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PHYTOCHEMICALS ANALYSIS AND ANTIMICROBIAL ACTIVITIES OF *ECHINOPS ECHINATUS* FROM CHOLISTAN DESERT, PAKISTAN

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ABSTRACT

Plants have been used as a source of food and medicines by man throughout history. *Echinops echinatus* was evaluated for phytochemicals and tested antibacterial activities against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and Anti-urease inhibition assay activities during the study. Cleaned the laminar flow cabin with 70% ethanol and performed α-glucosidase inhibition activity according to the standard method with slight modification. Carbohydrates, phenols, and tannins were positive in leaves, stems, and roots. Saponins and glycosides were found in flowers, leaves, roots, and stems. At the same time, roots and stems revealed the presence of steroids. Terpenoids were found in flowers and leaves. The leaves showed the maximum inhibition zone of 10mm against *K. pneumoniae*, while against *E. coli*, *S. aureus*, and *P. aeruginosa* was found inactive. *K. pneumoniae*, *E. coli*, and *P. aeruginosa* were resistant to flowers extract. The root extract showed a maximum zone of inhibition of 18mm against *S. aureus* while against *P. aeruginosa*, and *K. pneumoniae* inhibition zone was 15mm and 14mm, respectively. The extracts of leaves, flowers, roots, and stems showed positive results for α-glucosidase. The roots extract showed maximum inhibition with 75.3±1.5 with an IC50 value of 207.3±1.3 for α-glucosidase. Urease inhibitory activity of stem extracts showed maximum inhibition activity of 93.71±0.86 with IC50 value 15.63±0.42. In comparison, leaves and roots showed almost the same activity, 92.45±0.63 and 92.63±0.76, respectively. *Echinops echinatus* could be considered for further studies in the treatment of various ailments as a natural remedy.

Keywords: *Echinops echinatus*, *Klebsiella*, *Pseudomonas*, *Staphylococcus*, *E. coli*, Anti-urease, Anti-glucosidase

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1. INTRODUCTION

Plants are important source of food and medicine both for the man and animals. People used plants for treatments of ailments and their knowledge generations after generations. Those chemicals compound which are obtained from plants are called phytochemicals (Laxmi et al. 2015). These are divided as primary and secondary constituents; primary constituents include the common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids etc. Secondary constituents are the remaining plant chemicals such as alkaloids, terpenoids, flavonoids, lignans, steroids, saponins, phenolics, and glucosides. Terpenoids exhibit various important pharmacological activities i.e., anti-inflammatory, anticancer, anti-malarial, inhibition of cholesterol synthesis, anti-viral and anti-bacterial activities (Kappers et al. 2005). Plants produce different chemicals which are used for medicinal purpose (Rafay et al. 2020). Plants also have been used in ethno pharmacy for various diseases such as hypertension, diarrhea, eczema and cholesterol for centuries. Today scientific validation was provided by identification and isolation of bioactive phytochemicals (Laxmi et al. 2015). The crude juices of pomegranate leave and peels, fig leaves, guava leaves and olive leaves have been reported to possess large quantities of bioactive compounds. Pomegranate peels crude juice contained high amounts of phenols, flavonoids, tannins and anthocyanins, similarly, displayed strong antioxidant potential (Farag et al. 2020).

The modern age medicines are also discovered based on knowledge of medicinal plants. By the middle of the nineteenth century at least 80% of all medicines were derived from plants. Then, after the scientific revolution which leads to development of the pharmaceutical industry, the synthetic drugs dominated (Newman et al. 2000), but even; herbal drugs are prescribed widely because of their effectiveness, fewer side effects and are relatively low in cost (Odhav et al. 2013). In Pakistan 80% of the population belonging to the rural areas depends on the traditional

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medicines (Munir et al. 2013). Native natural vegetation found in desert areas has huge potential for different diseases treatment because of their medicinal properties.

Pathogens become resistant to regular use of medicines and create problems for scientist. Thus there is need to discover new drugs from medicinal plants which are better source for antibiotics. The plants are cheaper source of medicine for developing countries (Mariita et al. 2016). Many researchers are working on medicinal plants for discovering new antibiotics contained antibacterial properties (Reddy et al. 2001). *Echinops echinatus* is native to Cholistan desert and belongs to Asteraceae family. Locally it is called "Unt Kantara" and used as herbal medicine. It possesses antibacterial, antioxidant, anthelmintic and antidiuretic properties (Arshad et al. 2003). Traditionally it is used against many diseases like stomach disorder, antipyretic, eczema and appetizer (Parrotta 2001). *Echinops echinatus* is a plant that is widely used in traditional medicines for many years in the era. Various types of research experiments had shown that traditional use of this plant is given a considerable significance. Therefore, the objectives of the present study are to determine the phytochemicals founds in the *Echinops echinatus*, to evaluate the antimicrobial activity of different parts of plant and to determine the antioxidant properties of the plant part extract.

2. MATERIALS AND METHODS

2.1. Collection of plant materials

Fresh plants of *Echinops echinatus* were collected from the different areas of Cholistan desert Bahawalpur Punjab Pakistan. Plant parts were identified and authenticated from the Department of Forestry, Range and Wildlife Management, The Islamia University of Bahawalpur. Secondly all parts of plant were washed under running tap water and shade dried for 10-15 days. All parts of plant were grinded separately by grinder machine. Solidified powder was preserved in plastic bags after tagging.

2.2. Preparation of plant extracts

350g leaves powder was soaked in 1500ml methanol. 300gm stem powder was saturated in 1200ml methanol. 250g flower powder was dipped in 1000ml methanol while 225g of roots powder was drenched in 700ml methanol by using 4 beakers of 2500ml which were covered with aluminum foil. All extracts were stirred 3 times in a day. Extracts were filtered after a week by using silk cloth and Whatman's filter paper. Extracts were placed in open air to evaporate methanol. Dried extracts were stored in labeled glass jars. For further chemical analysis, dried extracts were kept in refrigerator at 4°C.

2.3. Qualitative phytochemical analysis

The extract was tested for the presence of bioactive compounds by using following standard procedures and methods as defined by Sofowara (1993), Harborne (1998) and Trease and Evans (2002).

2.3.1. Proteins Testing (Millon's Test): Crude extract of plant leaves was taken in a test tube with the help of spatula. 2 ml Millon's reagent was mixed with leaves extract by using 100ml pipet. White precipitates appeared in the test tube. Upon heating the test tube, white precipitates changed into red color indicating the presence of proteins in plant leaves.

2.3.2. Carbohydrates Testing (Fehling's Test): 5ml of each Fehling A and Fehling B reagents were mixed and used to screen the carbohydrates. Crude extract was added in Fehling reagents and boiled gently. Brick red color precipitate at the lowest part of the test tube revealed the occurrence of reducing sugars.

2.3.3. Test for Phenols and Tannins: 2% solution of Iron chloride was mixed with crude extracts of plant and gently heated. The presence of phenols and tannins was identified by blue-green or black coloration.

2.3.4. Test for flavonoids (Shinoda Test): Few fragments of magnesium ribbon were mixed with crude extracts of plant. Slowly drop wise concentrated hydrochloric acid was added. The presence of flavonoids was identified by revealing pink scarlet colour after few minutes.

2.3.5. Test for Saponins: 5ml distilled water and crude extracts of plant was mixed in a test tube. The mixture was shaken dynamically. The stable foam was appeared which revealed the presence of saponins.

2.3.6. Test for glycosides (Liebermann's Test): 2ml volume of each chloroform and acetic acid was taken. Then crude extracts were mixed in them. The mixture was chilled in ice. Gently concentrated H_2SO_4 was added in the mixture. Violet color appeared which changed into blue and green. Green color was an indication for the occurrence of steroidal nucleus for example glycine portion of glycoside.

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2.3.7. Steroid Testing: 2ml of chloroform was added in test tube containing crude sample. Concentrated sulphuric acid was added sidewise. At the bottom layer of chloroform, a red colour produced indicating steroids.

2.3.8. Terpenoids Testing: The samples were digested in 2 ml of chloroform and allowed to evaporate for dryness. 2ml of sulphuric acid was added in the test tube and heated gently for 2 minutes. Grayish colour revealed the existence of terpenoids.

2.3.9. Test for Alkaloids: 2ml of 1% HCl was added in a test tube containing crude extracts. This mixture was heated wisely. However, Mayer's and Wagner's reagents were also poured into the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

2.3.10. Tests for Microorganisms: Different strains of Bacterial culture were provided by Department of Biochemistry and Biotechnology, The Islamia University Bahawalpur. Three gram-negative bacteria and one gram-positive bacterium were used for microbial activity.

a) Preparation of Nutrient broth media: Five falcon tubes contained 5ml of autoclave broth media. Four different types of bacteria (*E. coli, K. pneumonia, P. aeruginosa, S. aureus*) were taken in different falcon tubes. After 24 hours' bacterial growth was analyzed.

b) Preparation of Nutrient Agar media: Nutrient agar media was prepared and poured into the petri plates.

c) Methanol Extraction: Plant extracts were mixed with methanol. For antibacterial activity 10µg methanol extract of each sample was thawed in 1µl methanol. The extracts were centrifuged at 2000 rpm for 15 minutes. Oven temperature was maintained 110°C in order to autoclave the discs.

d) Antibacterial Activity: The laminar flow cabin was cleaned with 70% ethanol. An inoculating loop was taken. It was heated till to red then cooled for 5 seconds. The cultured bacteria like *E. coli*, *S. aureus*, *P. aeruginosa* and *K. pneumoniae* was spread on the petri plates by loop wire. The discs containing extracts were transferred on petri plates to analyze bacterial growth. The discs containing Methanolic solvent were as negative control. The petri plates were covered with aluminum foil and placed in an incubator for 24 hours at 36°C. After incubation process inhibition zone of bacterial growth was measured with measuring scale.

3. RESULTS

Qualitative measures for phytochemicals analysis of *Echinops echinatus* in flowers, roots and stems are shown in Table 1. Proteins were absent in leaves of *Echinops echinatus* and carbohydrates were absent in flowers. But leaves, stems and roots revealed the presence of carbohydrates. Phenols and tannins were found in flowers, leaves and roots. While phenols and tannins were absent in stems. Flowers and leaves showed the presence of flavonoids compounds. Saponins and glycosides were found in flowers, leaves, roots and stems. Steroids were absent in flowers and leaves. While roots and stems revealed the presence of steroids. Terpenoids were found in flowers and leaves. This compound was absent in roots and stems. Flowers and roots revealed the presence of alkaloids. While alkaloids were absent in leaves and stems.

Table 1: Phytochemicals analysis of *Echinops echinatus*

Sr No.	Test	Flowers	Leaves	Roots	Stem
1	Protein	+	-	+	+
2	Carbohydrates	-	+	+	+
3	Phenols/Tannins	+	+	+	-
4	Flavonoids	+		+	-
5	Saponins	+	+	+	+
6	Glycosides	+	+	+	+
7	Steroids	-	-	+	+
8	Terpenoids	+	+	-	-
9	Alkaloids	+	-	+	-

3.1. Antibacterial Activity

Antibacterial activity of extracts of *Echinops echinatus* was performed. The result showed that the methanolic extracts of plant have the important characteristics against different bacteria. Methanol solvent was used as negative control. The results revealed that the methanolic extract of stem showed no activity against all bacteria which were used for testing. Methanolic extract of leaves revealed the maximum inhibition zone (10mm) against *K. pneumoniae.*

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While other bacteria like *E. coli*, *S. aureus*, *P. aeruginosa* showed resistance against methanolic extract of leaves. *Staphylococcus aureus* showed high sensitivity against methanolic extract of flowers which revealed inhibition zone of 19mm. However, *K. pneumoniae*, *E. coli*, *P. aeruginosa* were resistant to flowers extract. Methanolic root extract showed maximum zone of inhibition (18mm) against *S. aureus*. While *P. aeruginosa* (inhibition zone 15mm) and *K. pneumonia* (inhibition zone 14 mm) showed less sensitivity as compared to *S. aureus*. On the other hand, *E. coli* was found fully active against root extract as shown in Table 2.

Levofloxine showed the zone of inhibition of 16 mm against *S. aureus*, while *K. pneumonia*, *E. coli*, *P. aeruginosa* showed resistance against Levofloxine (positive control antibiotic disc). Cefaparzone showed maximum zone of inhibition of 25mm and 24mm against *P. aeruginosa* and *E. coli* respectively. *Staphylococcus aureus* was less sensitive (inhibition zone 17mm), while *K. pneumoniae* was least sensitive against Cefaparzone antibiotic (inhibition zone 14mm). Methanol (negative control) showed the inhibition zone of 15mm and 10mm against *S. aureus* and *P. aeruginosa* respectively, but *K. pneumoniae* and *E. coli* showed resistance against methanol (Table 2).

Table 2: Antibacterial activity of *Echinops echinatus* against *Klebsiella, Pseudomonas, Staphylococcus* and *E. coli*

Microorganisms	Zone of inhibition in mm						
	Stems	Leaves	Flowers	Roots	Levo	Cefa	Solvent
Klebsiella	-	10	-	14	-	14	-
Pseudomonas	-	-	-	15	-	25	10
Staphylococcus	-	-	19	18	16	17	15
E. coli	-	-	-	-	-	24	-

3.1.1. α-Glucosidase inhibitory assay activity: *E. echinatus* plant extracts of leaves, flowers, roots and stem showed positive results and maximum % enzyme inhibition for α-glucosidase. The roots extract showed maximum inhibition with 75.3±1.5 with IC_{50} value of 207.3±1.3. While stem showed 62.4±1.5 with IC50 of 302.7±1.2 enzyme inhibition as compared with leaves (45.2±1.2) and flowers (28.5±1.2) that showed no inhibition activity with IC_{50} value (Table 3).

Urease inhibitory activity of *Echinops echinatus* plant extracts of leaves, flowers, roots and stem is depicted in Table 4. The activity was comparable to that of Thiourea which was used as standard. The stem showed maximum inhibition activity 93.71±0.86 with IC_{50} value 15.63±0.42. While leaves and roots had same values 92.45±0.63 and 92.63±0.76 as compared with IC_{50} value 32.34±0.35 and 39.51±0.38, while the flowers showed 91.32±0.56 urease inhibition as compared with IC_{50} value 48.59±0.27. The percentage urease inhibition of standard Thiourea was 98.22%, IC50 21.7±1.98 (Table 4).

Table 3: Inhibition studies of yeast α-Glucosidase

Sr. No.	Code	Inhibition (%) at 1.0 mg/Ml	IC_{50} (µg/mL)
1	EE (stem)	62.4±1.4	302.7±1.2
2	EE (leaves)	45.2±1.2	-
3	EE (flowers)	28.5±1.2	-
4	EE (roots)	75.3±1.5	207.3±1.3
	Acarbose	65.7±1.9 Mm	375.8±1.7 µM

Mean±SE (n=3).

Table 4: Anti-urease inhibition activity (mean±SE) of urease enzyme

Sr. No.	Code	Inhibition (%) at 0.5 mg/mL	IC_{50} (µg/mL)
1	EE (stem)	93.71±0.86	15.63±0.42
2	EE (leaves)	92.45±0.63	32.34±0.35
3	EE (flowers)	91.32±0.56	48.59±0.27
4	EE (roots)	92.63±0.76	39.51±0.38
	Thiourea	Inhibition (%) at 0.25 mM	IC_{50} (µM)
		98.28±1.22	21.7±1.98

n=3; EE=*Echinops echinatus*

4. DISCUSSION

The results of preliminary phytochemical screening of hydro-alcoholic root extract of *Echinops echinatus* revealed the presence of alkaloids, carbohydrates, steroids, glycosides, flavonoids, phenols, tannins, proteins, saponins and terpenoids. Flavonoids are the compounds richly found in many plants. Anticancer and antioxidant properties have been reported (Siddhuraju et al. 2003). Heart ailments, bone problems and aging factors imposed

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degenerative diseases risk are reduced by the high ingestion of flavonoides (Havsteen 2002). The studies reported till date has shown that foods rich in vitamins have potential against many types of diseases. These include carcinogenic, cardiac and Alzheimer's diseases. Nature is blessed by plants that are main source of flavonoids, ascorbic acid and many more phenolics. These are the best constituents of antioxidants (Laandrault et al. 2001). Alkaloids are known for their role in bio- augmenting characteristics. A lot of alkaloids are also lethal in nature and are subsequently unfit for their utilization in medicines (Sreevidya and Mehrotra 2003). The recent research showed the presence of alkaloids in the roots and flowers and absence from stem and leaves. Terpenoids were found in flowers and leaves. This compound was absent in roots and stems. Steroids were absent in flowers and leaves. While roots and stems revealed the presence of steroids (Table 1). The preliminary phytochemical analysis of petroleum extracts showed that antidiarrheal and antidysetric properties of medicinal plants were due to the presence of alkaloids, flavonoids, saponins, tannins, terpenoids, steroids, and reducing sugars (Loganga et al. 2000). Plants contain tannins and alkaloids which have the ability against disease agents. Every plant revealed different antimicrobial activity like weak, medium or strong (Zaika et al. 1998).

Amino acids are the building blocks of proteins. Plants are rich source of many proteins. Out of 20 essential amino acids required for human body; 10 are synthesized by human body. While remaining amino acids must be supplied through food intake for optimum functioning of the body. When these are not provided through food intake the body muscles and proteins are degraded to continue proper functioning. Plants can be taken to cope with the need for essential amino acids. Proteins were found in the present study in the flowers, roots and stem but absent in leaves (Table 1). Phenolic compounds have found importance of varying degrees in all the fields of life. Diseases curing properties has resulted their potential significance against many diseases such as blood clotting, cancer and heart diseases (Prakash et al. 2010). In the present study, phenols were found in all parts except stem (Table 1).

Saponins are known for their soapy features. These are distinct class of glycosides (Fluck and Ahmed 1973). These possesses active role against fungal diseases (Sadipo et al. 1991). The key features associated are precipitating and coagulating RBCs, cholesterol binding properties and bitterness (Okwu 2004). Analysis of present study showed their presence in the whole plants parts (Table 1). Diuretic activity of root methanolic extracts of *E. echinatus* is because of occurrence of carbohydrates and phenols. Diuretic activity of aerial parts methanolic extract of *E. echinatus* is because of existence of carbohydrates, flavonoids and alkaloids (Patel et al. 2011). In the current study, carbohydrates were present in leaves, roots and stem but not found in flowers (Table 1).

Echinops echinatus extract has been found effective in improving the kidney and pancreas functions and reduced the lesions associated with diabetic state in alloxan – diabetic rats. Methanolic extracts of *E. echinatus* exhibited operational diuretic activity. This was exhibited during enlarged amount of excreted Na^+ and K^+ salts in overall urine production (Patel et al. 2011). Our findings were in line with the earlier research against *E. coli*, *S. aureus*, *P. aeruginosa*, *K. pneumonia* by phytochemicals (Ahmed et al. 2019). Plants are of significance importance because they possess certain antimicrobial traits. Plants produce secondary metabolites which are used as antimicrobials. These have got importance due to active substances such as phenols and tannins. These are excellent source of antimicrobial substances which are used against bacterial diseases. Alkaloids possessed analgesic, antispasmodic and antibacterial properties (Malik et al. 2017). In the recent study it was found that methanolic extract of different parts of *Echinops echinatus* revealed the excellent antimicrobial activity against certain strains of bacteria which was used in the various test as shown in Table 2.

Conclusion: In the light of these findings, we can conclude that methanol extracts were active against human pathogenic bacteria and showed maximum zone of inhibition. The inhibitory activity of all extracts against both the enzyme Urease and α-glucosidase showed inhibition activities. *Echinops echinatus* could be considered for further studies in the treatment of diabetes and ulcer as natural remedy. These extracts can also be used as antibiotic against many human pathogen diseases.

Author's Contribution: MR conceived and designed the study in addition to writing the manuscript and providing the critical revisions. MA conducted the research study and collection the data. MUG performed analysis while ZM provided technical support. MM wrote the manuscript. All authors approved the final version of the manuscript.

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