BIOACTIVIIES OF *SCORZONERA PARADOXA*FISCH & C.A. MEY. A MEDICINAL PLANT OF PUNJGUR, BALOCHISTAN, PAKISTAN

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غلاصہ

زیر تحقیق میں بلوچستان کے زلع پنجگور سے اکھٹی کی جانے والی خوردنی پودا *Scorzonera paradoxa* کا antibacterial, antifungal, antileishmanial, cytotoxicity, phytotoxicity and insecticidal کے خلاف طبی خصوصیات کے دو مختلف ذرائع یا ٹیکنیک Agar Well Diffusion اور Agar Tube Dilution کے تحت جانچ کی گئی۔ علاوہ ازیں پودے میں نمی اور راکھ کی مقدار بھی معلوم کی گئی۔ اس تجزیے میں دس بیکٹیریا اور آٹھ فنجائی کے علاوہ Lemna Aequinoctials اور Bine Shrimp استعمال کی گئیں۔ نتیجتا پودے میں تمام ٹیسٹ شدہ بیکٹیریا میں سے صرف ایک بیکٹیریا Salmonella Typhi میں 75% اور فنجائی میں *Trichophyton schoenleinii, T.simii, Fusarium oxysporum* and *F.solani* میں نمایاں خصوصیت پائی گئی سب سے زیادہ 84T, leinii فیصد مزاحمتی خصوصیت پائی گئی۔ پودے میں نمی کی مقدار 79 اور راکھ کی مقدار 21 فیصد پائی گئی۔ جبکہ Cytotoxic اور Phytotoxic خصوصیات نہیں پائی گئیں۔

Abstract

The present study is focused to determine antibacterial, antifungal, antileishmanial, cytotoxicity, phytotoxicity and insecticidal activity of medicinal plant *Scorzonera paradoxa*. The plant belongs to family Asteraceae collected from Punjgur, Balochistan. The plant is also studied for its ash and moisture content. Crude methanolic extract of the plant was tested against 10 bacterial and 08 fungal strains by using agar tube dilution method and agar well diffusion method respectively. Its toxicity is checked through Brine shrimp and *Lemna aequinoctialis* test using standard methods. The results shows 21% ash and 79% moisture content, the crude extract was significantly active against bacterial strain *Salmonella typhi* with 75% inhibition while it was inactive against other studied bacterial. Highly significant activity is observed against fungi *Trichophyton schoenleinii, T.simii, Fusarium oxysporum* and *F.solani*. Maximum inhibition 84% is achieved against *Trichophyton schoenleini*. Cytotoxicity and phytotoxicity are not observed.
Key words: *Scorzonera paradoxa*; Bioactivities; Antifungal; Panjgur, Balochisran.

Introduction

The plants have been utilized by human beings for the cure of many diseases; natural resources are providing an important contribution by producing metabolites that are effective against different diseases. Such plant metabolites are biologically active against some bacteria, fungi or other diseases. These are being used by the local people to treat diseases, this has been validated by various researchers it could replace the synthetically prepared medicines (Mahesh and Satish, 2008). Multidrug resistant microorganisms are responsible for a large number of deaths of human as well as animals.Therefore, there is a need to find out new resources to explore plants potential. *Scorzonera paradoxa* Fisch & Mey, commonly known as Putrunk in Balochi belongs to family Asteraceae.It is collected from Punjgur, Balochistan, Pakistan. It was found growing in spring season from March-May, 2018 usually after rainfall.

The genus Scorzonera L. representing the Cichorieae tribe and Asteraceae family comprising approximately one hundred and eighty species throughout the world (Shih and Kilian, 2011). It is reported from Mediterranean, central Asia and central Europe (Bermer 1994; Shih and Kilian, 2011). The genus Scorzonera remained controversial due to its poor details of floristic characteristics and taxonomic complexities.

Scorzonera paradoxa is a herbaceous plant with fleshy stem that is edible, the cooked plant taste is good when used as vegetable. All parts of the plant are used traditionally as medicine or as salad. However no comprehensive research has been conducted on this plant. The only paper so far published has reported its phenolic compounds, flavonoids, tannins and fatty acid composition. The research proved that the leaves contained more phenolic compounds than the roots. The root extracts showed high DPPH scavenging activity than the leaves. In addition, oleic arachidonic acid was found abundant in leaves while roots contain higher amount of stearic and linoleic acid (Nasseri *et al.,* 2014). Therefore, there was a need to check its efficacy as antibacterial, antifungal, anti-leishmanial and DPPH activity.

Materials and Methods

Plant material: Collection of 5kg of fresh plants of *Scorzonera paradoxa* was done from deserted area of district Panjgur during March-April, 2018. These were dried in dark (to increase its metabolites) at room temperature for ten days. Finally, the dried plant parts were ground with the help of electric grinder and was soaked in methanol for 20 days. The methanolic extract was evaporated through rotary evaporator it yielded 52gms of crude extract.

Biological Activities: The crude extract of *Scorzonera paradoxa* was checked for its efficacy and safety, Cytotoxicity bioassay was carried out by using the methods given by Meyer *et al*., (1982). Phytotoxicity, antibacterial, antifungal, insecticidal activities were carried out by following the methods described by Atta-ur-Rehman *et al*., (1991).The antifungal activity against 08 fungi was carried out by Agar Tube Dilution method. Antibacterial activity was checked against 10 bacteria by using agar Well Diffusion method. Antileishmanial activity was conducted by methods described by (Fournet *et al*., 1994) against *Leishmaniasis major* it is responsible for cutaneous leishmaniasis which is common in Balochistan.

Result and Discussion

Table 1. Ash and moisture (g % of Dry Weight)

S.No	Test sample	Ash	Moisture
1	*Scorzonera paradoxa*	21	79

Table 2. Brine shrimp cytotoxicity Bioassay of crude MEOH extract

S.No.	Dose µg/ml	No. of Shrimps	No. of survivors
1	1000	10 10 10	6 6 10
2	100	10 10 10	10 8 10
3	10	10 10 10	10 10 10

Table 3. Phytoxicity test of crude MeOH extract

S.No.	Name and no. of plants	Dose µg/ml	No. of fronds Sample	Negative Control No. of fronds	Growth regulation %	Positive control Paraquat No. of fronds	Growth inhibition %
1	*Lemna aequinoctialis* 20	500	12 15 14	20 20 20	41	0 0 0	100
2		50	16 16 18	20 20 20	50	0 0 0	100
3		05	15 15 16	20	53	0 0 0	100

Table 4. DPPH Scavenging Activity

Sample	Conc.µg/ml	% Inhibition	IC50±SE
S. paradoxa	0.5	69.3	376.9± 1.1
Std. Gallic acid	0.5	91.8	3.2± 0.1
Std. N-acetyl-L-Cystein	0.5	91.8	1.4± 0.1

No significant activity

Table 5. Antibacterial Assay of the crude MeOH extract

S.No.	Bacteria	Dose 200µg/100ml Crude extract	Zone of inhibition (mm)	Standard Antibiotics Zone of inhibition (mm)		*P* value
				Ampicillin	Tetracycline	
1	*Bacillus cereus*	MeOH	05	24	17	0.023*
2	*Corynebacterium diptheriae*	MeOH	02	28	30	0.004*
3	*Escherichia coli*	MeOH	14	40	30	0.001**
4	*Klebsiella pneumonia*	MeOH	04	32	30	0.032*
5	*Proteus mirabilis*	MeOH	02	24	22	0.032*
6	*Pseudomonas aeruginosa*	MeOH	12	16	17	0.042**
7	*Salmonella typhi*	MeOH	25	30	27	0.000***
8	*Shigellaboydii*	MeOH	04	35	22	0.000***
9	*Staphylococcus aureus*	MeOH	06	26	35	0.004*
10	*Streptococcus spp*	MeOH	03	40	16	0.004*
	P value		0.000***	0.023*	0.034*	

Table 6. Antifungal Activity of methanolic extract

S.No	Fungi	Linear Growth (in mm)			Inhibition %	Std. drug	Inhibition %
		Sample	Control	Confidence level (T-test)			
1	*Trichophyton schoenleinii*	59	70	***	84	Miconazol Ketoconazole	95 95
2	*Pseudallescheria boydii*	29	55	**	52	Miconazole Ketoconazole	90 90
3	*Candida albicans*	75	75	NS	0	Miconazole Ketoconazole	100 100
4	*Microsporum canis*	20	40	**	50	Miconazole Ketoconazole	100 100
5	*Trichophyton simii*	35	45	***	77.7	Miconazole Ketoconazole	100 100
6	*Fusarium oxysporum*	60	75	***	80	Miconazole Ketoconazole	100 100
7	*Fusarium solani*	43	60	***	71	Miconazole Ketoconazole	100 100
8	*Macrophomina phaseolina*	28	50	**	56	Miconazole Ketoconazole	100 100

Table 7. Anti-leishmanial Activity of crude extract

S.No	Test organism	Std. drug	Inhibition %	IC_{50}	Test sample	Inhibition %
1	*Leishmanisis major*	Amphotericin-B	100	0.8µg/ml	Crude MeOH	0

Experiment was repeated 03 times; No activity

Table 8. Insecticidal Activity of crude extract

S.No.	Samples	1	2	3	4
	Pests used	Triboliumc astaneum	*Sitophilus Oryzae*	*Rhyzopertha dominica*	*Trogoderma granarium*
1	No. of pests	10	10	10	10
2	Dose µg/cm^2	1571.33	1571.33	1571.33	1571.33
3	water % Negative Control	0	0	0	0
4	MeOH extract %	0	0	0	0
5	Survival no.	10	10	10	10
6	Std. insecticide (coopex)% (+) Control	100	100	100	100

Experiment was repeated 03 times; No activity

Results and Discussion

The plant *Scorzonera paradoxa* was studied to check its moisture, ash content and biological activities for the first time. Each value is the mean of three replicate; **significant and ***highly significant at 0.05 significant level.The moisture content was 79% with 21% ash content was recorded (Table No.1). The crude methanolic extract of *S.paradoxa* was not cytotoxic as brine shrimp cytotoxicity test showed no significant activity at any concentration (Table 2). However, it showed slight toxicity at very high concentration i.e. 1000µg/ml ($LD_{50\ \mu g/ml;}$ 4555.7020). The Phytotoxicity of the crude extract of *S. paradoxa* when checked through *Lemna aecquinoctialis* was also found to be non-toxic at all doses. Mild toxicity was shown at very high concentration i.e. 500µg/ml (Table 3). Therefore *S. paradoxa* being non toxic is safely recommended as food as compare to other medicinal plants commonly used have high phytotoxicity such as *Teucrium stocksianum, Chenopodium botrys* (Rauf *et al*., 2017).

No significant DPPH radical scavenging activity was found in *S. paradoxa* (Table 4) as compared to Rezaeian *et al.,* (2015) where medicinal plant *Berberis integrrima* showed highest DPPH inhibition with ($P<0.05$).The methanolic crude exract of *S. paradoxa* was also tested for antibacterial activity against ten different strains of bacteria including two gram +ve and eight gram-ve (Table 5). Results showed that it was significantly active against *S. typhi* and showed some activity against *E.coli* and *Pseudomonas aeroginosa*. However the crude extract was not active against these bacteria *Shigellaboydii, Bacillus cereus, Corynebacterium diphtheria, Escherichia coli, Proteus mirabilis, Pseudomonas aeroginosa, Staphyloccocus aureus, Streptoccocus sps*. It is concluded that it showed significantly high activity against an important bacterium *S. typhi* which is a dangerous typhoid causing bacteria. This might be the reason this plant is used as a remedy for fever. This activity was higher than that reported by Walter. *et al*., (2011) who worked on five important medicinal plant i,e. *Hyssopus latifolia, Mentha piperita, Glycyrrhiza glabra, Althaea officinalis* and *Justica adhatod* for their bioactivity against gram negative and gram positive bacteria.

The antifungal activity of crude methanolic extract of *S. paradoxa* (Table 6) showed highly significant activity *Trichophyton schoenleinii, T.simii, Fusarium oxysporum* and *F.solani.* Maximum inhibition 84% was achieved against *Trichophyton schoenleinii*. However significant activity was shown against *Macrophomina phaseolina* (plant pathogens), *Microsporum canis* and *Pseudallescheria boydii* (human pathogen). The crude extract exhibited no inhibition against a human pathogen *Candida albicans*. Similar results were reported by Ahmad *et al*., (2010), he found good activity while working on antifungal active constituents from legume seeds extract against six fungi species viz, *Microsporum canis, Candida albicans, Aspergillus* and *candida glabrata, Fusarium solani, Trychophyton longifusus.* This antifungal activity of *S. paradoxa* is very important as there are only few antifungal drugs available in the market and fungal infections are spreading fast and are becoming resistant to anti-fungal drugs. Therefore there is a need to find out new sources of antifungal drugs. The plant did not show any activity against *Leishmaniasis major (*Table 7). *Scorzonera paradoxa* did not show any insecticidal activity (Table 8) against different types of insects, checked at 157.33mg/cm^2dose of test sample. No activity was shown against all pests tested. Therefore, it is concluded that *S. paradoxa* is a safe plant that can be used as vegetable it is also effective bacterial infections caused by *Salmolnella typhi*. It is highly effective against a number of fungi tested. This justifies its use as medicinal plant by the local community. It has no toxicity so can be used safely as vegetable.

Conclusion

This study presented the efficacy of the plant *S. paradoxa* which actively found with significant inhibition zone against bacterial strain *Salmonella typhi* with 75% inhibition. Crude extract of the plant showed highly effectiveness against fungi *Trichophyton schoenleinii, T. simii, Fusarium oxysporum* and *F.solan.* Maximum inhibition zone 84% found against *Trichophyton schoenleinii* In addition, the plant showed 21% ash and 79% moisture content.

It is concluded that the plant *S. paradoxa* can be used medicinally against these bacteria and fungal strains but it needs `exploratory work for better result.

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