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PHYTOCHEMICAL AND IN VITRO EVALUATION OF ANTIMICROBIAL ACTIVITY OF *Andrographis paniculata* LINN

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ABSTRACT

A large number of medicinal plants have been used for years in daily life against diseases, whole over the world. Presently herbs are used as important materials in the health care system, create an herbal regeneration, spread with a superior speed throughout the world. The herbal products today used for safety in contrast to the synthetic drugs that are regard ed as unsafe to human environment. In Our current rework is leaves of Andrographis paniculata of family Acanthaceae with Pet ether extract (PEAP) and Methanol extract (MEAP) were used for photochemical investigations antimicrobial activity against bacteria and fungai.

PEAP and MEAP were tested positive for presence of phytochemicals as follows PEAP showed the presence of , alkaloids, tannins, steroids, proteins and fixed oils. The methanol extract shows presence of Alkaloids, carbohydrates, saponins etc, the literature studies showed that Alkaloids possess number of pharmacological activities. Both PEAP and MEAP were subjected to antimicrobial activities. Both extracts showed antimicrobial activity against Staphylococcus aurieus, Escherichia coli and Bacillus cereus

INTRODUCTION

Nature is the seventh heaven of medicinal principles offers to the humanity through plants Which act as richest source of Phytochemicals. An inspiring number of modern drugs have been isolated from the forest resources; many being strike basing on their use in the treatises of traditional medicines [1].The various living systems bear a rich biodiversity in nature. Since the earliest era before scientific knowledge would change plants performed important functions of the biosphere[2].

Medicinal plants play a significant role in the production of novel and valuable drugs used in modern medicine. Today we have a number of useful and life saving drugs and also drugs which can provide immediate therapeutic benefit[3].

Over 2000 plant species are found to have medicinal value and are used for medicinal purpose in different forms. Many common plants available in the kitchen gardens or in the forests are used by the tribe as medicines[4].

Herbal products today symbolize safety in contrast to the synthetic drugs that are regarded as unsafe to human environment. Then the conventional systems of medicine continue to be generally practiced on many accounts even at present. Population rise, insufficient supply of drugs, excessive cost of treatments, side effects of a number of allopathic drugs and development of resistance to currently used drugs for infectious diseases, have led to enlarge emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments.

India has been known to be a rich depository of medicinal plants. The forest in India is the principal repository of number of medical and aromatic plants, which are largely used as raw martial for manufacture of drugs and allied products.

Ayurveda is gaining momentum and prominence as the natural system of health care all over the world. Perusal of literatures reports the medicinal properties of most of the plants posture biological activity one of such species is ***Andrographis paniculata*** (F. *Acanthaceae*) which has been exploited for many traditi onal medicinal uses.

The plant extract also showed gastro protective activity. The inhibiting effects on formed protein non -enzymatic glycation an end product was studied from the ethanolic leaf extract.

The diversity of pathogenic bacteria is general and so is the variety of diseases caused by them. Despite the survival of many potent antimicrobial agents [5], multi- resistant phatogenic strains are continuously emerging, imposing the need for a continuous search and development of new drugs[6]. In the present study, solvent extract such as PEAP and MEAP were evaluated for the qualitative phyto chemical analysis and invivo antimicrobial activity which lead to the finding of more effective agent for the management of Diseases.

Preparation of the Extract (MEAP and PEAP)

Plant material of leaves were washed with distilled water and shade dried.

The dried leaves was ground together to a fine powder using blender. The coarsely powdered sample 50 gm was filled in the thimble and extracted with petroleum ether and ethanol using a Soxhlet extractor.

The filtrate was evaporated to dry under reduced pressur using a rotary vacuum evaporator. The extract were stored in ambient containers until further use[7].

Table 1: Preliminary Phytochemical Screening Of Both Extracts Of *Andrographis Paniculata*

Sr. No	PHYTO CHEMICAL CONSTITUENTS	METHANOL EXTRACT	PET ETHER EXTRACT
1	ALKALOIDS	+	_
2	SAPONINS	+	_
3	GLYCOSIDES	+	+
4	CARBOHYDRATES	+	+
5	TANNINS	+	_

6	FLAVONOIDS	+	_
7	STEROIDS	+	_
8	PROTEINS	+	_
9	TRITERPINOIDS	_	+
10	FIXED OILS & FATS	+	+
11	GUMS & MUCILAGE	_	_

+ PRESENT - ABSENT

Preliminary Phytochemical Screening
The freshly prepared crude PEAP and MEAP were qualitatively tested for the presence of phytochemical constituents such as alkaloids, flavones, terpenoids, steroids, tannins etc., by standard methods [8,9].

Bacterial Strains
Authenticated cultures of eight bacterias such as *Escherichiae coli* (MTCC 1687), *Klebsiella pneumoniae* (MTCC 7162), *Proteus mirabilis* (MTCC 9242), *Shigella flexneri* (MTCC 1457), *Streptococcus pyogenes* (MTCC 1926), *Streptomyces fulvissimus* (MTCC 7336), *Bacillus subtitis* (MTCC 736) and *Pseudomonus auruginosa* (MTCC 2488) were collected from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Sector 39-A, Chandigarh, India.

Fungal Strains
Two fungi *Aspergillus niger* and *Candida albicans* were selected for screening.

Preparation of Inoculums
Bacterial and fungal inoculums were prepared from the 24 h old pure culture grown on nutrient agar media for bacteria and one week old culture on potato dextrose agar media for fungi.

Bacterial colonies were pre-cultured in nutrient broth medium and kept overnight, then centrifuged at 10,000 rpm for 5 min.

Pellet was dissolved in sterilized distilled water and the cell turbidity was assessed spectroscopically incomparable to that of the 0.5 McFarland standards (approximately 1.5 × 108 CFU/ml) whereas the fungal spores were scraped from the mother culture and dispensed with sterilized distilled water.

Then the spore density was adjusted spectrophotometrically to obtain approximately 105 spores/ml final concentration.

Then the inoculums were used for the antibacterial and antifungal assays[10,16].

Disc Diffusion Bioassay
The disc diffusion test was carried out as described by [11] at 0.5 ml standardized inoculum suspension of each bacterial strain was spread on nutrient agar plates with a sterile bent glass rod spreader. Sterile 6-mm Whatman no. 1 filter paper discs were aseptically placed on plates.

PEAP and MEAP of standard concentrations (1mg/ml) were aseptically poured on the discs along with sterile double distilled water or 10% DMSO as negative and Oxytetracycline (1mg/ml) as positive controls.

Plates were allowed to stand for 30 minutes at room temperature prior to incubation at 35 -37°C for 24 hours.

The inhibition zone diameter were measured three times a and means were represented.

RESULTS AND DISCUSSION
In the present study ,preliminary chemical analysis of two different solvents viz. PEAP and MEAP revealed That the presence ofglycosides, flavonoids, tannins, steroids, proteins and fixed oils. The ethanol extract shows presence of Alkaloids, carbohydrates, saponins etc, the literature studies showed that Alkaloid posses number of pharmacological activities.

The disc diffusion assay that PEAP and MEAP extracts had different degrees of bacterial and fungal growth inhibition, depending on the microbial strains (Table 2).

Both extracts had shown similar antimicrobial activities against all tested strains. MEAP was very effective against bacteria like *E. coli, P. mirabilis, B. subtitis, and P. auruginosa.* But Petroleum ether extract showed moderate antimicrobial activities against *S. fulvissimus, P.mirabilis, B. subtitis and P.auruginosa.*[12] The antimicrobial activity of MEAP might be due the presence of secondary metabolites like tannin, alkaloids and anthraquinones which are known to have antimicrobial properties[13-15].

In the present investigation PEAP and MEAP Against suggest its antimicrobial potential which has also been studied previously agains phathogenic bacteria.

CONCLUSION
The present investigation concludes that MEAP exhibited maximum inhibition against Microbial strains. Crude extracts displayed significant antimicrobial activity, thus results obtained in the present investigation are promising enough for further isolation and characterization to reveal any novel metabolite of pharmaceutical importance.

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CONFLICT OF INTEREST
The authors hereby declare that there are no conflicts of interest.

Table 2: Antibacterial Activity Measured As Zone Of Inhibition At 1mg/ml of PEAP and MEAP (Leaf) And Standard Antibiotics

Extract 1mg/kg	Solvent type	E.coli	S. Flexneri	S. Pyogenes	S. fulvissimus	K. pneumonia	P. mirabilis	B. subtitis	P.auruginosa
Andrographis paniculata	*MEAP*	22 ± 1.1	16 ± 1.2	16 ± .23	17 ± 1.3	18 ± .1.5	19 ± 1.2	20 ± 1.4	20 ± .54
Andrographis paniculata	PEAP	9 ± 1.5	6 ± .75	8 ± .45	11 ± .56	7 ± .52	11 ± 1.3	13 ± 1.2	7 ± .56
Standard	Oxytetracyclin	26 ± 1.2	23 ± .62	21 ± .12	21 ± .24	17 ± .54	19 ± .52	25 ± .5	23 ± .16

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