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IN VITRO ANTI MICROBIAL AND ANTI OXIDANT ASSAY OF ROOT OF *ECHINOPSIS CALOCHLORA SCHUM*

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ABSTRACT

The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments of various ailments, including asthma, gastro-intestinal problems, skin disorders, respiratory and urinary complications, and hepatic and cardiovascular disease. Several pharmacological studies have confirmed that medicinal plants exhibit biological activities and that plant species can contain a diverse range of bioactive molecules responsible for a collection of pharmacological properties. The pharmacological benefits of medicinally important plants are primarily due to bioactive phytochemicals produced in the plant tissues as primary and secondary metabolites. These constituents have been identified as alkaloids, glycosides, flavanoids, phenolics, saponins, tannins, and essential oils, steroids etc. The present study investigates the phytochemical analysis and pharmacological activities of root of *Echinopsis calochlora schum* (ECS-R).

KEYWORDS: Secondary metabolites, alkaloids, glycosides, flavanoids, phenolics, saponins.

1. INTRODUCTION

Bioactive compounds originated from plants are called the secondary metabolites. They are distributed all over the plant body in different concentrations differing from one plant to another. These metabolites were the primary requirement in the synthesis of ancient medicines used worldwide.[1] The plants rich in these metabolites are economically cultivated and are used in several pharmaceutical industries. The synthetic drugs used today are the replica or the in vitro synthesis of the naturally available metabolite.[2] The extracted metabolites are of great importance because of its antimicrobial activities. The ancient remains proved that the plant metabolites were also used for preservation.[4] Plants belonging to the family cactaceae are known for its uses as fodder ,fruit, vegetable etc.[5] These plants are rich in antioxidants and also processes anticancer properties.[6] Not all the metabolites possesses medicinal properties, that is they may be active or inactive. The knowledge about such metabolites is necessary to discover valuable medicinal drugs.[7]

2. Experimental

2.1. Materials

2.1.1. Preparation of plant extract

The root of *Echinopsis calochlora Schum* (ECS-R) plant extract is prepared by taking 100g of root is soaked in 1L of ethyl alcohol for 3 days. Then it is filtered and the filtrate is used as stock solutions for further phytochemical and pharmacological studies.

2.2. Methods

2.2.1. Preliminary Phytochemical screening

Qualitative phytochemical analysis of the crude root extract is determined according to the standard procedures to identify the chemical constituents by Dragendorff test for alkaloids, Foam test for saponins, Salkowski test for triterpenoids, $FeCl_3$ test for tannins, NaOH test for glycosides, Molisch test for reducing sugars, Biuret test for proteins, and ammonia test for detection of flavanoids are performed to identify the constituents present in the extract of the leaves of the plant.

Test for Saponins

The acid extract is diluted with distilled water and shaken well for 15 minutes. Formation of foam indicates the presence of Saponin.

Test for Tannins

A 2ml of acid extract and 2ml of $FeCl_3$ are mixed. A blue or greenish black precipitate indicated the presence of tannins.

Test for Phenols

About 2ml of extract is dissolved in 2 ml of distilled water and is treated with a few drops of neutral 5% ferric chloride. Formation of dark green color indicates the presence of phenols.

Test for Flavanoids
About 3 ml of extract is treated with 2 ml of 10% ammonium hydroxide solution. A yellow fluorescence indicated the presence of flavanoids.

Test for Steroids
About 1ml of extract is treated with 2 ml of acetic anhydride. The side of the test tube is treated with 2-3 drops of concentrated sulphuric acid. An array of color showed presence of steroids.

Test for terpenoids
To 1 ml of extract, tin (one bit) and thionyl chloride are added. Appearance of pink color indicates the presence of terpenoids.

Test for carbohydrates
1ml of alpha-naphthol solution, and concentrated sulphuric acid through the sides of test tube are added to the extract .Formation of purple color at the junction of the two liquids revealed the presence of carbohydrates.

Test for proteins
Violet color formation on the addition of 40% sodium hydroxide to the extract indicates the presence of proteins.

Test for Cardiac glycosides
Aqueous sodium hydroxide is added to 2 ml of acid extract dissolved in 1ml water Yellow color formation indicates the presence of glycosides.

Test for alkaloids
To 1ml of extract, few drops of dragendroff's reagent is added and the color developed is noticed. Orange color is formed indicating the presence of alkaloids.

2.2.2. Antibacterial Activity
Analysis method: Agar diffusion method.

Bacteria analysed: E.Coli, S.aureus and P.aeruginosa.

Concentration screened: 20µl, 60 µl and 80 µl Std. antibiotic used for antibacterial activity: Ciprofloxacin

Description for antibacterial activity: Media Used: Peptone-10 g, NaCl-10g and Yeast extract 5g, Agar 20g in 1000 ml of distilled water.

Media used for anti fungal activity: czapek –dox agar composition (g/l) sucrose 30.0; sodium nitrate 2.0K_2HPO_4-1.0, MgSO4.7H2O-0.5; KCL-0.5; FeSO4-0.01; AGAR-20

Method: Initially, the stock cultures of bacteria are revived by inoculating in broth media and grown at for 18 hrs. Wells are made in the above prepared agar plate. Each plate is inoculated 37^oC with 18 hours old cultures (100 µl, 10-4cfu) and spread evenly on the plate. After 20 min, the wells are filled with of compound at different volumes. All the plates are incubated at 37^oC for 24 h and the diameter of inhibition zone are noted in mm.

2.2.3. Antioxidant assay
The antioxidant activity of the sample at different concentrations (20, 60, 80 µl) are taken in test tubes. The volume is reduced to 500µl by adding methanol. Five millilitres of 0.1 mm methanolic solution of 1, 1-diphenyl-2-picryl hydrazyl (DPPH) is added to these tubes and shaken vigorously. An equivalent amount of methanol is maintained. 517 nm is the absorbance at which the samples are measured. Butylated Hydroxy Anisole (BHA) is used as a reference standard. The following formula is used to calculate the free Radical scavenging activity % Radical scavenging activity = [Control OD-Sample OD]×100

2.2.4. FTIR STUDY
FT-IR Spectrum of the crude plant extract (ECS-R) is investigated for its characteristic functional groups in the spectral region between 4000-750cm^{-1} using Shimadzu make Spectrometer.

3. RESULTS AND DISCUSSION

3.1. Phytochemical Analysis
Results of the phytochemical constituents of ECS-R is reported in Table 1. The phytochemical screening showed the presence of flavonoids, alkaloids, glycosides, saponin and coumarins. Negative results are obtained for terpenoids, tannin, quinones, steroids, phenol and carbohydrates.

Table 1: Preliminary screening of ECS-R.

Phytochemicals	Observation
Alkaloids	+
Terpenoids	-
Coumarins	+
Steroids	-
Tannins	-
Saponins	+
Flavanoids	+
Quinones	-
Phenols	-
Carbohydrates	-
Glycosides	+
Carotenoids	-

+ Presence -Absence

3.2. Pharmacological activity
The root extract of different concentrations of *Echinopsis calochlora schum* in ethyl alcoholic extract is tested for its antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *P.aeruginosa* using disc diffusion method. The zones of inhibitions (mm) are mentioned in Table 2 and Fig 1. At 80 µL concentration of ECS-R, the diameter of inhibition zones (DIZ) against and *E.coli* was 4mm. The extract showed DIZ of 3mm and 5mm against P.aeruginosa at 60µL and 80µL respectively. Whereas, the drug showed total inactivity against the pathogen

Staphylococcus aureus. The standard drug Ciprofloxacin showed the considerable DIZ against all the pathogens. The results of antibacterial assay showed the moderate activity of the plant extract against the tested microorganisms.

Table 2: Antibacterial testing of ECS-R using Agar diffusion method.

Activity	Organism	Diameter of zone of inhibition(mm)			
		Standard Drug	Concentration of ECS-R		
		Ciprofloxacin 100 µg	20 µL	60 µL	80 µL
Antibacterial	S.aureus	31	0	0	0
	E.coli	32	0	0	4
	P.aeruginosa	30	0	3	5

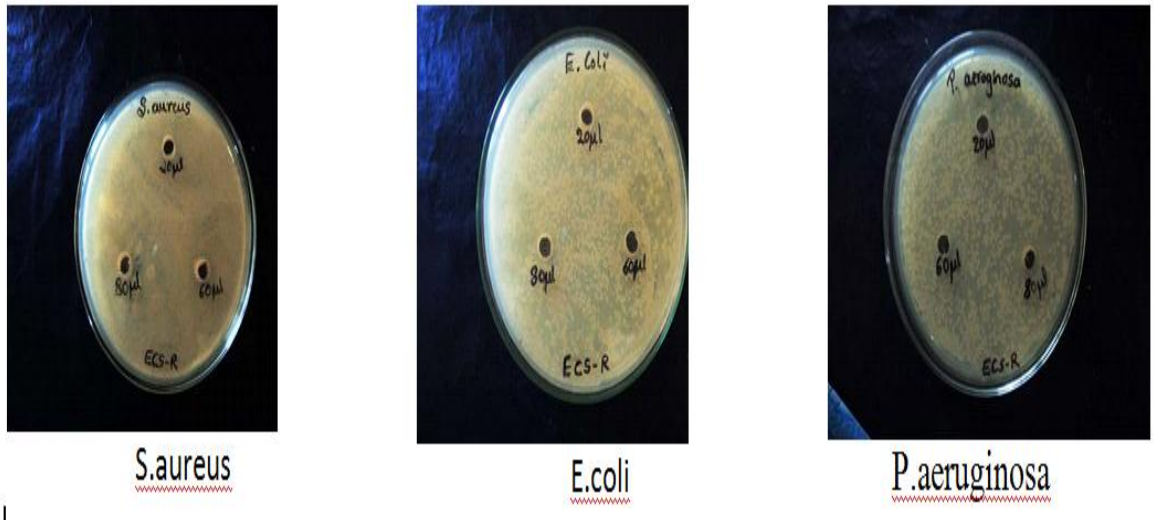

Fig. 1: Antibacterial activity of ECS-R.

3.3. Antioxidant activity

Flavanoids ability of scavenging hydroxyl radicals, superoxide anion radicals and lipid peroxy radicals, which highlights many of the flavanoid health-promoting functions in organisms, which are important for prevention of diseases associated with oxidative damage of membrane, proteins and DNA Antioxidants in human diet may reduce the risk of various cancers, prevents menopausal symptoms, prevents oxidative cell damage and have strong anticancer activity.[8-9] The 1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical is widely used as a model system to investigate the free radical scavenging activities of several plant extracts. DPPH is stable; nitrogen centered free radical which produces violet color in methanol solution. It is reduced to a yellow colored product with, diphenyl picryl hydrazine, with the addition of the extracts. The reduction in the number of DPPH molecules can be calculated with the number of available hydroxyl groups.[10] The increase in the DPPH scavenging activity of ethyl alcoholic extract of *Echinopsis calochlora schum* is depended completely in the concentration. The extract exhibited potent DPPH radical scavenging activity and is shown in Table 3, Figure 2 and Figure 3. IC50 is often used to express the amount of concentration of extracts to scavenge 50% of the free radicals. The IC50 value is inversely proportional to the scavenging activity of the extract. The anti oxidant assay showed that 9.72% and 22.92% of free radical scavenging activity is obtained at 20 µL and 60 µL of ECS-R extract respectively and also compared with standard BHA. The result of the present study showed that, the presence of flavanoids in the plant extract may be reason for the radical scavenging activity.

Table 3: Free radical scavenging activity of ECS-R.

	IC 50 values	
Concentration	ECS-R	BHA
20µl	9.72	54.27
60µl	22.92	70.10

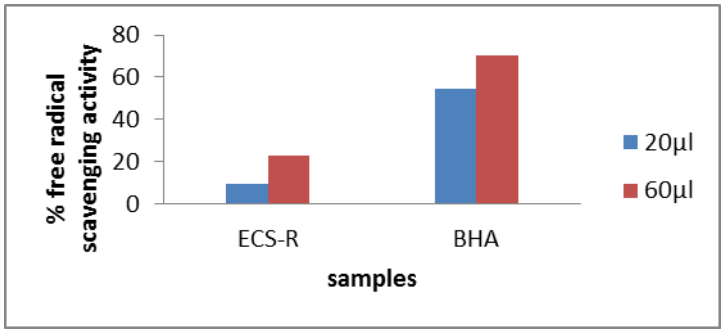

Fig. 2: IC 50 values of ethanolic extract of *Echinopsis calochlora schum.*

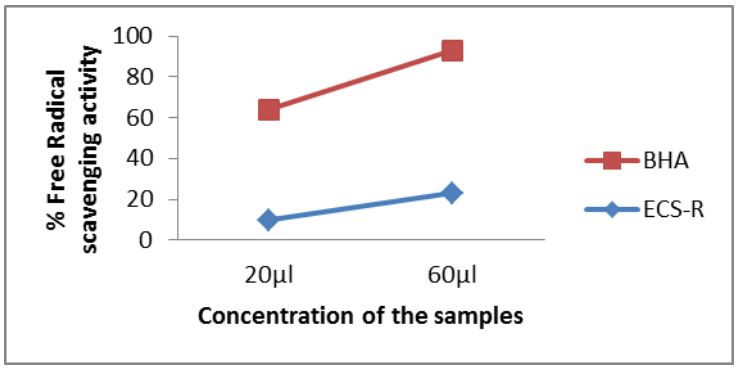

Fig. 3: DPPH radical scavenging activity of ethanolic extract of *Echinopsis calochlora schum.*

3.4. FTIR analysis

FTIR peak values and the spectrum for ECS-R are shown in Figure 4 and Table 4.The peak at2978.09cm^{-1} peak indicates the presence of -C=CH_2 allyl group. The peak at 1751.36cm^{-1} shows presence of cyclic 5-membered aldehydes or ketones. The peak at 1523.76cm^{-1} shows the presence of -N-O in nitro compounds. 1381.03cm^{-1}peak indicates the presence of -CH_3. The peak at 1238.30cm^{-1} indicates the presence of -C-O in aromatic ethers. The peak at 1087.85cm^{-1} shows the presence of-NH in aliphatic amines. 1041.56cm^{-1} shows the presence of-NH in primary amines. The peak at 875.68cm^{-1} shows the presence of –CH in aromatic meta distributed benzene.

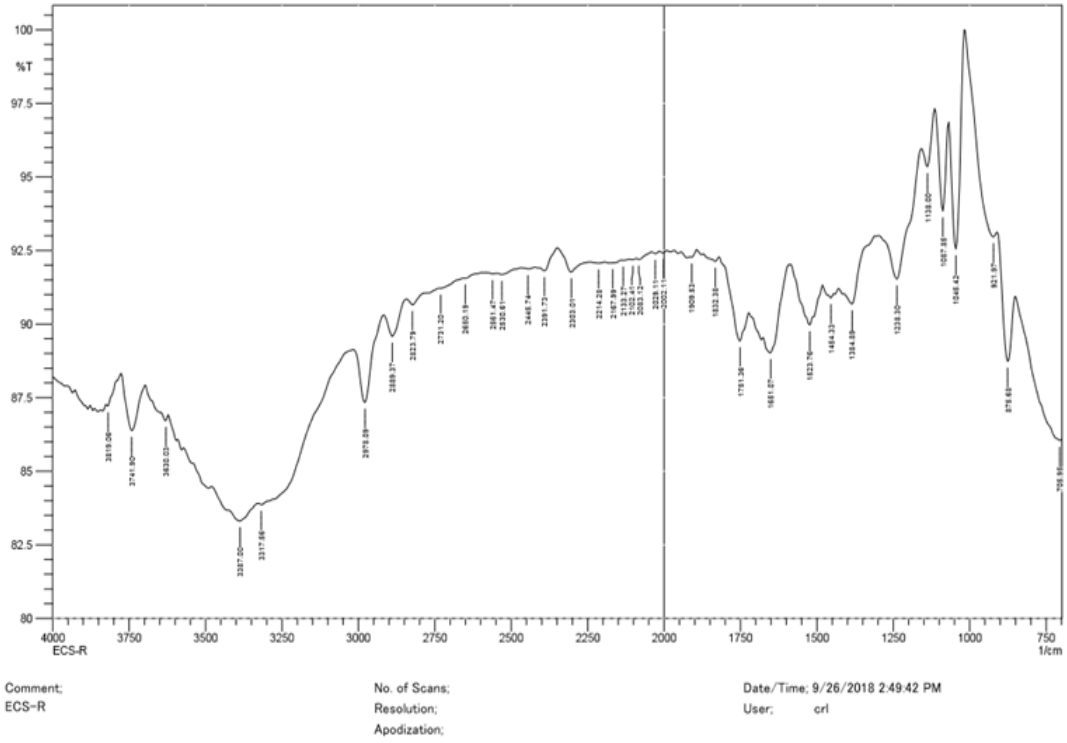

Fig. 4: FTIR spectrum of crude ECS-R.

Table 4: FTIR analysis of ECS-R extract.

FTIR peak values of ECS-R	Possible groups
2978.09cm^{-1}	-C=CH_2
1751.36cm^{-1}	-C=O in ketones
1523.76cm^{-1}	-N-O in nitro compounds
1381.03cm^{-1}	-CH_3
1238.30cm^{-1}	-C-O in aromatic ethers
1087.85cm^{-1}	-NH in aliphatic amines
1041.56cm^{-1}	-NH in primary amines
875.68cm^{-1}	-C-H in aromatic meta di substituted benzene

CONCLUSION

- Phytochemical screening of root of *Echinopsis calochlora schum* indicated that it contains the following medicinally important secondary metabolites like phenols, saponins, glycosides, alkaloids and coumarins are responsible for the tested pharmacological activities.
- The plant extract possesses moderate inhibitory activity against the tested bacterial strains.
- The alcoholic extract of ECS-R showed strong antioxidant potential by inhibiting DPPH free radical scavenging activity.
- FTIR analysis also supported the presence of different functional groups in the cactus plant.
- The present study explained the development of drugs from natural plant sources and to be used for the treatment of disease.

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