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IN VITRO EVALUATION OF MEDICINAL, ANTIOXIDANT ACTIVITIES AND PHYTOCHEMICAL SCREENING OF *IPHIONA GRANTIOIDES* AND *PLUCHEA ARGUTA* SUBSP. GLABRA QAISER

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Abstract

The crude ethanolic extracts (EE) derived from different parts of *Iphiona grantioides* (flower, leaf, stem and root) and *Pluchea arguta* subsp.*glabra* Qaiser (leaf, stem and root) were screened *In vitro* for possible antioxidant, antibacterial, antifungal activities and phytochemical profile. Results for phytochemical screening revealed the presence of various important primary and secondary metabolites including carbohydrates, protein, essential oils, tannins, flavonoids, saponins, glycosides, phytosterol, tri-terphenoids and volatile oil in the ethanolic extracts (EE) of different parts of *I. grantioides* and *P. arguta*. Significant antibacterial activity was observed for flower and leaf (EE) of *I. grantioides* against *Klebsiela pneumonia* and *Escherichia coli*, while moderate antibacterial activity was showed by extracts from other parts of this plant. Similarly *P. arguta* leaf (EE) showed significant inhibitory activity against *Escherichia coli*, *Pseudomonas aeroginosa* and *Micrococcus luteus* than its stem and root's extract. The crude ethanolic extract of all parts of *I. grantioides* and *P. arguta* proved a rich source of fungicidal potential. Both the plants proved to have antioxidant potential by exhibiting DPPH radical scavenging activity. So it may be concluded from the present investigations that *I. grantioides* and *P. arguta,* both have a wide range of antibacterial, antifungal and antioxidant properties and can be utilized for the development of antimicrobial drugs for human beings, animals and as safe pesticides in agricultural practices as well as could be an important source of natural radical scavengers.

Key words: Antibacterial, Antifungal, Antioxidant activity, *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*. Qaiser

Introduction

Preliminary phytochemical screening of medicinal plants is very important and useful test for the determination and isolation of pharmacologically active compounds present in plants, (Sugumaran & Vetrichelvan, 2008). This serves as an important tool for the quality assurance of plant for future studies. Till now almost all investigated plants showed to contain different active constituents of pharmacological importance in the form of secondary metabolites (Ming *et al*., 2005).

Plant species with medicinal properties have been playing a fundamental role in the efforts for drug discovery all over the world. 80% populations in developing countries are dependent on plants for their primary health care, and in spite of the significant progress in the field of synthetic organic chemistry of the twentieth century, more than 25% of prescribed medicines in developed countries are derived directly or indirectly from plant sources (Newman *et al*., 2000).

The indiscriminate use of antimicrobial drugs in the treatment of infectious diseases caused by pathogenic microorganisms has developed resistance in many bacteria and fungi against a wide range of antibiotics (Cowan, 1999). So it is need of the day that novel unconventional sources of antibiotics should be discovered or older antibiotics should be modified to overcome this health hazard (Tollefson & Miller, 2000). Medicinal plants represent a rich source of antimicrobial agents as they contain secondary metabolites which have been found inhibitory to microorganisms including bacteria, fungi, various parasites and viruses in a number of ways. There is a continuous and vital need to discover new antimicrobial drugs with diverse chemical composition and new mechanisms of action for new and re-emerging infectious diseases (Rojas *et al*., 2003). Therefore, researchers are keenly interested in herbal medicine to develop better drugs against microbial infections (Benkeblia, 2004).

The term 'antioxidant' refers to the activity of many phytochemicals to guard against the damage caused by reactive oxygen species (ROS). Plants represent an important source of natural antioxidants (Cai *et al*., 2003) and studies have revealed the significance of these antioxidant compounds possessing anti-atherosclerotic, anti-inflammatory, anti-mutagenic, antitumor, anti-carcinogenic, antibacterial, and antiviral activities (Sala *et al*., 2002). The DPPH radical scavenging assay is a standard, non-enzymatic procedure used to provide basic information on the ability of extracts to scavenge free radicals (Sanchez-Moreno *et al*., 1998).The role of antioxidant compounds in mitigating oxidative damage to biological systems has been reported by Perez *et al*. (2004). Various plants have been screened and reported to possess antioxidant potential e.g., *Phragmites karka* (Zainul Abideen *et al*., 2015); *Solanum surattense* (Muruhan *et al*., 2013); *Citrullus colocynthis* (Benariba *et al*., 2013) and wild olive nut galls (Ibrar *et al*., 2013).

As no such study is done on the *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*, so the aim of the present work was to investigate the phytochemical profile as well as antibacterial, antifungal and antioxidant potential of different parts of these two plants.

Materials and Methods

Fresh specimens of *I. grantioides* and *P. arguta* subsp. *glabra* were collected from District Karak in 2013 and identified by Curator Mr. Ghulam Jilani of Department of Botany, University of Peshawar according

to Qaiser & Abid (2003). Plant specimens were cleaned/washed, shade dried, grinded into fine powder and sieved. The fine powder drug of each specimen was preserved in airtight bottle to keep it safe from deterioration and moisture.

Qualitative phytochemical analyses was carried out for the detection of alkaloids, carbohydrates, glycosides, saponins, proteins, phytosterols, fixed oils and fats, Calcium Oxalate, tannin and flavonoids by the procedures of Kokate (1994); Trease & Evans 1985; Harborne, 1998; Khandelwal, 2004; Kumar & Kiladi, 2009 and Chitravadivu *et al*., 2009.

Antibacterial activity: Antibacterial study of the crude ethanolic extracts was carried out by agar well diffusion method following Carron *et al*. (1987).

Bacterial strains: Bacterial strains were obtained from the Microbiology Lab, department of Animal health and veterinary Sciences, Agriculture University, Khyber Pakhtunkhwa. Bacterial strains used were, *Bacillus licheniformis, Bacillus subtilis, Escherichia coli, Klebsiela pneumonia, Micrococcus luteus, Pasteurella multocida, Pseudomonas aeruginosa, Staphylococcus viridans, Staphylococcus aureus* and *Salmonella typhi.*

Antifungal activity: Antifungal activity of the crude ethanolic extracts of plants was evaluated by following the method of agar tube dilution method developed by Atta-ur-Rehman *et al*. (1991), against various pathogens including four human pathogenic strains i.e., *Trichophytonlongifusis*, *Candida albicans*, *C. glaberata, Microsporum canis*, two animal pathogenic fungi i.e., *Aspergilus flavus, A. niger* and three plant pathogenic types of *Fusarium solani,*a *Mucor* spp. and an *Alternaria* spp.

Growth inhibition was calculated with reference to negative control (Umadevi *et al*., 2003).

$$\frac{\text{\% Mycelia inhibition Gn-Gt}}{\text{Gn}} \text{ x } 100$$

where Gn = Mycelial growth in normal; Gt = Mycelial growth in test

1, 1-diphenyl-2-picrylhydrazyl (DPPH) Free radical scavenging activity for evaluating antioxidant potential: In the present study the DPPH radical scavenging activity for evaluating the antioxidant potential of different parts of *I. grantioides* and *P. arguta* extracts was carried out by following Kato *et al*. (1988).

The DPPH radical scavenging activity was determined by using the formula:

$$\text{DPPH\%} = \frac{A_0 - As}{A_0} \text{ x } 100$$

whereas A_0 is the absorbance of 0.001 M of DPPH solution only and As is the absorbance of the reaction mixture.

Results and Discussion

Qualitative phytochemical screening of leaf, stem, root and flower extracts of *I. grantioides* (Table 1) revealed that proteins, carbohydrates and tannins were present in both aqueous and ethanolic extracts. Alkaloids were detected in leaf and stem only while flavonoids were present in all parts of the two plants except root extract. Saponins, glycosides, phytosterol and tri-terphenoids were present in all parts of *I. grantioides*. Volatile oil was only detected in the ethanolic and aqueous extracts of flower and leaf of *I. grantioides* while spot test gave negative results for the presence of fixed oil. The result showed that *I. grantioides* is rich in bioactive compounds and hence could be a potential source of therapeutic properties. Almost similar results were obtained for the Phytochemical screening of leaf, stem and root of *P. arguta* subsp. *glabra* (Table 1). Proteins, carbohydrates, tannins, flavonoids and saponins were present in both aqueous and ethanolic extracts of all parts; however alkaloids were detected only in aqueous extract of *P. arguta* subsp. *glabra*. Glycosides were present only in stem and leaf extracts. Phytosterol, triterphenoids and volatile oil were present only in leaf of *P. arguta* subsp. *glabra*. Spot test gave negative result for extracts of all tested parts. So *P. arguta* subsp. *glabra* like *I. grantioides*, is also a source of therapeutically important metabolites.

Khan *et al*. (2010) reported the presence of same phytochemical constituents in the ethanolic extracts of *Pluchea lanceolata*. Similarly Naz *et al*. (2013); Kalyan *et al.* (2011); Hussain *et al*. (2011); Uddin *et al*., 2011 and Ibrar *et al*. (2013) emphasized the importance of phytochemical screening for obtaining firsthand information about metabolites of pharmacological significance by investigation of different medicinal plants for preliminary phytochemical screening.

The phytochemicals detected in our extracts are well known for their various pharmacological activities. For instance alkaloids are well known for antibacterial, antimalarial, cytotoxic and anticancerous properties (Wirasathien *et al*., 2006), saponins for insecticidal, antibiotic and fungicidal activities (Sparg *et al*., 2004), Flavnoids for antibacterial, anti-inflamatory, antiallergic, antineoplastic, antiviral, anti-diarrheal, anti-thrombotic, antioxidant and vasodilator properties (Nijveldt *et al*., 2001), Tannins for antiviral (Lin *et al*., 2004), antibacterial (Akiyama *et al*., 2001) and antioxidant potential (Shirahata *et al*., 1985; Bhagavathi, 1999; Yokozawa *et al*., 1998) and Cardiac glycosides for their cytotoxic and Na-K-ATPase inhibitory properties (Joseph *et al*., 2005). All these compounds are commonly present in medicinal plants and well known for their pharmacological activities.

Furthermore the present study revealed that different extracts of same plants may have different phytochemical constituents, suggesting that there may be variation in phytochemistry of different tissues. Evans *et al.* (2002) reported the presence of saponin in small quantities in roots only but absent from the leaves and inflorescence of *Euphorbia hirta*.

Table 1. Phytochemical investigation of different parts of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*.

	Constituents	Test	Ethanol crude extract							Aqueous crude extract						
			IF	IL	IS	IR	PL	PS	PR	IF	IL	IS	IR	PL	PS	PR
1.	Carbohydrates	Molish	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		Benedict	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		Fehling	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2.	Protien	Ninhydrin	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		Bieruit	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		Millon's test	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3.	Tanins	Lead Acetate	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		$FeCl_3$	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		Gilatin test	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		Alkali test	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4.	Alkaloides	Dragon Droff Hager	_	+	_	+	_	_	_	_	+	_	+	_	_	_
		Wager	+	+	+	+	+	_	_	+	+	+	+	_	_	_
		Mayer test	+	+	+	+	_	_	_	+	+	+	+	_	_	_
5.	Flavonoides	Shinoda test	+	+	+	_	+	+	+	+	+	+	_	+	+	+
		Alkali test	+	+	+	_	+	+	+	+	+	+	_	+	+	+
6.	Saponins	Forth Formation	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		$NaHCO_3$		+	_	_	+	_	_	_	+	_	_	+	_	_
7.	Glycosides	Killaerkilani test	+	+	+	_	+	+	_	+	+	+	_	+	+	_
		Borntrager	+	+	+	+	+	+	_	+	+	+	+	+	+	+
8.	Phytosterol/	Salkowski's test	+	+	+	+	+	_	_	+	+	+	+	+	_	_
	Triterpenoides	Libermann– Burchard's test	+	+	+	+	+	_	_	+	+	+	+	+	_	_
9.	Fixed oil	Spot test	_	_	_	_	_	_	_	_	_	_	_	_	_	_
10.	Volatiles oils		+	+	_	_	+	_	_	+	+	_	_	+	_	_

Key = I = *Iphiona grantioides* P = *Pluchea arguta* subsp. *glabra,* flower, F= flower, L = leaf, S = stem, R= root

Table 2. Antibacterial activity of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* (The figures show zones of inhibition in mm).

S. No.	Bacterial strain	IF	IL	IS	IR	PL	PS	PR	Ciprofloxacin
1.	*Micrococcus luteus*	15.67	11	10.67	11.67	12.00	10.67	10.33	27.00
2.	*Staphylococcus viridians*	15.00	10.2	×	11.67	10.67	×	×	25.00
3.	*Bacillus licheniformis*	16.67	10.21	×	×	11.67	×	×	21.67
4.	*Pasteurellamultocida*	17.47	11	11.50	11.33	11.33	10.33	×	23.67
5.	*Bacillus Subtilis*	12.33	×	×	10.33	11.67	×	10.67	21.67
6.	*Staphylococcusaureus*	19.67	×	×	×	11.67	×		23.00
7.	*Pseudomonas aeroginosa*	12.33	×	×	×	13.33	11.67	10.67	23.67
8.	*Escherichia coli*	15.67	14	11.33	×	15.33	10.67	×	25.00
9.	*Klebsiela.pneumoniae*	21.23	8.32	11.67	×	11.67	×	×	21.33
10.	*Salmonella typhi*	×	10.6	×	×	×	×	×	21.67

Key = I = *Iphiona grantioides* , P= *Pluchea arguta* subsp. *glabra,* F= flower, L = leaf , S = stem, R= root

Antibacterial activity: Results for antibacterial activity of *I. grantioides* are shown in Table 2 and Fig. 1a and Fig. 1b and for *P. arguta* subsp. *glabra* are shown in Fig. 2a and Fig. 2b. In the present study, flavonoids might be responsible for the antibacterial potential of *I. grantioides* and *P. arguta* subsp. *glabra*, as they have been reported for their significant antibacterial effects by Song *et al.*, 2002; Cushnie & Lamb *et al*., 2005;Akroum *et al*., 2009 and Rauf *et al*., 2012. Sesqueterpenes are the important constituents of *P. arguta* subsp. *glabra* having strong bactericidal effect against *Staphylococcus aureus* (Claeson *et al*., 1992). Results of the present study revealed that *I. grantioides* (flower and leaf) and *P. arguta* subsp. *glabra* (leaf) have significant antibacterial potential against important human pathogenic bacteria.

Antifungal activity: Crude ethanolic extracts of *I. grantioides* (flower, leaf, stem, root) and *P. arguta* subsp. *glabra* (leaf, stem, root) were tested against four human pathogenic fungal strains (*Trichophytonlongifusis*, *Candida albicans*, *Microsporumcanis*, *Candida glaberata*), two animal pathogenic fungi (*Aspergilus flavus*, *Aspergilus niger*) and three Plant pathogenic types (*Fusarium solani*, *Mucor* spp. and *Alternaria* spp.) for antifungal activity.

The results presented in Table 3 (Figs. 3 and 4) showed that in case of *I. grantioides* the most efficient extracts with highest percent inhibition values were in the order of i.e., leaf (54%) □ flower (53%) □ stem (27.56%) □ root (19%). It is clear that flower, leaf and stem extracts of *I. grantioides* were most effective, as they inhibited nine different pathogenic species at three different concentrations (125, 250 and 500 ug/ml).

In case of *P. arguta* subsp. *glabra*, ethanolic extracts of various parts (leaf, stem and root), at three different concentrations (125, 250, 500 ug /ml), showed promising effects against the tested fungal pathogens including *Trichophyton longifusis, Candida albicans, Microsporum canis, Candida glaberata, Aspergilus flavus, A. niger, Fusarium solani, Mucor* spp. and *Alternaria* spp (Table 3). The antifungal activity of the plant was found most effective on high concentration i.e., ethanolic extracts of stem, leaf and root at 500 ug /ml, had significant inhibitory effects against the tested fungal strains with highest percent inhibition values in the following order; leaf (43.6%) □ stem (35%) □ root (30%).

Presence of alkaloids, tannins, glycosides, saponins, terpenes and flavonoids, in the preliminary phytochemical screening are believed to be responsible for the observed antifungal effects of *I. grantioides* and *P.arguta*, which is further strengthen by the reports of Hussain *et al*. (2009); Uddin *et al*. (2010); Rajeswari *et al*. (2011); Mansour *et al*. (2012), who attributed the observed antifungal effects of plant extracts to the presence of secondary metabolites.

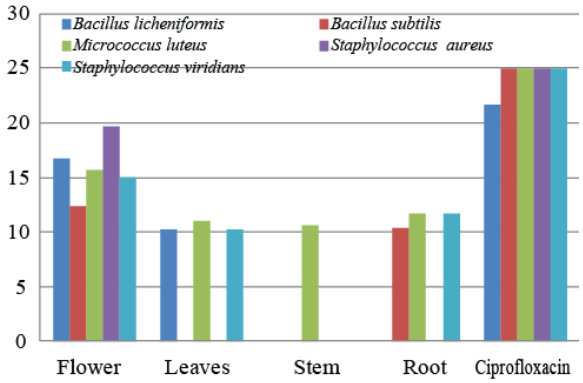

Fig. 1a. Antibacterial activity (gram positive strains) of *Iphiona grantioides*.

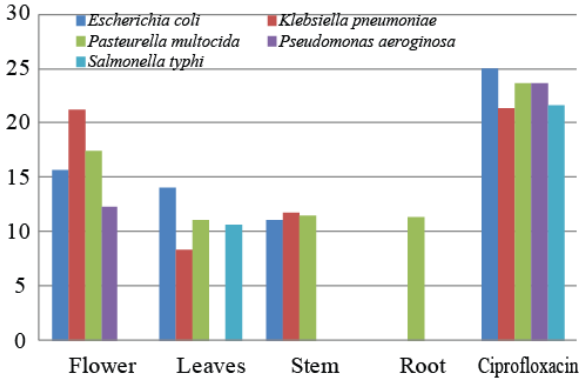

Fig. 1b. Antibacterial activity (gram negative strains) of *Iphiona grantioides*.

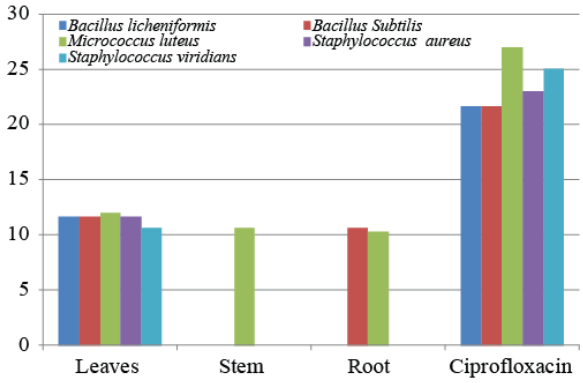

Fig. 2a. Antibacterial activity (gram positive strains) of *Pluchea arguta*.

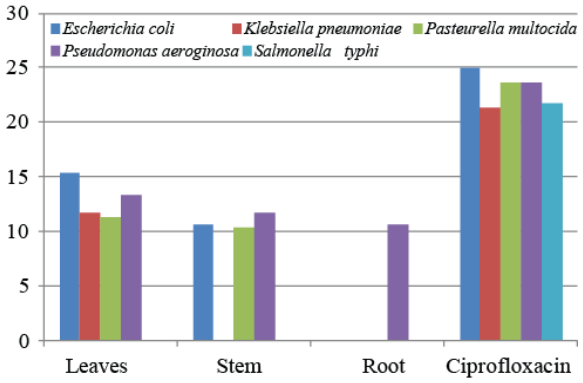

Fig. 2b. Antibacterial activity (gram negative strains) of *Pluchea arguta*.

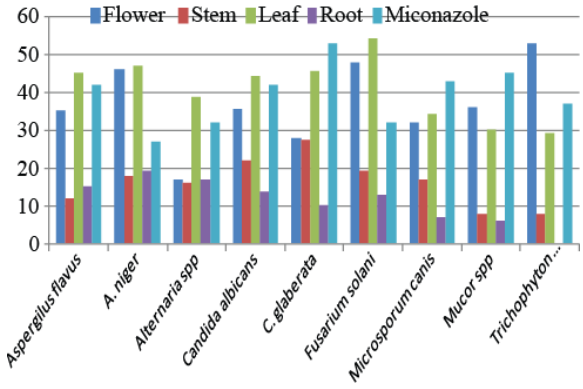

Fig. 3. Antifungal activity of *Iphiona grantioides*.

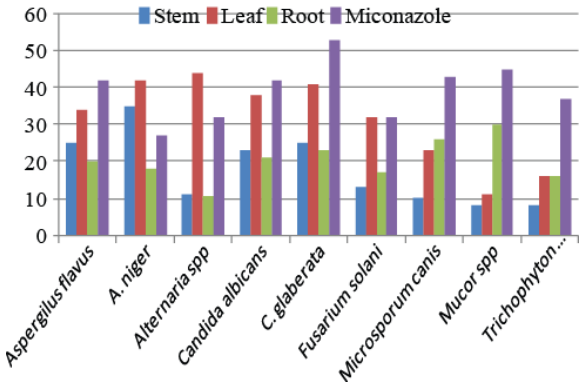

Fig. 4. Antifungal activity of *Pluchea arguta*.

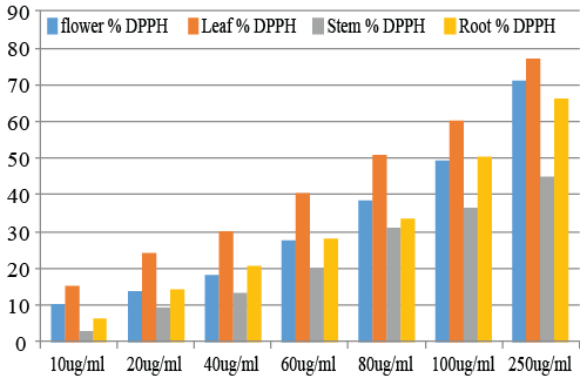

Fig. 5. Antioxidant activity of *Iphiona grantioides*.

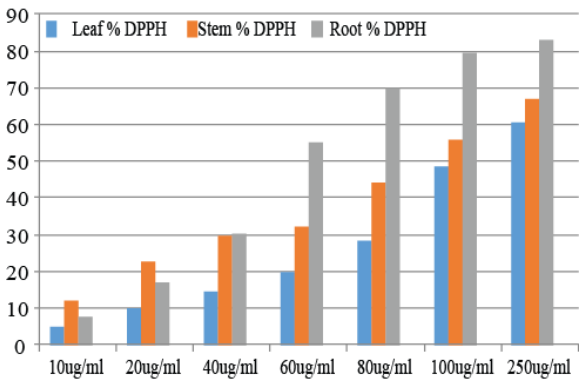

Fig. 6. Antioxidant activity of *Pluchea arguta* subsp. *glabra*.

Table 3. Antifungal activity of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* against different fungal pathogens (The figures show percent (%) inhibitions).

Extracts	Conc. ug/ml	*Aspergilus flavus*	*A. niger*	*Alternaria* spp.	*Candida albicans*	*C. glaberata*	*Fusarium solani*	*Microsporu mcanis*	*Mucor* spp.	*Trichophyto nlongifusis*
	125	6	12	7.6	18	12	27	1	12	28
IF	250	21	24	13	26.7	17.3	39	13	24	35
	500	35	46	17	35.3	28.00	48	32	36	53
	125	-	7	2	8.3	7.23	10	-	-	7
IS	250	10	13	1.6	13	15.3	14	-	-	-
	500	12	18	16	22	27.56	19	17	8	8
	125	13	18	11	13.7	26.9	33	16	16	13
IL	250	20	28	17.6	29.5	35.76	42	26	23	19
	500	45	47	38.9	44.2	45.67	54	34	30	29
	125	-	8	9.3	-	-	8	-	2	-
IR	250	9	13	11	7.9	-	11	-	5	-
	500	15	19	17	13.5	10	13	7	6	-
	125	18	19	17.3	25.8	23.2	17	11	9	10
PL	250	23	26	23	29	32	29	19	16	11
	500	34	42	43.6	38	41	32	23	11	16
	125	8	-	2	3.7	1	4	5	-	-
PS	250	17	18	1.6	12.4	12	9	8	3	-
	500	25	35	11	23	25	13	10	8	8
	125	-	7	1.7	4	1	-	16	12	-
PR	250	11	14	6.7	11	11	9	19	18	11
	500	20	18	10.3	21	23	17	26	30	16
Miconazole	(0.5mg/ml)	42±0.5	27	32	42	53	32	43	45	37

Key = I = *Iphiona grantioides*, P = *Pluchea arguta* subsp. *glabra*, *F*= flower, L = leaf, S = stem, R= root

Table 4. Antioxidant activity of *Iphiona grantioides*.

Concentration	% DPPH Scanvaging activity (Flower)	% DPPH Scanvaging activity (Leaf)	% DPPH Scanvaging activity (Stem)	% DPPH Scanvaging activity (Root)
10ug/ml	10.06	15.4	2.99	6.01
20ug/ml	13.58	24.1	8.97	14.07
40ug/ml	17.97	30.22	13.10	20.62
60ug/ml	27.41	40.3	20.03	28.27
80ug/ml	38.65	50.9	31.0	33.27
100ug/ml	49.42	60.2	36.36	50.48
250ug/ml	70.94	77.3	44.89	66.40

Table 5. Antioxidant activity of *Pluchea arguta* subsp. *glabra*.

Concentration	% DPPH Scanvaging activity Leaf	% DPPH Scanvaging activity Stem	% DPPH Scanvaging activity Root
10ug/ml	5.01	11.94	8.01
20ug/ml	10.07	22.79	16.97
40ug/ml	14.62	29.7	30.10
60ug/ml	20.27	32.06	55.03
80ug/ml	28.27	44.08	70.0
100ug/ml	48.48	55.8	79.36
250ug/ml	60.40	66.8	82.89

Antioxidant activity: Ethanolic extracts of *I. grantioides* (flower, leaf, stem and root) and *P. arguta* subsp. *glabra* (leaf, stem and root) were investigated for their free radical scavenging capacity. DPPH (1, 1- Diphenyl-2 Picrylhydrazyl) was used in the experiment. The bleaching of DPPH colour in the experiment by *I. grantioides* and *P. arguta* extracts indicated the scavenging capacity of the plants. Results of DPPH activity for *I. grantioides* are presented in Table 4 and Fig. 5 and for *P. arguta* extracts are shown in Table 5 and Fig. 6. respectively, showing that roots of *P. arguta* showed the highest DPPH scavenging activity (82.89%) followed by leaf (77.3%) and flower (70.94 %) of *I. grantioides*. The findings of Sen *et al*., 2002 and Ghosh *et al*. (2008) are in line with our findings who investigated the antioxidant activity of the root methanolic extract of tissue cultured *P. indica* (L.). Similarly Fernández & Torres (2006) evaluated the antioxidant activity of *P. carolinensis* leaves. Andarwulan *et al.* (2010) suggested that the plant may contribute to dietary antioxidant intake of *P. indica*as its extracts inhibited linoleic acid oxidation and had the DPPH, ABTS, and ferric cyanide antioxidant capacities.

Suggestions: In the present study results of phytochemical composition and pharmacological activities like antibacterial, antifungal, antioxidant revealed that flower and leaf of *I. grantioides* and leaf of *P. arguta* subsp. *glabra* have significant antifungal, antibacterial and antioxidant potential. Both can be used as potential sources of antifungal drugs not only for human and cattle diseases, but also as a safe and useful pesticide in agricultural practices too; however further research work is needed to explore, isolate and purify the active chemical compounds responsible for such activities.

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