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Anti-termite activity of certain plants against *Odontotermes obesus*

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ABSTRACT

Termites are found throughout the world and are a perennial economical issue. Some plants are bestowed with anti-termite compounds which prevent termite infestation. In our study, we examined the anti-termite potential of four plant species namely, *Achyranthes asapera*, *Sida acuta*, *Syzygium cumini* and *Terminalia arjuna* against *Odontotermes obesus*. Acetone, chloroform, n-hexane, methanol and distilled water extracts were prepared, their qualitative and quantitative phytochemical constitution was determined. Additional qualitative assays were conducted on the extracts for diterpenes, oils, phytosterols and terpenoids. Methanol extracts were found to be more diverse in phytoconstituents followed by chloroform and acetone extracts. Alkaloids, flavonoids, saponins, total phenols and tannins were found in higher quantities in *S. cumin*i and *T. arjuna* as compared to *A. aspera* and *S. acuta*. The anti-termite potential of each extract revealed that among the plants examined, *T. arjuna* stem methanolic extract exhibited the highest termiticidal potential (74.67%) followed by *S. cumini* leaf and stem methanolic extracts(70% ,67% respectively). Aqueous extract of *A. Aspera* was found effective against termites and exhibited 56% mortality over a period of 48hrs.

Key words: Termiticidal, *Odontotermes obesus, Terminalia arjuna, Syzygium cumini, Achyranthes asapera*, *Sida acuta*

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INTRODUCTION

Termites (*Isoptera*) are cellulophagous and eusocial insects found throughout the world. Termites damage standing crops, structural materials and even electric cables causing huge financial losses every year. 2800 termite species have been reported world-wide of which 185 species are known as pests (Verma *et al.*, 2009). In India 300 species of termite have been recorded and pestilence (in ecosystems) is caused by 35 species (Verma *et al.*, 2009) and about 15 to 25% maize crop loss has been reported due to termite infestation resulting in a 2.46-billion-rupee economic loss. Besides infesting wild plant species, termites cause major damage to various crops such as – wheat, sugar cane, paddy, cotton, ground nut, maize and soybean (Sileshi *et al.*, 2005). However, it has been found that certain plants have evolved specific defence mechanisms whereby the secondary metabolic pathways yield certain metabolites that could be toxic to parasites or function as repellents or deterrents to pest invasion. Several decades of usage of synthetic pesticides has not succeeded in curbing the termite infestation but has resulted in pesticide related toxicities in ecosystems. Discovery of plant defence systems and phytochemicals generated a new vista in the fields of medicine and pestology (Nerio *et al.*, 2010; War *et al.*, 2012). It is generally accepted that replacing the recalcitrant synthetic pesticides with biodegradable compounds that originate in living systems can prevent the adverse effects of synthetic pesticides on environment. Due to high degree of diversity in plant kingdom, plants provide a variety of phytometabolites, some of which can be used as pesticides/insecticides to curb the resistance of pests and ease the environmental burdens (Güçlü-Üstündağ and Mazza, 2007; Sahay *et al.*, 2014). The work reported here pertains to plant survey in our study area (Bakrol-Vallabh Vidyanagar, Gujarat, India) with reference to termite infestation. A total of 143 plants were recorded in the survey areas of which 87 plants were found to be uninfected while 56 plants were infected. For the present work, four plants were chosen from the uninfected plants and these are - *Achyranthes aspera*, *Sida acuta*, *Syzygium cumini* and *Terminalia arjuna* . These plants have been reported for their antibacterial, antimalarial and larvidcidal properties (Karou *et al.*, 2003; Bagavan *et al.*, 2008; Zahran *et al.*, 2017). The antitermite potential of these plants was investigated under laboratory conditions.

MATERIALS AND METHODS

Four plant species, *A. asapera*, *S. acuta*, *S. cumini* and *T. arjuna* were chosen due to their wide ranging distribution in the study areas encompassing Vallabh Vidyanagar and Bakrol, Anand district, Gujarat for examining their anti-termite potential against *Odontotermes obesus*.

Preparation of Extracts

Leaves and stem portions of *A. aspera*, *S. acuta, S. cumini* and *T. arjuna* were collected, washed and cut into small pieces and then extracted in five solvents - acetone (A), chloroform (C), n-Hexane (H), methanol (M) and distilled water (W). Solvents were selected on the basis of their polarity indices. 100gm freshly crushed plant material was soaked in 200ml solvent and shaken for 72 hrs. Extracts were obtained after centrifugation at 4000 rpm, filtered, evaporated and stored at 4ºC.

Qualitative Phytochemical Analysis

Presence or absence of alkaloids, carbohydrates, diterpenes, flavonoids, oil, phenols, proteins, saponins, phytosterols, tannins and terpenoids were determined using methods described by Tiwari *et al.* (2011).

Quantitative Phytochemicals Analysis

Total flavonoid content was estimated by the aluminum chloride method (Zhishen, 1999). The measurement of total phenols is based on the method described by Mallick and Singh (1980). Tannins and saponins were estimated by the method described by Sadasivam and Manickam (1992) and Makkar *et al*. (2007), respectively . Total alkaloid contents were extracted from the samples following the method of Harborne *et al.* (1973).

Termites

Termite species *Odontotermes obesus* were collected from nearby farms and brought to the laboratory in a humid thermocol box and were maintained in a hygrostat chamber, specially designed. Humidity (70% ± 5%) in the chamber was maintained by placing a wet paper towel and the chamber was placed at ambient temperature (27 ºC ± 2ºC) temperature. The chamber was also maintained at minimum light conditions using a black cotton cloth.

No-choice feeding test

The anti-termite effects of the extracts were tested on termites using a 'no-choice' test. Aliquots with a concentration range of 1000 $mgmL^{-1}$ - 4000 $mgmL^{-1}$ were applied to Whatman No. 1 filter papers and allowed to air dry completely and were placed in Petri plates. These treated filter papers were given as feed and 20 workers of *Odontotermes obesus* were placed and mortality was recorded at 24 hrs and 48hrs. Filter paper treated with solvent alone was used as a control.

RESULTS AND DISCUSSION

Long before the compounds were discovered, plant extracts have been widely used for insect control. Physico-chemical nature of constituents like polarity, electron density and number of carbon atoms affect the solubility of phytochemicals, hence solvents play a pivotal role in extraction. In our experiment five different extracts were selected according to polarity index. Preliminary qualitative phytochemical analysis revealed that various compounds were present in different extracts with diverse constitution ratio. Carbohydrates, oils, polysterols and proteins were present in all extracts. Methanolic extract of each plant was found to be rich in phytochemical diversity such as diterpenes, phenols and terpenoids. Alkaloids were found in all methanol extracts except *S. acuta* stem. Methanol is oxygenated organic solvent and has 0.762 relative polarity index which could be the cause of higher diversity. Chloroform (chlorinated organic solvent and 0.259 relative polarity) and acetone (0.355 relative polarity) showed the presence of diterpens in leaf extracts only, flavonoids in leaf and stem extracts of *S. cumini* and *T. arjuna* and phenols in leaf and stem extracts. Methanol, acetone and aqueous extracts of leaf and stem of *S. cumuni* and *T. arjuna* showed the presence of tannin. Terpenoids were present in chloroform, methanol and aqueous extracts of leaf and stem of *S. cumini* and *T. arjuna*, and also in *A. aspera* and *S. acuta* stem methanol extracts. n-Hexane and aqueous extracts exhibited least diversity of phytochemicals (Table 1 & 2).

Table 1. Qualitative screening of phytoconstituents

Plant name	Part	Alkaloid					Carbohydrates					Diterpenes					Flavonoids					Oils				
		A	C	H	M	W	A	C	H	M	W	A	C	H	M	W	A	C	H	M	W	A	C	H	M	W
A. aspera	Leaf	-	-	-	+	-	+	+	+	+	+	+	+	-	+	-	+	+	+	+	-	+	+	+	+	+
	Stem	-	-	-	-	-	+	+	+	+	+	-	-	-	+	-	-	-	-	-	-	+	+	+	+	+
S. acuta	Leaf	-	-	-	+	-	+	+	+	+	+	+	+	-	+	-	+	+	+	+	-	+	+	+	+	+
	Stem	-	-	-	-	-	+	+	+	+	+	-	-	-	+	-	-	-	-	+	-	+	+	+	+	+
S. cumini	Leaf	-	-	-	+	-	+	+	+	+	+	+	+	-	+	-	+	+	+	+	-	+	+	+	+	+
	Stem	-	-	-	+	-	+	+	+	+	+	-	-	-	+	-	+	-	-	-	-	+	+	+	+	+
T. arjuna	Leaf	-	-	-	+	-	+	+	+	+	+	+	+	-	+	-	+	+	+	+	-	+	+	+	+	+
	Stem	-	-	-	+	-	+	+	+	+	+	-	-	-	+	-	+	-	+	+	-	+	+	+	+	+

Acetone (A), Chloroform(C), n-Hexane (H), Methanol (M), Aqueous (W)
(+) indicates Presence and (-) indicates Absence of Phytochemical/s

Table 2 . Qualitative screening of phytochemical

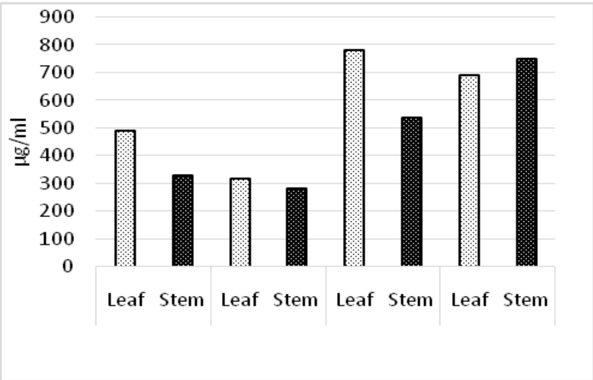
Plant name	Part	Phenols					Phytosterols					Proteins					Tannins					Terpenoids				
		A	C	H	M	W	A	C	H	M	W	A	C	H	M	W	A	C	H	M	W	A	C	H	M	W
A. aspera	Leaf	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	-	+	-	+	+
	Stem	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	+	-
S. acuta	Leaf	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	-	+	-	+	+
	Stem	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	-	+	-	-	-	-	+	-
S. cumini	Leaf	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	-	+	+
	Stem	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	-	+	+
T. arjuna	Leaf	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	-	+	+
	Stem	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	-	+	+

Secondary metabolites of plants serve many puroposes like medicinal, anti-bactarial, anti-fungal activities and as pesticides. The hexane dried leaf extracts of *Tagetes erecta* and the hexane extracts of *Flourensia cernua* were reported to possess termiticidal activity against formosan subterranean termite, *Coptotermes formosanus* and *Reticulitermes sp*. (Elango *et al.*, 2012; Tellez *et al.*, 2001). Ravikumar *et al*. (2010) also examined the hexane extracts of *Tagetes erecta* flowers and acetone extracts of *Argemone mexicana* and reported their termiticidal potencies. Significant mortality of *Helicoverpa armigera* was reported by Ramya and Jayakumararaj (2009) with aqueous extracts of *M. azedarach*, *A. indica* and *D.metel* leaves . An antitermite tertiary tricyclic alcohol, Cedrol was isolated from n-hexane: dichloromethane extracts of *Juniperous procera* (Kinyanjui *et al.* 2000). Besides, age dependant protcctive potential was assayed by Dungani *et al.* (2012) in Indonesian Teak wood (*Tectona grandin* L. f.). It was found that compared to younger wood, the elder wood exhibited a potential termiticidal properties against Holmgren sps. (*Coptotermes curvignathus*).

The phytochemical quantitative assays of various extracts (of leaf and stem samples) indicated that *S. cumini* and *T. arjuna* possess higher phytochemical content compared to *A. aspera*. *S. acuta* and *S. cumini* leaves were found to be a good source of alkaloids, flavonoids and tannins. *T. arjuna* leaf extracts contained larger amounts of saponins, phenols, carbohydrates and proteins.

The concentration of alkaloids varied from *S. cumini* leaves to S. *acuta* stem extracts (Fig. 1).

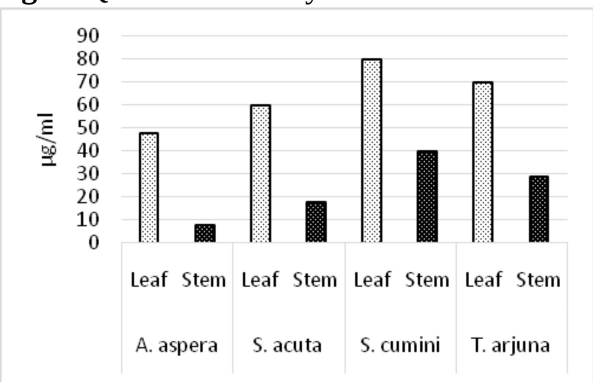
Fig. 1. Quantitative analysis of alkaloids



Amount of Alkaloids in µg per mL of extract (or per 500 mg of fresh plant material)

Flavonoid concentration was high in *S. cumini* leaf extracts (7.99 µg/mL) followed by *T. arjuna* leaf extracts, *S. acuta* leaf extracts, *S. acuta* and *A. aspera* stem extracts (Fig. 2).

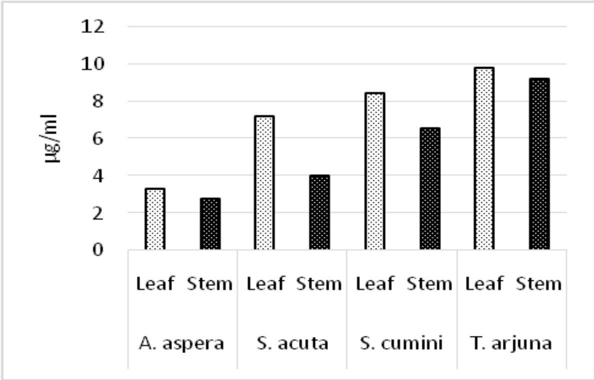
Fig. 2. Quantitative analysis of flavonoids



Amount of flavonoids in µg per mL of extract (or per 500 mg of fresh plant material)

The saponin content of stem and leaf extracts exhibited a range of 981.89µg/mL (in *T. arjuna* leaf extracts) to lowest in 276.77µg/mL (in *A. aspera* stem extracts) (Fig. 3).

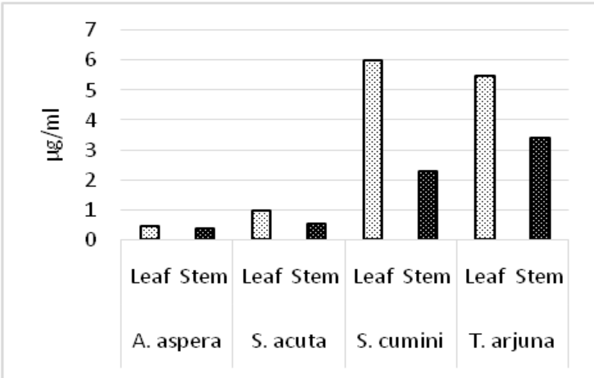
Fig. 3. Quantitative analysis of saponins



Amount of saponins in µg per mL of extract (or per 500 mg of fresh plant material)

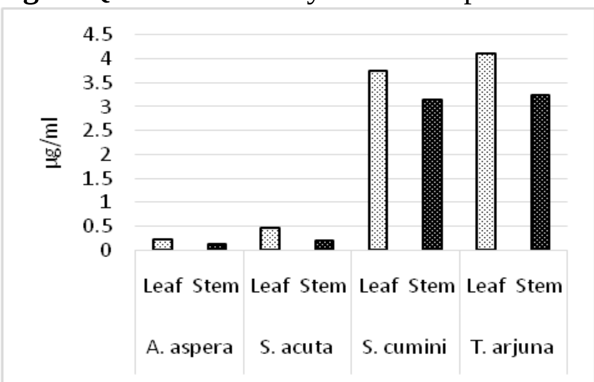
The total tannin and phenolic contents in both trees (*S. cumini* and *T. arjuna*) was higher than in both shrubs (*A. aspera* and *S. acuta*) (Figs. 4 and 5).

Fig. 4. Quantitative analysis of tannins



Amount of Tannins in µg per mL of extract (or per 500 mg of fresh plant material)

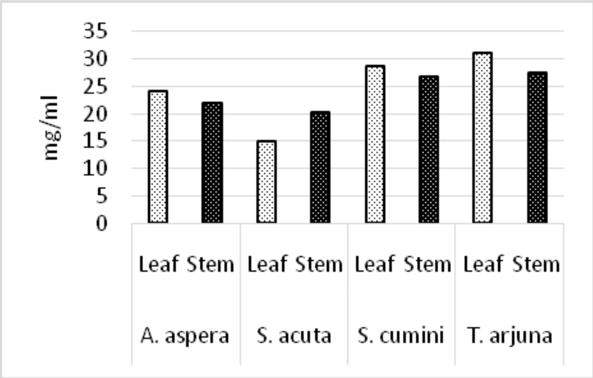
Fig. 5. Quantitative analysis of total phenols



Amount of total phenols in µg per mL of extract (or per 500 mg of fresh plant material)

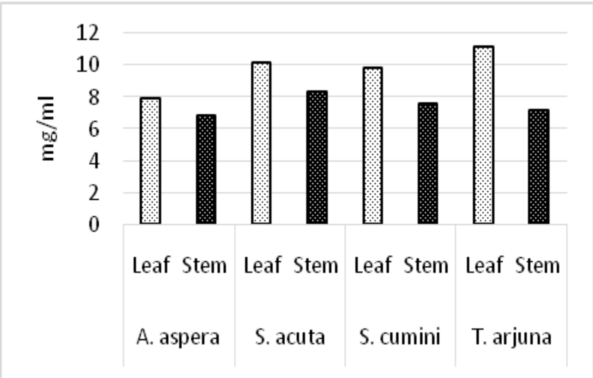
There is no considerable difference in carbohydrate and protein contents among the plants examined (Figs. 6 and 7).

Fig. 6. Quantitative analysis of carbohydrates



Amount of Carbohydrates in mg per ml of extract (or per 500 mg of fresh plant material)

Fig. 7. Quantitative analysis of protein

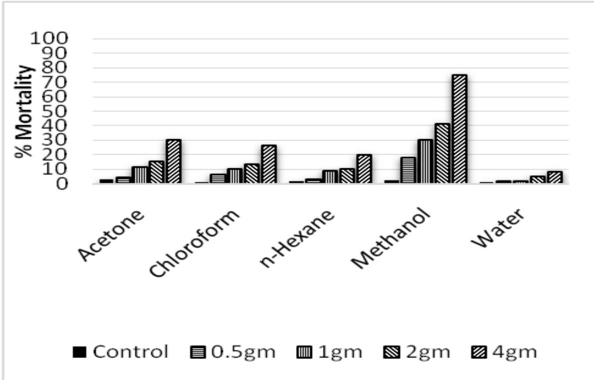


Amount of Protein in mg per mL of extract (or per 500 mg of fresh plant material) (The values are the mean of Triplicate)

Alkaloids of *Stemona collinsae* were reported for a toxic and feeding deterrent against *Spodoptera littoralis* (Brem *et al.,* 2002). In many cases flavonoid like phytochemicals function as inhibitors of moulting or reproductive hormones. Reduced fecundity in *Coptotermes formosanus* Shiraki was observed after oral feeding Bichanin A flavonoid (Boué and Raina 2003). Saponins and terpenoids have been reported for cytotoxic effects (Podolak *et al.*, 2010; Wang *et al.*, 2008). Carvon, cadinol, myrcene, limonene, phytol and pinene have been reported for generating insecticidal and cytotoxic effects (Abdelgaleil *et al.,* 2009; Thakor *et al.*, 2016).

In the present study, the phyto-constituent diversity in the leaf and stem extracts was found to be higher in methanol extracts as compared to other extracts and the methanol extracts were most effective against termites. On the basis of the results (Fig.8)

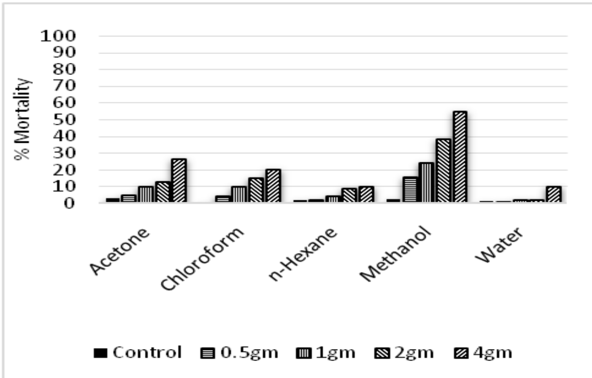
Fig. 8. Termiticidal potential of *Terminalia arjuna* stem



Mortality at various concentrations of extracts over a period of 48 hrs with comparison of control (solvent treated filter paper). % Mortality values indicated in Mean of Triplicate.

T. arjuna is found to be a good source for anti-termite compounds because methanol extracts of stem of *T. arjuna* exhibited the highest mortality and so did methanol extracts of *T. arjuna* leaves (Fig. 9).

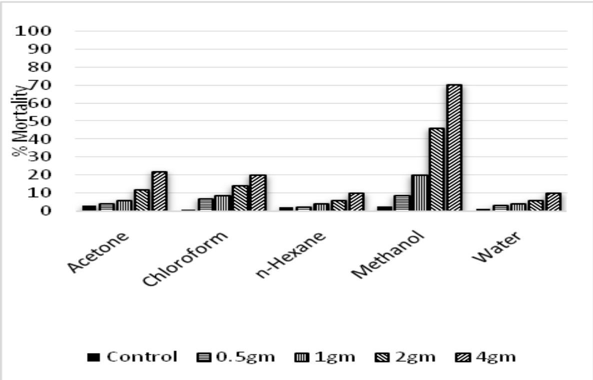
Fig. 9. Termiticidal Potential of Terminalia arjuna Leaves



The extracts of stems in acetone, chloroform and n-hexane were found more potent than the leaf extracts in the same solvents. A triterpenoid arjunolic acid, a metabolite isolated from stem and root of *T. arjuna* has been reported to be an inhibitor for fourth instar larvae of *Spilarctia oblique* (Hemrajbhai *et al.,* 2015; Amalraj and Gopi, 2017). Further, the bark extracts of *T. arjuna* were also shown to be antimicrobial to both gram positive and gram negative bacterial strains, namely *S. aureus*, *S. mutans*, *E. coli* and *K. pneumoniae* (Mandal *et al.*, 2013).

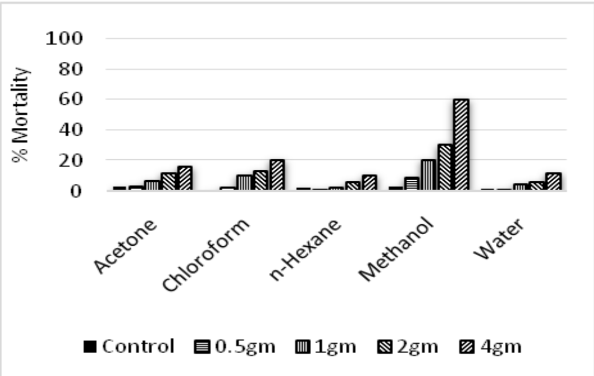
S. cumini leaf extracts were found more effective as they caused more mortality (Fig.10) than methanol stem extracts (Fig.11) did.

Fig. 10. Termiticidal potential of *Syzygium cumini* leaves



Mortality at various concentrations of extracts over a period of 48 hrs with comparison of control (solvent treated filter paper).

Fig. 11. Termiticidal potential of *Syzygium cumini* stem

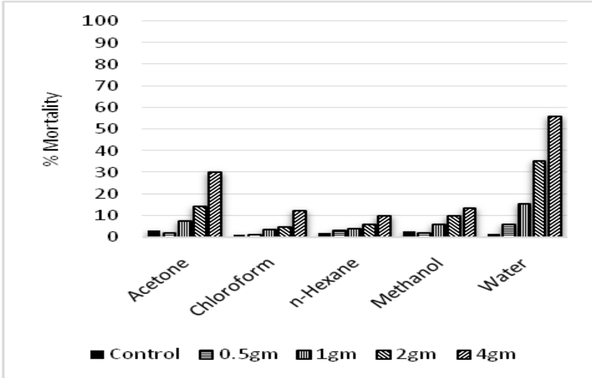


Mortality at various concentrations of extracts over a period of 48 hrs with comparison of control (solvent treated filter paper).

Rest of the extracts (of leaves and stems) though caused termite mortality, it was at a very low level of at about 20%. Antibacterial activity of *S. cumini* leaf methanolic extracts against various pathogenic bacteria has been reported by Gowri and Vasantha (2010). Other parts of *S. cumini* have also been reported for various effects. For instance, the acetone extracts and the saponins of the *S. cumini* buds are larvicidal (Murthy and Rani, 2009); Afify *et al*. (2011) reported that the fruits of this plant have acaricidal effects against *Tetranychus urticae* Koch; the methanolic fruit pulp extract has been found to be an effective antidote to an organophosphate pesticide, malathion induced cardiotoxicity (Atale *et al.*, 2014).

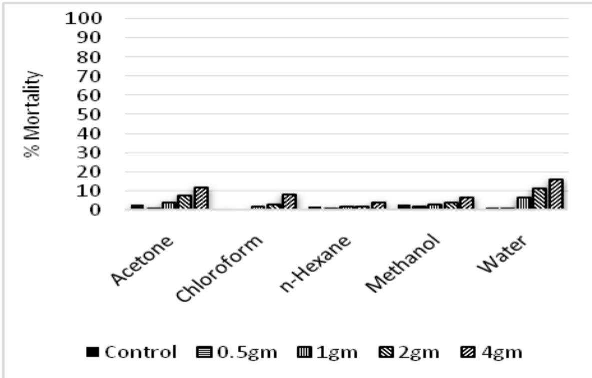
The aqueous extract of *A. aspera* leaves caused more mortality than acetone extract (Fig. 12).

Fig. 12. Termiticidal potential of *Achyranthes aspera* leaves



Mortality at various concentrations of extracts over a period of 48 hrs with comparison of control (solvent treated filter paper). However, the stem extracts of *A. aspera* did not display a similar anti-termite activity (Fig. 13).

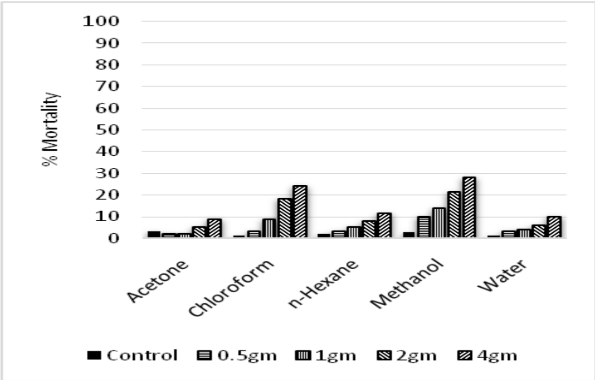
Fig. 13. Termiticidal potential of *Achyranthes aspera* Stem



Mortality at various concentrations of extracts over a period of 48 hrs with comparison of control (solvent treated filter paper).

A. aspera has been reported to be antibacterial, antiviral, insecticidal, anticancer, apoptotic with wound healing enhancing property. *A. aspera* has also been reported to possess antifeedant and insecticidal and larvicidal properties (Khandagle *et al.*, 2011; Jeyasankar *et al.*, 2014). Among the tested plants, *S. acuta* was found to be the least potent as an anti-termite plant with 24% mortality with chloroform extracts of leaves (Fig.14).

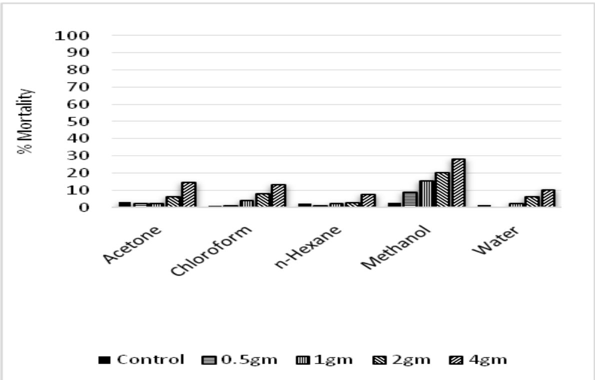
Fig. 14. Termiticidal potential of *Sida acuta* leaves



Mortality at various concentrations of extracts over a period of 48 hrs with comparison of control (solvent treated filter paper)

Among the tested plants, *S. acuta* was found to be the least potent as an anti-termite plant with 39% mortality with Methanol extracts of stem (Fig.15).

Fig. 15. Termiticidal potential of *Sida acuta* stem



Mortality at various concentrations of extracts over a period of 48 hrs with comparison of control (solvent treated filter paper).

However, the recent work on *S.acuta* indicates that this plant has antiplasmodial, insecticidal, larvidical and repellent activities (Banzouzi *et al.*, 2004; Adeniyi *et al.*, 2010; Govindarajan 2010; Wahab and Akinterinwa 2015).

The four plants selected for the present study *viz., A. asapera, S. acuta, S. cumini* and *T. arjuna* were resistant to termite attack and were found to have varying amounts of phytochemicals. The highest amounts of alkaloids, saponins, phenols, carbohydrates and proteins were found in the leaves and stem of *T. arjuna*. whereas *S.cumini* registered higher amounts of flavonoid and tannin contents. Among all the plants tested, methanol extracts of the stem of *T. arjuna* exhibited the highest potential for termite mortality followed by *S.cumini* leaf and stem methanol extracts. The aqueous and methanol leaf extracts of *A. aspera* and *T. arjuna* showed lower termiticidal effects. Considering the above, since all the selected plants have anti-termiticidal activities further work is in progress to isolate and identify the active compounds.

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