Anti-termite activity of *Sterculia foetida* L. seed extracts against Indian white termite, *Odontotermes obesus* Rambur

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***Abstract*: Phytochemical screening and anti-termite activity of the different solvent extracts of the Wild Indian almond, *Sterculia foetida* L. seed was studied in the present work. The seed powder of *S. foetida* was extracted by ethyl acetate, 98% methanol and water. All the extracts were subjected to phytochemical screening as per standard protocol and the results revealed that the presence of important secondary metabolites such as carbohydrate, amino acid, fats and oils, saponins, alkaloids, flavanoids and terpenoids. Later antibacterial activity of the all extracts were tested against two bacterial strains *Styphylococcus aureus* (G+ve) and *Proteus mirabillis* (G-ve) and the results revealed that the 98% methanolic extract was potent against both bacterial species at 30µg concentration. All extracts were subjected to anti-termite bioassay through Whatman filter paper method at different time duration. The results revealed that the 98% methanolic seed extract cause higher percentage of mortality compare than other extracts tested at 48 hours. Thus this study could help to develop potent plant based pesticide to control the insect pest like termites.**

***Keywords*: *Sterculia foetida,* seed extracts, phytoconstituents, antibacterial activity, antitermite activity.**

Introduction

The Indian white termite, *Odontotermes obesus* Rambur is extremely destructive polyphagous pest (Ravi Kant Upadhyay *et al*., 2012) which largely damage house hold wooden materials, papers, agricultural crops like sugarcane, barley and paddy. Termite colonies also heavily attack fodder crops and make tunnels in subtropical and tropical soil. For termite control within the sector various synthetic chemicals like cyclodiene (Sim *et al*., 1998), cypermethrin (Valles & Woodson 2002), hydroquinone and indoxocarb (Hu 2005) are used. Synthetic chemicals are extensively applied for controlling the termite population (Venkatesan *et al*., 2009) but in other hand it cause serious deleterious impacts on non-targeted biotic and abiotic factors of environment (Pimentel *et al*., 1992). The residues of the synthetic chemicals may persist in the environment for long time. Hence, their new alternatives should be discovered in kind of natural or plant origin insecticides. Thus, it is necessary to specialize in plant-based insecticides to manage the termites. These plant-origin pesticides are found much safer and easily biodegradable within the soil as compared to synthetic chemical insecticides. Few plant species like *Pseudotusuga menziesii*, *Lysitoma seemnii*, *Tabebina guaycan*, *Diospyros sylvatica*, *Curcuma aromatic* and *Euphorbia kansuii* (Ganapaty *et al*., 2004), are explored for their anti-feedant and insecticidal activities. Efficacy of various plants and their extracts are examined in in vitro and field conditions but still there is a requirement to develop suitable efficacious botanical based formulations to tackle the losses caused by termites (Sahay *et al*., 2014). *Sterculia foetida* is a large, straight, deciduous tropical plant belonging to the Sterculiaceae family. The tree growing maximum up to 115 feet in height with the branches arranged in whorls. The bark is grey color and smooth in nature. It is commonly called as Bastard poon tree, Hazel sterulia, Skunk tree, Java Olive, Sam-rong and Poon tree. In India it is called as Gorapu-badam in Tamil and Janglibadam in Hindi. Many researchers reported that this plant contain more secondary metabolites and chemical compounds like β-sitosterol, lupeol, n-triacontanol, stigmasterol and β-sitosterol-3-O-B-D-glucopyranoside (Desai *et al*., 1976; Shrivastava & Saxena, 1983). The presence of the chemical compound sterculic acid triglyceride in *S. foetida* seed was reported by Guerere *et al*., 1985. Pala Rajasekharreddy (2009) reported that the crude extract of *S. foetida* L. seed cause highest mortality on the common lepidopteran pests; *Achaea janata* L. and *Spodoptera litura*. In view of that present study focused to investigate the anti-termite activity of the seed of *S. foetida* L.

Materials and Methods

Collection & processing of plant sample

The dried fruits of *Sterculia foetida* were collected from the campus of Hindustan Institute of Science and Technology, Kelambakkam-603103, Tamil Nadu, India during the month of January. The collected seeds were authenticated by Prof. P. Jayaraman, Director, Institute of Herbal Science, Plant Anatomy Research Centre (PARC), Chennai-600045. The dried fruits were deseeded manually and then the seeds were shade dried for 2 weeks. The dried seed sample was pulverized into fine powder then sieved and stored in air tight container at room temperature for further analysis.

Collection of animal sample

The Indian white termite, *Odontotermes obesus* was collected from the infested logs and termitarium found in the Hindustan Institute of Science and Technology, Kelambakkam-603103, Tamil Nadu, India. The termites were collected in a jar during the month of February and fed with wood pieces and paper.

Preparation of extracts

Fifty gram of dried seed powder was subjected to consecutive organic solvent extraction by refluxing in the Soxhlet apparatus each for 24 hours. The present study, The organic solvents were selected for the extraction is based on the increasing polarity order like the order of ethyl acetate, methanol (98%) and water (Gopalasatheeskumar 2018). After 24 hours of extraction process all the extracts were concentrated by oven drying (Azhari *et al*., 2014). The dried extracts were stored at 4°C till further analysis.

Qualitative analysis of phytochemicals

Phytoconstituents from the seed of *Sterculia foetida* was extracted using three different solvents and qualitative phytochemical analyses were made using standard protocols. The different analysis such as Molisch's test & Fehling's test for carbohydrates, Ninhydrin test for amino acid, Stain test for fats and oils, Foam test for saponins, Dragendorff's test & Hager's test for alkaloids, Lead acetate test & Ferric chloride test for flavanoids and Salkowski test for terpenoids were performed (Brindha *et al*., 1977; Sofowora 1993, Harborne 1998).

Antibacterial activity

The antibacterial assay was carried out as per the method described by Murray *et al*., 1995 and Sudhama & Ramakrishnan 2019. Antibacterial activity of three solvent extracts of *Sterculia foetida* were determined by the agar well diffusion method. All extracts were tested against gram positive (*Styphylococcus aureus*) and gram negative (*Proteus mirabillis*) strains. Both bacterial strains were purchased from the Department of Microbiology, Hindustan College of Arts & Science, Padur, Kelambakkam-603103, Tamil Nadu, India. The standard drug Chloramphenicol act as a positive control (1 µg/ml concentration) and 10% DMSO act as a negative control. The antibacterial activity was assessed by measuring the diameter of zone of inhibitions formed after incubation. Triplicates were used for all test and control.

Antitermite activity bioassay

Antitermite bioassay was performed as per the methodologies proposed by the researchers Ranjith *et al*., (2017) and Krupal K. Patel & Narasimhacharya (2017). Three different solvent extracts were tested in various concentrations such as 0.5, 1, 1.5 and 2 ml were loaded in Whatman no. 1 filter paper and kept it in a separate petri dishes. The acetone treated filter paper act as a positive control and blank paper act as a negative control. The Whatman filter papers loaded with different concentrations of seed extracts were air dried and kept it in the petri plates. There are 10 number of adult worker white termites were introduced randomly on each petri plates contain Whatman filter paper impregnated with different seed extracts. Then, all the petri plates were kept it in an incubator at 25°C. After 24 and 48 hours of incubation period, the dead termites were identified and counted to determine the percentage of mortality. The percentage of mortality of test and control was calculated by using below formula:

$$\text{Mortality (\%)} = \frac{\text{Number of dead termites}}{\text{Number of initial termites in the test}} \text{ X } 100$$

FT-IR analysis

Fourier Transform Infrared Spectrophotometer (FT-IR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in chemical compounds. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. The present study KBr encapsulated sample pellet was loaded in FT-IR spectroscope (Shimadzu, IR Affinity 1, Japan), and scan the range from 400 to 4000cm^{-1} with a resolution of 4 cm^{-1} (Saranya *et al*., 2015).

Statistical analysis

All the experiment was conducted in triplicates. The statistical analysis of the data was expressed as mean values ± standard deviation.

Results

Qualitative analysis of phytochemicals

The preliminary phytochemical analysis was performed with seed extracts of *S. foetida* prepared by using ethyl acetate, methanol (98%) and aqueous solvents. The phytochemical screening results were shown in the Table 1.

Table 1: Phyto-constituents present in the seed extracts of *Sterculia foetida*

S. No.	Phyto-constituents	Solvents		
		Ethyl acetate	Methanol (98%)	Aqueous
1.	Carbohydrate			
	Molisch's test	+	+	+
	Fehling's test	+	+	+
2.	Amino acid			
	Ninhydrin test	+	+	+
3.	Fats and oils			
	Stain test	+	+	+

4.	Saponins			
	Foam test	+	+	+
5.	Alkaloids			
	Dragendorff's test	+	+	+
	Hager's test	+	+	+
6.	Flavanoids			
	Lead acetate test	+	+	+
	Ferric chloride test	+	+	+
7.	Terpenoids			
	Salkowski test	+	+	+

+ = Presence

Antibacterial activity

Table 2 shows the efficacy of three extracts of Sterculia foetida seed sample against the two pathogenic bacterial species. The results revealed that 98% methanolic extract showed most potent antibacterial activity against the *P. mirabillis* (14 ± 0.47) and *S. aureus* (13 ± 0.47) at 30 mg concentrations. The positive control exhibit the highest zone of inhibition diameter (18 ± 0.47) whereas there was no zone recorded in negative control.

Table 2: Antibacterial activity of *S. foetida* seed extract against *S. aureus* and *P. mirabillis*

Extracts	Conc. (µg)	Bacterial species		Control (+ve)	Control (-ve)
		S. aureus	*P. mirabillis*		
Ethyl acetate	10	6 ± 0.81	7 ± 0.81	10 ± 0.47	Nil
	20	9 ± 0.47	8 ± 0.81	14 ± 0.47	
	30	12 ± 0.47	11 ± 0.81	17 ± 0.47	
Methanol (98%)	10	9 ± 0.47	10 ± 0.81	9 ± 0.81	
	20	12 ± 0.47	12 ± 0.47	14 ± 0.47	
	30	13 ± 0.47	14 ± 0.47	18 ± 0.47	
Aqueous	10	6 ± 0.47	6 ± 0.81	10 ± 1.24	
	20	7 ± 0.81	8 ± 0.47	12 ± 1.24	
	30	8 ± 0.81	11 ± 0.47	16 ± 0.81	

All values are represented in the form of Mean ± SD.

Antitermite activity bioassay

The toxic responses of three different extracts of seed sample were evaluated against Indian white termites. The percentage of mortality calculated out of 10 termites was evaluated (Table 3). The antitermite activity results shows the highest mortality rate 90% was observed for the methanolic extract of seed sample at the concentration of 2% for the exposure period 48 hours when compared to negative control. The other extracts shows lesser antitermite activity when compare with methanol and positive control.

Table 3: Percentage of mortality of the termite, *O. obesus* during antitermite activity bioassay

Conc. (%)	Ethyl acetate		98% Methanol		Aqueous		Control (+ve)		Control (-ve)	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
0.5	0	0	0	10	0	10	10	30	0	0
1	10	30	20	40	10	20	20	40	0	0
1.5	30	50	50	70	30	40	60	70	0	10
2	50	60	70	90	60	70	90	100	0	10

FT-IR analysis

The phytochemical extract (98% methanol at 2% of concentration) showed highest termite mortality was subjected to FT-IR analysis. FT-IR is an effective tool to identify the functional groups present in the given sample. The FT-IR results of the present work presented in the Figure 1 and Table 4.

Figure 1: FT-IR graph of 98% methanolic seed extract

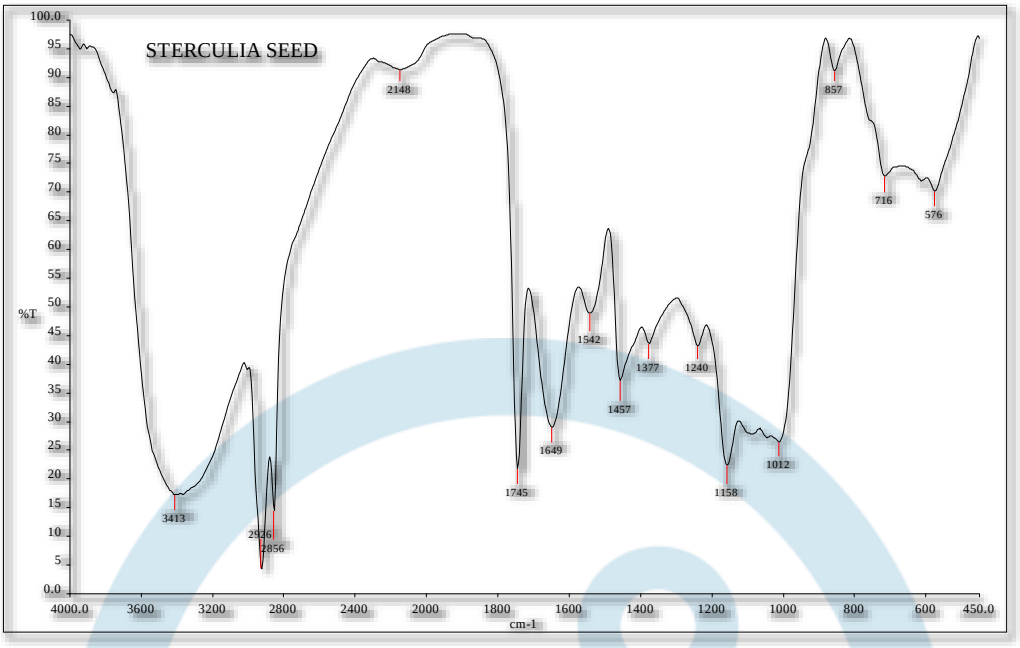

Table 4: FT-IR interpretation of 98% methanolic extract of *S. foetida* seed

S. No	Transmittance %	Functional group	Intensity	IR frequency Range cm^{-1}	Types of bond
1	3413	Amine (N-H)	Weak	3400-3500	Stretching vibrations
2	2926	Alknes (CH)	Medium	2850-3000	Stretching vibrations
3	2856	Aldehyde & Ketones (C-H) (C=O)	Medium	2690-3840	Stretching vibrations
4	2148	Alkynes (C=C)	Strong	2100-2250	Stretching vibrations
5	1745	Esters (O-C)	Strong	1735-1750	Stretching vibrations
6	1649	Amides (N-H)	Strong	1630-1695	Stretching vibrations
7	1542	Amides (N-H)	Medium	1500-1560	Bending vibration
8	1457	Alknes (CH)	Medium	1350-1470	Bending vibration
9	1377	Alknes (CH)	Medium	1370-1390	Bending vibration
10	1240	Alcohols & Phenols	Strong	970-1250	Stretching vibrations
11	1158	Carboxylic & derivatives	Strong	1100-1735	Stretching vibrations
12	1012	Amines (N-H)	Medium	1000-1250	Stretching vibrations
13	857	Arenes (C=C)	Strong-Medium	690-900	Bending vibration
14	716	Arenes (C=C)	Strong-Medium	690-900	Bending vibration
15	576	Halogen	Strong-Medium	~ 570-690	Bending vibration

Discussion

Phytochemical screening is highly essential to finding novel and effective drug and bio-control agents. In the present study, the phytochemical screening of all three seed extracts revealed that the presence of carbohydrate, amino acid, fats and oils, saponins, alkaloids, flavanoids and terpenoids. The researcher Shanthasubitha & Saravanababu (2016) reported that the secondary metabolites in the plant materials can control microbial diseases as well as insect pest. The antibacterial activity of the all extracts were

determined by measuring the diameter of zone of inhibition in millimeter. The methanolic (98%) extract of *Sterculia foetida* seed exhibited high antibacterial activity than ethyl acetate and water. Shanthasubitha & Saravanababu (2016), reported that the polar aprotic solvent acetone exhibited high antibacterial activity than antibiotic. In the present study polar protic solvent showed high antibacterial activity than ethyl acetate and water. Anti-termitic activity results revealed that the lower concentrations 0.5% did not show any higher percentage of mortality at both 24 and 48 hours when compared to positive control. The next concentrations 1, 1.5% exhibit significant percentage of mortality at 24 and 48 hours of when compared to negative control where as it showed lower or equal rate of mortality when compared to positive control. Among the all three extracts 98% methanolic extract showed higher percentage of mortality at 48 hours when compared to other extracts and controls and this result correlated with the findings of Osipitan & Oseyemi (2012). The researchers Krupal K. Patel & Narasimhacharya (2017) reported in their work that the methanolic extracts of T. arjuna stem showed the highest termite mortality and this report strongly support the present work. The final result could interpreted as the 98% methanolic extract of *Sterculia foetida* seed showed higher mortality against the Indian white termite, *Odontotermes obesus* under *in vitro* condition. The 98% methanolic extract showed the higher antibacterial activity against selected bacteria and it showed maximum percentage of mortality against termites species tested. Hence the methanolic extract was subjected to FT-IR analysis to identify the active functional group present in it and this may pave the way for identifying the important bioactive compounds from *S. foetida* seed. The FT-IR graph indicates the presence of amine, alknes, aldehyde, ketones, esters, amides, alcohols, phenols, halogen and arenes. The 98% methanolic extraction of *Sterculia foetida* seeds showed good antibacterial activity due to the presence of N-H, C=O, C=C and CH functional groups and these findings are matches with the work of Anitha Jabamalairaj *et al*., (2015). The overall results of the current work revealed that the 98% methanolic extract of *Sterculia foetida* seeds contain secondary metabolites and functional groups and these metabolites may provide high antibacterial activity and anti-termite activity.

Conclusion

The present work states that the presence of carbohydrate, amino acid, fats and oils, saponins, alkaloids, flavanoids and terpenoids are responsible for the antibacterial activity, the methanolic extract of *S. foetida* seeds have of N-H, C=O, C=C and CH bond stretching compounds exhibits maximum zone of inhibition against the tested organism when compared with control. The methanolic extract of the seed showed higher percentage of termite mortality than other extracted tested. Thus, the results obtained from the present experiments are encouraging and support the effectiveness of the *S. foetida* seed in control of Indian white termite. Further research is necessary to find out the exact compound which act on the termite control process.

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