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Phytochemical screening and *in vitro* antimicrobial activity studies of *Epipremnum aureum* Linn. leaves extracts

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ABSTRACT

This study was carried out with the objective of phytochemical screening of and to investigate the antibacterial and antifungal activity of aqueous and methanolic extract of Epipremnum aureum Linn..Antibacterial activity was carried out against Escherichia coli and Staphylococcus aureus and the antifungal activity was evaluated against Candida albicans. The testing was done by well diffusion method and evaluation was done by detecting zone of inhibition and minimum inhibitory concentration (MIC). Zone of inhibition were compared with standards like Gentamycin and ketoconazole. The results showed that aqueous extract has significant antimicrobial activity and no activity was reported with methanolic extract. MIC value of aqueous extract was found to be 50 µg/ml against bacterial strain and 25 µg/ml against fungal strain.

Keywords: aqueous extract, methanolic extract, well diffusion method, zone of inhibition, MIC.

INTRODUCTION

Infectious diseases are the second leading cause of death worldwide. [1] In industrialized nations, despite the progress made in the understanding of microbiology and their control, incidents of epidemics due to drug resistant microorganisms and the emergence of unknown disease-causing microbes, pose enormous public health concerns [2]. The emergence of multidrug-resistant bacteria has created a situation in which there are few or no treatment options for infections with certain microorganisms [3]. Along with bacterial infections, the fungal infections are also a significant cause of morbidity and mortality despite advances in medicine and the emergence of new antifungal agents [4]. Although the need for new antimicrobials is increasing, development of such agents faces significant obstacles [5]. A number of factors make antimicrobial agents less economically attractive targets for development than other drug classes [6]. Pharmaceutical research and development costs, which are estimated to be $400–$800 million per approved agent [7], pose a considerable barrier to new drug development in general.
Plants have been the traditional source of raw materials for medicines. The use of medicinal plants for the treatment of several diseases is a primary health care in India [8]. The potential of higher plants as a source of new drugs is still largely unexplored. The trend of using natural

products has increased and the active plant extracts are frequently screened for new drug discoveries and for the presence of antimicrobials [9]. Latest and previous studies have concluded the beneficial aspects of plant derived drugs as good source of antibiotics, antioxidants and anti-inflammatory agents [10]. In order to find new therapeutic agents, plants that have antimicrobial activity have attracted attention [11].

These facts motivate us to find out a new herbal moiety, which can act as an antimicrobial biomolecules to treat opportunistic microbial infections. This study has been conducted to uncover the phytochemicals present in the methanolic and aqueous extracts of leaves of *Epipremnum aureum* Linn. and to evaluate the antimicrobial effect of the extracts against clinical isolates of bacterial and fungal strains

Epipremnum aureum Linn. is a popular ornamental foliage plant used in hanging baskets and as a vine for totem poles. Pathos, *scindapsus aureus*, *Pathos aurea*, Silver vine, Devil's Ivy, Solomon Island's Ivy and money plant are some among the names by which *Epipremnum aureum* Linn. is mentioned. It belongs to the family of Araceae. It is a popular house plant with numerous cultivars and efficient at removing indoor pollutants such as formaldehyde, xylene and benzene. It is a scrambler shrub and it can climb by means of aerial roots over the trees and plants which hook over the tree branches [12, 13].

MATERIALS AND METHODS

➢ Collection and identification:

The leaves were collected from garden in Mumbai and identified and authenticated by Dr.Ganesh Iyer, Department of life science, Ramnarayan Ruia College, Matunga, Mumbai.

➢ Preparation of extract:

The leaves were collected, thoroughly washed and shade dried for 4-5 days and powdered in an electric mixer grinder.

- The 50 gm of powdered drug was then extracted using 300 ml of methanol as solvent by using soxhlet apparatus. After extracting all colouring matter, the solvent was removed by using vaccum evaporator which gave rise to a semisolid masses of extracts with 7.3% yield.
- Another 50 gm of powdered drug was extracted using 300 ml of distilled water as solvent by using soxhlet apparatus. After extracting all colouring matter, the solvent was removed by using vaccum evaporator which gave rise to a semisolid masses of extracts with 8.5% yield.

➢ Phytochemical screening of plant extract:

The phytochemical screening for both the extracts was done through standard test procedures [14].

➢ Antimicrobial assay:

For evaluation of antibacterial and antifungal activities of methanol and aqueous extract of *Epipremnum aureum* leaves, well diffusion method was used [15].

• Culture Media:

The media used for antibacterial test was Muller Hinton agar media and Sabouraud's dextrose agar of Hi media Pvt. Bombay, India.

Table-1: Phytochemical screening of extracts

Sr.No.	Chemical constituents	Test
1.	Test for alkaloids	Mayer's test
		Wagner's test
		Dragendroff's test
		Hager's test
2.	Test for glycosides	Keller Killiani test
		Modified Borntrager's test
		Legal's test
3.	Test for saponins	Foam test
		Bromine water test
4.	Test for steroids and terpenoids	Salkowski's test
		Liebermann Burchard's test
5.	Test for carbohydrates	Molisch's test
		Benedicts test
		Fehling's test
6.	Test for flavonoids	Ferric chloride test
		Lead acetate test
		Alkaline reagent test
		Shinoda test
7.	Test for tannins	Ferric chloride test
		Lead acetate test
		Potassium dichromate test
8.	Test for Phenols	$FeCl_3$ Test
9.	Test for amino acid	Ninhydrin test
10.	Test for fats and oils	Stain test
		Soap test
11.	Test for anthraquinones	Borntrager's test

- **Composition of Culture Media**

Preparation of Culture Media for Antibacterial Studies Mueller-Hinton Medium.

Content	**g/litre**
Acid Hydrolysate of Casein	17.5g
Beef Extract	2.0g
Starch	1.5g
Agar	17.0g

Mueller Hinton dehydrated media (38g) was dissolved in 1000 ml of purified filtered water and heated with frequent agitation. Media was sterilized at 121° C for 15 minutes and cooled to 45-50° C

Preparation of cultural media for antifungal studies SDA Medium (Sabouard Dextrose Agar)

Contents	**g/litre**
SDA	65 g
Distilled water	100 ml

Suspended 65.0 g of SDA in 100 ml distilled water and boiled to dissolve the medium completely. Sterilized by autoclaving at 15 1bs pressure and 121°C for 15 min.

- **Test organisms:**

Bacterial strains
1) Gram +ve bacteria: *Staphylococcus aureus*
2) Gram -ve bacteria: *Escherichia coli*
Fungal strains
1) Candida albicans

- **Antibacterial activity:**

For investigation of antibacterial activity, Sterile Muller Hinton agar media (Hi-media) was prepared and poured in Petri dishes 15 ml each aseptically. The bacteria (1×10^8 bacteria/ ml) were inoculated in the media. In each Petri dish four wells (diameter 6mm) were prepared under aseptic conditions. Dimethyl sulfoxide was used as control. In these wells, DMSO (1ml/well, as control), methanolic extract of *E. aureum* (1ml of 100 µg/ml solution in DMSO), aqueous extract of *E. aureum* (1ml of 100 µg/ml solution in DMSO) and Gentamycin (1ml of 50 µg/ml solution in DMSO) were added. All the dishes were incubated at 35ºC for 24 Hrs.

- **Antifungal activity:**

For investigation of antifungal activity, Sabouard Dextrose Agar media (Hi-media) was prepared and poured in Petri dishes 15 ml each aseptically. The fungal spores of *C.albicans* (1×10^6 spores/ ml) were inoculated in the media. In Petri dish four wells (diameter 6mm) were prepared under aseptic conditions. In these wells, DMSO (1ml/well, as control), methanolic extract of *E. aureum* (1ml of 100 µg/ml solution in DMSO), aqueous extract of *E. aureum* (1ml of 100 µg/ml solution in DMSO) and Ketoconazole (50 µg/ml solution in DMSO) were added. Petri plate was incubated at 25 ºC for 48 Hrs.

At the end of the incubation period, the media were observed for zone of inhibition. The zones of inhibition were measured in millimetre.

- **The minimum inhibitory concentration (MIC):**

The minimum inhibitory concentration (MIC) of the extracts were also determined according to standard method [16].

For determination of MIC values, test extracts was diluted to get final concentration ranging from 100- 0.2 µg/ml. Different concentration of the test extracts were added to sterile Muller Hinton broth and Sabouard Dextrose Agar in microtiter plates for detection of MIC against bacterial and fungal strain respectively. After addition of test extracts, bacterial suspension for selected strains(1×10^8 bacteria/ ml) strain and fungal suspension for single strain(1×10^6 spores/ ml) were inoculated in to respective microtiter plates. Bacterial and fungal suspension in media were used as positive control and test extract in media was used as negative control. After incubation at 37 ºC for 24 Hrs for bacterial strains and at 25 ºC for 48 Hrs for fungal strains plates were observed. MIC values were taken as the lowest concentration of the extract in the well of the microtiter plate that showed no turbidity after specified period of incubation. The turbidity of the well in the microtiter plate were interpreted as visible growth of microorganisms.

RESULTS AND DISCUSSION

Phytochemical screening of plant extract revealed the presence of alkaloids, flavonoids, tannins, terpenoids and anthraquinones in methanolic extract of plant; alkaloids, flavonoids, tannins, glycosides, anthraquinones, sterols and phenols in aqueous extract of plant.

Antibacterial and antifungal activity of methanolic and aqueous extract of leaves of *E. aureum* were observed using well diffusion method by measuring the diameter of the growth inhibition zone. The results are depicted in Table-2 and 3. It was observed that methanolic extract exhibit no antibacterial and antifungal activity while the values for zone of inhibition for aqueous extract indicates both activities at significant level. MIC values of aqueous extract against all test organisms are shown in Table-4. MIC values of aqueous extract were same against *E.coli* and *S. aureus* i.e. 25 µg/ml and it was to be 50 µg/ml against *C. albicans*.

Table-2: Antibacterial activity

Microorganism	Zone of inhibition(mm)		
	Methanolic extract (100 µg/ml)	Aqueous extract (100 µg/ml	Gentamycin (50 µg/ml)
E. coli	Resistant	15	25
S. aureus	Resistant	17	38

Table-3: Antifungal activity

Microorganism	Zone of inhibition(mm)		
	Methanolic extract (100 µg/ml)	Aqueous extract (100 µg/ml	Ketoconazole (50 µg/ml)
C. albicans	Resistant	14	22

Table-4: Determination of MIC values of aqueous extract of E. Aureum leaves against E.coli, S. aureus and C. Albicans

Concentration (µg/ml)	Aqueous extract of *E. aureum* leaves			Control	
	E. coli	*S. aureus*	*C. albicans*	Positive	Negative
100	-	-	-	+	-
50	-	-	-	+	-
25	-	-	+	+	-
12.5	+	+	+	+	-
6.25	+	+	+	+	-
3.125	+	+	+	+	-
1.6	+	+	+	+	-
0.8	+	+	+	+	-
0.4	+	+	+	+	-
0.2	+	+	+	+	-

+: Presence of growth; -:Absence of growth; Positive: microorganism and media; Negative: test extract and media.

From the above results it is observed that among the two only aqueous extract of the plant has anti bacterial and antifungal activity which may be attributable to the difference in the phytochemical constituents present in the two extracts.

CONCLUSION

From the above results it can be concluded that the aqueous plant extract have great potential as antibacterial and antifungal compound against microorganisms and that it can be used in the treatment of infectious diseases caused by resistant microorganisms. As aqueous extract has antimicrobial activity comparable to standard drugs, this extract can be used to discover bioactive natural products that may serve as leads for the development of new pharmaceuticals that address hither to unmet therapeutic needs. However, further investigation on isolation and characterization of the active principle(s) of the plant extract responsible for the antimicrobial

activity is necessary and it would give a comprehensive evidence of bioactive potential of medicinal plants.

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