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Research Article

PHYTOCHEMICAL, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF METHANOL EXTRACT OF LEAVES AND FLOWERS OF *IPOMOEA CAIRICA*

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ABSTRACT

Phytochemical, antimicrobial, and antioxidant investigation on the leaves and flower of *Ipomoea cairica* was traced in present investigation. Leaves and flowers of the plants were extracted with Methanol. Methanol Extracts of both leaves and flower of *Ipomoea cairica* were tested quantitatively for phytoconsituents. Methanol extract for both leaves and flower showed the presence of phytoconstituent Alkaloids, carbohydrates, tannins, phenolic compounds, proteins and amino acid, terpinoids and sterols and saponins. These extracts were further tested for their antimicrobial activity against the bacterial strains of *Eschirechia coli* (22 mm & 11 mm), *Klebsella pneumonia* (11 mm & 10 mm), *Salmonella typhi* (13 mm & 11 mm), *Bacillus subtilus* (10 mm & 15 mm) , *Staphylococcus aurius* (08 mm & 13 mm) and the fungal strains of *Aspergillus niger* (16 mm & 14 mm), *Penicillum chrysogenum* (20 mm & 18 mm), *Sacchomyces cerevisiae* (25 mm & 21 mm), *Candida albicans*(24 mm & 18 mm) of both methanol extract of leaves and flowers respectively. The results have shown that methanol extract of the leaves and flower of *Ipomoea cairica* showed very good activity against all the bacterial and fungus strains in comparison with chloramphenicol and ketoconazole. Methanol extracts of leaves and flowers showed remarkable antioxidant activity 82.58 % and 81.44 % at 500 µg/ml respectively.

Keywords: *Ipomoea cairica*, Antibacterial activity, Antifungal activity, Antioxidant activity, Ascorbic acid.

INTRODUCTION

Herbal medicine, sometimes referred to as Herbalism or Botanical Medicine, is the use of herbs for their therapeutic or medicinal value. Herb plants produce and contain a variety of chemical substances that act upon the body. Herbalists use the leaves, flowers, stems, berries, and roots of plants to prevent, relieve, and treat illness. The reality is, however, that herbal medicine has a long and respected history. With increased incidence of resistance to antibiotics, natural products from plants could be interesting alternatives [1,2]. Some plant extracts and phytochemicals are known to have antimicrobial properties, and can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to demonstrate such efficacy [3,4].

Ipomoea is the largest genus in the flowering plant family convolvulaceae, with over 500 species. The generic name is derived from the Greek words meaning "resembling". It refers to their twining habit. The genus occurs throughout the tropical and subtropical regions of the world. Humans use *Ipomoea* for their content of medical and psychoactive compounds, mainly alkaloids. The genus includes food crops; the tubers of sweet potatoes and the leaves of water spinachare commercially important food items. *Ipomoe amauritiana* is one of the many ingredients of *chyawanprash*, the ancient Ayurvedictonic called "the elixir of life" for its wide-ranging properties. The various species have wide medical application. They are used to treat blood disease, sterility in women, urinary infection, constipation, gynecological disorder [5]. The plant is also having laxative, psychedelic [6,7], anticarcinogenic, hepatoprotectivity, oxytocic [8], and an antioxidant properties [9]. They are also used in rheumatism and fungal infection [10].

Ipomoea cairica of ethanol extracts from medicinal plants commonly used by Governador Valadares people were tested for cytotoxicity (BST assay), antioxidant activity, antagonist properties [11-13]. Antinociceptic effect from *Ipomoea cairica* L. Sweet (Convolvulaceae) is used in Brazilian folk medicine for the treatment of rheumatism and inflammations [14]. Peroxidase was purified by gel filtration and SDS-PAGE homogeneity from *Ipomoea cairica* leaves using ammonium sulphate precipitation, acetone fractionation, and gel filtration chromatography on Sephadex G-100 and Sephadex G-200 column [15]. In continuation of our efforts in search of potential antimicrobial and antioxidant agent with no side effect, we have taken *Ipomoea cairica* and screened their leaves and flowers for antimicrobial and antioxidant activity.

MATERIALS AND METHODS

Collection of plant material

The plant leaves and flower was collected from area of Prem Nagar and outside the Indian military Academy (IMA), Dehradun during the month of November- December. Collected plant material was authenticated by Dr. S.K Srivastava (Scientist D/HOD), in Botanical Survey of India, Northern regional centre, Dehradun (BSD) and Acc. No. 113675 .The plant material was washed with water to removed and other undesirable material and dried under shade.

Preparation of extracts

The air-dried leaves (500 gm) and flower (500 gm) of *Ipomoea cairica* were crushed and powdered separately and subjected to soxhlet extraction with methanol. The extract was then evaporated and dried.

Qualitative Phytochemical test

The different extract of leaves of *Ipomoea cairica* were tested for various components by their specific tests viz. Mayer's test, Dragendroff's test, Wagner's test for alkaloids; Gelatin test, Ferric chloride test, Vanillin hydrochloride test for tannins & phenolic compounds; Million test, Ninhydrin test, Xanthoproteic test for proteins and amino acids; Salkowski test, Sulfur powder test for sterols and triterpenoids; Molisch's test, Benedict's test, Barfoed's test, Bromine water test for carbohydrates and Foam test for saponins.

Antimicrobial activity of extracts

The antimicrobial activity of the leaves and flower extracts of *Ipomoea cairica* were carried out. The leaves and flower extracts were screened for antibacterial and antifungal activities.

Antibacterial activity of leaves and flower extracts

The bacterial cultures used in the study were *E. coli, Klebsiella pneumonia, Bacillus subtilus, Salmonella typhi, S. aureus.* These bacteria's were provided by Department of Microbiology, Dolphin Institute of Biomedical and Natural Sciences, Manduwala, Dehradun and checked for purity by convention biochemical methods. These bacterial cultures were maintained on nutrient agar slants at first being incubated at 37ºC for about 18-24 hours and then stored at 4ºC as stock cultures for further antibacterial activity. Fresh culture

were obtained by transferring a loop full of culture into nutrient broth and then incubated at 37ºC overnight. To test antibacterial activity, the well diffusion method was used. The concentration used for antibacterial activity is 200 mg/ml.

Culture media preparation

The microbiological media prepared as standard instruction provided by the HI-MEDIA Laboratories Pvt. Ltd., Mumbai. The medium used for antibacterial activity were Mueller-Hinton Agar (MHA) and Nutrient Broth (NB). They were prepared and sterilized at 121ºC at 15 psi for 15-30 minutes in autoclave.

Plate preparation

25ml of pre autoclaved Mueller-Hinton agar (MHA) was poured into 90 mm diameter pre sterilized petri plates. These petri plates were allowed to solidify at room temperature.

Well diffusion method

After the plates solidified the freshly prepared microbial broth culture suspension (about 0.1 ml) was spreaded over the Mueller-Hinton agar (MHA) media using L-shaped sterilized glass spreader separately under aseptic condition using laminar air flow. Then wells were made in each plate with the help of borer of 8 mm diameter. In these well, about 0.1 ml of each leaves extracts individually was loaded. This method depends upon the diffusion of leaves extracts from hole through the solidified agar layer of petri dish to such an extent that the growth of added microorganism is prevented entirely in a circular area or zone around the hole containing leaf extract. Petri plates were incubated for 24 hrs at 37ºC in the incubator. After incubation, the diameter of clear zone of inhibition produced around the well or holes were measured in mm and compared with standard drug.

Antifungal activity of leaves and flower extracts

In this study, the antifungal activity was studied against the microorganism viz. *Aspergillus niger. Penicillum chrysogenum. Sacchromyces cerevisiae, Candida albicans.* These cultures were obtained from the standard cultures maintained in the Microbiology Department of DIBNS, Dehradun. These cultures were maintained on Sabouraud Dextrose Agar (SDA) at first being incubated at 25ºC for about 72-96 hours and then stored at 4ºC as stock cultures for further antifungal activity. Fresh cultures were obtained by transferring a loop full of cultures into sabouraud dextrose broth and then incubated at 25ºC for 72 hrs. To test antifungal activity, the well diffusion method was used. Here culture media preparation in sabouraud dextrose agar (SDA) and incubation period is 72 hours at 25ºC rest the method is same as that of antibacterial activity. The concentration used for antifungal activity is 200 mg/ml.

Antioxidant activity [16-17]

DPPH Method

Mechanism of DPPH method

The molecule of 1,1-diphenyl-2-picrylhydrazyl (α,α-diphenyl-β-picrylhydrazyl; DPPH:**1**) is characterized as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole, so that the molecules do not dimerise, as would be the case with most other free radicals. The delocalisation also gives rise to the deep violet colour, characterized by an absorption band in ethanol solution centered at about 517 nm. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet colour (although there would be expected to be a residual pale yellow colour from the picryl group still present).

Methodology of DPPH method

Preparation of DPPH

DPPH is a highly oxidisable compound. It oxidized in light, so DPPH is prepared in dark. Weigh accurately 20 mg DPPH and dissolved in solvent. Generally methanol and for some cases ethanol is used as a solvent for DPPH.

Preparation of standard Ascorbic acid solution

Ascorbic acid is a strong anti oxidizing agent. It is taken as standard. Standard solution of ascorbic acid is prepared.

Preparation of different concentration of *Ipomoea cairica* extract

Different concentrations of the test sample which is to be examined for antioxidant activity is prepared. *viz.* 50 µg/ml, 100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml, 500 µg/ml.

Preparation of test sample

3 ml of different concentration of test sample *Ipomoea cairica* extract was mixed with 1 ml of DPPH solution in dark.

Preparation of standard

3 ml of different concentration of standard solution of ascorbic acid was mixed with 1 ml of DPPH solution in dark.

Incubation

The prepared solution of ascorbic acid and test sample was incubated for 1/2 half an hour.

Measurement of absorbance

When procedure is done than absorbance is taken with the help of U.V. Spectrophotometer at 517 nm.

Calculation

We calculate the % activity of individual concentration of individual extract from the following formula:-

$$\% \, Activity = \frac{Abs.\,of\,controll - Abs.\,\,of\,individual\,concentration}{Abs.of\,controll} \times 100$$

Abs. = Absorbance.

RESULTS AND DISCUSSION

From Phytochemical analysis the extracts of leaves and flower of *Ipomoea cairica* undergoes various qualitative chemical tests. Methanol extract for both leaves and flower shows the presence of phytoconstituent tested viz. Alkaloids, carbohydrates, tannins, phenolic compounds, proteins and amino acid, terpinoids and sterols and saponins.

Anti microbial activity of extracts:

The antibacterial activity and antifungal activity of methanol extract of leaves of *Ipomoea cairica* and standard drug Chloramphenicol and ketoconazole were tested for different strains of bacteria and zone of inhibition was recorded in millimeter.

Antibacterial activity of leaves extract of *Ipomoea cairica*

Table 1: Showing antibacterial inhibition zone of methanol extract of leaves of *Ipomoea cairica* at concentration 200 mg/ml.

S. No.	Test organism	Inhibition zone (in mm)	
		Methanol	Chloramphenicol
1	*Escherichia coli*	22	25
2	*Klebsiella pneumonaie*	11	16
3	*Bacillus subtilus*	10	26
4	*Salmonella typhi*	13	25
5	*Staphylococcus aureus*	08	34

Antifungal activity of leaves extracts of *Ipomoea cairica*

Table 2: Showing antifungal inhibition zone of methanol extract of leaves of *Ipomoea cairica* at concentration 200 mg/ml.

S. No.	Test organism	Inhibition zone (in mm)	
		Methanol	Ketoconazole
1	*Aspargilus nigar*	16	19
2	*Candida albicans*	24	12
3	*Sacchromyces cerevisiae*	25	30
4	*Penicillium chrysogenum*	20	21

Antibacterial activity of flower extracts of *Ipomoea cairica*

Table 3: Showing antibacterial inhibition zone of methanol extract of leaves of *Ipomoea cairica* at concentration 200 mg/ml

S. No.	Test organism	Inhibition zone (in mm)	
		Methanol	Chloramphenicol
1	*Escherichia coli*	11	25
2	*Klebsiella pneumonaie*	10	16
3	*Bacillus subtilus*	15	26
4	*Salmonella typhi*	11	25
5	*Staphlococcus aureus*	13	34

Antifungal activity of flower extracts of *Ipomoea cairica*

Table 4: Showing antifungal inhibition zone of methanol extract of leaves of *Ipomoea cairica* at concentration 200 mg/ml

S. No.	Test organism	Inhibition zone (in mm)	
		Methanol	Ketoconazole
1	*Aspargilus nigar*	14	19
2	*Candida albicans*	18	12
3	*Sacchromyces cerevisiae*	21	30
4	*Penicillium chrysogenum*	18	21

Antioxidant activity by DPPH method

Antioxidant activity of leaves extracts of *Ipomoea cairica*

Table 5: Showing absorbance of methanol extract of leaves of *Ipomoea cairica* by DPPH method:

Concentration	Absorbance	
(µg/ml)	Methanol	Ascorbic Acid
50	0.295	0.091
100	0.245	0.091
200	0.191	0.090
300	0.175	0.092
400	0.171	0.094
500	0.167	0.092
Control	0.959	

Table 6: Showing % antioxidant activity of methanol extract of leaves of *Ipomoea cairica* by DPPH method

Concentration	% antioxidant activity	
(µg/ml)	Methanol	Ascorbic Acid
50	69.23	90.51
100	74.45	90.51
200	80.08	90.61
300	81.75	90.41
400	82.17	90.20
500	82.58	90.41

Antioxidant activity of flower extracts of *Ipomea cairica*

Table 7: Showing absorbance of methanol extract of leaves of *Ipomoea cairica* by DPPH method

Concentration	Absorbance	
(µg/ml)	Methanol	Ascorbic Acid
50	0.322	0.091
100	0.303	0.091
200	0.231	0.090
300	0.192	0.092
400	0.189	0.094
500	0.178	0.092
Control	0.959	

Table 8: Showing % antioxidant activity of methanol extract of leaves of *Ipomoea cairica* by DPPH method.

Concentration (µg/ml)	% antioxidant activity	
	Methanol	Ascorbic Acid
50	66.42	90.51
100	68.40	90.51
200	75.91	90.61
300	79.98	90.41
400	80.29	90.20
500	81.44	90.41

From Antibacterial studies of methanol extract of leaves of *Ipomoea cairica* showed inhibition zone against bacterial strains i.e., *Escherichia coli* (22 mm), *Klebsiella pneumonaie* (11 mm), *Bacillus subtilus* (10 mm), *Salmonella typhi* (13 mm), *Sacchromyces cerevisiae* (08 mm) in comparison with standard drug Chloramphenicol which showed inhibition zone against *Escherichia coli* (25 mm), *Klebsiella pneumonaie* (16 mm), *Bacillus subtilus* (26 mm), *Salmonella typhi* (25 mm), *Staphylococcus aureus* (34 mm) which is given in table 1.

In antifungal studies of methanol extract of leaves of *Ipomoea cairica* showed inhibition zone against fungus strains i.e., *Aspergilus nigar* (16 mm), *Candida albicans* (24 mm), *Sacchromyces cerevesie* (25 mm), *Penicillium chrysogenum* (20 mm) in comparison with standard drug Ketoconazole which showed inhibition zone against fungus strains i.e., *Aspergilus nigar* (19 mm), *Candida albicans* (12 mm), *Sacchromyces cerevisiae* (30 mm), *Penicillium chrysogenum* (21 mm) which is give in table 2.

Methanol extract of flower of *Ipomoea cairica* showed inhibition zone against bacterial strains i.e., *Escherichia coli* (11 mm), *Klebsiella pneumonaie* (10 mm), *Bacillus subtilus* (15 mm), *Salmonella typhi* (11 mm), *Sacchromyces aureus* (13 mm) in comparison with standard drug Chloramphenicol which showed inhibition zone against *Escherichia coli* (25 mm), *Klebsiella pneumonaie* (16 mm), *Bacillus subtilus* (26 mm), *Salmonella typhi* (25 mm), *S.aureus* (34 mm) which is given in table 3.

Methanol extract of flower of *Ipomoea cairica* showed inhibition zone against fungus strains i.e., *Aspargilus nigar* (14 mm), *Candida albicans* (18 mm), *Sacchromyces cervisiae* (21 mm), *Penicillium chrysogerum* (18 mm) in comparison with standard drug Ketoconazole which showed inhibition zone against fungus strains i.e., *Aspargilus nigar* (19 mm), *Candida albicans* (12 mm), *Sacchromyces cervisiae* (10 mm), *Penicillium chrysogenum* (21 mm) which is give in table 4.

Antioxidant activity of methanol extract of leaves of *Ipomoea cairica* showed maximum antioxidant activity of 82.58% at 500 µg/ml concentration and showed maximum antioxidant activity 81.44 % at 500 µg/ml concentration of methanol extract of flower of *Ipomea cairica* (results are shown in table 5, 6, 7 and 8).

The antimicrobial activity of various plants has been reported by many researchers. As the plant produce secondary metabolites in order to protect themselves from microorganism, herbivores and insects, thus antimicrobial effect is somehow expected from plants namely flavonoids, alkaloids, tannins, saponins and tri-terpenoids are producing a better opportunity for testing wide range of microorganism. The results obtained from this work revealed that the plants contained bioactive agents which are connected with antimicrobial properties in plants [18].

Antioxidants protect cells against damage caused by molecules known as free radicals the antioxidant effects of plant extracts are mainly due to the presence of phenolic compounds such as flavonoids, phenolic acids, tannins and phenolic diterpenes Phenolic are the largest group of phytochemicals and have been touted as accounting for most of the antioxidant activity of plants or plant products.

CONCLUSION

Infectious diseases are the world's leading cause of premature deaths. In recent years, drug resistance to human pathogenic bacteria has commonly been reported from all over the world. Even though pharmaceutical companies produce number of new antibacterial drugs, but gradual resistance to these drugs has increased which is matter of global concern besides synthetic drugs are normally associated with side effects (hypersensitive, immune suppression etc). Use of phytochemicals with known antimicrobial properties can be of great significance in therapeutic treatments. Present study is an effort towards this direction [19].

The present work also reveals that the extract from the leaves of *Ipomoea cairica* possesses good antioxidant potential presumably because of its phytochemical constituents. The DPPH scavenging activities of *Ipomea cairica* leaves extract showed a good correlation with its reductive potentials.

Based on the result of this study it can be said that *Ipomoea cairica* leaves is an effective antimicrobial and antioxidant agent that can be used for folk medicine and will be a good source to treat and control many diseases. These findings could also be of commercial interest to both pharmaceutical companies and research institutes in the production of new drugs.

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