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Full Length Research Paper

Antimicrobial and antioxidant activities of the plant *Heliotropium strigosum*

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Heliotropium strigosum **is an important medicinal plant and belongs to the Boraginaceae family. Traditionally, this plant is used as laxative and diuretic. The juice of the plant is used to treat gum boils, sore eyes and also as a cure for stings of nettles, insects and snake bites. The current study was carried out to evaluate the medicinal properties of this plant. The plant was collected from Malakand, Pakistan. It was dried in shade and crushed into powder. The shade dried plant powder was macerated for 15 days. A crude extract and ethyl acetate, n-hexane, chloroform and aqueous fractions were obtained. The crude extract and fractions were screened for antibacterial, antifungal and antioxidant properties. The plant showed excellent antimicrobial activity. Chloroform and n-hexane fractions inhibited the growth of all four fungal strains that were used in the antifungal assays. Crude extract showed antifungal activity against all fungal strains except *Aspergillus flavus.* The aqueous and ethyl acetate fractions had no antifungal activity. The plant exhibited excellent antibacterial activity against *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*, moderate activity against methicillin resistant *Staphylococcus aureus* (MRSA) and *Bacillus subtillus* but was inactive against *Escherichia coli* and *Klebsiella pneumonia*. The plant showed excellent diphenyl picryl hydrazine (DPPH) scavenging activity. Antioxidant activity was shown by ethyl acetate, n-hexane and aqueous fractions. Crude extract and chloroform fractions were lacking in DPPH scavenging activity.**

Key words: *Heliotropium strigosum*, antibacterial, antifungal, antioxidant.

INTRODUCTION

Medicinal plants are commonly used for treating diseases of the gastrointestinal tract (GIT) and skin in countries having poor socio-economic condition (Teklehaymanot and Giday, 2007). Ajwain (*Trachyspermum copticum*) is used for GIT discomfort (Hawrelak et al., 2009) and it also has antioxidant property (Nickavar and Abolhasani, 2009). Nariman et al. (2004) have reported anti *Helicobactor pylori* property of ajwain. Vincristine and vinblastine are potent anti-cancer agents obtained from *Catharanthus roseus* (Gutierrez-Lugo et al., 2002; Katzung, 2004).

The family, Boraginaceae, has hundred genera and eighteen hundreds species which are distributed through temperate regions but distributed more abundantly in the Mediterranean region. Heliotropium, Cordia, Arnebia, Martensia and Trichodesma are the important genera of the Boraginaceae family. Fruits of the Cordia are used as diaphoretic and sometimes as astringent. Most possess soft mucilaginous juices, which are added to beverages to impart them a refreshing taste. *Heliotropium strigosum* is an important plant from the medicinal point of view.

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Abbreviations: DPPH, Diphenyl picryl hydrazine; **GIT,** gastrointestinal tract; **MIC,** minimum inhibitory concentration; **%RSA,** percent radical scavenging activities; **MRSA,** methicillin resistant *Staphylococcus aureus*.

Traditionally, this plant is used as laxative and diuretic. The juice of the plant is used to treat gum boils, sore eyes and also as a cure for stings of nettles, insects and snake bit (Nasir, 1970; Iqbal and Shahida, 2005). The purpose of this study is to evaluate the medicinal value of *H. strigosum*.

MATERIALS AND METHODS

This study was conducted in the Department of Biotechnology and Genetic engineering and Institute of Pharmaceutical Sciences (IPS), Kohat University of Science and Technology, Kohat, from January 2009 to January 2010.

Collection and identification of plant

The plant was collected in January-February, 2009 from Manzaray Baba, Malakand; (Pakistan), identified by Department of Plant Sciences Kohat University of Science and Technology, Kohat and voucher was issued.

Preparation of crude extract and fractions

The whole plant was dried in shade by keeping in between old newspapers. The shade dried plant was crushed into small pieces and milled into a coarse powder. The coarse powder (15 kg) was macerated in methanol with agitation at intervals at room temperature (Allen and Ansel, 2006) for 15 days. After 15 days maceration, the methanol soluble fraction was filtered off. The filtrate was concentrated under vacuum at low temperature (40°C) with the help of a rotary evaporator. A crude extract (150 gm) was obtained from the filtrate. The crude extract (130 gm) was suspended in distilled water (500 ml) and sequentially portioned with n-hexane (3 x 500 ml), chloroform (3 x 500 ml) and ethyl acetate (3 x 500 ml), to yield the n-hexane (40 gm), chloroform (30 gm), ethyl acetate (25 gm) and aqueous fractions (35 gm), respectively. The crude extract and fractions obtained were tightly packed and stored in refrigerator at 4°C.

Antibacterial and antifungal assay

Antibacterial activity of crude extract and fractions was determined by using well assay and minimum inhibitory concentration (MIC) methods. Six bacterial strains *Escherichia coli* (NCTC 10418), *Pseudomonas aeruginosa, Klebsiella pneumonia* (ATCC 700603), *Staphylococcus epidermidis* (NCTC 11047), *Bacillus subtilis* and methicillin resistant *Staphylococcus aureus* (MRSA) (NCTC 13143) were used in antibacterial assay. Muller Hinton agar (Oxoid, UK) media was prepared in conical flask according to directions of manufacturer. The media along with petri dishes, pipette and metallic borer were sterilized in an autoclave for 15 min at 121°C and 15 psi pressure. The media was poured into petri dishes under aseptic conditions and allowed to solidify.

Well assay

Bacterial culture corresponding to 10^6 cfu was inoculated on the surface of the solidified media. Then, 6 mm wells were dug in the medium by using sterile metallic borer. Stock solutions of crude extract and fractions in dimethyl sulfoxide (DMSO) at concentration of 10 mg/ml were prepared and 200 ul from each stock solution was added into respective wells. The zones of inhibition were measured after 24 h of incubation at 37°C. Doxycycline was used as standard. The zone of inhibition of crude extract and fractions were compared with zones of inhibition of standard drug (Doxycycline).

MIC method

For this method, different dilutions of the crude and other extracts were prepared in DMSO. The upper limit was 10 mg/ml and subsequent ones were as 8, 6, 4 mg/ml with the lower being 2 mg/ml. Firstly, Muller Hinton agar media was prepared and cooled to 45°C. After complete solidification of the media, the six bacteria were inoculated as points on the media containing the sample at different concentrations. After 24 h incubation at 37°C, results were recorded.

Antifungal assay

The antifungal activity of the extracts was evaluated by the agar tube dilution method. Four fungal strains *Aspergillus niger*, *Aspergillus fumigates*, *Fusarium solani* and *Aspergillus flavus* were used for this assay. Four dilutions (4.5, 3.5, 2.5 and 1.5 mg/ml) of the crude extract and fractions were prepared in DMSO. These were incorporated into the sabourad dextrose agar media at 45°C and poured into sterile test tubes. A small piece of already grown fungus was placed in each tube and incubated at 25°C for 5 days. After 5 days, each tube was observed for the presence or absence of fungal growth and results were recorded. A negative control without any fungi and a positive control with only fungus strains were included.

Diphenyl picryl hydrazine (DPPH) radical-scavenging activity

The antioxidant activity of crude extract and fractions were determined by using the method of Blois (1958) with slight modification. Briefly, a 1 mM solution of DPPH radical (Fluka Germany) solution in methanol was prepared and 1 ml of this solution was added to 3 ml of sample solutions in ethanol (containing 20-100 *u*g) and control containing no sample. The absorbance was measured at 517 nm (SP-3000 PLUS Spectrophotometer, Optima, Japan) after 30 min. Decrease in the DPPH solution absorbance indicates an increase of the DPPH radical-scavenging activity. Scavenging of free radicals by DPPH as percent radical scavenging activities (%RSA) was calculated as follows:

%RSA = (control absorbance - sample absorbance) / control absorbance x 100

Triplicate assays were carried out and the results are expressed as mean values ± standard deviations. The extract concentration showing 50% inhibition (EC_{50}) was calculated from the graph of % RSA against extract concentration with quercetin, ascorbic acid, gallic acid and α-tocopherol used as standards.

RESULTS

Antibacterial activity

In this study, antibacterial activity of *H. strigosum* was determined. The zones of inhibition formed by the crude extract, fractions and doxycycline are given in Table 1. All the fractions were very active against *S. epidermidis* while no fraction showed any activity against *E. coli*. The activity against MRSA was shown only by ethyl acetate

Table 1. Antibacterial activity of crude extract and fractions of *Heliotropium strigosum.*

Microorganism	Zone of inhibition (mm)					
	Ethyl acetate fraction	Chloroform fraction	Aqueous fraction	Crude fraction	n-Hexane fraction	Doxycycline 30 ug
E. coli	0	0	0	0	0	16
P. aeruginosa	9	12	9	15	10	19
K. pneumoniae	0	0	0	0	0	24
MRSA	8	0	0	0	0	22
S. epidermidis	20	14	15	16	19	20
B. subtillus	0	10	0	12	0	17

Table 2. Minimum inhibitory concentrations (MICS) of crude extract and fractions against *Heliotropium strigosum.*

Microorganism	MICS (mg/ml)				
	Ethyl acetate fraction	Chloroform fraction	Aqueous fraction	Crude fraction	n-Hexane fraction
E. coli	NA	NA	NA	NA	NA
P. aeruginosa	8	8	8	6	8
K. pneumoniae	NA	NA	NA	NA	NA
MRSA	8	NA	NA	NA	NA
S. epidermidis	6	8	8	8	6
B. subtillus	NA	8	NA	6	NA

NA, Not active.

fraction while crude extract and other fractions did not show any antibacterial activity against methicillin resistant *S. aureus*. No fraction was completely inactive. The plant material showed good activity against *P. aeruginosa*. Crude extract and fractions of plant showed excellent antibacterial activity against *S. epidermidis*. The crude extract was active against the *aeruginosa*, *S. epidermidis* and *B. subtillus* while it had no activity against *E. coli*, MRSA and *K. pneumoniae*. The ethyl acetate fraction was active against *P. aeruginosa*, methicillin resistant *S. aureus* and *S. epidermidis* but was inactive against *E. coli*, *K. pneumoniae* and *B. subtillus*.

The chloroform fraction of plant showed activity against *P. aeruginosa, S. epidermidis, B. subtillus*, while antibacterial activity against *K. pneumoniae*, MRSA and *E. coli.* The aqueous fraction of *H. strigosum* was active against *P. aeruginosa, S. epidermidis* and was inactive against *E. coli, B. subtillus, K. pneumonia, B. subtillus* and MRSA. The antibacterial activity shown by n-hexane fraction were against, *P. aeruginosa, S. epidermidis* and was inactive against *E. coli,* MRSA, *K. pneumonia* and *B. subtillus.* The standard, doxycycline showed activity against all six bacteria used in the assay.

MICs of crude extract and fractions

The MICs of crude extract and fractions of *H. strigosum* were determined and are shown in Table 2. The MIC of crude extract against *P. aeruginosa*, *S. epidermidis* and *B. subtillus* were 6, 8 and 6 mg/ml, respectively. Ethyl acetate inhibited growth of *P. aeruginosa*, MRSA and *S. epidermidis* at 8, 8 and 6 mg/ml concentrations, respectively. The bacterial strains *P. aeruginosa, S. epidermidis, B. subtillu* were inhibited at 8 mg/ml concentration of chloroform. The minimum inhibitory concentration of aqueous fraction of plant was 8 mg/ml against *P. aeruginosa* and *S. epidermidis* while MIC of n-hexane fraction was 8 mg/ml against *P. aeruginosa* and 6 mg/ml against *S. epidermidis.*

Antifungal activity

H. strigosum was screened for antifungal activity. The results shown by crude extract and fractions of plant are shown in Table 3. The plant showed excellent antifungal activity. The chloroform and n-hexane fraction were active against all four fungal strains used, that is, A. *niger*, *A. fumigatus*, *F. solani* and *A. flavus*. Crude extract was active against *A. niger*, *A. fumigatus*, *F. solani* but was inactive against *A. flavus*. Ethyl acetate and aqueous fractions did not show activity against any fungal strain and were inactive. All the four fungal strains, *A. niger*, *A. fumigatus*, *F. solani* and *A. flavus*, were inhibited at 2.5 mg/ml concentration of chloroform and n-hexane fraction.

Table 3. Antifungal activities of crude extract and fractions of *Heliotropium strigosum.*

Microorganism	MIC (mg/ml)				
	Ethyl acetate fraction	Chloroform fraction	Aqueous fraction	Crude fraction	n-Hexane fraction
A. niger	NA	2.5	NA	2.5	2.5
A. fumigatus	NA	2.5	NA	3.5	2.5
A. flavus	NA	2.5	NA	NA	2.5
F. solani	NA	2.5	NA	3.5	2.5

MIC, Minimum inhibitory concentration; NA, not active.

Table 4. DPPH radical scavenging of crude extract and fractions of *Heliotropium strigosum.*

Test fraction (crude)	% Interaction DPPH 30 min
Crude extract	21.34
n-hexane fraction	90.70
Chloroform fraction	37.09
Ethyl acetate fraction	94.5
Aqueous fraction	94.68

Table 5. EC_{50} of crude extract and fractions of *Heliotropium strigosum.*

Test fraction (crude)	EC_{50} (ug/ml)
Crude extract	-
n-hexane fraction	35.53
Chloroform fraction	-
Ethyl acetate fraction	30.34
Aqueous fraction	20.51

The minimum inhibitory concentration of crude extract against *A. niger* was 2.5 mg/ml while against *A. fumigatus* and *F. solani* was 3.5 mg/ml.

Antioxidant activity

The plant crude extract and subsequent sub-fractions were screened for antioxidant activity. *H. strigosum* exhibited excellent antioxidant activity as shown in Tables 4 and 5. The DPPH scavenging activity was also shown by n-hexane fraction of plant and had an EC_{50} value of 35.53 ug/ml. The ethyl acetate fraction was active and showed good antioxidant activity and had an EC_{50} value of 30.34 ug/ml. Aqueous fraction showed an excellent antioxidant activity and was the most active as it had an EC_{50} value of 20.51 ug/ml. The crude extract did not show any antioxidant activity, same is true about the chloroform sub-fraction.

DISCUSSION

Plants possess bioactive compounds and are usually screened for different activities like antifungal, antibacterial, analgesic, antioxidant, enzyme inhibition, spasmolytic and many other activities of beneficial effects; they can also play important roles in the discovery of new compounds for diagnosis and treatment of various diseases (Choudhary et al., 2004; Modak et al., 2007; Urzua et al., 2008).

Antibiotic resistance is increasing globally (Murray et al., 2009; Ullah et al., 2009; Ullah et al., 2009). Pharmaceutical companies are always in search of new antimicrobials (Roblin et al., 2009; Rafie et al., 2010). One possible source for new antimicrobials can be plants (Angeh et al., 2007). Our study confirms that, the plant *H. strigosum* possess antibacterial, antifungal and antioxidant activities. No fraction of the plant was completely inactive against the six pathogenic bacteria, *E. coli*, *P. aeruginosa, K. pneumonia, S. epidermidis*, *B. subtilis* and MRSA, which were used in the antibacterial assays. MRSA is known as superbug commonly in the media.

Generally, MRSA are multi drug resistant (Dettenkofer et al., 2008). These pathogens cause infections of skin and soft tissues, ear, respiratory tract, blood and urinary tract (Furukawa et al., 2008; Gould, 2009; Hawser, 2009; Pereira et al., 2009). Our results are consistent with the antibacterial activities shown by other species of the genus *Heliotropium*. Urzua et al. (2008) have reported antibacterial activity of *Heliotropium filifolium*. They isolated chemicals namely 3'-hydroxy-2',2',6'-trimethyl-3*H*-spiro[1-benzo-furan-2,1'-cyclohexane]-5-carboxylic acid, methyl 3'-acetyloxy-2',2',6'-trimethyl-3*H*-spiro[1-benzofuran-2,1'-cyclohexane]-5-carboxylate, methyl 3'-isopentanoyloxy-2',2',6'-trimethyl-3*H*-spiro[1-benzofuran-2,1'-cyclohexane]-5-carboxylate and methyl 3'-benzoyloxy-2',2',6'-trimethyl-3*H*-spiro[1-benzofuran-2,1'-cyclohexane]-5-carboxylate in their study and then screened these chemicals for antimicrobial activity (Urzua et al., 2008). Similarly, Singh and Dubey, (2001) have also reported antibacterial and antifungal activities for *Heliotropium marifolium* (Singh and Dubey, 2001). They showed antimicrobial activity of *H. marifolium* against *E. coli*, *S. aureus*, *A. niger* and *Penicillium chrysogenum*.

In our study, plant showed excellent activity against *S.*

epidermidis as crude extract and fractions exhibited good activity against *S. epidermidis*. This is the first study confirming antibacterial activity of *H. strigosum* against *S. epidermidis*. *B. subtilis* is a gram positive, spore forming bacteria (Prescott et al., 2002). Plants constituents are tested to determine their activity against *B. subtilis*. Lawrence and Palombo (2009) reported antisporocidal activity of oil extracted from cardamom, tea tree, and juniper leaf (Lawrence and Palombo 2009). In our study, the plant showed activity against *B. subtilis*. A similar activity was shown by the *Heliotropium europaeum* as reported by Saeedi et al. (2009) from Iran. They extracted essential oil from *H. europaeum* that exhibited antibacterial activity against *B. subtilis, E. coli, S. aureus and Salmonella typhi* (Morteza-Semnani, 2009). *E. coli* and *K. pneumoniae* are gram negative bacteria causing diseases of GIT and urinary tract (Prescott et al., 2002). Our plant was inactive against these pathogens.

Fungi cause a variety of infections, that is, infection of mouth, lungs, liver, blood, etc (Fung, 2002; Ker et al., 2002; Danziger-Isakov et al., 2008). Fungi mostly infect skin (Sogair et al., 1991). Fungal pathogens have become drug resistant (Baddley et al., 2009). Various plants have been tested exhibiting antifungal activity (Moghaddam et al., 2009). *A. niger* mostly cause infection in lungs and has also been detected on skin of burnt patients (McGinnis, 1980; Singhal et al., 2005). *A. fumigates, A. flavus* and *F. solani* have been implicated in lung and eye infections (Kang et al., 2008).

Hood et al. (2009) in a recent study in Australia, found antifungal activity of volatile oil extracted from *Leptospermum petersonii*. The plant under study was screened for antifungal activity. The n-hexane and chloroform fractions were very active and inhibited the growth of all four fungal strains used, while crude extract was active against *A. niger*, *A. fumigatesi* and *F. solani*. Ruiz-Bustos et al. (2009) have reported strong activity of medicinal plants against *A. niger* from Mexico. An excellent antifungal activity was shown by the crude extract, chloroform and n-hexane fractions against *A. niger* and inhibited its growth at a concentration of 2 mg/ml. A related specie *Heliotropium europaeum* was studied for antifungal activity against *A. niger* by Saeedi et al. (2009) in Iran. They conducted an antifungal activity test of *essential* oil of *H. europaeum* and reported antifungal activity exhibited by the essential oils against *A. niger* (Morteza-Semnani, 2009). *H. strigosum* inhibited the growth *A. fumigatus*, *F. solani* and *A. flavus*. This is the first report confirming antifungal study of *Heliotropium* against fungi.

The plant, *H. strigosum,* showed excellent DPPH scavenging activity. The antioxidant activity was shown by other plants of the genus, *Heliotropium*. Aqueous fraction of plant under study was most active as it has an EC_{50} value of 20.51 ug/ml. In 2009, Modak isolated three flavonoids, naringenin, 3-O-methylgalanginand and 7-O-methyleriodictiol from the plant, *Heliotropium taltalense* and flavonoids islolated exhibited antioxidant activity which suggest that *H. strigosum* may possess flavonoids responsible for antioxidant activity (Modak et al., 2009). Similarly, the ethyl acetate fraction also showed DPPH scavenging activity and is consistent with antioxidant activity shown by *Heliotropium sinuatum.* Modak et al., (2003) islolated, 4-(3',5'-dihydroxynona-decyl) phenol 1, and eight flavonoids from *H. sinuatum* and reported the antioxidant activity of these compounds (Modak et al., 2003).

Modak et al. (2009) isolated filifolinol, one flavanone: naringenin and 3-oxo-2-arylbenzofuran derivative from *Heliotropium sclerocarpum* and reported the antioxidant activity of plant *H. sclerocarpum.* The n-hexane fraction of plant under the present study was active and exhibited antioxidant activity but least active as compared to ethyl acetate and aqueous fractions. The antioxidant activity was reported by Murugesh et al. (2006) of the plant, *Heliotropium zeylanicum*.

Similarly, Modak et al. (2007) isolated a new aromatic geranyl derivative and three flavonoids: 5,3'-dihydroxy-7,4'-dimethoxyflavanone, 5,4'-dihydroxy-7-methoxyflavanone and 4'-acetyl-5-hydroxy-7-methoxyflavanone from *Heliotropium glutinosum* and reported the antioxidant activity of compounds isolated from *H. glutinosum*. Thus, from the above, *H. strigosum* may possess flavonoids as islolated from other species of the genus *Heliotropium* that may be responsible for the DPPH scavenging activity.

Conclusion

H. strigosum was screened for the first time for different biological activities. This plant has antioxidant potential as shown by high DPPH scavenging activity. The plant was active against the bacterial and fungal strains tested. The plant showed antibacterial activity against both gram positive and gram negative bacteria. The chloroform and n-hexane fractions completely inhibited the growth of all the four fungal strains used in the assay. Crude extract exhibited antifungal activity against *A. niger*, *A. fumigates* and *F. solani.*

In conclusion, *H. strigosum* is an important plant from the medicinal point of view and can be a potential candidate for further bio-assays which would lead to the synthesis of safe herbal drugs of global interests.

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