

# Termiticidal Activity of Ethanolic and n- Hexane Leaf Extraction of *Calotropis procera*, *Cannabis indica* and *Mentha longifolia* Against a Higher Termite *Microtermes obesi* Holmgren (Blattodea: *Termitidae*)

Khalid Z Rasib<sup>1\*</sup>, Amir Khan<sup>1</sup>, Ayesha Aihetasham<sup>2</sup> and Arif Malik<sup>1</sup>

<sup>1</sup>University of Lahore. Institute of biology and biotechnology (IMBB). Defence Campus Road, Lahore, Pakistan

<sup>2</sup>Department of Zoology, University of the Punjab, New Campus, Lahore, Pakistan

## Research Article

Received: 08/03/2021  
Accepted: 22/03/2021  
Published: 29/03/2021

### \*For Correspondence:

Khalid Z Rasib,  
University of Lahore. Institute of  
biology and biotechnology (IMBB).  
Defence Campus Road, Lahore,  
Pakistan

E-mail: [Khalidrasib786@gmail.com](mailto:Khalidrasib786@gmail.com)

**Keywords:** Biological control;  
*Microtermes obesi*; Plant extracts;  
Anti-termiticidal effects

## ABSTRACT

A comprehensive study was carried out to determine the chemical constituents of plant extract from *Calotropis procera*, *Cannabis indica* and *Mentha longifolia* against termite *Microtermes obesi* (Blattodea: *Termitidae*) under laboratory conditions in University of Lahore (Pakistan) in June 2016. Extraction was done by rotary evaporator. Subsequently the analysis of these compounds was performed by Gas Chromatography-Mass Spectrometry (GC-MS). Gas Chromatography-Mass Spectrometry of plant extracts. Plant extractives of *Calotropis procera* in ethanol and n-hexane detected multiple Phyto compounds. Leaf extracts of three concentrations 25, 50, 100% (low medium and high) were tested against *Microtermes obesi*. *Calotropis procera*, *Mentha longifolia* and *Cannabis indica* showed maximum 98, 92 and 95% mortality at 100, 50 and 25% concentration after 14 days (2 weeks) of exposure under no choice trials respectively. *Calotropis procera*, *Cannabis indica* and *Cannabis indica* in n-hexane caused 100% mortality at each concentration, after 14 days of exposure. so increasing the concentration the greater mortality was recorded. Based on results, extracts of these plants proved repellent and termiticidal in action except *Mentha longifolia* as non-repellent. Under choice feeding trials the feeding on treated filter paper in each concentration was observed that termite repeated its

instinct and fed more on untreated paper than treated one under both solvent conditions. Repellency tests indicated all concentrations proved repellent except 25% concentration of *Mentha longifolia* against termite. Therefore, these plants may be environmentally friendly and could prove useful in developing soil barrier to obstruct termite activity. It was concluded that the plants studied were rich in phytochemicals with significant termiticidal potential and could be used as a replacement of synthetic insecticides which are source of health concern and bio magnification.

## INTRODUCTION

Phytochemicals are generally plant originated and in fact bioactive compounds called secondary metabolites. They are of two type's i.e., primary and secondary metabolites and both have different functions. Primary metabolites are important for the plants such as growth and development. Whereas Secondary metabolites are required primarily in little need of the plant [1]. Syntheses of these metabolites in plants is in almost all parts of the plant like bark, leaves, stem, root, flower, fruits, seeds, etc. In past few years phytochemicals have been extensively used by researcher, Phytochemicals are widely applied in the traditional herbal medicine. These herbal medicines are used by the local people to cure the various diseases which include the major diseases such as Diabetes Mellitus, Cancer, HIV etc., pharmaceutical industry and laid emphasis on their application against diverse pests of crops and allied services [2]. Apart from pharmaceutical role of Phyto-compounds they are being in practices in agriculture, production of drugs, flavor and fragrances, dye and pigments, pesticides and food additives [3]. Biological control is providing an everlasting and bio rational solution to the pest problem with less degradation of the environment.

Termites are major source of wood degradation in both tropic and subtropical regions [4] and being predominantly members of order isopteran and now blattodea with more than 2600 species around the world. Ecologically termites are divided in three main groups based on their feeding and nesting behavior as, damp wood termites, dry wood termites and subterranean [5-7] Termites are of great economic importance as they damage a diverse variety of wood in buildings, crops and play havoc in plantations and agro forests. In Pakistan around 51 species are well documented in different ecological zones of with 11 pest species with their drastic devastation to diverse cellulose containing materials [8-9]. The subterranean termites is highly infesting wood pests causing an economic loss of billions throughout the world, especially in the tropical and subtropical regions? It is highly significant to formulate insecticides from plant-based chemicals rather than synthetic ones. Previously termite control was based on persistent organochloride [9-11].

*Calotropis procera* is a flowering plant of the family *Asclepiadaceae*, inherited to North Africa, tropical Africa, Western Asia, South Asia, and Indochina. The extensive pharmacological profile shown by this plant should be operated by the pharmaceutical industry for the development of new drugs, so the therapeutic arsenal for many diseases could be extended to benefit humanity. The green globes of the plant is fleshy comprising of a toxic milky sap extremely bitter and turns into a gluey coating resistant to soap. Medicinal plants have been used to treat various ailments of the poor population around the world [12]. *Cannabis indica* is a strain of cannabis, used for various medical drives. Distributed in Southeast Asia, South- and East Africa, and even the Americas. Advanced ratio of Cannabidiol (CBD) to Tetra Hydro Cannabinol (THC) is believed to produce lower levels of anxiety than other strains of cannabis. The wild mint (*Mentha longifolia* L. family *Lamiaceae*) grows extensively in Mediterranean

regions, Europe, Australia, and North Africa [13] used in the pharmaceutical, tobacco and food industries and particularly in cosmetology. Used widely in traditional medicines. Antimicrobial, carminative, stimulant, antispasmodic for the treatment of headaches and digestive and development of new drugs. Besides this it demonstrates antibiotic activity against various bacteria, yeasts, insects, etc. [14]. Keeping in view the importance of these plants the aims and objectives were to determine their termiticidal potential and feeding activities along with their mortality.

## MATERIALS AND METHODS

### Selection of plant species

All plants area collected and selected from Malakand Division is an administrative division of the Khyber Pakhtunkhwa (North West Frontier Province now KPK) of Pakistan specifically lower Dir selected for experimentation. Subsequently, all plants were brought in university of Lahore laboratory Pakistan. All plants around 1.5 kg were subjected to washing and dryness in the laboratory under extremely uncontaminated environment.

### Extraction method for crude extracts of the plants

Crude extracts of the selected plants, a non-polar and polar solvents, methanol, was used by incubating plant leaves of *Calotropis procera*, *Cannabis indica* and *Mentha longifolia* to remove moisture contents. Plants subsequently ground well to prepare them in to fine pieces for extraction and preserved in a zip lock bags to avoid contamination. One hundred gm. of extract of each plant species with solvent were placed separately in a rotary evaporator and extracted with 150 ml of methanol and n -hexane separately. Extraction achieved through rotary evaporator was refrigerated at 4°C and subsequently checked the bioactivity of the extracted compounds separately against termites *Microtermes obesi* using different concentrations of 100, 50 and 25% n-hexane and methanolic extractions.

### Gas chromatography-mass spectrometry

To carry out analysis and identifications of the compound present in three plant extracts, further processed through rotary evaporator in ethanolic and n- hexane and subsequent hydro distillation process and then filtered through 0.20 µm pore size for GC-MS analysis. The gas chromatography temperature ranged of 60 to 310°C with 10°C/min, with a solvent delay of 4 min. The temperature of the injector was 240°C. The inert gas was helium at a flow rate of 1.0 mL/min, and 2 µL of sample was injected sample in the split less mode. The percent composition of the samples was calculated. The quantitative analysis was based on the percent area of each peak of the sample compounds. The mass spectrum of each compound was compared with those of NIST 98 (Mass Spectral Library, National Institute of Standards and Technology, MD, USA). The phytochemicals obtained from all extracts were verified from NIST Mass Spectrometry Data Center.

### Collection of *Microtermes obesus*

Collection was made possible through different sites in Lahore mainly from botanical garden of university of Lahore and side by side from open fields located towards Raiwind site Pakistan. Tissue papers, wetted roll and card boards even installed at the site of infestation as bait and subsequently collected them. Bait of more palatable woods like *Populus euramericana* was buried in to the soil and hence that served as a source of collection. Termite collected from field was first acclimatized under laboratory conditions for 3 days prior to subsequent bioassays.

**Repellent responses of plant extracts** Repellency bioassay was tested of three plant extracts separately against termites. The plant extracts of *Calotropis procera*, *Cannabis indica* and *Mentha longifolia* at concentrations of 100,

50, and 25% were tested against termite. Similarly same procedure was adopted for n-hexane concentrations against termite. Responses of these extracts checked using treated filter placed and cut from the middle in a petri plate dimension (70 × 10 mm). Half of the whatmann1 filter paper in a plate was treated with respective plant extract at specific concentration separately and other half was kept treated with distilled water as control. Each bioassays for each concentration was replicated three time and 10 active termite worker along with soldier in a ratio of (10 w: 3 S) placed at the middle of the petri plate and filter papers were allowed to dry prior to the release of termites. Observations were made at 15 min intervals. Termites oriented towards treated and untreated zones were counted and a concentration considered repellent when 21 of the 30 termites (sum of three replicates) were on untreated zone in each case.

**Feeding bioassays Choice trials**

Under choice trials whatmann filter paper 1 was cut at the middle and treated with concentration separately, Half of the portion treated with plant extracts at specific concentration and remaining half was kept as untreated and only treated with distilled water only, so a sort of choice was allocated to termites. 50 termites along with 5 soldier in a ratio of (5W:1S) were released in a petri plate and kept in a dark room covered with black cloth to minimize the effect of light. Observations were made for 7 days under laboratory conditions and mortality was observed and dear or moribund termites were separated daily and kept under record for each concentration and plant extracts. Set up were dismantled after 7 days and termite feeding and mortality was observes. Percentage mortality was calculated using the following formula,

$$Mc (Mo - Me) / (100 - Me) \times 100$$

Where, Mo = mortality rate of treated termite (%), Me = mortality rate of control (%), Mc =corrected mortality rate (%).

**No choice test under laboratory conditions**

All three plant extracts were also evaluated under no choice condition under lab. Under no choice conditions only a single filter paper was placed in a petri plate and subsequently placed in a plate treated with concentrations at 100, 50, and 25% separately. Three replicates for each concentration were made and 50 termite workers and 5 soldiers were released in the plate to maintain their natural population size ratio. Experimentation was culminated on 7 days and after that entire set up was dismantled and mortality and feeding was observed against each concentration.

**Statistical analysis**

Data were analyzed statistically by using Minitab (version 19) Anova one way and paired comparison t- test were applied and results were statistically significant in all cases (P<0.05).

**RESULTS**

**Table 1:** Bioassays on feeding choice trials for 2 weeks under laboratory conditions using extract from ethanol and n-hexane as solvent.

Plants	Concentrations%	Mean feeding in (mg) and mean SE		Mean Survival% and mean SE	Mean Mortality% and mean SE
		(Treated)	(Untreated)		
Solvent as Ethanol					

<i>Calotropis procera</i>	100	15+2.10	35+2.31	10.0+0.21	90.0+11.0
	50	20+4.23	30+2.14	30.0+5.8	70.0+8.0
	25	22+3.25	28+2.18	42.0+10.2	58.0+10.0
<i>Cannabis indica</i>	100	18+0.23	32+2.36	20.0+0.35	80.0+12.8
	50	21+1.47	29+2.15	32.0+5.3	68.0+ 10.5
	25	24+2.33	26+3.45	48.0+4.63	52.0+12.5
<i>Mentha longifolia</i>	100	16+3.21	24+2.35	17.0+0.23	83.0+2.0
	50	18+3.54	32+2.75	38.0+4.30	62.0+9.0
	25	23+4.25	27+2.34	40.0+1.23	60.0+5.0
Solvent as n-hexane					

Plants	Concentrations%	Mean Feeding in (mg) and mean SE		Mean Survival% and mean SE	Mean Mortality% and mean SE
		(Treated)	(Untreated)		
		Filter paper 1	Filter paper 2		
<i>Calotropis procera</i>	100	10+2.10	30+2.31	5.0+0.21	95.0+11.0
	50	12+4.23	28+2.14	25.0+5.8	75.0+8.0
	25	15+3.25	26+2.18	35.0+10.2	65.0+10.0
<i>Cannabis indica</i>	100	13+0.23	31+2.36	15.0+0.35	85.0+12.8
	50	11+1.47	26+2.15	22.0+5.3	72.0+ 10.5
	25	16+2.33	23+3.45	35.0+4.63	58.0+12.5
<i>Mentha longifolia</i>	100	14+3.21	20+2.35	8.0+0.23	92.0+2.0
	50	15+3.54	30+2.75	34.0+4.30	66.0+9.0
	25	18+4.25	24+2.34	31.0+1.23	69.0+5.0

### Feeding choice trials

Three plants *Calotropis procera*, *Cannabis indica*, and *Mentha longifolia* extracts were tested at concentrations of 100, 50, and 25% against termite in a bioassay using two different solvents i.e. ethanol and n-hexane extracts, where a choice was given. One half of the filter paper was treated with each plant concentration at specific concentration and remaining half of the filter paper was treated with distilled water only which also worked as an control. 100 termites were released in the center of a Petri plate and observation was made for 14 days. Termite has a choice to feed on treated or untreated filter paper. Mortality and feeding activity was monitored for 2 weeks. After two weeks maximum feeding activity of termites was noticed at a concentration of 25% in each concentration as under ethanoic plant extracts termite fed more on the untreated filter paper than treated one. Similarly, under n-hexane extraction from all three plants, termite repeated its instinct and fed more on untreated paper as seen in the both Table 1 and 2 respectively. Maximum mean % mortality was recorded at 100% in each concentration as 90, 80 and 83% as compared to 95, 85, and 92% under solvent n-hexane respectively after 2 weeks. Minimum mortality was observed at concentration of 25% as 58, 52 and 60% respectively in case of plant extracts of *Calotropis procera*, *Cannabis indica*, and *Mentha longifolia*. Under n-hexane solvent extraction the results were entirely amazing results as after two weeks maximum feeding activity of termites was noticed at a concentration of 25% in each concentration as under n-hexane plant

extraction the feeding activity was 26,23 and 24mg respectively and mortality mean was 65, 58 and 69% respectively. Maximum mean % mortality was recorded at 100% in each concentration of three plants as 98, 92 and 95% after 2 weeks. So comparing extract using n hexane is most effective and an ideal for managing termites. Data were analyzed statistically using paired comparison showed significant ( $P>0.05$ ,  $t=1.43$  and  $p=0.289$ ) (Table 1).

**No Choice feeding trials**

When no choice was bio assayed in which only a single type of filter paper was place in the petri plate and all three plant extract concentration was applied at 25, 50 and 100% level, then at concentration of 25% in each plate, maximum feeding was recorded a 56,57 and 58mg respectively. The minimum feeding was observed at concentration of 100% as 55, 50, and 45%. Although termite fed on filter paper aggressively but mean mortality was 98, 92, and 95% as compared to 70, 80 and 90% respectively. Statistically data when applied anova one way test it was significant ( $P<0.05$ ) ( $F=1.73$  and  $P=0.226$ ) (Table 2and3).

**Table 2:** No choice feeding trial of three plants extracts using ethanol extract under laboratory conditions against termite for 2 weeks.

Plants	Concentrations%	Mean feeding (mg) and mean ( $\pm$ SE)	Average mean mortality% and mean ( $\pm$ SE)
<i>Calotropis procera</i>	100	55.0+6.23	70.0+17.0
	50	45.0+4.32	64.0+10.0
	25	70.0+3.21	56.0+11.0
<i>Cannabis indica</i>	100	50.0+2.31	80.0+22.0
	50	40.0+2.14	62.0+9.0
	25	53.0+3.15	57.0+13.0
<i>Mentha longifolia</i>	100	45.0+2.69	90.0+17.0
	50	33.0+3.57	67.0+8.5
	25	35.0+2.28	58.0+14.1

**Table 3:** No choice feeding trial of three plants extracts using n-hexane extract under laboratory conditions against termite for 2 weeks.

Plants	Concentrations%	Mean feeding (mg) and mean ( $\pm$ SE)	Mean mortality% and mean ( $\pm$ SE)
<i>Calotropis procera</i>	100	35.0+6.23	98+17.0
	50	28.0+4.32	77+10.0
	25	25.0+3.21	67+11.0
<i>Cannabis indica</i>	100	30.0+2.31	92+22.0
	50	23.0+2.14	75+9.0
	25	26.0+3.15	60+13.0
<i>Mentha longifolia</i>	100	22.0+2.69	95+17.0
	50	22.0+3.57	70+8.5
	25	28.0+2.28	69+14.1

**Repellency bioassays** The aims of this study were to determine the repellency of *Calotropis procera*, *Cannabis indica* and *Mentha longifolia* crude extracts against subterranean termite, *Microtermes obesus*. Bioassays were conducted by applying varying concentrations of the plant extracts at 100, 50 and 25% concentration under laboratory conditions using solvent n-hexane and ethanol separately. All extracts exhibited a significant repellency activity in time- and concentration-dependent manners after 14 days of exposure. The highest repellency of *Microtermes obesus* was noted in all ethanoic extracts of *Calotropis procera*, *Cannabis indica* and *Mentha longifolia* as. Indicated at 100% as 27 and 29 under both solvent applications. All concentrations proved repellent with the exception of concentration 25% in case of *Mentha longifolia* was non repellent. As a rule when 21 or more of the termite (sum of three replicates) were recorded on untreated area then it was considered as repellent. Comparing both solvents n-hexane and ethanol extraction it was found that n-hexane solvent extraction is more repellent than ethanol extract, so more suitable for termite control (Table 4).

**Table 4:** Repellency bioassays of *Calotropis procera*, *Cannabis indica* and *Mentha longifolia* against a higher termite *Microtomes obesi* for 2 weeks using ethanol and n hexane extracts.

Solvent ethanol			
Plants	Concentrations%	Repellent	Non- repellent
<i>Calotropis procera</i>	100	27	-
	50	24	-
	25	21	-
<i>Cannabis indica</i>	100	25	-
	50	23	-
	25	21	-
<i>Mentha longifolia</i>	100	26	-
	50	24	-
	25	19	11
Solvent n -hexane			
<i>Calotropis procera</i>	100	29	-
	50	27	-
	25	26	-
<i>Cannabis indica</i>	100	25	-
	50	23	-
	25	20	-
<i>Mentha longifolia</i>	100	27	-
	50	25	-
	25	21	-

**Table 5:** Phytochemicals identified in ethanoic of *Mentha longifolia*, *Calotropis procera* and *Cannabis indica* extract of by GC-MS.

Plant species	Retention time	Phytochemicals	Extraction solvent
---------------	----------------	----------------	--------------------

<i>Calotropis procera</i>	9.934	7 Oxabicyclo[4.1.0]heptan-2-one,6-methyl-3-(1-methylethyl)	Ethanol
	10.786	5-Hydroxymethylfurfural	
	11.592	cyclohexanone,2,5-dimethyle-2-(1methylethyle)-	
	18.479	Hexadecanoic acid, ethyl ester	
	18.735	n-Hexadecanoic acid	
	19.775	phytol	
	20.205	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)	
	20.514	9,12,15-Octadecatrienoic acid, (Z,Z,Z)	
	22.782	Cannabidiol;	
	23.633	Dronabinol	
	24.824	Lup-20(29)-en-3-ol,acetate, (3β)	
	25.351	Squalene	
	25.811	Oleic acid, 3-(octadecyloxy)propyl ester	
	28.041	Vitamin E	

	29.262	Campesterol	
<i>Calotropis procera</i>	9.67	Benzene, 1,3-bis(1,1 -dimethyl ethyl)	n-hexane
	9.942	7Oxabicyclo[4.1.0]heptan-2-one,6-methyl-3-(1-methylethyl)-	
	11.599	3-Cyclopenten-1-one,2-hydroxy-3-(3-methyl-2-butenyl)	
	13.604	Phenol,2,4-bis(1,1-dimethylethyl)-	
	17.605	9-Hexadecenoic acid, methyl ester,(Z)-	
	17.952	Hexadecanoic acid, methyl ester	
	18.811	Heptadecanoic acid, methyl ester	
	19.828	9,12,15-octadecatrienoic acid, methyl ester,(Z,Z,Z)	
	19.881	Methyl stearate	
	20.205	9,12,15-Octadecatrienoic acid, ethyl ester,(Z,Z,Z)-	
	21.531	Eicosanoic acid ,methyl ester	
	22.97	13-Docosenoic acid ,methyl ester	
	24.342	i-propyl 9,12,15-octadecatrienoate	
	24.666	Tetracosanoic acid, methyl ester	

	25.366	Squalene	
	25.819	Nonacosane	
	26.188	1-Heptatriacotanol	
	27.303	Tetratetracontane	
	28.026	Vitamin E	
	29.255	Campesterol	
	12.195	Caryophyllene	
12.654	Humulene		
14.357	Caryophyllene oxide		
15.299	$\alpha$ -acorenol		
15.533	7-epi-cis-sesquisabinene hydrate		
	19.745	Phytol	
	21.184	p-Heptylacetophenone	
	21.486	DELTA. 8-Tetrahydrocannabinol	
	22.119	$\delta$ 9-Tetrahydrocannabivarin	

	22.375	1H-4-Oxabenzo(f)cyclobut(cd)inden-8-ol, 1a- $\alpha$ ,2,3,3a,8b- $\alpha$ ,8c- $\alpha$ -hexahydro-1,1,3a-trimethyl-6-pe	
	23.061	Cannabidiol	
	23.98	Dronabinol	
	24.161	1,3-Benzenediol,2-(3,7-dimethyl-2,6-octadienyl)-5-pentyl-	
	24.289	Cannabinol	
	25.397	Squalene	
	25.683	1H-Cyclopropa[3,4]benz[1,2,e]azulene-5,7b,9,9a-tetrol,1a,1b,4,4a,5,7a,8,9-octahydro-3-(hydro);	
	25.866	Nonacosane	
	26.55	Pregn-4-ene-3,20-dion,6,16-dimethyl-,(6 $\alpha$ ,16 $\beta$ );	
	28.034	Vitamin E	
<i>Mentha longifolia</i>	4.253	$\alpha$ -Pinene	n-hexane
	4.539	Camphene	

	5.036	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-,(1S)-	
	6.023	Limonene	
	6.882	<i>Cyclohexanol, 1-methyl-4-(1-methylethenyl)-,cis</i>	
	8.691	<i>Bicyclo[2.2.1]heptan-2-ol,1,7,7-trimethyl-,(1S-endo)-</i>	
	10.492	7-Oxabicyclo[4.1.0]heptan-2-one,6-methyl-3-(1-methylethyl)	
	12.232	3-Cyclopenten-1-one, 2-hydroxy-3-(3-methyl-2-butenyl)	
	12.361	Caryophyllene	
	12.783	Bicyclo[3.1.0]hexane-6-methanol, 2-hydroxy-1,4,4-trimethyl	
	13.137	1H-Cyclopenta[1,3]cyclopropano[1,2]benzene,octahydro-7-methyl-3-methylene-4-(1-methylene)-,[3];	
	13.664	Phenol,2,4-bis(1,1-dimethylethyl)	
	14.395	Caryophyllene oxide	
	19.7	Phytol	
	22.759	Cannabidiol	
	23.618	Dronabinol	

	24.409	Heptaacosane;	
	25.359	Squalene	
	25.917	Nonacosane	
	26.195	Ethyl iso-allocholate	
	27.017	Hexatricontane	
	27.522	Octacosane	
	28.072	dl- $\alpha$ -Tocopherol;	
	28.245	<i>Hexatriacontane</i>	
	30.362	$\gamma$ -Sitosterol	
<i>Mentha longifolia</i>	8.699	Bicyclo[2.2.1]heptan-2-ol,1,7,7-trimethssyl,(1S-endo)	Ethanol
	10.228	7-Oxabicyclo[2.1.0]heptan-2-one,6-methyl-3-(1-methylethyl)	
	11.359	Phenol,2-methyl-5-(1-methylethyl)	
	11.969	3-Cyclopenten-1-one,2-hydroxy-3-(3-methyl-2-butenyl)	
	12.278	Caryophyllene	

	12.406	Ledol	
	12.662	1-Acetoxy-p-menth-3-one	
	13.039	1H-Cyclopenta[1,3]cyclopropano[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-, [3a]	
	22.752	Resorcinol, 2-p-mentha-1,8-dien-3-yl-5-pentyl-, (-)-(E)	
	23.596	Dronabinol	
	24.756	Lup-20(29)-en-3-ol, acetate, (3 $\beta$ )	
	27.974	Vitamin E	
<i>Cannabis indica</i>	6.061	Eucalyptol	n-hexane
	12.293	Caryophyllene	
	12.572	cis- $\beta$ -Farnesene	
	12.722	Humulene	
	13.235	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2 $\alpha$ ,4 $\alpha$ ,8 $\alpha$ $\beta$ )]	

	13.499	Y-Muurolene	
	14.403	Caryophyllene oxide	
	15.345	2-Naphthalenemethanol,1,2,3,4,4a,5,6,8a-octahydro- $\alpha,\alpha,4a,8$ -tetramethyl-,[2R-(2 $\alpha,4\alpha,8a\beta$ )]	
	15.578	$\alpha$ -Bisabolol	
	19.775	phytol	
	21.343	$\delta 9$ -Tetrahydrocannabivarin	
	21.629	DELTA.8-Tetrahydrocannabinol	
	22.511	1H-4-Oxabenzo(f)cyclobut(cd)inden-9-ol, 1a- $\alpha,2,3,3a,8b,-\alpha,8c-\alpha$ ,hexahydro-1,1,3a-trimethyl-6-pe.	
	23.181	Cannabidiol	
	24.078	Dronabinol	
	25.532	Squalene	
	25.804	1HCyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol, 1a,1b,4,4a,5,7a,8,9-octahydro-3-(hydro).	
	26,037	Nonacosane	

Phytochemicals identified in chromatogram of *Mentha longifolia*, *Calotropis procera* and *Cannabis indica* using methanolic extraction solvent.

## DISCUSSION

Previous control of termites was dependent upon synthetic insecticides and persistent organochloride, organophosphate insecticides [15]. The application of pesticides/insecticides triggered pest resistance, environmental degradation and health concerns forced an alternative solution of the problem to more precise and environmentally friendly bio rational program on plant extracts against pest species [14]. Since bio rational application of insecticide is accepted and accepted worldwide [15], and bio rational options are much better strategy using plant extracts along with their bioactive compounds in managing termites [8]. Since plant extracts are investigated in other parts of the world with multi-dimensional parameters comprising of repellency, anti feedant properties [13]. The chemicals defense in plants offer such promise of developing them as insecticides could prove effective against termites and other arthropods in management and replacement of prevalent application of insecticides in the environment [12]. In recent years, the biorational botanical insecticides gaining impetus in terms of the claim along with termite control. The antitermitic activity is seen in different plants like Lemon grass (*Cymbopogon citratus*), Cassia leaf (*Cinnamomum cassia*), Vetiver (*Vetiveria zizanioides*), Eucalyptus (*Eucalyptus citrodora*, *Eucalyptus globules*), Cedar wood (*Cedrus atlantica*), Clove bud (*Syzygium aromaticum*), *Coleus amboinicus*, Isoborneol and *Calotropis procera* [9-13].

The plant extracts along with oil of *J. curcas* with many steroids. Terpene alcohol may contribute to termite mortality as a result of bioactive compounds [9-12] and termite protection comprising *O. obesus* and *Microcerotermes beelsoni*, *Reticulitermes santonensis* De Feytaud, *Reticulitermes virginicus* (Banks), *Coptotermes formosanus Shiraki*, and *Schedorhinotermes intermedius Breinli*. Termite investigators in other parts of the world highlighted on Leaf and seed extracts of *Jatropha curcas* Linn. used against workers and soldiers of subterranean termites, *Odontotermes obesus* (Ramb) to determine mortality and tunneling behavior noted that quinones extracted from the roots of *Diospyros sylvatica* caused higher mortality in termite, *Odontotermes obesus*. Methanol extract of *Eugenia caryophyllata* bud (5.2 mg/cm<sup>2</sup>) caused 100% mortality of *Atta* genus unicolor Japonicus during seven days treatment. Similarly, the crude water extracts of *Larix keptolepis* holding flavonoids in great quantities exhibited effectively termite, *Coptotermes formosanus*, feeding deterrent activities. Extracts of *Lantana camara*, *Calotropis procera*, *Ocimum sanctum*, *E. helioscopia* and *S. incanum*, *W. somnifera* were found effective against termite workers and soldiers. Essential oils as vital natural resource of insecticides development could be larvicides in action and facilitating insect repellency behavior [10-16].

The use of bio pesticides in agriculture to manage pests with less risks with synthetic compounds which are toxicologically and environmentally desirable. In future various experimentation with plant extracts for protection of agriculture and household pests' management is promising and acceptable. The plant extracts of *Calotropis procera*, *Cannabis indica* and *Mentha longifolia* against a higher termite *Microtomes obesi* with complex mixtures of such compounds have been investigated for their insecticidal, repellent, and anti-feedant properties. The harmful effects of photochemical or crude plant extracts on insects are established in several methods, including suppression of calling behavior, growth retardation, toxicity, oviposition deterrence, feeding

inhibition and reduction of fecundity and fertility. Screening for anti-termite activity of plant extracts with medicinal attributes could lead to the discovery of new agents for termite control. So we can conclude our study that n-hexane extraction of *Calotropis procera*, *Cannabis indica* and *Mentha longifolia* could be used as a potential natural termiticide against termites, Isolation of antitermitic constituents to find out their mode of action against termites. However, the results of our study revealed that leaf extraction of *Calotropis procera*; *Cannabis indica* and *Mentha longifolia* have remarkable toxic effect against notorious termites' species *Microtermes obesi*. So this study clearly indicating that n-hexane extraction is much better in terms of managing termites and furthermore suggested to use all plants extracts against different species of termite inhibiting diverse ecological zones of the globe.

### ACKNOWLEDGEMENTS

We wish to thank KPK administration along with Amir Friend for providing samples. Also thanks to Dr Arif Malik for their technical support and assistance. We thank Dr Dildar Ahmad Alavi Dean Forman Christian College University Lahore for assistance with GCMS analyses and anonymous referees for their comments.

### AUTHORS CONTRIBUTION

KZR AM conceived and designed the project. AK collected the data. KZR AM, and MZM conducted the data analysis. KZR wrote the manuscript with support from all authors.

### REFERENCES

1. Akhtar MS. Zoogeography of termites of Pakistan. Pakistan J of Zoology.1974.6:85-106.
2. Global IPM Facility Expert Group on Termite Biology and Management: Finding Alternatives to Persistent Organic Pollutants (PoPs) for Termite Management. Stockholm Convention. Food and Agriculture Organization, Rome.2000.118-68.
3. Ahmed MT. Efficacy of insecticides on subterranean termites in sugarcane. International Journal of Agriculture and Biology.2006.8:508-510.
4. Blaske VU. Repellent and toxic effects of plant extracts on subterranean termites (Isoptera: *Rhinotermitidae*). Journal of Economic Entomology.2001.94:1200-1208.
5. Blaske VU. B.T.Repellent effect of isoborneol on subterranean termites (Isoptera: *Rhinotermitidae*) in soils of different composition. Journal of Economic Entomolog.2003.96:1267-1274.
6. Breuer M, et al. Influence of a short period treatment with *Melia azedarach* extract on food intake and growth of the larvae of *Spodoptera frugiperda* (Lepidoptera; *Noctuidae*). Journal of Plant Diseases and Protection.1995.102:633-654.
7. Cheng S, et al. Antitermitic and antifungal activities of essential oil of *Calocedrus formosana* leaf and its composition. Journal of Chemical Ecology.2004:1957-1967.
8. Cheng S, et al. Anti-termite activities of essential oils from coniferous trees against *Coptotermes formosanus*. Bioresource Technology.2007.98: 456-459.
9. Commission Implementing Regulation (EU) No 872/2012 of 1 October 2012 adopting the list of flavoring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No 1565/2000 and Commission Decision 1999/217/EC Text with EEA relevance, 2012-10-02, retrieved 2018-06-25 Paulo: BASF; 1999
10. Chung K M, et al. Pest Control - A Malaysian Perspective: Termites. In: Lee CY, Yap HH, Chong NL, Jaal (editors). Universiti Sains Malaysia, Penang, Malaysia.2009:107-114.
11. Elango G, et al. Efficacy of medicinal plant extracts against Formosan subterranean termite,

*Coptotermes formosanus*. Industrial Crops and Products.2012.36:524-530.

12. Elsayed G. The effectiveness of certain plant extracts in controlling the potato tuber moth, *Phthorimea operculella*. Proceeding of the 6th International Conference on Environment Protection is a Must, Organized by N.I.O.F. and I.S.A.2012.
13. Ganapaty S, et al. Antitermitic quinines from *Diospyros sylvatica*. Photochemistry.2004.65:1265–1271.
14. Gary G, et al. Abou-zaid.(z,z)-6,9-Henecosadien-11-one,1 Labile se pheromone of the white marked tussock moth, *Orgyia leucostigma*,Journal of Chemical Ecology.2003.
15. Gbolade AA. Plant-derived insecticides in the control of malaria vector. Journal of Tropical Medicinal Plants.2001. 2: 91-97.
16. Giridhar G, et al. Antitermites' properties of *Calotropis latex*. Pesticides.1988.22: 31-33.