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Elemental Analysis and Evaluation of Phytochemical and Biological Properties of *Orbanche agyptica*

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Abstract: Medicinal plants possess the antibiotic properties in the traditional system and are also used as a folk medicines by the tribal people worldwide. It is now believed that medicinal plants have given the cure of chronic diseases in one way or another. The present study was undertaken to evaluate the phytochemical, elemental analysis and biological activity of crude extract and the fractions of *Orbanche Agyptica*, which was collected from the hilly area of the North Waziristan, province Khyber Pakhtunkhwa, Pakistan. All the fractions and crude of *Orbanche Agyptica* were found to be active against all bacterial species except ethyl acetate fraction. The phytochemicals like alkaloids, reducing sugar, Anthraquinones, Flavonoids, Terpenoids, tannins and glycosides were present while saponins, proteins and coumarin glycosides were found absent. These organic compounds and the presence of heavy metals including Fe, Zn, Cu, Pb, Cd, Ni and Na, K, Ca, indicates the promising potential of this plant as medicine.

Key words: Phytochemicals · Alkaloids · Flavonoids · Anthraquinones · Terpenoids and tannins' etc

INTRODUCTION

Medicinal plants are the rich source of organic constituents and have been used since long time ago,provide about 75-80% drugsWorldwide.Medicinal value of the plant is due to presence of various phytochemical and elemental composition [1,2]. Medicinal plants possess phytochemicals are of more importance to the health of humans and animals. The curative purposes are often accounted for medicinal plants in terms of their organic compounds including alkaloids, glycosides, essential oil, flavonoids and tannins etc. [3-5].

Trace elements are concern with environment and harmful to humans and animals when present in large amount. Metals accumulate in the soil from industrial and atmospheric pollution and can affect the standard life of humans and animals, because of their accumulation in food chain, for this reason it is very important to investigate trace elements in plant samples in term of environmental pollution and particularly for plants with nutritional requirements [6]. Essential (Fe, Cu, Zn etc) and non-essential elements (Pb, Ni, Cd etc) influence biochemical processes (metabolism) in the human body. Bioactive constituents of plants and other mineral elements play a key role in the metabolism. Some mineral elements make chelated ligands and make them available to the body system. These elements also used in homoeopathic system and neurochemical transmission [7].

Bacteria are single cell microorganism that can act as antigen for human, animals and plants, causes various diseases and infections. These microorganisms spread through blood and saliva. For examples Staphylococcus aurous (a gram positive bacterium) causes necrosis of skin and Staphylococcus epidermidis causes nosocomial infections [8].

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The plant extracts having antimicrobial potential can be used to treat many pathogenic diseases. Natural found bioactive compound in pants display a key role in defense system of plant and their physiological action on human body [9].

This study was conducted to find out the elemental analysis, phytochemical screening and biological activities, of crude extract n-hexane, ethyl acetate, chloroform, butanol and aqueous fractions of *orbanche agyptica.*

MATERIALS AND METHODS

Collection of Plant: The whole plant of *Orbanche agyptica* was collected in the month of May, 2014 from North Waziristan area KPK Pakistan. Chloroform, n-hexane, ethyl acetate, butanol, ethanol, sulphuric acid, ammonium hydroxide, acetic acid, cefoxime were present in the department of chemistry kohat university of science and technology, kohat, Pakistan. Nutrient agar, dimethyl sulphoxide (DMSO) and bacterial strains including *Staphylococcus aureus*, *Shigela, proteus*, *Pseudomonas auriginosa*, *Salmonella typhi* and *Vibrio cholera* were purchased from pharmaceutical department of Kohat University, kohat, Pakistan.

Extraction and Fractionations: The plant was first washed with tap water then with distils water to remove the dust particles and dried under shadow. The plant was crushed into powder so that the bioactive compounds extracted easily. The plant powder (2 kg) was soaked in 5 L of methanol for 15 days to extract all bioactive compounds and filtered. This process was repeated for three times to abstracted more and more compounds. Ethanolic extract (130g) was obtained by using rotary evaporator. 130 grams of crude extract was dissolved in 600 ml distilled water and partitioned with n-hexane, chloroform, ethyl acetate, butanol and aqueous fractions were obtained.

Antibacterial Assay: Nutrient agar medium was prepared by dissolved 28 grams nutrient agar in 1 L distil water. The medium was autoclaved at 121 c for 16 mints to kill all microbes. The medium was poured into petri dish and then placed in freezer at 0 °c. This can be used for inoculation of bacteria and zone of inhibition of different fractions.

Methods for Elemental Analysis: The whole plant of *Orbanche agyptica* was cleaned with tap water and then with distilled water to remove the dust particles and dried by using oven at 60°C for 72 h. After drying, the plant was grinded into fine powder using an electric grinder. Specified amount of powder was placed in 100ml conical flasks to which 30ml nitric acid (HNO_3) were added. The flask was placed on magnetic stirrer heater for more than three hours at 250ºC as a result colour solution was changed to milky solution which was cooled for 15 minutes and then 15ml conc. Perchloric acid were added and heat the solution until the solutions became colourless. The colourless solution was filtered and subjected for trace elements analysis by Atomic Absorption Spectrometer (Perkin Elmer 400).

Test for Phytochemicals Screening: The phytochemicals tests of *Orbanche agyptica* were analyzed after extraction. The crude of the given plant was subjected for qualitative characterization of phytochemicals by using the standard methods [10, 11].

Test for Tannins

Gelatin Test: sodium chloride with 1% gelatin solution was added to the plant extract. Appearance of white precipitate clearly shows the presence of tannins.

Test for Phenols

Ferric Chloride Test: Extract was treated with 4 drops of ferric chloride ($Fecl_3$) solution. Formation of bluish colour indicates the presence of phenols.

Test for Flavonoids (12)

Lead Acetate Test: Extract was treated with few drops of lead acetate solution. Yellow colour ppt. was appeared which indicated the presence of flavonoids. Or Few drops of 1% NH3 solution was added to the plant sample in a test tube. A yellow coloration was appeared which indicates the presence of flavonoid compounds.

Test for Saponins (12)

Foam Test: 0.5 g. of extract was dissolved in 2 ml of distill water and shaken it for 10 minutes. Foam was produced which indicated the presence of saponins.

Test for Glycosides (12): Extract was hydrolyzed with dil. hydrochloric acid and then subjected to test for glycosides. Legal's Test: filtrate was treated with legal's solution (sodium nitropruside in pyridine and sodium hydroxide). Formation of pink or red colour indicates the presence of cardiac glycosides.

Test for Alkaloids (12): 0.5g of extract was added to10ml of water in a test tube, boiled and filtered the solution, then added 2 ml dil, NH_3 and 5 ml $CHCl_3$ and stunned quietly to extract the alkaloidal base. 10ml acetic acid was also added to the same solution. a) Mayer`s reagent (Potassium Mercuric Iodide) was added and was given cream color ppt with Mayer`s reagent. The appearance of cream color ppt. clearly indicates the presence of alkaloids. b) Wagner's Test: Wagner's reagent (Iodine in Potassium Iodide) was mixed with the filtrate. Formation of reddish ppt. indicates the presence of alkaloids. c) Hager's Test: Hager's reagent (saturated picric acid solution) was treated with the filtrate. The formation of yellow coloured ppt. clearly indicates the presence of alkaloids.

Test for Coumarin Glycosides (12): Dissolve the ethanolic crude in 2ml of deionized water and divide the volume into two equal parts. Half of the volume is for witness and add 0.5ml 10% NH4OH into another volume. Take two spots on TLC plates and study under UV light. Intense fluorescence indicates the presence of coumarins.

Test for Triterpenoids (12): The ethanolic crude of plant was mixed with 0.5ml of chloroform and subjected for phytosterols. a) Liebermann Burchard's test: filtrate was treated this with the 0.5ml of acetic anhydride and few drops of Conc. Sulphuric acid was added carefully. Formation of brown ring at the junction indicates the presence of sterols. b) Salkowski's Test: The filtrate and few drops of Conc. Sulphuric acid was shaken and allowed to stand. Formation of golden yellow colour indicates the presence of triterpenes.

Test for the Carbohydrates (12)

Reducing Sugars: Ethanolic extract was dissolved in 5ml distilled water and filtered. a) Molisch's Test: Few drops of alcoholic α-naphthol solution were added to the filtrate in a test tube. Violet ring color formation indicates the presence of Carbohydrates. b) Benedict's Test: Benedict's reagent was treated with filtrate and heated gently. Orange red ppt. indicates the presence of reducing sugars. c) Fehling's test: 20 drops of hot Fehling's solution (A and B) in a test tube was added to filtrate, red-brick colouration was formed in the bottom of the tube which indicates the presence of reducing sugars.

Test for Starch (12)

Iodine Test: The 5ml aqueous extract was mixed with the iodine reagent of starch. Formation of blue violet color indicates the presence of starch.

Test for Protein(12)

Xanthoproteic Test: The plant extract was treated with few drops of conc. HNO_3. Yellow colour appeared which indicates the presence of proteins. b) Ninhydrin Test: 0.25% weight/volume ninhydrin reagent was added to the extract and boiled it for few minutes; blue colour appeared which shows the presence of proteins.

Anthraquinone Glycosides (12) Borntrager's Test: Ferric Chloride solution was treated with the extract in test tube and placed on hot water bath for about 5 minutes. Equal volumes of benzene were added to the cooled mixture. Two layers were formed from which benzene layer was separated and treated with ammonia solution. Appearance of rose-pink colour indicates the presence of anthranquinine glycosides.

RESULTS AND DISCUSSION

The present study was carried out to investigate the antibacterial activity, phytochemical screening and elemental analysis of *Orbanche agyptica*. The study revealed the presence of phytochemical active compounds including alkaloids, tannins, flavonoids and terpenoids, glycosides; phenols etc. of *Orbanche agyptica*, results are given in Table 1. Alkaloids, flavonoids, terpenoids, tennins, Bufadinoloids glycosides were found present and saponins, coumarins, anthraquinones glycosides and proteins were found absent in the plant sample. Alkaloids were determined by three different methods and were given positive results in all methods. Flavonoids, triterpenoids and tannins, each of them was analyzed by two different methods, given positive results, for this reasons it can be concluded that this plant may be considered a good source of pharmaceutical and therapeutically applications, naval drugs synthesis.

The medicinal value of plant is due the presence of some phytochemicals which may be helpful in protection against chronic diseases due to their physiological action on the human body. For example, alkaloids is one of the chemical present in plants protect the body against chronic diseases, reducing headaches associated with hypertension. Steroids and terpenoids show the analgesic properties. The steroids and saponins are used against central nervous system diseases. Similarly Saponins have anti-hypercholesterolemia and antibiotic properties [12].

Bactericidal Action: The plant extract and its fractions showed good antibacterial effect against different bacterial species and zone of inhibition measured in mm.

Table 1: Phytochemical screening of *Orbanchi agyptica*

Alkaloids:			
S.No	Test		Indication
1	Mayer's test		+
2	Wagner's test		+
3	Hager's test		+
Carbohydrates:			
S.No	Test		Indication
1	Molish test		-
2	Benedict test		-
3	Fehling's test		-
Flavanoids:			
S.No	Test		Indication
1	Shinoda test		+
2	Lead acetate test		+
Triterpenoids and Steroids:			
S.No	Test		Indication
1	Libermann-burchards test	Terpenoids	+
		Steroids	-
2	Salkowshi test	Steroids	-
		Triterpenoids	+
Tennins and Phenolic compounds:			
S.No	Test		Indication
1	$FeCl_3$ test		+
2	lead acetate test		+
Proteins:			
S.No	Test		Indication
1	Xanthoproteic Test		-
2	Ninhydrin Test		-
Glycosides:			
S.No	Test		Indication
1	Legal,s test		+
Anthraquinone glycosides:			
S.No	Test		Indication
1	Borntrager,s test		-
Saponine:			
S.No	Test		Indication
1	Foam test		-
Coumarin glycosides:			
S.No	Test		Indication
1	$FeCl_3$ test		-
Starch:			
S.No	Test		Indication
1	Iodine		+

Indication: Absence (-) Present (+)

The zone of inhibition of Crude extract, n-hexane, chloroform, ethyl acetate, butanol and aqueous fractions were 12.0 ± 1.25, 9.0+ 0.50, 11.0 1.0±0, 8.0 ±0.50, 1±1.25 and 9.0±0.25 mm against *Staphylococcus aurous*, 11.0 ±0.25, 8.0 ±0.75, 10.0± 0.25, 0.0, 10.0±0.50 and 18.0± 1.50 mm against shigela, 22.75 ±1.75, 13.0 ± 1.00, 15.25 ±0.61, 10.0 ±0.25, 23.0 ±0.75 and 18.0 ±0.50 mm against proteus, 8.0 ±0.25, 9.0 7±5, 17.0 ±1.00, 8.0 ±0.00 9.0 ±0.25 and 11.0 ±0.40 mm against *Pseudomonas auriginosa*, 15.0 ±1.25, 11.0 + 0.75, 12. ±0 0.40, 8.0 ±0.00, 15.0 ±1.25 and 10.5± 0.75 mm against *Salmonella typhus* and 10.5 ±1.00, 9.5 ±0.60, 12.0 ±0.50, 0.0, 11.0 ±0.30 and 9.5 ±0.40 mm against *Vibrio cholera* (Table 2).

Orbanche agyptica extract and its fractions played good bactericidal effect against all sex bacterial species but particularly against proteus, *Salmonella typhus* and *Pseudomonas auriginosa* at their extent. Ethyl acetate fraction showed no activity against shigela and vibrio cholera at all. These bacteria causes many diseases including soft tissues, skin, respiratory, ear and urinary tracts [13].

The crude extract was found to be the most effective against proteus, n-hexane fraction showed higher activity against *Salmonella typhus*, Ethyl acetate fraction displayed effective inhibition against proteus, chloroform fraction much effectively inhibited the proteus and *Pseudomonas auriginosa*, butanol fraction inhibited against proteus and *Salmonella typhus* while Aqueous fraction showed good inhibition against shigela and proteus in equal amount.

Essential and non-essential elements were analyzed by using Flame Atomic Absorption Spectrophotometer (Perkin Elmer 400) in the three different parts (stem, leaves and roots) of *Orbanche agyptica*, different concentration of elements were observed in all the three parts, appended in Table 3.

From the results given in table, higher concentration of K, Ca and Na was recorded in all three parts while other heavy metals showed moderate concentration. The elements present in Leaf contained Na-23, K-147, Ca-104, Pb-10.12, Cu-29.85, Zn-18.16, Cd-0.0, Fe-29.76 and Ni-2.89 mg/kg respectively. The stem contained Na-99, K-143, Ca-115, Pb-13.5, Cu-3.95, Zn-1.67, Cd-0.0, Fe-17.26 and Ni-11.14 mg/ kg, followed by root of Na, K, Ca, Pb, Cu, Zn. Cd, Fe and Ni in the concentration of 25, 125,153, 0.5, 11.3, 0.45, 0.95, 28.03 and 8.07 mg/kg respectively. Higher concentration in leaf and in stem was observed of K (147 and 143 mg/kg) and in root Ca was present in large amount (153mg/kg). Ca was found to be present in large amount (153mg/kg) followed by K (147 and 143 mg/kg) in all the three parts of *Orbanche agyptica* while Zn was found present in low concentration. Cadmium (Cd) was absent in leaf and stem while present in low concentration in root.

Table 2: Antibacterial activity (mm) of *Orbanche Agyptica*

Bacterial Species	Crude Extract	n-Hexane fraction	Chloroform fraction	Ethyl acetate fraction	Butanol fraction	Aqueous fraction	IMP
Staphylococcus aurous	12.0 1±.25	9.0 ±0.50	11.0 ±1.00	8.0 ± 0.50	11±1.25	9.0±0.25	13
Shigela	11.0 ± 0.25	8.0 ± 0.75	10.0± 0.25	0.0	10.0±0.50	18.0±1.50	50
Proteus	22.75±1.75	13.0± 1.00	15.25±0.61	10.0±0.25	23.0±0.75	18.0±0.50	45
Pseudomonas auriginosa	8.0±0.25	9.0±75	17.0±1.00	9.0 ±0.25	8.0±0.00	11.0±0.40	26
Salmonella typhy	15.0±1.25	11.0± 0.75	12.0 ±0.40	8.0±0.00	15.0±1.00	10.5±0.75	24
Vibrio cholera	10.5±1.00	9.5±0.60	12.0±0.50	0.0	11.0±0.30	9.5±0.40	28

Table 3: Elemental analysis of leaf, stem and root of *Orbanche agybtica*

Parts	Na	K	Ca	Pb	Cu	Zn	Cd	Fe	Ni
Leaf	23	147	104	10.12	29.85	18.18	Nd	29.76	9.89
Stem	99	143	115	13.5	3.95	1.67	Nd	17.26	11.14
Root	25	125	153	0.5	11.3	0.45	0.95	28.03	8.07

CONCLUSION

This study reveals that the plant sample showed good phytochemical, elemental analysis and good bactericidal effect of the crude extract and the fractions of *Orbanche agyptica*, this plant can be considered a valuable source of potential therapeutic applications in the remedy of different microbes-originated infections.

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