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PHYTOCHEMICAL ASSESSMENT OF *IPHIONA AUCHERI* (BIOSS.) ANDERB. AND ITS CYTOTOXIC, ANTIOXIDANT AND ANTIDIABETIC ACTIVITIES

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Abstract

The presence of phytochemical constituents and estimation of total phenolic contents in *Iphiona aucheri* (Bioss.) Anderb. stem and assessment of their cytotoxic, total antioxidant and anti-diabetic activity were investigated. All fractions were assessed for phytochemicals, cytotoxic activity, total phenolic contents, antioxidant and anti-diabetic characteristics. Saponins, glycosides, protein and amino acids, carbohydrates and flavonoids were found in aqueous fraction and methanolic extract while they were absent in *n*-hexane fraction except glycosides and protein. The crude methanolic extract (70.3 ± 1.9%) revealed highest brine shrimp mortalities. Except *n*-hexane fraction others indicated considerable antioxidant activities via DPPH, $ABTS^{\bullet+}$ and H_2O_2 assays. Crude methanolic extract expressed higher inhibition of α-amylase (60.71 ± 0.89%) than glucophage (54.92 ± 0.56%). Non significant correlation of total phenolic contents with percentage antioxidant and anti-diabetic activities of crude methanolic extract and its various fractions was observed in all cases.

Introduction

Plants having medicinal properties have been used long ago for the treatment of various diseases. Virtually the presence of thousands of bioactive constituents, generally known as phytochemicals in these plants are responsible for their therapeutic characteristics (Afanas'ev 2010). The phytochemicals may be present in fruit, seeds, flowers, leaves, stems, roots and bark (Gurib-Fakim 2006).

Iphiona aucheri belonging to Asteraceae is scattered in Pakistan, Oman, North-East Africa, the Arabian Peninsula and Iran (Anderberg 1985). In Pakistan, it is distributed in Khuzdar, Chaghi, Mekran, Lasbela, and Loralai districts (Kakar *et al*. 2012). Previously, antibacterial activities of *Iphiona aucheri* against *S. aureus*, *E. coli*, *S. pyogenes* and *K. ponumoniae* were reported (Kakar *et al.* 2012). The minerals, namely Na, K, Ca, Fe and Ni were reported in *Iphiona aucheri* while Al was found absent (Lanjwani *et al.* 2016).

The aim of this study was is to carry out the phytochemical assessment and estimation of total phenolic contents, cytotoxic, antioxidant and antidiabetic properties of methanolic extract and its *n*-hexane, chloroform and aqueous fractions of *Iphiona aucheri* stem by using different standard assays. This investigation will provide the base for isolation and purification of natural therapeutic compounds for the treatment of cancer, diabetics and oxidative stress-related diseases in the future.

Materials and Methods

Stems of *Iphiona aucheri* (Bioss.) Anderb. (AR-123) were collected in March, 2017 from district, Bannu, Pakistan. Its taxonomic study was carried out by Prof. Abdur Rehman, Govt. Postgraduate College Bannu, Khyber Pakhtoon Khawa (KPK) Pakistan.

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The collected stems were washed with tap water, dried under shade and pulverized into fine powder by using pestle and mortar. A 500 g powder was extracted in 70% methanol (1.5 liter), evaporated at room temperature and finally was stored 14.37 g of extract in falcon tube for further use. The said extract (10 g) was sequentially extracted at room temperature with *n*-hexane, chloroform and aqueous (300 ml each) using separating funnel to elude any kind of damages to the filtrate. The resultant extract of each solvent; *n*-hexane (1.3 g), chloroform (2.19 g) and water (4.98 g) was stored for further use.

Standard methods *viz.*, foam test, ferric chlorides, alkaline reagent test, Millon's test and Fehling test were employed to conduct phytochemicals screening of methanolic extract of *Iphiona aucheri* stems and its different fractions to investigate the presence of saponins, flavonoids, protein and amino acids, glycosides and carbohydrates.

Brine shrimp lethality bioassay was opted to find out the cytotoxic activity of methanolic extract of *Iphiona aucheri* stems and its different fractions with the help of Meyer protocols (Meyer *et al.* 1982).

Total phenolic contents contained in the plant extract and its various fractions was determined by using Folin-Ciocalteu reagents following the method of Singleton and Rossi (1965).

Antioxidant assays indirectly measure the ability of compounds to interrupt free radicals by scavenging or trapping. Methanolic crude extract of *Iphiona aucheri* stem and its various fractions were subjected to DPPH, ABTS and H_2O_2 free radicals scavenging assays to analyze their antioxidant activities by engaging the free radicals. DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging assay is a very refined method for estimation of *in vitro* antioxidant activity.

The DPPH, ABTS and H_2O_2 free scavenging by plant extract were determined according to protocol of Gyamfi *et al.* (1999), Re *et al*. (1999), Wettasinghe and Shahidi (2000).

The potential of the samples to scavenge the free radicals was calculated by using the following equation:

$\%$ inhibition $= (A_1 - A_2/A_1) \times 100$

where, A_1 = The absorbance of the control and A_2 = The absorbance of the samples.

The α-amylase inhibitory activities of methanolic extract and its different fractions were determined through Worthington Enzyme Manual (Kwon *et al.* 2007) protocol.

Amylase inhibition (%) = Control absorbance (blank) – Sample absorbance/control absorbance × 100.

Statistical analysis was carried out through GraphPad Prism software.

Results and Discussion

Phytochemical screening of *Iphiona aucheri* stem crude methanolic extract and its various fractions revealed the presence of saponins, glycosides, protein, amino acids, carbohydrates and flavonoids in aqueous fraction and methanolic extract while they were absent in *n*-hexane fraction except glycosides and protein. Glycosides, protein and amino acids and flavonoids were also found in chloroform fraction while saponins and carbohydrates were not found. The results are presented in Table 1. Further study of the desired plant extract depends on the phytochemicals present in the plant extract. In the recent era cancer is an emerging group of life treating diseases. The side effects of synthetic commercially available medicines have boosted the search of phytochemicals having cytotoxic/anti-cancer properties. In this experiment, the highest cytotoxic properties expressed by crude methanolic extract (70.3 ± 1.9%) while lowest by *n*-hexane fraction (30.8 ± 1.2%) at a concentration of 1000 µg/ml in brine shrimp lethality bioassay. The percentage mortalities of shrimp larvae of different applied samples are shown in Table 2. Similar results

were observed during cytotoxic activities of *Hapllophyllum tuberculatum* (Al-Muniri and Hossain 2017) and *Coccinia grandis* L. (Laboni *et al.* 2017). This investigation uncovered the fact that the *Iphiona aucheri* stem extract possesses phytochemical components having cytotoxic properties.

The *n*-hexane (9.18 ± 1.92 mg GAE/g), an aqueous fraction (9.81 ± 1.69) and crude methanolic extract (16.09 ± 1.52) had lesser phenolic contents when compared with chloroform fraction (21.30 ± 2.04 mg GAE/g). The results are comparable with earlier reports of total phenolic contents of plant extracts of *Salvia officinalis* (7.78 ± 0.0041), *Teucrium polium* (8.29 ± 0.0064), *Ocimum basilicum* (13.1 ± 0.021), *Mentha pulegium* (16.34 ± 0.011), *Agaricus campestris* (18.96 ± 0.0079), *Thymus algeriensis* (18.73 ± 4.59), *Mentha spicata* (19.65 ± 0.001), *Hedysarum scoparium* (26.71 ± 0.15) (Roukia *et al.* (2013), Soumia *et al.* (2014). The results ensure that *Iphiona aucheri* stem is a potent source of phenolic compounds.

Table 1. Phytochemical screening of methanolic extract, chloroform and aqueous fractions of *Iphiona aucheri* stems.

Sl. no.	Phytochemicals and tests		Crude methanolic exract and its fractions			
	Phytochemical	Tests	Aqueous fraction	Methanolic fraction	Chloroform fraction	*n*-hexane fraction
1	Saponins	Foam test	+	+	-	-
2	Flavonoids	Alkaline reagent test	+	+	+	-
3	Glycosides	Fehling	+	+	+	+
4	Carbohydrates	Milisch's test	+	+	-	_
5	Protein and amino acids	Biuret test	+	+	+	+

Table 2. Percentage lethality of brine shrimps caused by crude methanolic extract and its various fractions of *Iphiona aucheri* stems.

Conc. (µg/ml)	Crude methanolic extract and its fractions (%) lethality				Control
	Methanolic extract	Chloroform fraction	Aqueous fraction	*n*-Hexane fraction	
100	30.5 ± 1.7	20.2 ± 1.3	30.6 ± 1.7	10.6 ± 1.9	00
250	40.2 ± 1.3	40.4 ± 1.6	40.4 ± 1.1	10.3 ± 1.6	00
500	60.7 ± 1.4	50.1 ± 1.5	50.9 ± 1.3	20.7 ± 1.8	10.3 ± 1.1
1000	70.3 ± 1.9	50.5 ± 1.1	60.7 ± 1.6	30.8 ± 1.2	00

Table 3. Phenolic content (mg/g gallic acid equivalent) of crude methanolic extract and its various fractions of *Iphiona aucheri* stems.

Extracts	Methanolic extract	Chloroform fraction	Aqueous fraction	*n*-Hexane fraction
Total phenolic contents	16.09 ± 1.52	21.30 ± 2.04	9.81 ± 1.69	9.18 ± 1.92

The highest antioxidant activity (76.68%) was revealed by crude methanolic extract followed by an aqueous fraction (62.93%) while *n*-hexane expressed lowest antioxidant activity (23.55%) at the concentrations of 2 mg/ml. Antioxidant activity of ascorbic acid recorded was 80.43% at the same concentration. Fig. 1 indicates antioxidant properties of the mentioned standard and extracts

samples. The results showed that the antioxidant activities of the *Iphiona aucheri* stem methanolic extract and its different fractions are concentration dependent. Similar results were observed in previous studies of *Leucas aspera* (Willd) link (Annapandian and Rajagopal 2017) and *Pogostemon* species (Kaliyappan *et al.* 2017).

In ABTS radical cation assay, it was observed that the methanolic extract exhibited maximum antioxidant activity (80.52%) followed by *n*-hexane (54.65%), aqueous (47.43%) and chloroform fractions (45.11%, Fig. 2)

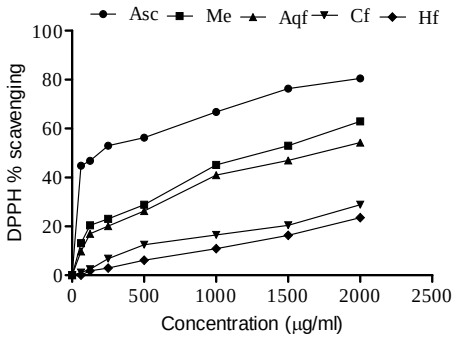

Fig. 1. DPPH free radical scavenging capability of *Iphiona aucheri* stem's methanolic extract and its fractions. Cf : Chloroform fraction, Me : Methanolic extract, Aqf: Aqueous fraction, Hf : *n*-Hexane fraction and Asc: Ascorbic acid.

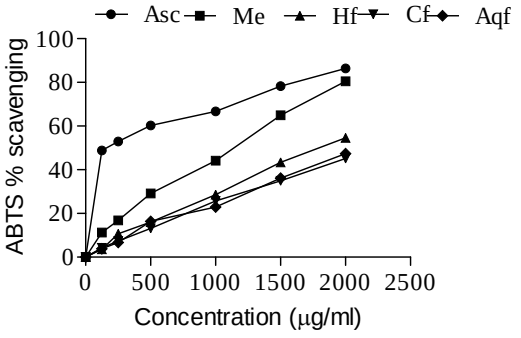

Fig. 2. ABTS free radical scavenging capability of *Iphiona aucheri* stem's methanolic extract and its fractions. Cf : Chloroform fraction, Me : Methanolic extract, Aqf : Aqueous fraction, Hf : *n*-Hexane fraction and Asc : Ascorbic acid.

Hydrogen peroxide (H_2O_2) is transformed into more toxic hydroxyl radical in the presence of transition metal and can cause cellular damages. The generation of singlet oxygen by reacting with hypochlorous acid (HOCl) or with superoxide anion or chloramines in living systems makes it more reactive and deteriorative (Karadag *et al.* 2009). Methanol extract and its water, chloroform and *n*-hexane fractions expressed 32.85, 31.55, 28.06 and 19.16% scavenging activity, respectively on hydrogen peroxide at the concentration of 2000 µg/ml whereas ascorbic acid showed 61.04% scavenging activity at the same concentration (Fig. 3). Previous studies have also uncovered the connection between the scavenging characteristic of plant extracts and phytochemicals, such as flavonoids and total phenolics (Tiwari *et al*. 2014)**.**

The assessment of anti-diabetic activities of crude methanolic extract and its various fractions of *Iphiona aucheri* stems was carried out by α-amylase enzyme inhibition assay. Results are presented in Fig. 4. The said property of glucophage (commercially available medicine, used as a standard) methanolic extract and aqueous fraction were recorded 54.92, 60.71 and 32.70%, respectively. Its chloroform and *n*-hexane fractions did not show anti-diabetic activities. The recent research project suggests that the *Iphiona aucheri* stem contain substantial cytotoxic, antioxidant and anti-diabetic properties. The methanolic crude extract has moderate phenolic contents when compared with its fractions but expressed maximum antioxidant activities (DPPH, ABTS and H_2O_2) and antidiabetic activities (α-amylase inhibition) while the chloroform fraction with highest phenolic contents exhibited lowest antioxidant and anti-diabetic activities. Moreover, among all antioxidants methods (DPPH, ABTS and H_2O_2) the methanolic extract has the highest antioxidant activities followed by its water and chloroform fractions. In case of *Iphiona aucheri* stem extracts indirect correlation of phenolic contents with antioxidant and antidiabetic activities was observed. The higher anti-diabetic activities of methanolic extract than Glucophage (merck®)

ensure that the applied extract has a significant amount of active naturally occurring anti-diabetic component.

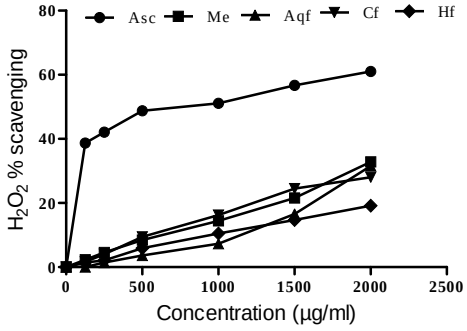

Fig. 3. H_2O_2 free radical scavenging capability of *Iphiona aucheri* stem's methanolic extract and its fractions. Cf : Chloroform fraction, Me : Methanolic extract, Aqf : Aqueous fraction, Hf: *n*-Hexane fraction and Asc : Ascorbic acid.

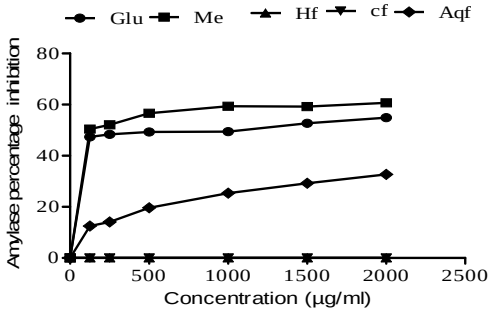

Fig. 4. Anti-diabetic activity of *Iphiona aucheri* stem's methanolic extract and its fractions, Cf : Chloroform fraction, Hf : n-Hexane fraction, Me : Methanolic extract and Glu: glucophage *(Cf : Chloroform fraction and Hf : *n*-Hexane fraction did not show anti-diabetic activities).

Table 4. *Iphiona aucheri* stems methanol extract and its different soluble fractions were used in the correlation, p value (two tailed) and ns: Nonsignificant.

Sl. no.	Assays	Correlation R^2 phenolics	Significance
1	% DPPH radical scavenging ability	0.3129	ns
2	$ABTS^{\bullet+}$ % scavenging ability	0.1857	ns
3	% H_2O_2 scavenging	0.0962	ns
4	% amylase inhibition	0.5810	ns

Non-significant correlation of total phenolic contents with percentage antioxidant and anti-diabetic activities of crude methanolic extract and its various fractions was observed in all cases (Pearson correlation). Analogous results were reported in previous studies (Sahreen *et al.* 2017). DPPH, ABTS and H_2O_2 assays revealed that chloroform fraction containing maximum phenolic content (21.30 ± 2.04 mg GAE/g) expressed minimum antioxidant and anti-diabetic properties. The said correlations are indicated in Table 4.

Finally, it may be concluded that the *Iphiona aucheri* stem extract is a potent source of antioxidant, cytotoxic and anti-diabetic compounds. Its further analysis will depict its pharmacological properties.

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