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PHYTOCHEMICAL AND *IN-VITRO* PHARMACOLOGICAL EVALUATION OF *IPOMOEA SEPIARIA* AGAINST SELECTED PATHOGENIC MICROORGANISMS.

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ABSTRACT

Background: Convolvulaceae - The Morning Glory Family, the genus *Ipomoea* since time immemorial have been in continuous use for different purposes, such as nutritional and somany importand medicinal values. There is no much more work was carried out on this species. **Objective:** To Study the preliminary phyto chemical parameters and in-vitro antimicrobial and anthelmintic acitvity on selected medicinal plant crude extract. **Materials and Methods:** In the present study antimicrobial activity of petroleum ether and ethyl acetate extracts from the leaves of *Ipomoea sepiaria* and phytochemicals was evaluated with Gram-positive and Gram-negative bacteria. The fungi employed for screening were *Aspergillus niger* and *Candida albicans*. Leaves were dried, powdered and extracted with soxhlet apparatus. *In-vitro* antibacterial and antifungal activity was performed by the agar diffusion method at concentrations of 50mg/mL to 150mg/mL. Ciprofloxacin (10 µg/mL) and Fluconazole (10µg/mL) was employed as antibacterial and antifungal reference standard to compare the results. Anti-helminthic activity also performed by using *Pheretima posthuma*. paralysis and death time was monitored. Albendazole was used as standard. **Results:** It has been observed that all the tested extracts showed mild to moderate activity against the bacteria and fungi whereas higher concentration of petroleum ether extract and ethyl acetate extract 150mg/mL were found to be most promising antibacterial activity and shows highest activity towards gram positive organisms. Plant extract shown comparable anthelmintic activity to that of albendazole as standard. **Conclusion:** We concluded that the petroleum ether and ethyl acetate extracts of *Ipomoea sepiaria* leafhave mild activity against bacteria and fungus but it revealed significant anthelmintic activity.

KEYWORDS: Albendazole, Ciprofloxacin, Fluconazole, *Ipomoea sepiaria*, *Pheretima posthuma.*

INTRODUCTION

India is one of the largest producers of medicinal herbs in the world. The Indian traditional healthcare system, Ayurveda provides relatively organized database and more exhaustive description of botanical materials, many of which have been used as templates for novel drug development.[1] Due to the increase of resistance to antibiotics, there is a need to develop new and innovative antimicrobial agents. Among the potential sources of new agents, plants have long been investigated. Because, they contain many bioactive compounds that can be of interest in therapeutic.The genus Ipomoea since time immemorial have been in continuous use for different purposes, such as nutritional and medicinal uses. *I. aquatic* and *I. batatus,*is consumed as food in Sri Lanka, Hong Kong, Taiwan and China. The most common use of the roots of Ipomoea species is to treat constipation and is also used in treatment of diabetes (*I. aquatic*), Leaves decoctions are used as alterative, aphrodisiac, astringent (*I. batatas*), Against Immunodeficiency Syndrome (AIDS) and to treat hypertension (*I. carnea*).[2-5] From the review of literature we identified that less biological activity was examined. So we have made an attempt for antimicrobial and anti-helminthic activity.

MATERIALS AND METHODS

Identification and authentification of selected plant species

The leaves were collected from Warangal district, A.P and it was authenticated by professor Dr.S.M.Khasim, Department of Botany, Acharya Nagarjuna University, Guntur.

Collection of plant material

Fresh plant material was collected in bulk, washed under running tap water to remove adhering material, dried under shade and pulverized in a mechanical grinder. The coarse powder was passed through sieve no. 60#. Care was taken to select healthy plants and for normal organs.

Standard Drugs Used for Activity Comparison

I. Ciprofloxacin (10 µg/mL) was used as reference standard for activity comparison in antibacterial study[6, 7]
II. Fluconazole (10 µg/mL) was used as reference standard for activity comparison in antifungal activity[7]
III. Albendazole (25mg/mL)used as reference standard for activity comparison in anthelmintic study.[8]

Chemicals Used

I. Normal saline.
II. Dimethyl sulfoxide (AR grade): The test samples were dissolved in Dimethyl sulfoxide and used for the antibacterial activity study.
III. Gum acacia: The test samples were suspended in 1% gum acacia in normal saline and used for the anthelmintic activity study.

Preparation of extracts

The techniques commonly used in the field of photochemistry were extraction, isolation and structural elucidation of natural products, as well as chromatographic techniques. The solvent extraction of any botanical materials may yield very less quantity of volatile oils and a large yield of non-volatile components like resins, pigments, waxes and fatty acids.

Petroleum Ether Extract

This extract was prepared by using Soxhlet apparatus. About 150gm of dried leaves powder was taken in a muslin cloth bag. The purified petroleum ether was passed through the tube where the powder bag was kept. The petroleum ether was passed through siphon tube to reach the round bottom flask in which porcelain chips were provided. The vapourof the solvent pass through the condenser and reach the tube containing powder bag and the process was repeated. This was continued for 24hrs. Then the round bottom flask containing extract was transferred to a beaker and was allowed to evaporate in a water bath. This concentrated petroleum etherextract was used for further studies.

Ethyl Acetate Extract

This extract was prepared by using Soxhlet apparatus. Same as that of petroleum ether extract but Ethyl acetate used as solvent.

Recovery of Solvent

For purification of solvents the distillation apparatus was first set. Then 40% of solvents were added to the bottom flask kept in electrical mantle. The respective solvents were allowed to distil and pure solvents were collected in a vessel from the adapter attached to the condenser.

Phytochemical screening

Preliminary phytochemical identification.[9, 10]

The chemical tests were performed to identify the phytochemical constituents present in petroleum ether and ethyl acetate extracts of *Ipomoea sepiaria*as per already reported procedure.

Biological Screening

***In-vitro* screening of petroleum ether and ethyl acetate extract**

In view of varied biological and pharmacological importance of petroleum ether and ethyl acetate extract, it has been prompted us to evaluate for antimicrobial, antifungal and anthelmintic activities for the extracts.

Antibacterial Activity

The petroleum ether and ethyl acetate extract were studied for antibacterial activity[11, 12] on microorganisms by using cup plate method. Gram-Positive Bacteria: *Bacillus subtilis (NCIM 2699), Micrococcus luteus (NCIM 2721), Staphylococcus aureus (NCIM 2672) and* Gram-Negative Bacteria*: Escherichia coli (NCIM 2685), Pseudomonas aeruginosa (NCIM 5029), Klebsiella pneumonia (NCIM 5082) were used.* Ciprofloxacin (10 µg/mL) was employed as reference.Nutrient broth was used for the preparation of inoculum of the bacteria and nutrient agar was used for the screening method. The test organisms were sub cultured using nutrient agar medium. The tubes containing sterilized medium were inoculated with respective bacterial strain. After incubation at 37 °C± 1°C for 18 hours, they were stored in the refrigerator.Meanwhile the nutrient agar medium was sterilized by autoclaving at 121°C (15 Ib /sq.inch) for 15 min. The Petri plates, tube and flasks plugged with cotton were sterilized in hot-air oven at 160 °C, for an hour. Into each sterilized Petri plate (20 cm diameter), 125 mL of molten nutrient agar medium was poured which was already inoculated with the respective strain bacteria (5mL of inoculum to 250 mL of nutrient agar medium) aseptically. The plates were left at room temperature aseptically to allow the solidification. After solidification the cups of each of 7 mm diameter were made scooping out medium with a sterilized cork boresr from a Petri dish and were labelled accordingly.

Each test compound was dissolved in dimethyl sulfoxide (5 mL, AR grade) at a concentration of 50,100 & 150 mg/mL. ciprofloxacinsolution was also prepared at a concentration of 10 µg/mL in dimethyl sulfoxide. All the compounds were tested at a concentration of 0.1 mL (100 mg/mL) and 0.15 mL (150 mg/mL) level and DMSO as the control did not show any inhibition.The solutions of each test compound, control and reference standard (0.1mL) was added separately in the cups and the plates were kept undisturbed for at least 2 hours in the refrigerator to allow the diffusion of the solution properly into nutrient agar medium. Petri dishes were subsequently incubated at 37 ± 1°C for 24 hrs. After incubation, the diameter of zone of inhibition surrounding each of the cups was measured with the help of an antibiotic zone reader. All the experiments were carried out in triplicate. The results are presented in Table-2.

Antifungal activity

The petroleum ether and ethyl acetate extractwere studied for antifungal[11, 12]activity. The fungi employed for screening were *Aspergillus niger* and *Candida albicans*. Fluconazole (10µg/mL) was employed as standard to compare the results.Sabourad dextrose agar medium used as culture media. The test organisms were subcultured using Sabourad dextrose agar medium (SDA) medium. The tubes containing sterilized medium were inoculated with test fungi and kept at 24^0c temperature for obtaining growth. After that they were stored at 4 °C in a refrigerator. The inoculum was prepared by taking a loop full of stock culture to about 5 mL of sabourad dextrose broth in a test tube. The tubes were incubated at 25º C for 48 hours before use.The solution of test compound was prepared by a similar procedure described under the antibacterial activity. A reference standard solution of Fluconazole (10 µg/mL) was prepared by dissolving 10 mg of Fluconazole in 10 mL of dimethylsulfoxide (DMSO) stock.The SDA medium was sterilized by autoclaving at 121°C (15 lb/sq. Inch) for 15 min. the Petri-plates were sterilized in hot-air oven at 160º C for an hour. Into each sterilized Petri-plate about 27 mL of molten SDA medium was added. Incubated at 30º C for 2 days. After 2 days of incubation, the medium free of contaminations was spreaded with 50 µl of 48 hours culturing. After solidification of the cups of 7 mm diameter were made in each plate with sterile bores. Accurately 50 µl of 50 mg, 100 mg, and 150 mg concentrations of test solution was transferred to the respective Petri-plates aseptically and labelled accordingly. The reference standard 50µlwas also added to the boresin each plate.The plates were kept in refrigerator for one hour to allow the solution to diffuse properly into the SDA medium. Then the plates were incubated at 25º C for 48 hours at inverted position. The diameter of zone of inhibition was read with help of an antibiotic zone reader. The experiment was performed in triplicate and the results were represented in Table-3.

Anti-helmenthic activity

The petroleum ether and ethyl acetate extract were studied for the anti-helminthic activity[13] by using Indian earthworm *Pheretima posthuma*(Annelida). On adult Indian earthworm *Pheretima posthuma*as it has anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. Each group consists of 6 earthworms and 10 groups are selected as the following control, standard and petroleum ether and ethyl acetate extracts of test groups of concentrations of 20, 30 and 50 µg /mL and Albendazole was taken as standard.The earthworms in each group were released into desired formulation. Observations were made for the time taken to paralyze and death of individual worms. Paralysis was said to occur when the worms do not receive even in normal saline. Death was concluded when the worms lose their motility followed with fading away of their body colour.

RESULTS

"Table 1: Phytochemical constituents present in petroleum ether and ethyl acetate extracts of *Ipomoea sepiaria*"

Phyto constituents	Petroleum ether	Ethyl acetate
Alkaloids	--	--
Cardiac Glycosides	--	--
Saponin Glycosides	--	--
Coumarin Glycosides	--	--
Anthraquinone glycosides	--	++
Tannins	--	--
Steroids and terpenoids	++	++
Carbohydrates	--	--
Protein	--	++

Qualitative chemical tests were performed to identify the phytochemical constituents present in petroleum ether and ethyl acetate extracts of *Ipomoea sepiaria*. Steroids and triterphenoids present in the petroleum ether. Anthraquinone glycosides, Steroids and terpenoids positive in ethyl acetate extract.

"Table 2: Antibacterial activity of petroleum ether and ethyl acetate extractof *Ipomoea sepiaria* and antibiotic against bacterial species tested by cup plate method."

Solvent extract	Concentration (mg/mL)	Zone of inhibition (mm)					
		B. subtilis	K. pneumonia	P.vulgaris	S.aureus	S.luteus	E. coli
Petroleum ether	50	12±0.12	13±0.23	12±0.33	11±0.22	11±0.33	12±0.21
	100	14± 0.13	14±0.12	15±0.22	12±0.23	13±0.45	13±0.22
	150	18±0.21	19±0.31	17±0.42	14±0.41	14±0.42	14±0.32
Ethyl acetate	50	14±0.12	11±0.21	13±0.42	10±0.30	11±0.35	14±0.34
	100	16±0.33	12±0.34	13±0.31	12±0.32	11±0.12	14±0.30
	150	18±0.33	14±0.32	15±0.32	14±0.22	12±0.21	15±0.42
Ciprofloxacin	10µg/mL	25±0.24	24±0.23	23±0.23	26±0.23	21±0.22	22±0.25

Values are mean inhibition zone (mm) ± SEM of three replicates.

The petroleum ether and ethyl acetate extract were tested for *In-vitro* antibacterial activity by the agar diffusion method and the *Average zone diameter of triplicates in mm.* are presented in table 2, Ciprofloxacin was employed as reference standard to compare the results.It has been observed that all the tested compounds showed mild to moderate activity against the bacteria, whereaspetroleum ether extract and ethyl acetate extract

150mg were found to be most promising antibacterial activity among the series of extracts and shows highest activity towards gram positive organisms.

"Table 3: Antifungal activity of petroleum ether and ethyl acetate extractof *Ipomoea sepiaria* and antibiotic against bacterial species tested by cup plate method."

Solvent extract	Concentration (mg/mL)	Zone of Inhibition (mm)	
		Candida albicans	*Aspergillus niger*
Petroleum ether	50	9±0.23	10±0.22
	100	11±0.21	10±0.43
	150	13±0.30	12±0.14
ethyl acetate	50	10±0.45	10±0.23
	100	12±0.33	13±0.33
	150	14±0.28	14±0.42
Fluconazole	10	16±0.12	15±0.13

All extracts were tested for *In-vitro*antifungal activity by the agar diffusion method and the *Average zone diameter of triplicates in mm.* values were tabulated in table 3.Fluconazole was employed as reference standard, It has been observed that all the tested compounds showed mild to moderate activity against the fungiAmong them,ethyl acetate extract (150mg/mL) were found to be most active against the both *C. albicans* and *A. niger*.

"Table 4: Anthelmintic activity of petroleum ether and ethyl acetate extractof *Ipomoea sepiaria* and antibiotic against*Pheretima posthuma.*"

Test substance	Concentration (mg/mL)	Time taken for Paralysis (min.)	Time taken for Death (min.)
Vehicle	---	No paralysis (upto72min)	No death (upto 72min)
Albendazole (Std.)	25	31.86±0.22**	42.50±0.26**
Petroleum etherextract	25	36.35±0.23**	72.10±0.33**
	50	23.59±0.29**	64.1±0.24**
	75	18.09±0.07**	43.91±0.26**
	100	8.50±0.37**	32.10±0.53**
Ethyl acetate extract	25	34.94±0.93**	69.05±0.28**
	50	28.43±0.18*	48.83±0.29**
	75	19.29±0.20**	51.6±0.21**
	100	8.55±0.10**	32.25±0.25**

*Each value represents mean ± SEM (N=6). *P<0.05, **P<0.01. This activity was Concentration dependent. The potency was found to be inversely proportional to the time taken for paralysis and time of death of the worms.*

From the observations made, ethyl acetate and petroleum ether extracts exhibited anthelmintic activity in dose-dependent manner giving shortest time of paralysis (P) and death (D) with 100 mg/mL concentration. Results are shown in table 4**.** The petroleum etherextract of *Ipomoea sepiaria* caused paralysis (P) of 8.50 min. and time of death (D) of 32.1 min. while ethyl acetate revealed paralysis of 8.55 and death of 32.25 min. respectively against the earthworm *Pheretima posthuma*. The reference drug Albendazole showed the same at 31.86 and 42.50 minutes.

DISCUSSION

India is one of the largest producers of medicinal herbs in the world. The Indian traditional healthcare system, Ayurveda provides relatively organized database and more exhaustive description of botanical materials, many of which have been used as templates for novel drug development. Nature has provided a complete store-house of remedies to cure all ailments of mankind by providing us drugs in the form of herbs, plants and algae to cure the incurable diseases without any toxic effects.Now days allopathic system usage was decreased due to side effects, adverse reactions, so now a day's herbal drugs usage was increased due to less side effects and patience acceptance in these way herbal drugs usage was increased.

In the present study, the attempt is made to phytochemical investigation of the petroleum ether and ethyl acetate extracts of *Ipomoea sepiaria* leaves and performed antibacterial, antifungal and anthelmintic activities. The different phytochemical constituents are steroids, triterphenoid, Anthraquinone glycosides, proteins are found in the extracts of *Ipomoea sepiaria* leaves. Ethyl acetate extract showed mild to moderate activity and better anthelmintic activity when compared to petroleum etherextract. Mechanism of the

anthelminthic activity of *Ipomoeasepiaria* cannot be explained on the basis of our present results. From the observations made, higher concentration of extract produced paralytic effect much earlier and the time to death was shorter for all worms. So further investigation have to be carried to identify the active chemical constituents present in the extracts of *Ipomoea sepiaria*.

CONCLUSION

The present study enabled us to conclude the potential use of petroleum ether and ethyl acetate extracts of *Ipomoea sepiaria* as mild to moderate antibacterial and antifungal agent but it possess significant anthelmintic activity against Pheretima posthuma. Extensive research is needed to determine the individual component responsible for the anthelmintic activity and molecular mechanism responsible for the same.

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