

Antioxidants, antimicrobial and cytotoxic potential of *Swertia chirata*.

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Abstract

The current study was designed to evaluate the properties of various fractions of methanolic extract prepared from aerial parts of *Swertia chirata*. Five fractions, RA-78, RA-91, RA-112, RA-14 and RA-15 showed significantly high DPPH scavenging capacity. In finding out antimicrobial activities, different fractions have shown mild activities against *Bordetella bronchiseptica* and *Micrococcus luteus*, while all fractions showed significant results against *Aspergillus fumigatus* followed by *Mucor* species and five fractions were active against *Aspergillus niger*. In measurement of brine shrimp cytotoxic potential of different fractions, three fractions RA-12, RA-34 and RA-78 had shown the best cytotoxic results with zero LD₅₀. In determining antileishmanial properties of different fractions, the best results were exhibited by fraction RA-78 and RA-112 with zero percent survival of *Leishmania* parasite. Various fractions of methanolic extract prepared from aerial parts of *Swertia chirata* promisingly possess antioxidant, antimicrobial, brine shrimp cytotoxic, and antileishmanial activities.

Keywords: *Swertia chirata*, Phytochemical evaluation, DPPH free radical scavenging activity, Antimicrobial properties, Cytotoxicity assay, Antileishmanial activity.

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Introduction

Medicinal plants are as assumed as major source for active constituents which are used for development of drugs. There are about half million plants around the world, and most of their medicinal activities have not explored yet [1]. According to WHO, about 80% of world population relies on plant based conventional medicines especially for their primary health care need [2]. It was a common perception on the basis of tradition, that there is no harm in the uses of natural medicinal plants [3].

The medicinal plant *Swertia chirata* (Roxb.) Buch-Ham. ex C. B. Clarke belongs to the family gentianaceae. Its folk use names are Chirata, Chareta, and chirayta. It is being used in different remedies as antipyretic and anthelmintic [4] and in arthritis [5]. It is also used for gastrointestinal and urogenital tract disorders [4]. This plant is also being used traditionally for treatment of cancer [6].

The objectives of present study were to prepare crude methanolic extract and its different fractionations obtained from the aerial parts of *Swertia chirata* and evaluate their phytochemical, antioxidant, antimicrobial, cytotoxic, and antileishmanial potential.

Materials and Methods

Extract preparation and its fractionation

Swertia chirata plant material was collected from Galyat region of Khyber Pakhtunkhwa, Pakistan and identified by the taxonomist and verified by comparing with available literature and herbarium specimens. The specimen was deposited in the herbarium and given the voucher number "PAC1009".

The aerial parts of the plant material were shade dried, crushed into fine powder. The powdered plant material (4.3 kg) was macerated in methanol (10 L) for 10 d. Methanolic extract was

filtered through muslin cloth and filter paper and dried under reduced pressure for further analysis.

Fractionation of methanolic extract (RA-16) was carried out by using gravity column chromatography. For column packing, slurry of silica gel (400 g) prepared in chloroform was poured into it, chloroform was further added with continuous tapping to develop the column and methanolic extract was applied slowly on the top. The solvent systems of 5% CH₃OH/AHCl₃-10%, 15%, and 20% CH₃OH/AHCl₃ were run and different fractions were collected, labeled as RA-12, RA34, RA-56, RA-78, RA-91, RA-112, RA-13, RA-14, and RA-15.

Total phenolic and flavonoid contents

The procedure used to determine the total phenolic contents, using Folin-Ciocalteu reagent [7]. The absorbance of reaction mixture was recorded at 715 nm. Gallic acid was used as standard and total phenolic contents were express as gallic acid equivalent. In addition, the method utilized for evaluation of total flavonoid contents was aluminium chloride colorimetric method [8]. The absorbance of the reaction mixture was taken at 415 nm. Quercetin was used as a standard and flavonoid contents were expressed as quercetin equivalent. The experiments were repeated in triplicate.

DPPH radical-scavenging activity

The antioxidant activities of crude methanolic extract and its fractions were determined by using DPPH free radical scavenging assay, as described previously [9]. The absorbance was taken at 517 nm by micro plate reader and scavenging percentage was calculated. The experiment was performed in triplicate. Ascorbic acid was used as a positive control, whereas methanol was used as a negative control.

Antimicrobial assay

The antibacterial activity of each fraction was determined by using disc diffusion method [10] using three strains of bacteria. One strain *Micrococcus luteus* was gram-positive while two strains *Bordetella bronchiseptica* and *Salmonella typhi* were gram-negative. The inoculum of each bacterium was spread smoothly on surface of sterilized solidified nutrient agar media present in the petri plate. Then 5 µl of each fraction was loaded on autoclaved filtered paper discs and placed on the respective places in the petri plates. Each plate contained two disc of positive and one disc of negative control. The plates were incubated at 37°C for about 24 h. The zone of inhibition (mm) was measured and assay was performed in duplicate.

The antifungal activity of each plant fraction was evaluated by using disc diffusion method [11]. Three different fungal strains were used, which were *Mucor* specie, *Aspergillus fumigates*, and *Aspergillus niger*. After solidification of sterilized sabouraud dextrose agar media in the petri plate, inoculum of each fungus was swabbed on its surface to produce the lawn. Then 5 µl of each fraction was loaded on filter paper disc and each disc was placed on its respective place in labeled plate. Each plate contains two disc of positive and one disc of

negative control. The plates were then incubated at 28°C for 24 h. After incubation, zones of inhibition were measured and experiment was performed in triplicate.

Brine shrimp lethality assay

The cytotoxic effects of all fractions were measured by using brine shrimp lethality bioassay [8]. Three concentrations of each fraction (1000 µg/ml, 100 µg/ml, and 10 µg/ml) and three concentrations of standard drug were prepared (10 µg/ml, 1 µg/ml, and 0.1 µg/ml). 96 plate was used for this assay and 250 µl of sea water was added to the well so that the sample was dissolved in the sea water. Then 10 shrimps (*Artemisa salina*) were transferred to each well. The volume of the wells was raised up to 300 µl to attain desired concentration of each well. The plates were incubated for 24 h at room temperature. Then shrimps were taken from each well and counted the survivors under magnifying glass. The lethal dose of crude fractions against brine shrimps was calculated by using Finny software.

Antileishmanial assay

Pre-established *Leishmania tropica* KWH23 strain was incubated at 24 ± 1°C for 6-7 d in 199 medium containing 10% fetal bovine serum. The *in vitro* antileishmanial assay was performed according to the protocol previously described previously [12] with a slight modification. Stock solution of each fraction (4,000 ppm) was prepared by dissolving 4 mg in 1000 µL of distilled water. The stock solutions were serially diluted in 96 well plates. Positive and negative control was Amphotericin B and distilled water, respectively. The microtiter plates were incubated at 24°C for 72 h. The experiment was performed in triplicate. After 72 h, about 15 µl of test culture were then transferred to improved Neubauer counting chamber and live promastigotes were counted under light microscope. For LC₅₀ calculation, probit regression analysis of SPSS Ver. 21 was used.

Results and Discussion

Total phenolic and flavonoid contents

According to results of fractions and crude methanolic extract of *Swertia chirata*, high total phenolic contents were found in four fractions, RA-15, RA-13, RA-34 and RA-14 (8.61 ± 0.08, 7.92 ± 0.07, 7.16 ± 0.51 and 7.16 ± 0.06 µg EQ/mg respectively) (Figure 1). Similar range of total phenolic contents was found in *Polygonatum* species [13]. Moreover, these concentrations may depend on variation in collection time and site [14]. High total flavonoid contents (Figure 1) were found in three fractions, RA-56, RA-78 and RA-34 (232.5 ± 7.4, 118.855 ± 3.5 and 93.185 ± 3.2 µg EQ/mg, respectively). The highest total flavonoid contents were found in the fraction RA-56 followed by RA-78 and RA-34. These concentrations of total flavonoid contents present in the plant were higher than that present in *Torilis leptophylla* [15].

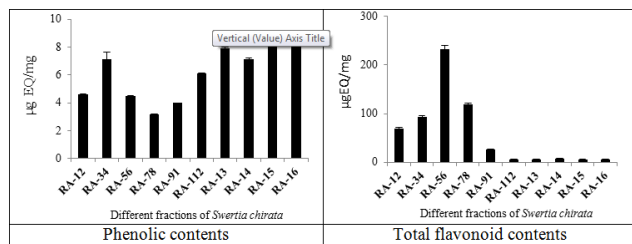


Figure 1. Phytochemical analysis of *Swertia chirata*.

DPPH radical-scavenging activity

All fractions showed statistically significant (P<0.05) DPPH scavenging capacities (%). DPPH scavenging activity (Figure 2) of five fractions RA-78, RA-91, RA-112, RA-14, and RA-15 had shown significantly (P<0.05) high scavenging potential (74.3 ± 3.4 %, 85.77 ± 2.49 %, 77.17 ± 2.1 %, 85.29 ± 2.61 % and 78.81 ± 2.1 %, respectively). RA-91 fraction showed 10.4% more potential with respect to extract, showing significantly (P<0.05) higher antioxidant potential. As compared to DPPH results of *Thymus serpyllum*, it was found that methanolic extract of plant showed better antioxidant results than the aqueous and n-hexane plant extract [16].

Table 1. Antibacterial and antifungal activity of crude extract and its fractions.

Sample code	Zone of inhibition (mm)					
	<i>S. typhi</i>	<i>M. luteus</i>	<i>B. septica</i>	<i>A. niger</i>	<i>Mucor Sp.</i>	<i>A. fumigatus</i>
RA-12	0	0	6.5	0	14 ± 1.41	13 ± 1.41
RA-34	0	0	6	0	10.5 ± 0.71	10 ± 2.83
RA-56	0	0	6	0	16 ± 1.41	11 ± 1.41
RA-78	0	0	6	7 ± 0.15	13 ± 1.41	7 ± 0
RA-91	0	0	6	7 ± 0.17	14.25 ± 3.18	10 ± 0
RA-112	0	6	6	7 ± 0.19	9 ± 1.41	6 ± 0
RA-13	0	6	6	7 ± 0.21	15 ± 4.24	9 ± 1.41
RA-14	0	6	6	7 ± 0.21	15.5 ± 0	21 ± 1.41
RA-15	0	7	6	0	10 ± 0	8 ± 2.83
RA-16	0	6	6	0	10.5 ± 4.95	14.5 ± 0.71
Control (Cefotaxime/terbinafine)	23	21	17	33 ± 1.52	30 ± 1.0	30 ± 1.52

Brine shrimp lethality assay

In brine shrimp lethality assay, LD₅₀ value lower than 1000 µg/ml is considered cytotoxic [18]. Three fractions RA-12, RA-34 and RA-78 showed the best cytotoxic results with zero LD₅₀. The other significant results were obtained by the fraction RA-13 with 16.54 µg/ml LD₅₀ and by fraction RA-112 with 17.37 µg/ml LD₅₀ (Table 2). On correlation of our work with brine shrimp lethality of *Artemisia dubia* extract, our all fractions showed encouraging cytotoxic activities [8].

Table 2. Results of brine shrimp lethality assay.

Antimicrobial assay

All fractions did not show any activity (Table 1) against *Salmonella typhimurium*. However, RA-112, RA-13, RA-14, RA-15 and RA-16 were active against *Micrococcus luteus*. The encouraging activities were shown by all fractions against *Bordetella bronchiseptica*. These results are contrary to the results in which methanolic extract prepared from bark, fruits and leaves of *Ficus microcarpa* was used and high antibacterial activity was reported [17].

Five fractions (RA-78, RA-91, RA-112, RA-13, and RA-14) were slight active against *Aspergillus niger* (Table 1). However, all fractions have shown significantly (P<0.05) results against *Mucor* species where RA-56 showed highest inhibition followed by RA-91. Comparative fungal growth inhibition cause by all fractions against *Aspergillus fumigatus* was highest. Fraction RA-14 showed highest growth inhibition in the present antifungal study. Our results are better than those published in another study performed previously [16].

Sample code	Brine shrimp lethality assay (percentage death)			
	1000 µg/ml	500 µg/ml	250 µg/ml	LD ₅₀ µg/ml
RA-12	43	35	23	0
RA-34	23	20	10	0
RA-56	53	33	13	901.2
RA-78	33	25	15	0
RA-91	60	56	53	92.91
RA-112	96	83	73	17.37

RA-13	96	86	77	16.54
RA-14	86	80	75	69.5
RA-15	100	70	56	144.94
RA-16	100	73	50	249.52
Doxorubicin	100	85	70	1.98

Antileishmanial activity

All fractions were also subjected to antileishmanial activities and found positive against leishmaniasis disease (Figure 3). The best results were shown by fraction RA-78 and RA-112 with 0% survival of *Leishmania* parasite. The second best results were shown by fractions RA-16 and RA-91 by 12% survival rate. Amphotericin-B used as a standard drug against *Leishmania* protozoa. The results of current study were quite better than the previous work done by *in vitro* assessment of antcutaneous leishmaniasis activity of Sudanese plants [19,20].

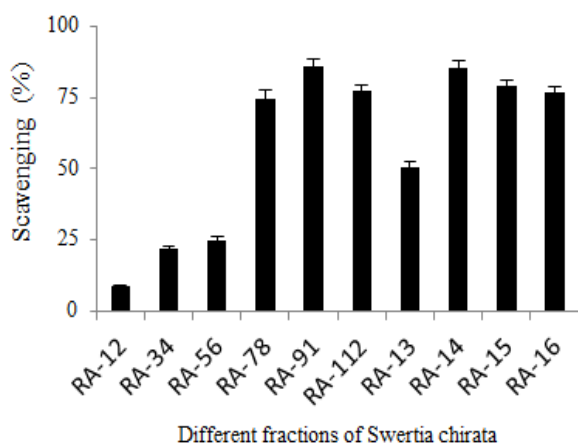


Figure 2. DPPH scavenging assay of various fractions of crude methanolic extract.

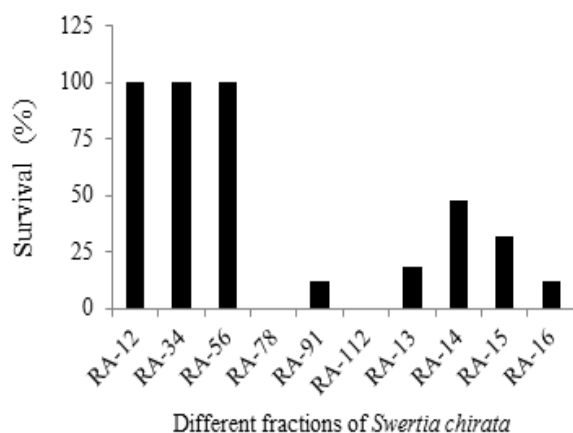


Figure 3. Antileishmanial activity of different fraction of *Swertia chirata*.

Conclusion

It can be concluded that various fractions of methanolic extract prepared from the aerial parts of *Swertia chirata* promisingly possess various chemical and biological properties including antioxidant, antimicrobial, brine shrimp cytotoxic, and antileishmanial activities.

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