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BIOLOGICAL SCREENING OF POLARITY BASED EXTRACTS OF LEAVES AND SEEDS OF *SISYMBRIUM IRIO* L.

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Abstract

Different solvent extracts of leaves and seeds of *Sisymbrium irio* were tested against human pathogenic bacterial (*Staphylococcus aureus, Streptococcus epidermidis, Escherichia coli, Escherichia coli Gravitus, Klebsella pneumonia* and *Pseudomonas aeruginosa*) and fungal strains (*Fusarium oxysporium* and *Aspergillus flavus*). Antibacterial and antifungal activities were performed by using agar well diffusion method and tube dilution methods, respectively. Minimum Inhibitory Concentration (MIC) was calculated by using serial dilution method. The susceptibility pattern of microbes varied with the change in solvent polarity and type of plant part used. The chloroform crude extract of seeds proved potent against all tested bacterial strains except *E. coli* G. The methanolic crude extract of both leaves and seeds showed superior antibacterial activities than other solvents used for extraction. The leaves n-hexan crude extract was inactive against *A. flavus* and *F. oxysporium* while seeds extract did well against them. Seeds extracts proved potent antifungal irrespective of the solvent polarity except seed extract in water. This research will be helpful for the isolation of bioactive compounds and drug development.

Keywords: Sisymbrium, Antifungal, Weed, Screening, Extract

Introduction

Sisymbrium irio L. is known as London rocket, rocket mustard, rocket mustard in local languages. Most of the plants belonging to this family are of economic importance, being either common food plants (Care, 1955) or constitute important articles in oil production (AL-Qudah & Zarga, 2010). According to Ashfaque (2011), *S. irio* is not effective against *S. typhi* but it showed strong inhibition against *S. aureus* and according to Vohora *et al.,* (1980) this certainly favors their indiscriminate use in the treatment of such bacterial infections where it is usually accompanied by high fever. Due to the high nutritional value and the use of *S. irio* in folk medicine with no published data, it was found that it is worthy to carry out phytochemical and pharmacological study on it (Collenete, 1999). Aqueous extract of *S. irio* L. has significant anti-pyratic activity **(**Finland, 1978). Moreover, the seeds of *S. irio* were also active against *Echerichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*. Surprisingly, it is not effective against *Salmonella typhi* (Khan, *et al*., 1991).

Phytochemical investigation of antimicrobial agents from *Sisymbrium irio* L. and study of their antimicrobial activity was carried out by several researchers (Khan & Saeed, 2000; Gehan *et al*., 2009). Three common solvents i.e., petroleum ether (40-60 degree), chloroform and methanol were used successively for the extraction of antimicrobial principles from its various parts. Five major compounds (namely C1, C2, C3, C4 and C5) were isolated and purified from the active methanol extract of the roots of this species by silica gel column and thin layer chromatography (Raie *et al*., 1983). Three gram (+) bacteria (*Bacillus pumilus, Bacillus subtilis* and *Staphylococcus aureus*), three gram (-) bacteria *Proteus vulgaris, Escherichia coli* and *Pseudomonas aeruginosa*) and a fungus (*Candida albicans*) were used as test microorganisms.

Aqueous extract effects of 64 weed species on growth and development of *Alternaria solani* Sorauer. *Helminthosporium satirum* King & Bakke and *Rhizodonia solani* Kuhn. Plant pathogenic fungi were studied *in vitro*. Extracts varied in the strength and persistence of their antifungal effects against the three fungi species. Some stimulated, others inhibited or had no effect (Shah *et al*., 2013) while others have reported antibacterial activity of plants (Shah *et al*., 2014). Among all species tested, extracts of *Chenopodium murale, Falearia vulgaris. Ranunculus asiaticus* and *Sisymbrium irio* were the most toxic to *A. solani*. *Anagallis arlensis. Atriptex leucoclada, Crepis aspera. Notobasis syriaca. R. asiatieus, Rumex crispu. S. irio. Sonehu.s oleraceous* and *Vieia narhonensis* to *H. satirum* and *R. asiatieus. S. oleraceous* and *Mercurialis annua* to *R. solani*. However. *R. asiatieus* extract was the most effective and completely inhibited the growth and sporulation of the three fungi species at all incubation periods (Qasem, 1996). In view of the importance of the above mentioned plant, the present study was conducted to investigate the antifungal activity of *S. irio*.

Materials and Methods

Plant Samples extract preparation: Leaves of *Sisymbrium irio* were collected from Islamabad and Rawalpindi while seeds were collected from Rawalpindi food market in well pliable bags which were labeled accordingly with code and date of samples collection. Samples were branded by skillful taxonomist of Botany Department PMAS AAUR and checked specimens were submitted in the Department of Botany, PMAS AAUR for further reference. The samples were washed gently to remove clinging fibrous material and were dried at 40^0C. The powdered (5g) samples were dissolved in 50ml n-Hexane (80%) and kept on shaker for 24-hr at 30^0C and

140 rpm. The supernatant was collected in pre-weighed falcon tubes by filtration on Watman filter paper and the residues were re-suspended in chloroform followed by butanol, ethanol, methanol and distilled water, respectively. The falcon tubes were left opened in fume hood till the complete evaporation of solvents. The stock solutions of the extract were prepared at the concentration of 15 mg/ml in DMSO for the biological screening.

Antibacterial assay: Antibacterial activity of different polarity based extracts (n-hexane, ethyl acetate, chloroform, butanol, ethanol, methanol and water) of *Sisymbrium irio* was tested against two different gram positive (*S. aureus*, *S. epidermidus*) and four gram negative (*E.coli*, *K. pneumonia*, *P.aeroginosa* and *E. coli G*) bacterial strain (Table 1). Zone of inhibition of leaves and seeds samples of *Sisymbrium irio* for tested bacterial strains were compared with antibiotic ampicillin.

Table. 1 Bacterial strains used.

Strain used	ATCC No.	Type
E. coli	PEO 12536	gram-ve
K. pneumonia	PEO 12537	gram-ve
S. aureus	PEO 118536	Gram +
P. aeruginosa	PEO 12538	gram-ve
S. epidermidus	PEO 112983	Gram +
E. coli G	PEO 12537	gram-ve

The antibacterial activity was performed by the ascribed protocol of Linton (1983). The bacterial strains were rejuvenated in pre-sterilized Lauria Bertini (LB) brothand were labeled with care for future use. Sterilized LB-agar medium was poured into autoclaved petri plates and allowed to solidify. The 6mm diameter wells were created in solidified LB-agar medium with the help of sterilized borer. The 30µl bacterial inoculums of 0.5×10^5CFU/ml turbidity were transferred and spread on LB-agar medium with cotton swab. The agar well was filled with 60µl of each extract, whereas one well was filled with ampicillin as positive control. The petri plates were wrapped with paraffin film and placed in incubator at 37^0C for 24-hr. The results of antibacterial activity with clearance zone were aspersed as '+' while extract with no clearance zone were expressed as '-'. The zone of inhibition was measured with Vernier caliper in millimeter (mm).

Minimum inhibitory concentration of samples were determined according to ascribed protocol of Bibi *et al.*, (2011). Through serial dilution method different concentrations (15- 0.46mg/ml) of effective extracts were prepared in LB broth. Sterility control (SC) was used as negative control by taking LB broth in sterile environment while growth control (GC) was used as positive control by taking LB broth and bacterial strain. O.D of culture was adjusted to 0.5×10^5CFU/ml at 615nm. Then 100ul bacterial cultures were added in all concentrations and mixture was incubated at 37^oC for 24 hours. Then 100ul from above mixture was spread over LB agar plates separately to check bacterial inhibition. Plates with minimum inhibitory concentration were selected as minimum bactericidal concentration (Kuar & Arora, 2009).

Antifungal activity: The agar dilution method was used against *Fusarium oxysporium* (*F. oxysporium*) and *Aspergillus flavus* (*A. flavus*) according to the described protocol of Fatima *et al.*, (2009). Sterilized LB media fortified with samples extracts was prepared and poured in dully labeled autoclaved test tubes. Prior to antifungal activity, fungal inoculums were prepared according to Hemaiswarya *et al.*, (2009) protocol. Each fungal colony was placed over the solidified slant (mixture of plant extract and LB agar). Over one slant safradine was poured as positive antifungal control while 30% DMSO was used as negative control. Than test tubes were wrapped with cotton plugs and aluminum foil and placed in incubator at 37^oC for 48 hours (Hemaiswarya *et al*., 2009). Results were expressed as positive or negative antifungal symbols (+ and – respectively) and inhibition was measured in terms of diameter of inhibition (mm).

Statistical analysis: All the collected data was subjected to Mean, Standard deviation and ANOVA by using Completely Randomized Design (One factor and 2 factor). More over LSD and DMRT was done to compare the results of leaves and seeds of *S. irio* with each other.

Results and Discussion

Plants are considered to be a rich source of phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections (Balandrin *et al*., 1985). Table 2 summarize the antibacterial activity of 7 different extracts of *S. irio* against 6 bacterial strains. Various extracts of leaves and seeds showed the inhibition against the bacterial strains while some did not show any activity. However, negative results do not indicate the absence bioactive constituents, nor the plant is inactive. Active compound (s) may be present in insufficient quantities in extract to show activity with the dose level employed (Taylor *et al*., 2001).

Antibacterial activity of polarity based extracts of leaves and seeds of *S. irio* were active to inhibit the growth. *n*-Hexane extract of leaves of *S. irio* inhibited the growth of *K. pneumonia* and *S. epidermidus.* While seed showed marked inhibition against *P. aeroginosa* and *S. epidermidus.* Ethyl acetate fraction of leaves was active against the bacterial strains of *E. coli*, *K. pneumonia* and *P. aeroginosa* and of seeds was active against the *K. pneumonia, S. aureus* and *S. epidermidus.* Methanolic leaf extract showed the best inhibition as compared to all other extract used in the study. Followed by ethanolic seed extract, water extract of leaves and seeds inhibited majority of the bacterial strains used in the study (Table 2).

Antibacterial potential of each extract used in the study was calculated in term of their zone of inhibition (mm) against each bacterial strain used. Leaves methanolic extract showed the best result among the all other extract used for *Sisymbrium irio.* Water extract for both leaves and seeds also displayed a remarkable inhibition after the methanolic extract. *n*-Hexane extracts of both leaves and seeds were not much active in comparison to all other tested extracts (Table 3).

Table 2. Antibacterial activity of *S irio* extracts.

Solvents	Plant parts	Gram negative				Gram positive	
		E. coli G	*E. coli*	*P. aeroginosa*	*K. pneumonia*	*S. epidermidus*	*S. aureus*
n-Hexane	Leaves	-	-	-	+	+	-
	Seeds	-	-	+	-	+	-
Ethyl acetate	Leaves	-	+	+	+	+	-
	Seeds	-	-	+	+	+	+
Chloroform	Leaves	-	+	-	+	-	-
	Seeds	-	+	+	+	+	+
Butanol	Leaves	+	+	+	+	-	-
	Seeds	+	-	+	+	+	-
Ethanol	Leaves	-	+	+	-	+	+
	Seeds	+	+	+	+	+	-
Methanol	Leaves	+	+	+	+	+	+
	Seeds	+	-	+	+	+	-
Water	Leaves	+	+	+	-	+	+
	Seeds	+	-	+	+	+	+

- = No inhibition + = Inhibition by plant extract

Table 3. Zone of inhibition (mm) of different solvents extracts of *S irio*.

Solvents	Plant parts	*E. coli*	*K. pneumonia*	*S. aureus*	*P. aeroginosa*	*S. epidermidus*	*E. coli* G
n-Hexane	Leaves		0.56± 0.51	-	-	0.74± 0.64	-
	Seeds	-	-	-	0.84±0.15	0.74±0.23	-
Ethyl acetate	Leaves	0.4± 0.69	0.57± 0.49	-	-	0.66± 0.57	-
	Seeds	-	0.67±0.57	0.70±0.63	0.84±0.21	0.6±0.1	-
Chloroform	Leaves	0.57± 0.98	1.1± 0.26	-	-	-	-
	Seeds	0.467±0.404	0.2±0.35	0.53±0.53	0.267±0.46	0.13±0.23	-
Butanol	Leaves	0.9± 0.26	0.64± 0.56	-	0.34± 0.57	-	0.2± 0.35
	Seeds	-	0.74±0.75	-	0.44±0.37	0.54±0.53	0.4±0.34
Ethanol	Leaves	0.57± 0.51	-	0.86±0.75	0.24± 0.41	0.9± 0.1	-
	Seeds	-	0.87±0.12	-	0.43±0.37	0.87±0.15	0.94±0.12
Methanol	Leaves	1.3± 0.17	0.57± 0.49	1.37±0.12	1.34± 0.32	1.134± 0.057	1.07± 0.12
	Seeds	0.267±0.42	0.6±0.53	-	1.1±0.36	0.54±0.50	0.9±0.17
Water	Leaves	1.2± 0.21	-	0.4± 0.69	1.4± 0.17	1.13± 0.15	0.6± 0.55
	Seeds	-	0.84±0.76	0.67±0.15	1.1±0.17	0.77±0.67	0.87±0.15

Methanolic extract showed best inhibition of *E. coli*, and *E. coli* G. with MIC value 15mg/ml and for *P. aeroginosa*, *S. epidermidus* and *S. aureus* with maximum zone of inhibition (Table 3) and MIC value of 7.5mg/ml for respectively (Table 4). Cholorform for *K. pneumonia*e with MIC value 15mg/ml (Table 4), Ethanol extract was for *K. pneumonia* with MIC 15mg/ml (Table 5).

Antifungal assay against *A. flavus* and *F. oxysporium* showed that all extracts of leaves except water extract, while among seed extracts, ethanolic, butanolic and chloroform extracts were inactive against *A. flavus* (Table 6). Similarly only ethyl acetate, methanol and water extracts of leaves and n- Hexane, ethyl acetate and ethanol extracts of seeds were active against *F. oxysporium* (Table 6). Many plants provide useful chemicals that can be used as antifungal or for suppression of other plants (Afridi & Khan, 2014; Afridi *et al*., 2014). Therefore the present results revealed that this plant should be explored for further research to be used for different pathogens.

These activities of *Sisymbrium irio* is accounted by the variety of phytochemical in its composition and especially the high content of flavonoids reported (Al-Jaber 2011; AL-Qudah & AbuZarga, 2010; Khan *et al*., 1991). These made this plant to combat the various infectious diseases including bacterial and fungal infections.

Table 4. Minimum Inhibitory Concentration (MIC) of leaves of *S irio*.

Strains	Solvents	15mg/ml	7.5mg/ml	3.75mg/ml	1.87mg/ml	0.93mg/ml	0.46mg/ml
E. coli	Methanol	+	-	-	-	-	-
	Water	+	-	-	-	-	-
P. aeroginosa	Methanol	-	-	+	-	-	-
	Water	-	+	-	-	-	-
S. epidermidus	Methanol	-	+	-	-	-	-
	Water	+	-	-	-	-	-
S. aureus	Methanol	-	+	-	-	-	-
K. pneumonia	Chloroform	+	-	-	-	-	-
E. coli G	Methanol	+	-	-	-	-	-

- = No inhibition, + = Inhibition by plant extract

Table 5. Minimum Inhibitory Concentration (MIC) of seeds of *S irio*.

Strains	Solvents	15mg/ml	7.5mg/ml	3.75mg/ml	1.87mg/ml	0.93mg/ml	0.46mg/ml
E. coli	Chloroform	-	-	+	-	-	-
	Methanol	-	+	-	-	-	-
P. aeroginosa	Methanol	-	+	-	-	-	-
	Water	-	+	-	-	-	-
S. epidermidus	Ethanol	-	+	-	-	-	-
S. aureus	Ethyl acetate	+	-	-	-	-	-
*K. pneumonia*e	Ethanol	+	-	-	-	-	-
E. coli G	Methanol	+	-	-	-	-	-

- = No inhibition, + = Inhibition by plant extract

Table. 6 Antifungal activity of leaves and seeds of *S irio*.

Solvents	Plant part	*A. flavus*	*F. oxysporium*
Positive control	Ampicillin	+	+
Negative control	30% DMSO	-	-
n-Hexane	Leaves	-	-
	Seeds	+	+
Ethyl acetate	Leaves	-	+
	Seeds	+	+
Chloroform	Leaves	-	-
	Seeds	+	-
Butanol	Leaves	-	-
	Seeds	+	-
Ethanol	Leaves	-	-
	Seeds	+	+
Methanol	Leaves	-	+
	Seeds	+	-
Water	Leaves	+	+
	Seeds	-	-

- = No inhibition, + = Inhibition by plant extract

Conclusion

The comparative screening of various extract of leaves and seed of *Sisymbrium irio* showed the clear picture of antibacterial as well as antifungal activities. It could be a promising source of new and effective antimicrobial to treat infections caused by the multi drug resistant strains of the microorganism. However, need of the day is to determine the toxicity of extracts used, their side effects and pharmaco-kinetic properties.

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