Bioactivity of medicinal plants *Mentha arvensis* and *Peganum harmala* extracts against *Heterotermes indicola* (Wasmann) (Isoptera)

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**Abstract**

As termites are hazardous causing damages to wooden structures leading to huge economic loss, efforts are directed towards the study of plant based products for effective and eco-friendly termite control. Present study was performed to determine the chemical constituents of the seed extracts from two medicinal plant species of *Peganum harmala* and *Mentha arvensis* against *Heterotermes indicola*. An impregnated filter paper no-choice bioassay method was applied. In Gas Chromatography Mass Spectrometry (GC-MS) analysis of *M. arvensis*, eleven different compounds were identified. Compounds in seed extractives of *P. harmala* were Cyclopropanepentanoic acid, 2-undecyl-, methyl ester, trans 5-Octadecenoic acid, methyl ester, Linoleic acid ethyl ester, Leptaflorine and Harmine. The biological activity of these seed extracts was investigated against *H. indicola* in laboratory bioassay. Results revealed that extracts of *P. harmala* exhibited anti-termitic activity in a dose-dependent manner and showed a significant activity after 40 hours of exposure while the extract of *M. arvensis* was toxic and also attractant. The LT₅₀ value of *M. arvensis* was 38.48, 67.54 and 83.19 hour respectively for 10%, 5% and 3% respectively. However, in case of *P. harmala* the LT₅₀ was 76.56, 142.6 and 147.9 hours for 10%, 5% and 3% respectively. Gas Chromatography Mass Spectrometry (GC-MS) identified components could be further utilized to explore antitermitic activity of each component against other pest termite species in Pakistan.

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Introduction
Termites cause serious damage to household materials, plants and agricultural crops such as sugar cane, millet, barley and rice (Sattar et al., 2014). There are more than 2,800 described species of termites, but the nuisance species are about 185 (Lewis, 1997; Verma et al., 2009). From Pakistan 50 species of termites have been described, and 11 have been recorded damaging man made wooden structures, in buildings (Akhtar, 1983). One of the most important termites in urban and rural areas is Heterotermes indicola (Wasmann).

In the past, the fight against termites was completely relied on synthetic insecticides (Hertel, 2000; Venkateswara et al., 2005; Sattar et al., 2014). These insecticides were carcinogenic, toxic to mammals and environmental pollutants. With the increasing spread of termite infestation, there is an increased need to find out human and environment safe treatments (Meepagala et al., 2006). There is a growing interest in natural toxic substances from plants (Chang et al., 2001; Elango et al., 2012).

Substitution of synthetic by biological insecticides is an acceptable procedure and common practice worldwide (Logan et al., 1990; Nisar et al., 2012). Plant extracts with complex mixtures of these compounds were tested for their insecticide, repellent and devoured properties (Zhu et al. 2001; Isman et al., 2006; Nisar et al., 2012). Many researchers tried plant extracts against termites (Sakasegawa et al., 2003, Park and Shin, 2005, Jembere et al., 2005, Cheng et al., 2007, Ding and Hu, 2010, Supriadi and Ismanto, 2010; Ahmed et al., 2011). Extracts repellent to the termites include Eucalyptus globules, lemon grass, clove bud and vetiver grass (Zhu et al., 2001a, b; Ahmed et al., 2011). Extract of some other plants such as Veliveria nigeitana, C. schoenanthus, Digitari sp, Pennicetum purpurerum, Sanseviera libercum, and Ocimum basilicum have also been reported to have chemical substances that are repellent to termites (Oliver, 1960; Benner, 1993; Delat and Grace, 1995; Malaka, 1996; Ajayi et al., 2012).

The present study was undertaken to assess the toxicant potential of the extracts from P. harmala (harmal) and M. arvensis (mint) against H. indicola. It includes: Ethanolic extraction of selected plant seeds using soxhlet extractor. Collection of H. indicola (Wasmann) to determine the feeding bioactivity in extracts under laboratory bioassays. Structural characterization of compounds in seeds extracts through GC-MS.

Materials and methods
Collection of termites
Termite workers and soldiers of species H. indicola were collected from old trees of Lahore. The termites were maintained for at least 1 week by placing water soaked filter papers and 5 gram oven dried soil in each petri-plate.

Seeds Collection
Seeds of locally used medicinal plants, Mentha arvensis (Podina) and Peganum harmala (Harmal) were purchased from local market.

Extract preparation
The seeds of the medicinal plants were ground into fine powder using a grinder. Twenty grams of each seed powder was taken separately for extraction in Soxhlet extractor with 200ml of ethanol. Rotary evaporator was used to obtain dried residues and stored in refrigerator for making stock solution. Stock solution was prepared for each plant extract by taking 1 g dried extract in 10ml of absolute ethanol to get a solution of 10% concentration. 10%, 5% and 3% concentrations were prepared from it.

Gas chromatography mass spectrometry conditions
All seed samples were analyzed by gas chromatography coupled with mass spectrometry. The gas chromatography conditions include a temperature range of 50 to 250°C with 4°C/min, with a solvent delay of 5 minutes. The temperature of injector was maintained at 250°C. Helium was used as an inert gas with a flow rate of 1.0 mL/min. and the volume of injected sample in the splitless mode was 2μL. The MS conditions were the following:
ionization energy, 70 eV; quadrupole temperature 100°C; scanning velocity, 1.6 scans /s; weight range, 40-500 amu.

The percent composition of the samples was calculated. The qualitative analysis was based on the percent area of each peak of the sample compounds. The mass spectrum of each compound was compared with the mass spectrum from the spectra library NIST 98 (USA National Institute of Science and Technology software).

**Anti termitic assay**

Tests were conducted following the procedure adopted by Abbas et al., 2013. Circular filter papers were cut and the bottom of each sterilized glass petri dish was provided with one. Each filter paper was soaked with 0.5 ml of the 10%, 5% and 3% extracts concentrations. The filter papers were dried at ambient temperature and placed in petri dish separately. Then population of 50 workers and five soldier of *H. indicola* was added to each petri plate. Initially after every 2 hours observations were taken up to 12 hours then data for the mortality of the termite was recorded after an interval of 12 hour up to 96 hours.

\[
\text{Mortality Rate } (%) = \frac{\text{Number of dead termites after test}}{\text{Number of initial termites used in test}} \times 100
\]

**Repellency assay**

For the estimation of repellency filter papers of 9cm in diameter were cut into two equal halves. One half of each filter paper was treated with 10%, 5% and 3% concentration of both extracts as treated and second half was served as untreated. The two halves were placed into the petri dishes with a cut space in the middle. 10 termites were released into the middle space. Repellency was noted after every 15 minutes by counting the number of termites on treated (T) and untreated (UT) filter paper discs and experiment was conducted for 2 hours. Three replicates were prepared for each concentration of all four plant extracts. A treatment concentration was considered repellent when 21 (sum of three replicates) of 30 termites were present on untreated area against respective % treatment.

**Statistical analysis**

Percentage mortality of termites was calculated and analyzed by using one way ANOVA at P= 0.05 were considered statistically significant (P<0.05). LT50 was calculated by Probit analysis (Finney, 1971; Qureshi et al., 2015).

**Results and discussion**

**Gc-Ms analysis**

In GCMS analysis of *M. arvensis* 11 different compounds were identified i.e N-ethyl-hexahydro-1H-azepine, Isoborneol, Tricyclo[4.2.1.1(2,5)] deca-3,7-dien-9-one, 10-hydroxy-10-methyl-1, stereoisomer, 7-Oxabicyclo[4.1.0]heptan-2-one, 6-methyl-3-(1-methylethyl), Phenol, 2-methyl-5-(1-methylethyl), 3-Cyclopenten-1-one, 2-hydroxy-3-(3-methyl-2-buteryl), 2-Cyclopenten-1-one,2-(2-butenyl)-4-hydroxy-3-methyl, 5,5,6-Trimethyl-2-phenylethynylbicyclo[2.2.1]heptan-2-ol,1-(1-chloro-2,3 dimethylcyclopropyl)-3, 3-dimethyl-1-butene,5,8,11,14-Eicosatetraenonic acid, methyl ester and 2-Methyl-E-E-3,13-octadecadien-1-ol as shown in table 1 and Fig. 1.

The GC-MS analysis of *Mentha arviensis* revealed that it mainly composed of menthol (63.2%), menthone (13.1%), limonene (1.5%), β pinene (0.7%), α pinene (0.6 %), and linalool (0.2%). Among these menthone showed 8.1 times more insecticidal properties and has an inhibitory effect on acetylcholinesterase in rice weevil (Lee et al., 2001; Qureshi et al., 2012).

GC-MS analysis identified many compounds in extract of seed of *P. harmala* were Cyclopropanepentanoic acid, 2-undecyl-, methyl ester, trans 5-Octadecenoic acid, methyl ester, Linoleic acid ethyl ester, Leptaflorine and Harmine as shown in figure b2 and table 2.

**Anti termitic assay**
The ethanolic seed extracts of *M. arvensis* and *P. harmala* were used to determine their efficacy against subterranean termite *H. indicola*. Greater mortality was observed in 10% concentration it decreased in 5% and low in 3% concentration. The result showed that ethanolic extract of *M. arvensis* caused 100% mortality in workers of termite *H. indicola*. The rate of mortality at 10%, 5% and 3% concentration was 100%, 72% and 60% respectively after 96 hour with LT$_{50}$ value 38.48, 67.54 and 83.19 hour respectively. The % mortality of *P. harmala* at 10%, 5% and 3% was 64%, 33%, 24% with LT$_{50}$ 76.56, 142.6 and 147.9 hours respectively.

**Table 1.** Compound obtained from GC-MS analysis of *Mentha arvensis*.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>R$_{t}$ (min.)</th>
<th>R(%)$_{b}$</th>
<th>Structural formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-ethyl-hexahydro-1-H-azepine</td>
<td>5.200</td>
<td>7.31</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>Isoborneol</td>
<td>7.001</td>
<td>8.23</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>Tricyclo[4.2.1.1(2,5)]deca-3,7-diene-9-one10-hydroxy-10-methyl</td>
<td>7.918</td>
<td>8.64</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>7-oxabicyclo[4.1.0]heptan-2-one, 6-methyl-3-(1-methylethyl)-</td>
<td>8.131</td>
<td>10.57</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>Phenol, 2-methyl-5-(1-methylethyl)</td>
<td>8.887</td>
<td>8.31</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>3-cyclopenten-1-one, 2-hydroxy-3-(3-methyl-2-butenyl)</td>
<td>9.031</td>
<td>10.07</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>2-cyclopenten-1-one, 2-(2-butenyl)-4-hydroxy-3-methyl</td>
<td>9.685</td>
<td>100</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>5,5,6-Trimethyl-2-phenylethynyl-bicyclo[2.2.1]heptan-2-ol</td>
<td>10.170</td>
<td>35.47</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>1-(1-chloro-2,3-dimethylcyclopropyl)-3,3-dimethyl-1-butyne</td>
<td>11.139</td>
<td>9.58</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>5,8,11,14-Eicosatetraenoic acid, methyl ester</td>
<td>12.429</td>
<td>57.35</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>2-Methyl-E,E-3,13-octadecien-1-ol</td>
<td>17.629</td>
<td>12.89</td>
<td><img src="image" alt="Structure" /></td>
</tr>
</tbody>
</table>

*a*=Retention time in minutes, b*= Relative percentage composition.
Table 2. Compound obtained from GC-MS analysis of *P. harmala*.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>R&lt;sub&gt;t&lt;/sub&gt; (min.)</th>
<th>R (%)</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclopropanepentanoic acid, 2-undecyl methyl ester, trans</td>
<td>15.90</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>5-octadecenoic acid methyl ester</td>
<td>17.60</td>
<td>1.15</td>
<td></td>
</tr>
<tr>
<td>Linoleic acid ethyl ester</td>
<td>18.17</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td>Leptaflorine</td>
<td>19.30</td>
<td>3.51</td>
<td></td>
</tr>
<tr>
<td>Harmine</td>
<td>20.41</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

Repellency assay

When termites workers were exposed to treated with 10%, 5% and 3% concentrations of *M. arvensis* along with untreated filter papers. Result showed that all the three concentrations were non repellent to *H. indicola* as less than 21 termites were present on untreated filter paper, However all concentrations of *P. harmala* i.e. 10%, 5% and 3% were repellent against *H. indicola*.

Fig. 1. GC-MS chromatogram of essential oils obtained from *Menthaarviensis*. 
Discussion

The termites (Isoptera) are social and highly organized insects. They live in colonies in soil or woods and damage valuable products of humans (Alawy and Mehsin, 1987; Thamer, 2008). The plants used in this study to control termites are *M. arvensis* and *P. harmala* and contained biological active compounds. *M. arvensis* showed highest mortality against *H. indicola*. Many components have been recognized by GC-MS analysis. The major components in *M. arvensis* were 3-Cyclopenten-1-one, 2-hydroxy-3-(3-methyl-2-butenyl), 2-Cyclopenten-1-one, 2-(2-butenyl)-4-hydroxy-3-methyl and 1-(1-Chloro-2,3-dimethylcyclopropyl)-3, 3-dimethyl-1-butyne at retention time of 9.685, 10.170 and 12.429 min. respectively. Several species of *Mentha*, belonging to the Lamiaceae family, have been investigated for essential oils produced by their leaves (Sartoratto et al., 2004; Bertini et al., 2005; Gende et al., 2014). The aqueous extract of *M. piperita* has considerable antibacterial activity against *Helicobacter pylori*, the main etiological agent of chronic gastritis and peptic ulcer disease (Castillo-Juarez et al., 2009; Pramila et al., 2012). Matias 2005; Qureshi et al., in 2012 explained insect pest repellent activities of menthol propylene glycol carbonate and its analogs. They also exhibit anti-inflammatory and anti-antigenic effects on human. Similarly chemicals like menthol, α-terpineol, p-menthone, menthol acetate and others were isolated from *M.arvensis*(Deschamps et al. 2006; Brito et al. 2007; Qureshi et al., in 2012). Menthol, the main component of *M.arvensis*, has been used for centuries in medicines, proven to have anti allergic, anti-ulcer antispasmodic and antimicrobial activities (Nascimento et al. 2009; Qureshi et al., in 2012). The extract of *M.arvensis* has fumigant toxicity and proven effective against *Sitophilus oryzae* L, a rice weevil.

Fig. 2. GC-MS chromatogram of essential oils obtained from *P. harmala*.

The major essentail oil constituents of mint 1,8-cineole, carvone, limonene, linalool, linalyl acetate, menthol, menthone, menthyl acetate, and piperitenone oxide, were detected in GCMS analysis by Gracindo et al., 2006. The study was designed by Qureshi et al., in 2012 to check the insecticidal properties of *M.arvensis*. 25mg, 50mg and 100 mg concentration of extracts applied against termite workers, soldiers and gut flagellates was observed depending upon a lethal dose over time, in both termite species Thus *M.arvensis* extract can be safely used to control termites and other pests.

The chemical compostion of *P. harmala* was revealed by GCMS was cyclopropanepentanoic acid, octadecenoic acid, linoleic acid, leptaflorine and harmine. The leaf extract and its fractions of *P.
P. harmala have shown pronounced mortal effect, decreased percent pupation and adult emergence of the cotton leaf worm, Spodoptera littoralis Boisd. The third instar larvae fed for two days on treated leaves were more susceptible to plant extract and its ethyl acetate and chloroform fractions. The active lowest concentration (5%) of the leaf fractions of P. harmala showed significant effect on the percentage of emerged adult parasitoids, Microplitis srufiventris Kok. GC/MS analysis showed the major constituent in ethyl acetate fraction was (23S) ethylcholest-5-en-3 beta-ol (28.04%) while those of chloroform fraction were hydroxycoumarin (Bergaptol) (15.68%), piperidinone (12.08%), thymol (11.82%), phosphoric acid, tributylester (9.80%) and trimethyl-nonanol (9.66%). The medicinal plant P. harmala could be carefully applied in integrated pest management due to its strong effect on cotton leaf worm pest (Shonouda et al., 2008). M. arvensis has also the potential for use against control of termites because its 10% concentration was toxic and at the same time attractive to H. indicola.

![Fig. 3. LT50 of all concentrations of M. arvensis and P. harmala extracts against H. indicola.](image)

GC-MS identified components could be used to further explore antitermitic activity of each component of these seed extracts, against other pest termite species in different ecological zones of Pakistan.

![Fig. 4. Repellency test of M. arvensis and P. harmala against H. indicola.](image)
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