts, suggesting that polar solvents more effectively extract bioactive secondary metabolites. The harmful environmental and health effects of synthetic insecticides necessitate sustainable alternatives, with botanical insecticides emerging as a promising solution. The Rutaceae family is well-documented for its antimicrobial and insecticidal properties. In particular, *Zanthoxylum armatum* has demonstrated significant anti-termite activity due to its bioactive compounds, including 2-undecanone, 2-tridecanone etc[34]. Because of the environmental and health hazards associated with synthetic insecticides, botanical alternatives such as *Z. armatum* could serve as eco-friendly and sustainable substitutes for termite control if an industrial-scale production protocol is developed.

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