academicJournals

Vol. 7(22), pp. 1608-1617, 10 June, 2013
DOI: 10.5897/JMPR12.683
ISSN 1996-0875 ©2013 Academic Journals
http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Antitermitic activity and phytochemical analysis of fifteen medicinal plant seeds

Mazhar Abbas[1], Muhammad Shahid[1]*, Munawar Iqbal[1], Fozia Anjum[2], Sumaira Sharif[1], Sohail Ahmed[3] and Tajnees Pirzada[4]

[1]Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad-38040, Pakistan.
[2]Department of Chemistry, Government College University, Faisalabad, Pakistan.
[3]Department of Entomology, University of Agriculture, Faisalabad-38040, Pakistan.
[4]Department of Chemistry, Shah Abdul Latif University, KhairPur, Pakistan

Accepted 6 June, 2012

The present study was designed to evaluate the phytochemical composition (qualitative and quantitative) as well as the antitermitic activity of EtOH (ethanol) seed extracts of fifteen medicinal plants. Different bioassay were used for the determination of phytochemical constituents and flavonoids, saponins, tannins and alkaloids contents were found to ranged from 0.65 to 15.18%, 0.54 to 8.60%, 0.08 to 27.71% and 0.34 to 12.47%, respectively; while the qualitative analysis of methanolic extracts also revealed the presence of glycoside, steroid, cardiac glycosides and terpenoid. The EtOH extracts of fifteen medicinal plant seed showed excellent antitermitic activity and the LT_{50} of *Foeniculum vulgare, Peganum harmala, Psoralea corylifolia, Ricinus communis, Croton tiglium, Mentha* species*, O. sativum* and *Capsicum frutescens* was found lower than 10 h, while *Nigella sativa, Allium sativum*, *Plantago ovata*, *Azadirachta indica* and *Melia azadirachta* showed LT_{50} values above 35 h in 10% extracts. The antitermitic activities were found significantly different for 3, 5 and 10% extracts. It is concluded from the results that the medicinal plant seed extracts tested, may provide a renewable source of safe natural antitermitic agent. These findings suggest the use of seed extracts of medicinal plant to control termites population. The results reported here open the possibility of further investigations of efficacy of these medicinal seed extracts to control termites population practically.

Key words: Medicinal plant, phytochemical analysis, antitermitic activity, ethanolic extract.

INTRODUCTION

Plants are not only the source of food, feed, energy and raw material, but also a potent source of medicines. The history of using plant based medicines is embedded into the histories of people and the civilizations. Early human recognized their dependence on nature in both health and illness. Led by instinct, taste, and experience, primitive man treated illness by using plants, animal parts and minerals that were not part of their usual diet. Physical evidence of the use of herbal remedies goes back some 60,000 years to a burial site of a Neanderthal man uncovered in 1960 (Solecki, 1975). All cultures have long folk medicinal histories that include the use of plants. Even in ancient cultures, people methodically and scientifically collected information on herbs and developed well-defined herbal pharmacopoeias. Earlier efforts were confined to the collection of the wild plants for medicinal use and there were no well planned and organized efforts for cultivation of herbs.

The concept of herbs growing has largely developed in ancient Egypt, Christian and Islamic religious traditions and flourished from 10 to 13 countries with the Islamic civilization. The 17th century is said to have created great

*Corresponding author. E-mail: mshahiduaf@yahoo.com.

change and expansion in styles and patterns in France and other European countries (Shah et al., 2009).In Pakistan, the Greco-Arab System, commonly known as Unani system of medicine or Tibb are considered as the oldest systems of medicine. Plants are generally referred to and published in folk literature by their vernacular or common names rather than botanical names. Due to this confusion, genuine plant drugs are adulated with closely related species. The main difficulty in fixing the botanical identity of medicinal plant in ancient Unani literature and traditional systems arises due to local name(s) of medicinal plants, nomenclatural controversy attributed to more than one plant species.

The botanical sources of large number of folk medicine found therapeutically effective in indigenous system are still unknown or doubtful. Many studies (Ahmad and Khan, 1998; Fahey, 2005; Khan et al., 2008; Ahmad et al., 2008, 2009) have stressed the need for authentic botanical identification of medicinal plant used in the Unani system of medicine, in order to maintain their efficacy. In last decade, the use of local botanicals has gained much importance researchers, because of their high bio-efficacy against termites. A considerable antitermitic activity has been reported from various plant extracts. It caused not only mortality but also made changes in behavior of these insects (Ahmed et al., 2006, 2007; Sobia et al., 2012; Zubair et al., 2012). The traditional uses of medicinal plant used in the present study are shown in Table 1 along with botanical, family and local names.

From the earlier discussion, it was hypothesized that the medical plant may have a considerable antitermitic activity. So, the present study was designed to determined the phytochemical constituents (quantitative and qualitative) and antitermitic activity of fifteen medicinal plant.

MATERIAL AND METHODS

Seeds collection

Seeds of locally used medicinal plants; *Nigella sativa* (*N. Sativa*), *Foeniculum vulgare* (*F. vulgare*), *Peganum harmala* (*P. harmala*), *Psoralea corylifolia* (*P. corylifolia*), *Ricinus communis* (*R. communis*), *Croton tiglium* (*C. tiglium*), *Mentha* species, *Ocimum basilicum* (*O. basilicum*), *Allium sativum* (*A. sativum*), *Cichorium intybus* (*C. intybus*), *Moringa oleifera* (*M. oleifera*), *Capsicum frutescens* (*C. frutescens*), *Plantago ovata* (*P. ovata*), *Azadirachta indica* (*A. indica*) and *Melia azadirachta* (*M. azadirachta*) were purchased from local market and authenticated from a taxonomist, Department of Botany, University of Agriculture, Faisalabad, Pakistan.

Preparation of extracts

The seeds of the medicinal plants were ground into fine powder using a Mill grinder. Twenty grams of each medicinal plant seeds powdered material were separately extracted with 100 ml of absolute EtOH for 7 days.

Each mixture was shaked regularly after every 24 h. The extracts were filtered through Whatman filter paper no. 1 and evaporated at room temperature, and residue thus obtained was suspended in 10% dimethyl sulfoxide and stored until phytochemical screening and antitermitic activity (Gangadevi et al., 2008).

Phytochemical screening

Qualitative analysis

Chemical tests were carried out on the ethanolic extract and on the powdered specimens using standard procedures to identify the constituents as described by Harborne (1973), Trease and Evans (1989) and Sofowara (1993). For tannins, 0.5 ml of extract solution, 1 ml of water and 1 to 2 drops of ferric chloride solution was mixed. Blue color was observed for gallic tannins and green black for catecholic tannins (Iyengar, 1995). Saponin foam test was performed as small amount of the extract was shaken with water and foam produced persists for 10 min indicating the presence of saponins (Roopashree et al., 2008). For flavonoids, alkaline reagent test were performed as the extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow color which becomes colourless on addition of dilute acid indicated the presence of flavonoids (Roopashree et al., 2008) and alkaloids were determined by Mayer's reagents test (Siddiqui and Ali, 1997). Glycoside; glacial acetic acid was added to the sample along with few drops of ferric chloride and concentrated sulphuric acid, and a reddish brown coloration at the junction of two layers and the bluish green color in the upper layer was observed (Siddiqui and Ali, 1997). Steroid; 4 mg of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then, concentrated solution of sulphuric acid was added slowly and green bluish color for steroids was noted (Siddiqui and Ali, 1997). Cardiac glycosides; 0.5 g of dried extract was dissolved in 2.0 ml of glacial acetic acid along with one drop of ferric chloride solution and on addition of 1.0 ml of concentrated H_2SO_4 brown ring obtained at the interface indicated the presence of a cardenolides (Oloyede, 2005). Terpenoid; salkowski test was performed. A 0.5 g extract was mixed with 2 ml of chloroform and on addition of H_2SO_4, a reddish brown color on the interface indicates the presence of terpenoids (Ayoola et al., 2008).

Quantification of phytochemical constituent

Harborne (1973) method was used to determine alkaloid. Five grams of the defatted sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitated matter was collected and washed with dilute ammonium hydroxide, filtered, dried and weighed. Van-Burden and Robinson (1981) method was used to determine the quantification of tannin. For this, 500 mg of the sample was weighed into a 50 ml plastic bottle and mixed with 50 ml of distilled water for 1 h in a mechanical shaker. This was filtered and 5 ml of the filtrate was taken out in a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 605 nm within 10 min. Obadoni and Ochuko (2001) method was adopted for saponin quantification. The samples were ground and 20 g of each were put into a conical flask and 100 ml of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel

Table 1. Selected medicinal plant and their traditional uses.

Botanical Name	Family	Local Name	Traditional uses	Reference
Nigella sativa	Ranunculaceae	kalonji	Asthma, diarrhoea and dyslipidaemia	Ali and Blunden (2003)
F. vulgare	Apiaceae or (umbelliferae)	Saunf	Indigestion, carminative, colic, respiratory congestion, cough remedies	Kaur and Arora (2010)
P. harmala	Nitrariaceae	Harmal	Infusion for fever, diarrhoea, abortion and subcutaneous tumours and is widely used as a remedy of dolorous events (rheumatic pain, painful joint and intestinal pain)	Bellakhdar (1997)
P. corylifolia	Fabaceae	Babchi	Stomachic, deobstruent, diaphoretic in febrile conditions, and also in leucoderma and other skin diseases	Saxena (1983)
R. communis	Euphorbiaceae	Arind	Anasarca, arthritis, asthma, boils, burns, cancer, carbuncles, catarrh, chancre, cholera, cold, colic, convulsions, corns, craw-craw, deafness, delirium, dermatitis, dog bite, dropsy, epilepsy, erysipelas and fever	Duke and Wain, 1981
C. tiglium	Euphorbiaceae	JamaalGota	Cancer, carbuncles, colds, dysentery, fever, flux, paralysis, ranula, scabies, schistosomiasis, skin, snakebite, sore, throat, and toothache	Duke and Wain (1981)
Mentha spp.	Lamiaceae	Pudina	Gastrointestinal and hepatic-intestinal	Estomba, 2006
O. basilicum	Lamiaceae	Niazbo	Antimicrobial, antimalarial, antiallergic and immunomodulator, antistress/adaptogenic, anti diabetic effect, heart ailments and others	Barghava and Singh (1981)
A. sativum	Amaryllidaceae	Garlic	Atherosclerosis and ischemic heart disease and reduction on serum cholesterol levels	Singh et al. (2008).
C. intybus	Asteraceae	Kasni	Liver tonic, cardiotonic, diuretic, stomachic, cholagogue, depurative, emmenagogue, hepatomegaly, cephalalgia, inflammations, anorexia, dyspepsia, flatulence, colic, jaundice, splenomegaly, amenorrhea dysmenorrhea, and asthma	Sala (1994); Jabeen et al. (2009)
Moringa oleifera	Moringaceae	Suhanjana	Antibiotic, antitrypanosomal, hypotensive, antispasmodic, antiulcer, anti-inflammatory, hypocholesterolemic, and hypoglycemic	Fahey (2005)
C. frutescens	Solanaceae	Lal Mirch	Anaesthetic, antihaemorrhoidal, antirheumatic, antiseptic, carminative, diaphoretic, digestive, irritant, rubefacient, sialagogue, stimulant and stomachic	Palevitch and Craker, (1996)
P. ovata	Plantaginaceae	Isapgohol	Laxative in stomach disorder and abdominal disorder	Yadav et al. (2006)
A. indica	Meliaceae	Neem	Leprosy, intestinal helminthiasis, respiratory disorders, constipation and also as a general health promoter	Chattopadhyay (1997)
M. azadirachta	Meliaceae	Bakain	Leprosy, scrofula, anthelmintic, antilithic, diuretic, deobstruent, resolvent, deobstruent and rheumatism	Khan et al. (2008)

Table 2. Qualitative phytochemicals analysis of fifteen medicinal plant seeds ethanolic extracts.

Plant name	Alkaloid	Flavonoid	Steroids	Terpenoids	Cardiac glycoside	Tannic acid	Glycoside	Saponins
N. sativa	+	+	-	+	+	-	+	+
F. vulgare	+	+	+	+	+	+	-	+
P. harmala	+	+	-	+	+	+	-	-
P. corylifolia	-	+	-	+	-	+	-	+
R. communis	+	-	-	-	-	-	+	+
C. tiglium	+	-	+	+	-	-	+	+
Mentha spp.	+	+	+	-	-	+	-	-
O. basilicum	+	+	+	+	+	+	-	+
A. sativum	+	-	-	+	+	+	+	-
C. intybus	+	+	+	+	+	+	+	+
M. oleifera	+	-	+	+	+	-	+	-
C. frutescens	+	+	-	+	+	+	-	-
P. ovata	-	-	+	-	+	+	+	-
A. indica	+	+	-	+	+	+	+	-
M. azadirachta	+	+	-	+	+	+	+	-

The data presented is the mean ± SD of three independent experimental values. The sign (-) and (+) represent the absence and presence of phytoconstituents present in each medicinal plant.

and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage. Bohm and Kocipai-Abyazan (1994) method was used to determine flavonoid; for this, 10 g of the sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature and filtered through Whatman filter paper No 42. The filtrate was then transferred into a crucible and evaporated into dryness form over a water bath and was weighed.

Antitermite activity

Termite collection and soil preparation

The termite species, *Odontotermes obesus* were collected from damaged canes and from sugar cane fields from experimental plots of the Department of Entomology, University of Agriculture, Faisalabad, Pakistan. The soil used for antitermitic bioassays was of sandy clay loam nature (52.6, 24.8 and 20.6% sand, silt and clay, respectively).

The soil was sieved through a 30-mesh size and moisture contents were determined by a moisture meter and water was added to simulate 50% of water holding capacity and to avoid mortality of termites due to dehydration.

Bioassay procedure

Soils (40 g) were spread in a Petri dish (8.75 cm diameter) using a sterilized spatula. Different concentrations of seed extracts of the plants were added to the Petri dishes separately. The soil in the dishes was shuffled and layer was made with a sterilized spatula. Sugarcane strips were placed in each Petri dish to prevent termites from starvation. Twenty termites were released in each Petri dish (contains 3, 5 and 10% medicinal plant seed extracts) and untreated soil dishes were used as control. The dishes were placed in a growth chamber at 28±2°C and 80±5% relative humidity. Termite's mortalities were examined after every 2 to 12 h and then after 12 to 96 h. The extracts having antitermite activity were tested for there lethal time to insect by application of different concentrations.

Statistical analysis

The data obtained for phytochemical analysis and antitermitic activity were reported as mean ± standard deviation (SD) of triplicate experiment and LT_{50} values were determined by Probit analysis (Steel et al., 1997).

RESULTS

The ethanolic extracts were used for the evaluation of phytochemical qualitative analysis and seed extracts showed the presence of medicinally active constituents like flavonoids, saponins, tennins, alkaloids, glycoside, steroid, cardiac glycosides and terpenoid (Table 2). Furthermore, some important phytochemical constituent like flavonoids, saponins, tannins and alkaloids were quantified and results are shown in Table 3. The EtOH seed extracts of medicinal plants showed phytochemicals constituents qualitatively as *N. Sativa*, *P. harmala*, *P. corylifolia*, *R. communis, A. sativum*, *C. frutescens*, *A. indica*, *M. azadirachta* and *P. ovata* (steroid), *R. communis*, *Mentha* spp. and *P. ovata* (terpenoids), *P. corylifolia*, *R. communis*, *C. tiglium, Mentha* spp. (cardiac glycoside), *N. sativa*, *R. communis, C. tiglium* and *M. oleifera* (tannic acid), *F. vulgare*, *P. harmala*, *P. corylifolia, Mentha* spp., *O. basilicum*, *C. frutescens* (glycoside) and *P. harmala*, *Mentha* spp., *A. sativum*, *M. oleifera*, *C. frutescens*, *A. indica* and *M. azadirachta*

Table 3. Quantitative (percent) phytochemical components of seeds of selected medicinal plants in ethanolic extracts.

Plant species	Flavonoids	Saponins	Tannins	Alkaloids
N. sativa	12.95±0.05	4.54±0.04	0.00±0.000	3.19±0.070
F. vulgare	15.18±0.11	0.54±0.03	27.71±0.14	2.73±0.080
P. harmala	7.49±0.230	0.00±0.00	20.30±0.06	12.47±0.21
P. corylifolia	11.31±0.08	6.57±0.06	16.58±0.04	0.00±0.000
R. communis	0.00±0.000	2.30±0.00	0.00±0.000	11.53±0.15
C. tiglium	0.00±0.000	2.54±0.03	0.00±0.000	9.40±0.260
Mentha spp.	11.08±0.07	0.00±0.00	7.54±0.060	02.50±0.20
O. basilicum	2.26±0.050	3.66±0.04	21.26±0.08	04.58±0.10
A. sativum	0.00±0.000	0.00±0.00	0.08±0.010	02.48±0.07
C. intybus	10.07±0.07	8.60±0.10	11.20±0.07	03.46±0.12
M. oleifera	0.00±0.000	2.33±0.06	0.00±0.000	0.34±0.030
C. frutescens	6.44±0.100	0.00±0.00	1.75±0.020	13.35±0.13
P. ovata	0.00±0.000	0.00±0.00	17.89±0.05	0.00±0.000
A. indica	0.65±0.030	0.00±0.00	09.30±0.20	0.53±0.020
M. azadirachta	5.54±0.040	0.00±0.00	11.47±0.38	1.46±0.060

The data presented is the mean ± SD of three independent experimental values.

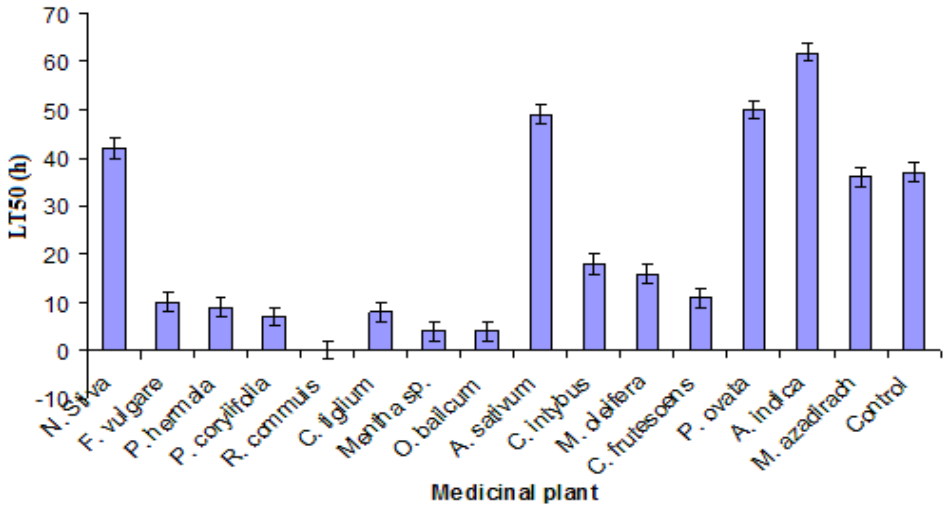

Figure 1. The LT_{50} value of fifteen medicinal plant ethanolic extracts.

(saponins). The quantitative values of phytochemical constituents from EtOH extracts are shown in Table 3. The *C. frutescens* showed the highest percentage of crude alkaloids (13.35%) followed by *P. harmala* (12.47%), while *M. oleifera* furnished the lowest alkaloid value (0.34%). In case of flavonoids, *F. vulgare* (15.18%) showed the highest value and the lowest was found in *A. indica* (0.65%). *F. vulgare* showed higher value of tannins (27.71%), while in *A. sativum* (0.08%) showed lower value. The saponins content was also found considerable which were greater in *C. intybus* (8.60%) than others and lowest (0.54%) in *F. vulgare*. The antitermitic activity has also been tested along with the LT_{50} value of 10% diluted EtOH extracts. *F. vulgare*, *P. harmala*, *P. corylifolia*, *C. tiglium*, menthe spp., *O. basilicum*, *C. intybus*, *M. oleifera*, and *C. frutescens* showed LT_{50} < 20 h, while *N. sativa, A. sativum, P. ovata* and *M. azadirachta* showed LT_{50} values > 35 h, but < 50 h and *A. indica* LT_{50} was found to be > 60 h (Figure 1). The antitermitic activity of EtOH extracts of the fifteen medicinal plants seed were checked at 3, 5 and 10% concentration of the extracts and results are shown in Tables 3 to 5, respectively with mean ± SD values. The 3% extracts of EtOH extracts showed low antitermitic activity as compared to 5% and 10 extracts.

The medicinal plant seed extracts of *F. vulgare*, *P. harmala*, *Mentha* spp. and *O. basilicum* showed complete

Table 4. The antitermitic activity of 3% ethanolic extracts of fifteen medicinal plant seeds.

Plant name	N	Extract (%)	2 h	4 h	6 h	8 h	10 h	12 h	24 h	36 h	48 h	60 h	72 h	84 h	96 h
N. sativa	20	3	2±0.11	-	-	1±0.04	-	-	3±0.14	-	-	-	-	-	-
F. vulgare	20	3	2±0.10	1±0.03	-	2±0.09	1±0.02	1±0.04	-	2±0.10	1±0.05	-	2±0.10	1±0.03	2±0.11
P. harmala	20	3	2±0.11	2±0.12	-	1±0.04	2±0.09	-	1±0.02	2±0.11	1±0.03	1±0.02	-	1±0.03	3±0.15
P. corylifolia	20	3	1±0.04	-	2±0.10	1±0.04	-	1±0.03	2±0.10	-	1±0.09	2±0.10	3±0.14	1±0.04	-
R. communis	20	3	1±0.03	-	-	1±0.02	-	-	1±0.02	-	-	-	-	-	-
C. tiglium	20	3	2±0.11	-	1±0.01	-	1±0.02	1±0.01	-	1±0.01	1±0.02	-	1±0.01	2±0.09	-
Mentha spp.	20	3	2±0.09	1±0.02	1±0.03	2±0.10	2±0.09	1±0.02	2±0.11	1±0.03	2±0.12	3±0.14	1±0.02	2±0.09	-
O. basilicum	20	3	2±0.10	1±0.03	-	2±0.11	2±0.09	-	2±0.10	3±0.15	1±0.03	2±0.09	-	5±0.24	-
A. sativum	20	3	1±0.02	-	-	2±0.09	-	-	2±0.10	1±0.03	-	1±0.02	2±0.11	1±0.02	2±0.09
C. intybus	20	3	2±0.09	-	2±0.10	-	1±0.02	-	2±0.09	-	1±0.03	2±0.08	1±0.04	1±0.03	2±0.10
M. oleifera	20	3	3±0.16	2±0.11	-	2±0.10	1±0.03	-	1±0.02	1±0.01	-	2±0.10	1±0.02	-	2±0.11
C. frutescens	20	3	2±0.10	1±0.02	-	1±0.01	1±0.03	2±0.11	-	2±0.11	1±0.03	2±0.09	1±0.01	2±0.08	-
P. ovata	20	3	-	1±0.03	-	2±0.09	2±0.10	-	1±0.11	2±0.08	-	1±0.02	-	2±0.09	1±0.02
A. indica	20	3	-	-	-	-	-	-	-	1±0.03	1±0.02	2±0.09	1±0.02	2±0.11	1±0.02
M. azadirachta	20	3	1±0.03	1±0.04	2±0.09	-	1±0.04	2±0.09	1±0.03	-	2±0.10	-	2±0.10	1±0.02	1±0.01
Control	20	D-water	-	1±0.02	-	-	-	-	1±0.02	-	-	-	-	1±0.01	-

The data presented is the mean ± SD of three independent experimental values. The sign (-) represent the value not detected.

eradication of termites, while all other extracts were found to be unable to eradicate termites in stipulated period of time. Among active EtOH extracts at 3%, the *Mentha* spp. and *O. basilicum* eradicated the termites after 84 h impregnation, while *F. vulgare, P. harmala* species took 96 h (Table 3). By increasing the extracts concentration form 3 to 5% the antitermitic activity increased significantly. At 5% concentration, medicinal plant like *F. vulgare, P. harmala, P. corylifolia, C. tiglium, Mentha* spp., *O. basilicum, C. intybus, M. oleifera* and *C. frutescens* completely eradicated the termites after 72, 60, 36, 60, 12, 10, 96 and 96 h, respectively. Species such *as N. Sativa, R. communis, A. sativum, P. ovata, A. indica* and *M. azadirachta* remained unable to eradicate the termites population completely at 5% extracts composition (Table 4). By increasing further extracts concentration to 10%, all medicinal plant seed EtOH extracts completely eradicated the termites population except *N. Sativa* (Table 5). *N. Sativa* eradicated only 70% population of termites at 10% extracts concentration after 96 h of impregnation (Table 6). The antitermitic activity of fifteen medicinal plant seed EtOH extracts was found to be correlated with phytochemical constituents. The seed extracts that showed tannins contents also showed higher antitermitic activity.

DISCUSSION

Phytochemicals constituents are non-nutrient, bio-active, secondary metabolites, naturally occurring plant compounds found in seeds, fruits, spices and vegetables.

In the present study, all the seed extracts of medicinal plant revealed the presence of alkaloids, flavonoids, terpenoids, cardiac glycosides, tannins, glycoside and saponins which are considered very important components from medicinal point of view. Plants have an almost limitless ability to synthesize aromatic substances and derivatives which form the phytochemical constituents (Olukoya et al., 1992; Okarter et al., 2009). Phytochemical constituents and derivatives are commonly used for medicinal purposes against number of disease such as analgesic, antimalarial, bactericidal and antiseptic (Stray, 1998). In the present study, we observed high tannins and alkaloid content in *C. frutescens, C. tiglium, P. harmala* and *R. communis* which may be responsible for antitermitic activity. Saponins are produced by plants as a defense mechanism

Table 5. The antitermitic activity of 5% ethanolic extracts of fifteen medicinal plant seeds.

Plant name	N	Extract (%)	2 h	4 h	6 h	8 h	10 h	12 h	24 h	36 h	48 h	60 h	72 h	84 h	96 h
N. sativa	20	5	2±0.1	-	-	1±0.05	-	-	3±0.15	-	-	-	-	-	-
F. vulgare	20	5	3±0.14	1±0.06	1±0.05	2±0.1	3±0.16	1±0.05	-	2±0.11	3±0.013	1±0.05	3±0.14	-	-
P. harmala	20	5	2±0.09	3±0.013	1±0.1	1±0.06	3±0.14	2±0.1	-	3±0.13	3±0.15	2±0.11	-	-	-
P. corylifolia	20	5	3±0.16	2±0.09	4±0.16	1±0.04	3±0.016	2±0.1	3±0.13	2±0.11	-	-	-	-	-
R. communis	20	5	1±0.03	-	-	1±0.04	-	-	1±0.05	-	-	-	-	-	-
C. tiglium	20	5	5±0.25	-	-	2±0.09	3±0.13	2±0.11	2±0.1	1±0.04	3±0.15	2±0.11	-	-	-
Mentha spp.	20	5	4±0.17	3±0.013	4±0.16	2±0.09	4±0.15	3±0.14	-	-	-	-	-	-	-
O. basilicum	20	5	5±0.23	3±0.12	2±0.09	6±3.0	4±0.17	-	-	-	-	-	-	-	-
A. sativum	20	5	1±0.03	-	-	2±0.1	-	-	2±0.11	1±0.04	-	1±0.03	4±0.16	3±0.14	2±0.12
C. intybus	20	5	2±0.1	1±0.04	2±0.09	1±0.03	1±0.03	-	2±0.11	2±0.1	1±0.05	2±0.12	1±0.01	3±0.14	2±0.1
M. oleifera	20	5	2±0.11	2±0.09	-	2±0.1	1±0.04	-	1±0.03	3±0.14	2±0.1	2±0.11	2±0.09	2±0.1	1±0.12
C. frutescens	20	5	2±0.09	4±0.18	1±0.05	-	3±0.014	1±0.04	-	2±0.1	1±0.03	2±0.1	4±0.17		
P. ovata	20	5	-	-	-	2±0.11	-	2±0.12	1±0.02	2±0.02		1±0.04	2±0.11	3±0.15	2±0.09
A. indica	20	5	-	-	-	-	-	-	-	1±0.03	1±0.04	2±0.11	1±0.05	2±0.1	1±0.03
M. azadirachta	20	5	1±0.02	1±0.04	-	1±0.03	1±0.03	1±0.04	2±0.1	2±0.1	2±0.11	-	2±0.09	1±0.4	1±0.04
Control	20	D-water	1±0.04	-	-	-	1±0.03	-	-	-	-	-	-	-	-

The data presented is the mean ± SD of three independent experimental values. The sign (-) represent the value not detected

to stop attacks by foreign pathogens, which makes them natural antibiotics (Okwu and Emenike, 2006). Saponins were also demonstrated to have potential to kill or inhibit cancer cells (Nwinuka et al., 2005; Okwu and Nnamdi, 2008). Flavonoids are known to protect inflammation, platelet aggregation, allergies and microbial infection (Okwu and Omodimiro, 2005).Similar results have also been reported by Tellez et al. (2001) for tarbush (*Flourensia cernua*) leaves hexanes, diethyl ether, and ethanol extracts as an antitermitic agent, while Kareru et al. (2010) reported the antitermitic activity of *Thevetia peruviana* (Pers.) K. Schum seed oil used in surface paint. The repellent action of paint against subterranean termites (*Microtermes* species) was significant and suggested the use of *T. peruviana* based oil paint as a self preserving agent against subterranean termite attack.

These findings corroborate the application of selected plant seeds ethanolic extracts as an antitermitic agent. In this regard, recently, Elango et al. (2012) reported the antitermitic (*Formosan subterranean*) activity of medicinal plant extracts and discovered new agents for termite control. They investigated the antitermitic activity of crude leaf hexane, ethyl acetate, acetone and methanol extracts of *Andrographis lineata* Wallich ex Nees. (Acanthaceae), *Andrographis paniculata* (Burm.f.) Wall. ex Nees. (Acanthaceae), *Argemone mexicana* L. (Papaveraceae), *Aristolochia bracteolata* Lam. (Aristolochiaceae), *Datura metel* L. (Solanaceae), *Eclipta prostrata* L. (*Asteraceae*), *Sesbania grandiflora* (L.) Pers. (Fibaceae) and *Tagetes erecta* L. (*Compositae*) against *Coptotermes formosanus*. All the crude extracts showed antitermitic activity in a dose-dependent manner and exhibited a significant activity after 24 and 48 h of exposure; the highest termite mortality was found in leaf hexane extract of *A. bracteolata*, ethyl acetate extract of *A. paniculata*, *D. metel*, *E. prostrata*, methanol extract of *A. lineata* and *D. metel* after 24 h, respectively. The hexane extract of *T. erecta*, acetone extract of *A. mexicana*, methanol extract of *S. grandiflora* and *T. erecta* showed activity after 48 h, respectively.

In a study to asses the antitermitic activities of 11 essential oils from three species of coniferous tree against *C. formosanus* Shiraki, Cheng et al. (2007) demonstrated that at the dosage of 10 mg g^{-1} of essential oils of *Calocedrus macrolepis* var. *formosana* and *Cryptomeria japonica* and the leaf Essential oil of *Chamaecyparis obtuse v*ar. *formosana* showed 100% reduction in termite

Table 6. The antitermitic activity of 10% ethanolic extracts of fifteen medicinal plant seeds.

Plant name	N	Extract (%)	2 h	4 h	6 h	8 h	10 h	12 h	24 h	36 h	48 h	60 h	72 h	84 h	96 h
N. sativa	20	10	5±0.25	-	1±0.02	-	3±0.15	-	-	1±0.01	-	-	-	2±0.09	1±0.02F
F. vulgare	20	10	7±0.35	3±0.14	1±0.02	3±0.13	6±0.31	T	-	-	-	-	-	-	-
P. harmala	20	10	3±0.30	11±0.60	3±0.29	1±0.02	2±0.09	T	-	-	-	-	-	-	-
P. corylifolia	20	10	2±0.10	4±0.20	8±0.41	5±0.26	1±0.01	T	-	-	-	-	-	-	-
R. communis	20	10	-	-	1±0.02	-	1±0.03	S-	-	-	-	-	-	-	-
C. tiglium	20	10	10±0.51	5±0.24	3±0.16	1±0.01	1±0.02	S	-	-	-	-	-	-	-
Mentha spp.	20	10	8±0.39	11±0.58	1±0.01	F	-	-	-	-	-	-	-	-	-
O. basilicum	20	10	17±0.80	3±0.14	T	-	-	-	-	-	-	-	-	-	-
A. sativum	20	10	1±0.02	-	-	2±0.10	-	-	2±0.10	1±0.01	-	3±0.16	8±0.37	3±0.14	T
C. intybus	20	10	1±0.02	2±0.09	3±0.15	3±0.16	1±0.02	-	4±0.21	6±0.33	T	-	-	-	-
M. oleifera	20	10	2±0.12	-	1±0.02	-	1±0.01	-	2±0.12	14±0.69	F	-	-	-	-
C. frutescens	20	10	5±0.27	12±0.62	1±0.03	-	2±0.12				F	-	-	-	-
P. ovata	20	10	-	-	-	3±0.14	-	4±0.19	5±0.23	7±0.34	1±0.02	T	-	-	-
A. indica	20	10	-	-	-	-	-	-	-	1±0.01	3±0.15	6±0.32	3±0.31	2±0.09	5±0.26T
M. azadirachta	20	10	-	1±0.01	-	1±0.02	-	2±0.10	3±0.14	4±0.18	9±0.44	T	-	-	-
Control	20	D-water	1±0.01	-	-	-	1±0.01	-	-	-	-	-	-	-	-

The data presented is the mean ± SD of three independent experimental values. The sign (-) represent the value not detected.

mortality after 5 days. Among the tested essential oils, *C. macrolepis* var. *formosana* killed all termites after 1 day of test, with an LC_{50} value of 2.6 mg g^{-1}, exhibiting the strongest termiticidal property. In another study, Kinyanjui et al. (2007) revealed antitermitic activity of *Juniperus procera* extracts and attributed the activity to the presence of alcoholic and phenolic compounds together with acids such as cedrol and a tertiary tricyclic alcohol. In another study, Ahmad et al. (2007) showed that that the seed extracts of *Withania somnifera*, *C. tiglium* and *Hygrophila auriculata* has potential as antitermitic agent. These plant extracts changed the tunneling behaviour, number of microbial count and enzymes activities in midgut of *O. obesus*. 50% concentration of the extracts showed no significant activity, while 100% affects the termites tunneling behaviour, number of microbial count and enzymes activities. The results of the present study show that the seed extracts of medicinal plant offer a source of naturally occurring chemicals that could be used as termite controlling agents and this activity is attributed with the presence of phytochemicals of diverse chemical structure that had repellent, antifeedant, or toxic effects on termites in feeding assays (Ahmad et al., 2007).

Conclusion

The present study revealed the antitermitic activity of some Pakistani medicinal plants traditionally used in local remedies. These plant seed ethanolic extracts have the potentials to develop new and safe products for *O. obesus* control, a naturally occurring termite. These plants derived materials could be useful as an alternative for synthetic insecticides controlling field populations of *O. obesus*, because these plants are easily available, accessible and affordable, therefore, the usage of traditional plants should be promoted among the local residents. The screening results suggest that *F. vulgare, P. harmala, Mentha* spp. and *O. basilicum* have promising capability in termite control. These plants may be used as sustainable antitermitic agent and seed extract could be exploited to develop new wood preservatives to protect wooden structures, agricultural crops, plants and trees. Furthermore, there is

need to conduct field studies to use them as commercial antitermitic agent.

ACKNOWLEDGEMENTS

The authors are thankful to the Lab staff of Termite Control Lab, Department of Entomology, University of Agriculture, Faisalabad, Pakistan and Institute of Chemistry, University of The Punjab, Lahore, Pakistan for providing technical assistance during the study.

REFERENCES

Afaq SH (1998). An overview of pharmacognostical identification of some herbal drugs, In: Proceedings of the national seminar on research methodology in Unani Medicine, Department of History of Medicine and Science. New Delhi: Jamia Hamdard.

Ahmad I, Khan NA (1998). Methodology for the identification of controverstial and Unestablished drugs of Unani repository, In: Proceedings of the national seminar on research methodology in Unani Medicine, Department of History of Medicine and Science. New Delhi: Jamia Hamdard.

Ahmad M, Khan MA, Hasan A, Zafar M, Sultana B (2008). Chemotaxonomic standardization of herbal drugs Milk thistle and Globe thistle. Asian J. Chem. 6(20):4443-4459.

Ahmad M, Khan MA, Rashid U, Zafar M, M Arshad, Sultana B (2009). Quality assurance of herbal drug valerian by using chemotaxonomic markers. Afr. J. Biotechnol. 8(6):1148-1155.

Ahmed S, Riaz MA, Shahid M (2006). Response of *Microtermes obesi* (Isoptera: Termitidae) and its gut Bacteria towards some plant extracts. J. Food Agric. Environ., 4(1):317-320.

Ahmed S, Riaz MA, Malik AH, Shahid M (2007). Effect of seed extracts of *Withania somnifera*, *Croton tiglium* and *Hygrophila auriculata* on behavior and physiology of *Odontotermes obesus* (Isoptera, Termitidae). Biologia. 62(6):770-773.

Ali BH, Blunden G (2003). Pharmacological and toxicological properties of *Nigella sativa*. Phytother. Res. 17(4):299-305.

Ayoola GA, Coker HAB, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC, Atangbayila TO (2008). Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. Trop. J. Pharm. Res. 7(3):1019-1024.

Bellakhdar J (1997). La pharmacopee marocaine traditionnelle. Medecine arabe ancienne et savoirs populaires. Paris: Ibis Press. pp. 529-30.

Chattopadhyay RR (1997). Effect of Azadirachtaindica hydroalcoholic leaf extract on the cardiovascular system. General Pharmacol: Vascular System. 28(3):449–451.

Cheng SS, Chang H-T, Wu C-L, Chang S-T (2007). Anti-termitic activities of essential oils from coniferous trees against Coptotermes formosanus. Bioresource Technol. 98(2): 456-459.

Estomba D, Ladio A, Lozada M (2006). Medicinal wild plant knowledge and gathering patterns in a Mapuche community from North-western Patagonia. J. Ethnopharmacol. 103:109–119.

Duke JA, Wain, KK (1981). Medicinal plants of the world. Computer index with more than 85,000 entries. 3 vols.

Elango G, Abdul Rahuman A, Kamaraj C, Bagavan A, Abduz Zahir A, Santhoshkumar T, Marimuthu S, Velayutham K, Jayaseelan C, Vishnu Kirthi A, Rajakumar G (2012). Efficacy of medicinal plant extracts against Formosan subterranean termite, *Coptotermes formosanus*. Ind. Crops Prod. 36: 524–530.

Gangadevi V, Yogeswari S, Kamalraj S, Rani G, Muthumary J (2008). The antibacterial activity of Acalypha indica L. Indian J. Sci. Technol. 1(6):1-5.

Harborne JB (1973). Phytochemical methods, London: Chapman and Hall, Ltd.

Iyengar MA (1995). Study of Crude Drugs. 8th ed. Manipal Power Press.

Jabeen A, Khan MA, Ahmad M, Zafar M, Ahmad F (2009). Indigenous uses of economically important flora of Margallah Hills National Park, Islamabad, Pakistan. Afr. J. Biotechnol. 8:763-784.

Fahey JW (2005). Moringa oleifera: A Review of the Medical Evidence for Its Nutritional, Therapeutic, and Prophylactic Properties. Part 1. Johns Hopkins School of Medicine, Department of Pharmacology and Molecular Sciences, Lewis B. and Dorothy Cullman Cancer Chemoprotection Center, 725 N. Wolfe Street, 406 WBSB, Baltimore, Maryland, USA.

Kaur GJ, Arora DS (2010). Bioactive potential of Anethum graveolens , Foeniculum vulgare and Trachyspermum ammi belonging to the family Umbelliferae- current status. J. Med. Plants Res. 4:87-94.

Kinyanjui T, Gitu PM, Kamau GN (2000). Potential antitermite compounds from *Juniperus procera* extracts. Chemosphere 41(7):1071-1074.

Kareru PG, Keriko JM, Kenji GM, Gachanja AN (2010) Anti-termite and antimicrobial properties of paint made from Thevetia peruviana (Pers.) Schum. oil extract. Afr. J. Pharm. Pharmacol. 4(2):87-89.

Khan AV, Khan AA, Shukla I (2008). *In vitro* Antibacterial Potential of Melia azedarach Crude Leaf Extracts Against Some Human Pathogenic Bacterial Strains. Ethnobotanical Leaflets 12:439-445.

Nwinuka NM, Ibeh GO, Ekeke GI (2005). Proximate composition and levels of some toxicants in four commonly consumed spices. J. Appl. Sci. Environ. Manage. 9(1):150-155.

Obdoni BO, Ochuko PO (2001). phytochemical studies and comparative efficacy of the crude extracts of some Homostatic plants in Edo and Delta States of Nigeria. Global J. Pure Appl. Sci. 8:203-208.

Okwu DE, Omodamiro DO (2005). Effect of Hexane extract and phytochemical content of *Xylopia aethiopica* and *Ocimum gratissimum* on uterus of guinea pig. Bio-Res. 3(2): 40-44.

Okwu DE, Emenike IN (2006). Evaluation of the phytonutrients and vitamin contents of citrus fruits. Int. J. Mol. Med. Adv. Sci. 2(1):1-6.

Okwu DE, Nnamdi FU (2008). Evaluation of the chemical composition of Dacryodes Edulis and Raphia Hookeri Mann and Wendl exudates used in herbal medicine in South Eastern Nigeria. Afr. J. Trad. Comp. Alt. Med. 5(2):194-200.

Okarter N, Liu C, Sorrells ME, Liu RH (2009). Phytochemical content and antioxidant activity of six diverse varieties of whole wheat. Food Chem.119(1):249-257.

Oloyede OI (2005). Chemical profile of Unripe Pulp of Carica Papaya. Pak. J. Nutr. 4(6):379-381.

Olukoya DK, Idiaka N, Odugbemi T (1992). Anti-Microbial Activity of some medicinal Plants from Nigeria. J. Ethnopharmacol. 39:69-72.

Roopashree TS, Dang R, Rani RHS, Narendra C (2008). Antibacterial activity of antipsoriatic herbs: Cassia tora, Momordica charantia and Calendula officinalis. Int. J. App. Res. Nat. Prod. 1: 20-28.

Sala AV (1994). Indian Medicinal Plants: A Compendium of 500 Species, 1st ed. Origent Longmen Ltd., Chennai p 74.

Saxena AP (1983). In vitro antibiotic activity of compounds of remedy used for the treatment of leprosy and leucoderma by the tribals of Central India. Abstract of papers presented at the Asian Cont. Trad. Asian Med. Bombay, 6–9 March.

Shah GM, Khan MA, Ahmad M, Zafar M, Khan AA (2009). Observations on antifertility and abortifacient herbal drugs. Afr. J. Biotechnol. 8(9):1959-1964.

Siddiqui AA, Ali M (1997). Practical Pharmaceutical chemistry. 1st ed. New Delhi: CBS Publishers and Distributors.

Sofowara A (1993). Medicinal plants and Traditional medicine in Africa. Ibadan, Nigeria; Spectrum Books Ltd.

Sobia, Zubair M, Rasool N, Shahid M, Iqbal M, Mushtaq M (2012). Antioxidant, antibacterial, antifungal activities and phytochemical analysis of dagger (*Yucca aloifolia*) leaves extracts. J. Med. Plant Res. 7(6):243-249.

Solecki RS (1975). Shanidar IV, a Neanderthal flower burial of northern Iraq. Science 190:880-888.

Steel FGD, Torrie JH, Dikey DA (1997). Principles and procedures of Biometrical approach. 3rd ed. New York: McGraw Hill.

Stray F (1998). The natural guide to medicinal herbs and plants. London: Tiger Books International.

Tellez M, Estell R, Fredrickson E, Powell J, Wedge D, Schrader K, Kobaisy M (2001). Extracts of *Flourensia cernua* (L): volatile

constituents and antifungal, antialgal, and antitermite bioactivities. J. Chem. Ecol. 27(11): 2263-2273.
Trease GE, Evans WC (1989). pharmacognsy. 11th ed. Brailliar Tiridel Can: Macmillian publishers.
Van-Burden TP, Robinson WC (1981). Formation of complexes between protein and tannin acid. J. Agric. Food Chem. 1: 77.
Yadav N, Singh N, Siwach P, Yadav OP (2006). Nutraceutical composition of selected genotypes of *Plantago ovata* for vigorous genotype selection as health food. Int. J. Plant Sci. 1(2): 167-171.
Zubair M, Mehmood N, Rızwan K, Rasool N, Jamil M, Shahid M, Ahmad VU (2012). Antioxidant, Antimicrobial and Phytochemical analysis of *Cichorium intybus* seeds extract and various organic fractions. Iran. J. Pharm. Res. 11(4):1145-1151.