Journal of Medicinal Plants Research Vol. 4(20), pp. 2108-2112, 18 October, 2010
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2010 Academic Journals

Full Length Research Paper

Evaluating biological activities of the seed extracts from *Tagetes minuta* L. found in Northern Pakistan

Irum Shahzadi, Amjad Hassan, Ummara W. Khan and Mohammad Maroof Shah*

Biotechnology Program, COMSATS Institute of Information Technology, Abbottabad, Pakistan.

Accepted 3 August, 2010

Wild marigold (*Tagetes minuta L.*) is grown over a wider range of climatic conditions starting from 3000 to 11000 feet of altitude in the north and Northwestern parts of Pakistan. The plant yield strongly aromatic essential oils and other compounds, reported to be of great medicinal importance. The information regarding biological and biochemical activities of the compounds present in *Tagetes* grown in Northern parts of Pakistan is unknown. In current study our main goal was to carry out bioassays of bioactive extracts from the seeds of *Tagetes* naturally growing in north of Pakistan. The essential oils from the seeds were extracted using solvent extraction method and the crude fractions were prepared for biological (malaricidal, phytotoxic and insecticidal) activities. The crude fractions in n-hexane and ether of *T. minuta* were applied on *Plasmodium falciparum* 3D7, *Lemna minor* and three species of common grain pests of fungus namely *Tribolium castaneum*, *Rhyzopertha dominica*, *Callosobruchus analis*. The n-hexane fraction showed significantly better results tahn ether-fraction for anti-malarial activity. Both fractions showed low or no phytotoxic activity but n-hexane fraction was effective at the concentration of 1000 µg/ml. Insecticidal activity (~70%) was observed for both n-hexane and ether fractions against common grain pests especially for *Rhyzopertha dominica.* The present information may provide foundation for further study and improvement of wild *Tagetes* for extraction of more valuable compounds, decreasing the cost incurred nationally on the treatment of malaria and chemical control of insects and adding to national income by exporting the plant or its products. These results provided the first preliminary findings of anti-malarial activity of the seed extract of *T. minuta.* The findings would be useful in promoting research aiming at the development of new drugs against mosquitoes based on the use of bioactive chemical compounds present in this wild plant of Northern Pakistan.

Key words: *Tagetes minuta,* essential oils, anti-malarial activity, phytotoxicity, insecticidal activity.

INTRODUCTION

Medicinal plants are used by almost 80% of the world's population for their basic health care because of low cost and ease in availability. From the dawn of human civilization, peoples' have great interest in plant-based drugs, pharmaceuticals, perfumery products, cosmetics, aroma compounds and natural colors used in food around the globe. There is a definite trend to adopt plant based products as the allopathic medicines are known to produce serious side effects and resistance against antibiotics which makes these drugs non potent. *Tagetes* is a genus of flowering Marigold and a member of the family Asteracea (Compositae). Three species (*Tagetes erecta, Tagetes patula, Tagetes minuta*) were previously reported from Pakistan. In tropical regions, *T. minuta* oil has numerous applications, as an insect repellent and in treatment of certain illnesses such as smallpox, earache, colds and fevers. In addition, it has been recognized to possess hypotensive, spasmolytic, anti-inflammatory, antimicrobial, antifungal and nematicidal properties

*Corresponding author. E-mail: mmshah@ciit.net.pk. Tel: 0092-992-383591-6. Ext. 304.

(Tereschuk, 1997; Mangena and Muyima, 1999).

Chemical composition and yield of *Tagetes* essential oil has been well investigated (Singh et al., 1992; Chalchat et al., 1995; Bansal et al., 1999; Gil et al., 2000). The main constituents of *Tagetes* oil are limonene and (Z)-ocimene (monoterpenes); dihydrotagetone (E)-and (Z)-tagetone (E) - and (Z)-tagetenone (also known as (E) - and (Z)-ocimenone) (Kaul et al., 2005). Fractions of *T. minuta* oil have potential for aphid control and it also has potential as natural herbicide for managing rice weeds (Tomova et al., 2005; Batish et al., 2007). Due to the competitive nature *T. minuta* is resistant to the natural, drought tolerant and easily survives on poor soils as weed (Hulina, 2008) but now it is also cultivated as crop for agrochemical and pharmaceutical properties. Grain and other seed pests damage substantial quantities of economically valuable food. Development of resistance against conventional pesticides is another problem associated with the use of insecticides in grain protection. Weeds are one of the major factors of poor agriculture productivity in developing world and control by synthetic weedicides (herbicides) is an expensive venture. The search for new weedicides requires an assay, which may predict the general phytotoxic effects of the tested samples. Weedicides from natural sources have promising future. In this regard allelopathic plants can be used for managing the weeds (Singh et al., 2003). Over the last thirty years the number of reports describing chemical and biochemical characteristics of this plant have increased. Recently, interest grew in the bioactivity of various plant extracts and their isolates. *T. minuta*- a wild plant of hilly areas of north and Northwestern of Pakistan, is well known for a wide range of biological activities but no attention so far has been focused on ascertaining the biological activity of essential oils from *Tagetes* species found in Pakistan. The current study was performed to evaluate the anti-malarial, phytotoxic and insecticidal activities of essential oil from seeds of *T. minuta* L. wildly grown in Northern Pakistan.

MATERIALS AND METHODS

Plant material

T. minuta L. was collected at the fruit stage in the month of October from Abbottabad, a district of North West Frontier Province of Pakistan. Seeds (780 g) were separated and crushed to fine powder.

Extraction and fractionation

The air dried seeds (780 g) of *T. minuta* were ground to fine powder with a pestle and mortar which were exhaustively extracted with n-hexane (2.5LX3) at room temperature for 21 days. The solvent of combined extract was evaporated under reduced pressure, yielding an oily dark brownish residue (96 g). Due to less polar nature the essential oils are soluble in n-hexane; therefore, the use of n-hexane was appropriate. The crude oil was extracted with distilled water (100 ml) to get only essential oil and the inorganic part was dissolved in water. After extraction the two fractions of essential oil were made, which were partitioned between ether/water, n-hexane/water, yielding ether-soluble (78 g) and n-hexane soluble (11 g) fractions.

Biological assays

Anti-malarial bioassay

Plant extracts were assessed for antiplasmodial activity *in vitro* in human blood, following the lactate dehydrogenase method (Makler et al., 1993). A drop of blood infected with malarial parasite was taken and at least 500 erythrocytes were counted, making a note of the number that contains parasites. The culture medium contained RPMI 1640 (Gibco, Life Technologies) buffered with 25 mM HEPES (Gibco, Life Technologies), 0.2% sodium bicarbonate and 10% human serum (Chan et al., 2004). The culture was diluted to final parasitemia of 2.0% and grown at 37°C in the presence of 5% CO_2 for 72 h. 100 µl aliquots were distributed in to sterile 96 well micro-titer plate and 10 µl containing various concentrations of the crude extracts (solublised in 0.5% Dimethyl sulfoxide) and culture was placed in humidified CO_2 (5%) incubator at 37°C. Negative control was administered 10 µl PBS in place of the drug while positive control contained standard drug chloroquine diphosphate. Thin blood films of the culture were prepared after 24, 36 and 72 h. The slides were stained with Giemsa stains and the number of parasites per 1000 erythrocytes was determined by microscopic examination. Total parasitemia was calculated and plated as percentage control against concentration and IC_{50} was determined.

Phytotoxicity assay

Phytotoxic bioassay was carried out for n-hexane and ether fractions of *T. minuta* against *Lemna minor* (Tinny plant containing leaves with 1 - 8 mm long and 0.6 - 5 mm wide). The medium was prepared containing various inorganic components in1000 ml distilled water and pH was adjusted approximately 5.5 - 6.0 by adding KOH pellets. The medium was autoclaved at 121°C for 15 min. 30 mg each of crude extract fractions of *T. minuta* were dissolved in 1.5 ml organic solvent (methanol) serving as stock solutions. Three sterilized flasks were inoculated with 1000, 100 and 10 µg of solutions from the stock solution for 500, 50 and 5 ppm. The solvent was allowed to evaporate overnight in sterilized conditions. 20 ml of medium was added to each flask containing the plant extract under investigation. Ten plants of *L. minor* each containing a rosette of three fronds was added to each flask. Other three flasks serving as control were also supplemented with ten plants of *L. minor* in 20 ml of medium each. The flasks were kept in the growth cabinet for seven days. Plants were observed during incubation. Number of fronds per flasks were counted and recorded on the seven day (Atta-ur-Rehman, 1991). Results were analyzed as growth regulation in percentage, calculated with reference to the negative control.

Insecticidal activity assay

Crude extracts as well as the fractions of *T. minuta* were subjected to insecticidal bioassay to determine the insecticidal activities of this plant extract and fractions. Three different insects *Tribolium castaneum* (Red flour beetle), *Rhyzopertha dominica* (Lasser grain

Table 1. Anti-malarial activity of *T. minuta* oil in the n-Hexane and ether soluble fraction.

Test organism	Standard drug	IC_{50}(µg/ml)	Test samples IC_{50}(µg/ml)	
			n-Hexane fraction	Ether fraction
P. falciparum $3D_7$	Chloroquine diphosphate	0.025	2.78	>25

Table 2. Phytotoxicity bioassay of *T. minuta* oil in the n-Hexane and Ether soluble fractions.

Name of plant	Conc. of compound (µg/ml)	No. of fronds			Growth regulation (%)		Conc. of standard drug paraquat (µg/ml)
		Sample		Control	n-Hexane fraction	Ether fraction	
		n-Hexane fraction	Ether fraction				
L. minor	1000	14	16	18	22.22	11.11	0.015
	100	15	17	18	16.66	5.5	
	10	16	18	18	11.11	0	

borer) and *Callosobruchus analis* (Pulse beetle) were used to test insecticidal activity of the *T. minuta* extracts. The stored grain pests were reared in the laboratory under controlled temperature and humidity, so that the insects of uniform age and size were available for the experiments. The pair of insects was reared in 9.0 cm diameter and 11 cm high plastic bottles containing 250 mg of breeding media. The bottles were covered with muslin cloth tied by means of rubber bands. The media was sterilized at 60°C for one hour.

For fractions 100 mg each was taken and was dissolved in methanol. Insecticidal studies were carried out by exposing the insects to test sample by contact method using filter paper. The fractions of *T. minuta* prepared by dissolving in 3 - 4 ml of methanol were used for the determination of solvent effect using filter paper absorption method. One filter paper was absorbed by the methanol only used to dissolve the samples, to be used as check for determination of solvent effect and by placing it overnight for evaporation. Next day 10 adults of the same size and age were transferred to Petri dishes containing samples. A check batch of insects was transferred to Petri dishes containing solvent only, (which had then evaporated). Another batch supplemented with reference insecticide Coopex in the same quantity was used. All the insects were kept without food throughout 24 h exposure period. Mortality counts were done after 24 hours exposure period.

RESULTS AND DISCUSSION

The results of the anti-malarial assay are presented in Table 1. The n-hexane fraction showed better anti-malarial activity at 2.78 µg/ml than did ether fraction at 25 µg/ml which was inactive against *Plasmodium falciparum* 3D7 strain (Table 1). The chloroquine diphosphate was used as standard drug with 0.025 µg/ml. The ratio of n-hexane fraction and chloroquine diphosphate showed commonly applied to analyze the phytochemicals of that it was moderately active. Biological activities are medicinal plants. Like most other developing countries of the world the practice of herbal treatment is well established in Pakistan. Few studies have been made to investigate phytotoxic and insecticidal activities but no report on anti-malarial activity of *T. minuta* extracts is seen. In the present study malaricidal, phytotoxic and insecticidal activities of *T. minuta* seeds extracts were evaluated.

In the previous study it has been found that the direct burning and thermal expulsion of plant as mosquito-repellent was the most common method of application for *T. rninuta* (54.8 and 56.0%) (Seyoum et al., 2002). The results presented in this study confirm the usefulness of *T. minuta* extract in determining the anti-malarial activity against *P. falciparum* 3D7 strain.

Phytotoxic effect of *T. minuta* oil on *L. minor* of different concentrations (1000, 100 and 10 µg/ml) was significantly lower in n-hexane soluble and ether soluble fractions (Table 2). Paraquat was used as standard drug and incubation condition was 28 ± 1°C. It was observed that n-hexane fraction extract showed high activity as compared to ether soluble extract. The n-hexane fraction showed 11.11, 16.66 and 22.22% growth inhibition at 10, 100 and 1000 µg/ml, respectively. As the concentration of sample fraction was increased the inhibition effect also increased proportionately. The ether fraction extract showed low inhibition activity 11.11% at 1000 µg/ml.

It has been found that secondary metabolites including terpenes especially monotrepenes like tagetones and ocimenones are abundant in essential oil of *T. minuta*. These secondary metabolites of plant inhibit the germination of cohabitant species and thus cause their delayed in germination (Zygadlo et al., 1993; Simon et al., 2000; Lopez et al., 2009). Further more, phytotoxicity of the plant inhibits or reduced the growth of weeds rather then causing any negative effect on growth of crop (Batish et al., 2007).

The insecticidal activity of crude fraction extracts of the *T. minuta* were also tested against three species of common grain pests namely *T. castaneum* (Red flour beetle), *R. dominica* (Lasser grain borer), *C. analis* (Pulse beetle) (Table 3). In treated samples 70% mortality was

Table 3. Insecticidal activity of *T. minuta* oil in the n-Hexane and ether soluble fractions.

Insect used	Mortality (%)			
	n-Hexane soluble fraction	Ether soluble fraction	Control	Coopex
T. castaneum	20	40	0	100
R. dominica	70	70	0	100
C. analis	80	0	0	100

considered as significant and active effect of the extract. The n-hexane fraction proved highly active and caused 70 - 80% growth inhibition of *C. analis* and *R. dominica*, respectively. On the other hand the same n-hexane fraction showed nearly 20% inhibitions against *T. castaneum* proving virtually ineffective. Ether soluble fraction showed significant inhibition activity (70%) against *R. dominica*, and it showed non significant inhibition activity of 40% against *T. castaneum* and no activity at all against *C. analis.*

Previous studies on *T. minuta* extract showed weak repellant effect against *Callosobruchus maculatus* (Boeke, 2004). Krishna et al. (2005) found that essential oil of *T. minuta* is also toxic against stored product beetle species, *C. maculatus* (Fabricius) and *Sitophilus oryzae* (Linnaeus). The composition of essential oil varies with plant parts. A fine powder leaves of *T. minuta* were moderately toxic against *Acanthoscelides obtectus* and *Zabrotes subfasciatus* as compared to whole leaves for the management of sorted beans (Paul et al., 2009).

The results of the present study provided convincing evidence that bioactive extracts of wild *T. minuta* that grows as weed is potentially valuable plant containing economically important compounds effective against human disease and seed pests. The study paved the way for further exploration of the compounds in the plant that still are mystery. These results also justify the traditional use of *T. minuta* as insecticide.

ACKNOWLEDGEMENTS

The authors greatly appreciate and acknowledge the contributions of COMSATS Institute of Information Technology, Islamabad for generously funding this study through a competitive grant to young researcher under CIIT Research Grant Program (CRGP).

REFERENCES

Atta-ur-Rehman (1991). Studies on natural products chemistry; Netherland, Elsevier Science Publishers, B.V. pp. 383-409.

Bansal RP, Bahl JR, Garg SN, Naqvi AA, Sharma S, Ram M, Kumar S (1999). Variation in quality of essential oil distilled from vegetative and reproductive stages of *Tagetes minuta* crop grown in North Indian plains. J. Essent. Oil Res., 11: 747-752.

Batish DR, Aroraa K, Singh HP, Kohli KR (2007). Potential utilization of dried powder of *Tagetes minuta* as a natural herbicide for managing rice weeds. Crop Prot., 26: 566-571.

Boeke SJ (2004). Efficacy of plant extract against the cowpea beetle, Callosobruchus maculatus. Int. J. Pest Manage., 50: 251-258.

Chalchat LC, Garry RP, Muhayimana A (1995). Essential oil of *Tagetes minuta* from Rwanda and France: chemical composition according to harvesting location, growth stage and part of plant extracted. J. Essent. Oil Res., 7: 375-386.

Chan KL, Choo CY, Abdullah NR, Ismail Z (2004). Antiplasmodial studies of *Eurycoma longifolia* Jack using the lactate dehydrogenase assay of *Plasmodium falciparum*. J. Ethnopharmacol. 92: 223-227.

Gil A, Ghersa CM, Leicach S (2000). Essential oil yield and composition of *Tagetes minuta* accessions from Argentina. Biochem. Syst. Ecol., 28: 261-274.

Hulina N (2008). Wild Marigold-*Tagetes minuta* L., New Weed on the Island of Hvar, and New Contribution to the Knowledge of its Distribution in Dalmatia (Croatia). Agriculturae Conspectus Scientificus. 73: 23-26.

Kaul PN, Bhattacharya AK, Rao BRR, Syamasundar KV, Ramesh S (2005). Essential oil composition of *Tagetes minuta* L. fruits. J. Essent. Oil Res., 17: 184-185.

Krishna A, Prajapati V, Bhasney S, Tripathi AK (2005). Potential toxicity of new genotypes of *Tagetes* (Asteraceae) species against stored grain insect pests. Int. J. Trop. Insect Sci., 25: 122-128.

Lopez LM, Bonzani EN, Zygadlo JA (2009). Allelopathic potential of *Tagetes minuta* terpenes by a chemical, anatomical and phytotoxic approach. Biochem. Syst. Ecol., 36: 882-890.

Makler MT, Ries JM, Williams JA, Bancroft JE (1993). Parasite lactate dehydrogenase as an assay for *Plasmodium falciparum* drug sensitivity. Am. J. Trop. Med. Hyg., 48: 739-741.

Mangena T, Muyima MYO (1999). Comparative evaluation of the antimicrobial activities of essential oils of *Artemisia afra*, *Pteronia incana* and *Rosmarinus officinalis* on selected bacteria and yeast strains. Lett. Appl. Microbiol., 28: 291-296.

Paul UV, Lossini JS, Edwards PJ, Hilbeck A (2009). Effectiveness of products from four locally grown plants for the management of *Acanthoscelides obtectus* (Say) and *Zabrotes subfasciatus* (Boheman) (both Coleoptera: Bruchidae) in stored beans under laboratory and farm conditions in Northern Tanzania. J. Sorted Prod. Res., pp. 1-11.

Seyoum A, Palsson K, Kung'a S, Kabiru EW, Lwande W, Killeen GF, Hassanali A, Knols BGJ (2002). Traditional use of mosquito-repellent plants in western Kenya and their evaluation in semi-field experimental huts against Anopheles gambiae: ethnobotanical studies and application by thermal expulsion and direct burning. T. Roy. Soc. Trop. Med. H., 96: 225-231.

Simon PM, Katinas L, Arambarri AM (2002). Secretory structures in *Tagetes minuta* (Asteraceae, Heleniae). Bol. Soc. Argent. Bot., 37: 181-191.

Singh B, Sood RP and Singh V (1992). Chemical composition of *Tagetes minuta* L. oil from Himalachal Pradesh (India). J. Essent. Oil Res., 4: 525-526.

Singh HP, Batish DR, Kohli RK (2003). Allelopathic interactions and allelochemicals: New possibalities for sustainable weed management. Crit. Rev. Plant Sci., 22: 239-311.

Tereschuk ML, Riera MV, Castro GR, Abdala LR (1997). Antimicrobial activity of flavonoids from leaves of *Tagetes minuta*. J. Ethnopharm.

56: 227-232.
Tomova BS, Waterhouse JS, Doberski J (2005). The effect of fractionated *Tagetes* oil volatiles on aphid reproduction. Entomol. Exp. Appl., 115: 153-159.
Zygadlo JA, Lamarque AL, Maestri DM, Guzman CA (1993). Composition of the inflorescences oils of some *Tagetes* species from Argentina. J. Essent. Oil. Res., 5: 679-681.