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Research Article

IN VITRO ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY SCREENING OF ANDROGRAPHIS PANICULATA LEAF ETHANOLIC EXTRACT IN TAMIL NADU

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ABSTRACT

This study is a positive demonstration on the utility of antimicrobial and antioxidant activities of leaf extracts of *Andrographis paniculata* Nees. From the leaf were extracted using various solvents such as Chloroform, Petroleum ether, Acetone, Ethyl alcohol, Isoamyl alcohol and Water (according to the non polar to high polar used for the extraction). The antibacterial activity was carried out against *Micrococcus luteus, Staphylococcus aureus, Escherichia coli and Klebsiella pneumonia* by agar well diffusion method. The ethanolic extract was able to inhibit *Escherichia coli, Staphylococcus aureus and Micrococcus luteus*. The ethanolic extracts were screened for their in vitro antioxidant potential. Inhibition of oxygen derived free radicals, viz., assays for free radical scavenging by 2, 2- diphenyl -1 picryl hydrazyl (DPPH), reducing power ability and nitric oxide scavenging were performed. The antioxidant activity was compared with standard antioxidant such as D- ascorbic acid. The ethanolic extract elucidated agreeable antioxidant and antimicrobial activity against four human pathogenic bacterial strains experimented.

Keywords: Ethnopharmacology, *Andrographis paniculata*, Antioxidant and Antibacterial microbial activity

INTRODUCTION

Medicinal plants are considerably useful and economically essential. They contain active constituents that are used in the treatment of many human diseases[1]. The plant extracts have been developed and proposed for use as antimicrobial substances[2]. Phytochemicals from medicinal plants showing antimicrobial activities have the potential of filling this need, because their structures are different from those of the more studied microbial sources, and therefore their mode of action may too very likely differ[3]. There is growing interest in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity[4, 5, 6, 7,] and [8]. Oxidation is essential to many living organisms for the production of energy to fuel biological processes. However, oxygen-centred free radicals and other reactive oxygen species that are continuously produced *in vivo*, result in cell death and tissue damage. Oxidative damage caused by free radicals may be related to aging and diseases, such as atherosclerosis, diabetes, cancer and cirrhosis[9]. The degenerative diseases associated with aging include cancer, cardiovascular disease, immune-system decline, brain dysfunction and cataracts[10]. As carcinogenic properties have been reported for some synthetic antioxidants, recent research on the potential applications of natural antioxidants from spices and herbs, for stabilizing foods against oxidation, have received much attention[11].

Andrographis paniculata also known commonly as "King of Bitters (English) or Hempedu Bumi (Malay)," is a member of the plant family Acanthaceae. This is an herbaceous plant in the family Acanthaceae, native to India and Sri Lanka. It is widely cultivated in southern Asia, where it is used to treat infections and some diseases, often being used before antibiotics were created. Mostly the leaves and roots were used for medicinal purposes.

This plant has been used for long without any known toxicity and has a strong traditional usage from safety point of view[12]. The herb has been used for the almost hundreds of years Asia for treating upper respiratory track infection, herpes and gastrointestinal track infection. It has also been used to treat hypertension, diabetic or as an anti-malarial[13]. The primary medicinal component of *A.paniculata* is andrographolide, which is a diterpene lactone. Andrographolide has been reported for its anti-cancer[14], anti-HIV[15], cardioprotective[16] and hepatoprotective[17] properties among others. The aim of the present investigation was to identify the compounds active for the antioxidant and antimicrobial activity of the leaf extract of *A.paniculata.*

MATERIALS AND METHODS

Andrographis paniculata Nees is an Herbal plant were collected from area of Elaikadambur village twenty one Kilo meter from Ariyalur District, Tamilnadu, India. The collected plants were identified and authenticated by Prof. P.T. Kalaichelvan, Life Science Department, University of Madras, Maraimalai Campus, Guindy and Chennai. The collected plant leaves were washed with running tap water and shade-dried for 3 days. After they were powdered and kept in an airtight container prior to solvents extraction. The extraction procedure is powder and each solvent in the ratio of 1:4. About the 100 g of dried leaves powder was extracted using 400ml of the extraction solvents with continuous shaking on a rotary shaker at 150 rev/min for 48 h and repeated three times. The filtrates were brought to slimy solid state by using hot water bath at 55 ºC and stored away at 4 ºC in air tight containers for further use. Slimy solid state paste dissolve with phosphate buffer saline then it was centrifuged at 5000 rpm for 5 minutes and the supernatant were subjected to further analysis.

Test organisms and cultures

Human pathogenic bacterial cultures were obtained from the culture collection centres (Centre for Advanced Studies in Botany lab, MTCC and ATCC). Two Gram-positive bacteria and two gram negative bacteria like *Micrococcus luteus* (CAS culture), *Staphylococcus aureus* (MTCC96), *Escherichia coli* (MTCC1687) and *Klebsiella pneumoniae* (MTCC109) respectively. All the microbial cultures were subcultured and maintained on Mueller Hinton Agar.

Determination of Antibacterial Activity

The agar well diffusion method was used to screen the antimicrobial activity. In vitro antimicrobial activity was screened by using Mueller-Hinton agar medium was purchased from HiMedia, Laboratories Limited, Mumbai, India. The Agar plates were prepared by pouring 15-20 ml of molten media into sterile Petri plates; they were allowed to solidify for 5 minutes. Then 100 µl of the inoculum suspension was swabbed uniformly and it was allowed to dry for 5 minutes. Wells, with diameter of 7 mm, were cut on the surface of the plates; different solvent of extracts 100 µl were loaded into the wells. In the compound was allowed to diffuse for 60 minutes and the Mueller-Hinton agar medium plates were kept for incubation at 37°C for 24 hrs.

Determination of Antioxidant Activity

The antioxidant activity was used this method described by Hatano[18]and Bhuiyan[19]. Crude ethanolic extract of *Andrographis paniculata* leaf was determined on the basis of their free radical scavenging activity was measured in vitro by using of the stable 1, 1-diphenyl-2-picryl hydrazyl (DPPH). A solution of DPPH (0.1 mM) in ethanol was prepared, and DPPH was added to solvent dissolved test solution at different concentrations (200-1000 µg/mL). After 30 min; the absorbance was measured at 517 nm using Beckman spectrophotometer. The percentage of free radical scavenging abilities at different concentrations was determined. D-Ascorbic acid was used as a standard. The DPPH absorbs at 517 nm, and its concentration is reduced by the existence of an antioxidant. The difference in absorbance on DPPH and the percentage of inhibition was calculated as a function of antioxidant activity.

Then the % inhibition was calculated by the following equation

$$\% \text{ radical scavenging activity} = \frac{\text{absorbance of blank - absorbance of scavenging activity sample}}{\text{absorbance of blank}} \times 100$$

RESULTS AND DISCUSSION

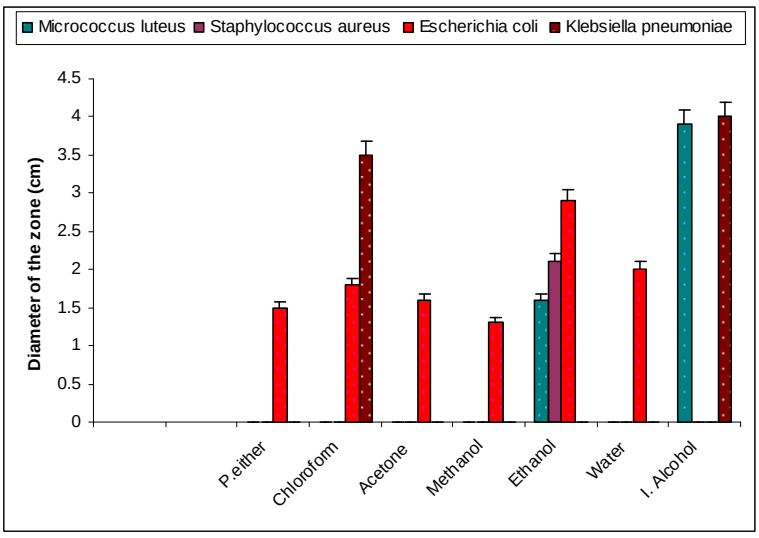
The present attempt Isoamyl alcohol and Ethyl alcohol extracts showed the maximum antibacterial activity against all the four tested bacterial strains (Table 1 & Graph 1). Both gram positives and gram negative bacteria have much large diameter 3.9cm in *Micrococcus luteus* and 4.0cm in *Klebsiella pneumonia* than that of the tested bacteria. The Isoamyl alcohol extract having highest antibacterial activity was observed for the *Klebsiella pneumonia* (4.0 cm) followed *by Micrococcus lettuce* (3.9 cm). Another one Ethyl alcohol was observed *Escherichia coli* (2.9 cm), *Staphylococcus aureus* (2.1) *and Micrococcus luteus* (1.6). There was no inhibition was observed by water; acetone and petroleum ether extracts against *Micrococcus lettuce, Klebsiella pneumonia and Staphylococcus aureus; Escherichia coli* having activity (petroleum ether 1.5 cm, chloroform 1.8 cm, acetone 1.6 cm, Ethyl alcohol 2.9, water 2cm).

Isoamyl alcohol extract there is no inhibition against *Escherichia coli.*

Table 1: Effect of different solvent using Antimicrobial activity on human pathogens

Micro organisms	P.ether	Chloroform	Acetone	Ethanol	Water	I. alcohol
Gram positive bacteria						
Microococcus lettuce	0	0	0	1.6	0	3.9
Staphylococcus aureus	0	0	0	2.1	0	0
Gram negative bacteria						
Escherichia coli	1.5	1.8	1.6	2.9	2	0
Klebsiella pneumoniae	0	3.5	0	0	0	4.0

The above the results were measured in centimetre and triplicates



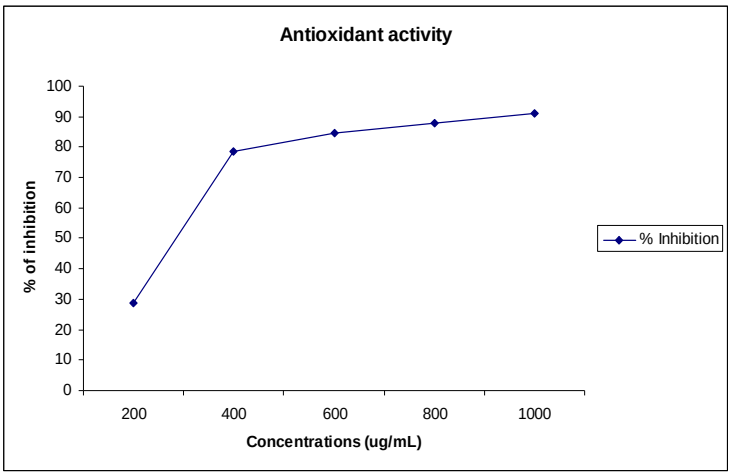
Graph 1: Effect of Antimicrobial activity on different solvents

Decolouration due to reaction of antioxidants in samples with the stable free DPPH radical was measured spectrophotometerically. Results (Table 2 & Graph 2) show the free radical scavenging potential of *A. paniculata* leaf extract 91.01% (1000 µg/ml) is significantly lower DPPH free radical scavenging activity compared to the positive control 61.71% (6µg/ml).

Table 1: Effect of Antioxidant activity of *Andrographis paniculata* L.

Concentration (g/ml)	Percentage of Inhibition
200	28.74

400	78.44
600	84.73
800	87.72
1000	91.01



Graph 1: Effect of Antioxidant activity of *Andrographis paniculata* L.

Among the different solvents Isoamyl alcohol were gave most large zone of the inhibitions compare to ethanol not large size of the zone but no of bacterium was inhibited. Other solvents moderate diameter of the results was observed. Zone of the results organisms' vize, *Micrococcus lettuce and Staphylococcus aureus bacteria* ethyl alcohol extract only inhibited zone was observed. (1.6 cm, 2.1cm) but all among them does not present the results 100 and 150µl concentrations.

Escherichia coli tested all the solvents present the results (1.5, 1.8, 1.6, 2.9 and 2 cm) and I. alcohol not shown the result at 100 and 150µl concentrations.

Klebsiella pneumonia chloroform and Isoamyl alcohol solvent extract showed the results. (3.5 and 4.0 cm) but not inhibit petroleum ether, Acetone, ethanol and water solvent extracts at 100 and 150µl concentrations.

Different µg/ml concentration of ethanolic extract (200µg to 1000µg/ml) was used for the estimation of antioxidant activity. Initially 200 to 400 µg/ml increases the working sample and inhibition also increased. Further 400 -1000µg/ml concentration decreased ratio of the activity was showed. Finally 91.01% of maximum antioxidant activity was observed at the concentration of 1000µg/ml.

Present results showed interesting results it can concluded that the crude ethanolic extracts of *Andrographis paniculata* herbal plant leaves collected from Tamil Nadu unravelled are promising medicinal value like antibacterial and antioxidant activities. Further phytochemical work need to be done on these extracts including fractionation to isolate active constituent and subsequent pharmacological evaluation.

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