Int J Pharm Bio Sci 2014 July ; 5 (3) : (B) 509 - 514

Research Article **Microbiology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

AN ASSESSMENT OF *IN-VITRO* ANTIMICROBIAL ACTIVITY OF *ANDROGRAPHIS PANICULATA* -A SCREENING STUDY

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ABSTRACT

Andrographis paniculata (Acanthaceae) is a potent medicinal plant in the Indian systems of medicine. Traditionally the leaves are used to treat influenza, bronchitis, gonorrohea, cholera, fertility and anti-bacterial, anti-cancer, anti-diabetic, anti-inflammatory and anti-snake venom etc. The present study describes the phytochemical profile and antimicrobial activity of *Andrographis paniculata*. For the present investigation, samples of *A. paniculata* extracts, obtained by extraction in ethanol,were used for their antimicrobial activity. The antimicrobial activities were assessed by measuring the diameter of the inhibition zones and MIC. This is the first report on analysis of antimicrobial components from *A. paniculata*, and our results confer the utility of this plant extract in developing a novel broad spectrum antimicrobial agent. Antimicrobial activity of extract of *Andrographis paniculata* was studied using ethanol against bacterial strains like Staphylococcus *aureus, Klebsiella pneumonia, Proteus sp., Pseudomonas aeruginosa. E.coli and* strains of fungi which are *Aspergillus fumigatus*, *Aspergillus flavus*, *Mucorindicus, Rhizopus sp., Trichophyton mentagrophytes, Trichophyton rubrum, Candida albicans.* The antimicrobial activity was determined by well diffusion method and MIC for fungal strains.

KEYWORDS : ANDROGRAPHIS PANICULATA, ANTIMICROBIAL ACTIVITY, WELL DIFFUSION METHOD, MINIMUM INHIBITORY CONCENTRATION (MIC)

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INTRODUCTION

Plants have been an essential part of human society for the cultivation. The herb known as *Andrographis panicualata* is found growing in the forests of Asian countries such as China, India, Pakistan and Thailand. It is an erect and branched herb mostly seen in the wild. Traditional practitioners use this plant to treat hyperdipsia, wounds, ulcers, chronic fever, malaria cough, bronchitis, skin diseases, leprosy, flatulence, colic, diarrhoea,and dysentery[17]. It is used to overcome the *sannipata* type of fever, difficulty in breathing, homeopathy burning sensation, cough, skin diseases, fever, ulcer and worms. It is also useful in acidity and liver complaints[2]. The use of *Andrographis* during recent researches produced favorable results, these observations during the particular study suggests that the *Andrographis* herb possesses a very potent cell and antiulcerogenic effects of Andrograpolide[12]. The risk of being affected by the common cold is reduced by fifty percent in all patients who took 200 mg every day of *Andrographis paniculata* preparation commonly marketed as KanJang, these doses were carried out throughout the duration of the cold season when common colds typically tend to affect susceptible individuals[4]. Infectious diseases are the number one among all causes of death, accounting approximately one-half all deaths throughout the world. About 50-75% of hospital deaths are reported due to infectious diseases[9]. These numbers are still increasing due to the development of resistance in microorganisms to the existing first line drugs. Scientists from divergent fields are investigating plants with a new eye for their antimicrobial usefulness and as an alternative source to existing drugs. Plants, with their wide variety of chemical constituents offer a promising source of new antimicrobial agents with general as well as specific activity[6].

Andrographis paniculata is an herbaceous plant belongs to family Acanthaceae, native to India and Sri Lanka. Mostly the leaves and roots were used for medicinal purposes. *Andrographis paniculata* is used in traditional Siddha and Ayurvedic systems of medicine as well as in tribal medicine in India and some other countries for multiple clinical applications. The plant extract exhibits anti-typhoid, anti-fungal, anti-fertility and anti-nematicidal activities. It is also reported to possess anti-hepatitis, anti-thrombogenic, anti-inflammatory, anti-snake venom, anti-pyretic and anti-cancer properties, etc.. The primary medicinal component of *Andrographis* is and rographolide, which is a 'diterpene lactone' water soluble substance and is distributed all over the plant body in different proportions[3]. Recent research has thrown light on cultivated of this plant on large because of its high medicinal value. Hence, the present investigation was taken up with an objective to evaluate the antimicrobial potential against the microorganisms.

MATERIALS AND METHODS

The leaves of the medicinal plant *Andrographis paniculata* were collected from Tamil Nadu Agricultural University (TNAU), Chennai. The plant material is identified by Dr.P.Jayaraman, Director, Institute of Herbal Science, Plant Anatomy Research Centre, Chennai. A Voucher specimen (PARC/2014/2245) has been preserved in the Institute for future reference. The leaves were dried under shade and made in to coarse powder using an electrical grinder. The powdered plant material was taken in different aspirator bottles and totally extract with solvent ethyl alcohol. After extraction, the solvent was evaporated under reduced pressure using high vacuum condition.

TEST MICROORGANISMS

Five bacterial cultures, namely *Staphylococcus aureus*, *Klebsiella pneumonia*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Escherichia coli bacterial Strains* were used in this investigation. The following seven fungal strains used for our study were *Aspergillus fumigatus*, *Aspergillus flavus*, *Mucor indicus,*

Rhizopussp, Trichophyton mentagrophytes, Trichophyton rubrum, and *Candida albicans*.

MEDIA AND INOCULUM PREPARATION

The media used for antibacterial test were Muller Hinton agar. All the media were obtained from Himedia Pvt Ltd, Mumbai, India. The test bacterial strains were inoculated into nutrient broth and incubated at 37ºC for 6hrs. After the incubation period, the culture tubes were compared with the turbidity (opacity) standard. The media used for antifungal test was Sabouraud's dextrose agar/broth of Hi media Pvt. Mumbai, India. The fungal strains were inoculated separately in Sabouraud's dextrose broth for 6 hrs and the suspensions were checked to provide approximately 10^5 CFU/ml[1].

ANTIBACTERIAL ASSAY

DETERMINATION OF ANTI-BACTERIAL ACTIVITY (WELL DIFFUSION METHOD)

Bioassay was carried out by Agar well diffusion method[8]. Fresh bacterial culture of 0.1ml having 10 CFU was spread on Mueller-Hinton agar plate with cotton swab the bacterial colonies was streaked onto the surface of the agar four times in the different directions by rotating the plate each time to ensure that the bacterial distribute evenly on the agar medium. In addition, around the agar should also be swabbed with bacterial colonies. A well of 6mm diameter was punched off into agar medium with sterile cork borer and filled with 100µl of different concentration of ethanol extracts by using micro pipette in each well in aseptic condition. Plates were then kept in a refrigerator to allow pre-diffusion of extract for 30minutes and further incubated in an incubator at 37ºC for 24hrs. The antibacterial activity was evaluated by measuring the zone of inhibition. The experiment was done in triplicate and the mean diameter of the inhibition zone was calculated. 100% DMSO (Dimethyl sulphoxide) as a negative control were used[14].

ANTIFUNGAL ASSAY

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC)

The MIC was regarded as the lowest possible concentration of the extract that does not show any visible growth after 14 days of incubation (compared with positive control, which shows growth in 3-4 days). For MIC tube dilution and plate dilution methods were followed.

TUBE DILUTION METHOD

About 1ml of extract with different dilutions and 0.1ml of each fungal suspension present in normal saline was mixed with 9ml of pre-sterilized Sabouraud's Dextrose Agar (SDA) medium for reaching the bearing temperature (45˚c - 50˚c approximately), to make the total volume up to 10ml and kept for slant preparation and on solidifying, the tubes were wrapped with paraffin tape and incubated at room temperature for 7-14 days. With fungal suspension and without plant extract serves as Positive control. SDA alone or SDA with DMSO serves as a Negative control. SDA with fungal suspension and standard drug dilution serves as test control.

PLATE DILUTION METHOD

About 2ml of each extract and 0.1ml of each fungal suspension in normal saline were mixed with approximately 18ml of pre-sterilized SDA medium for reaching the bearing temperature and poured onto sterile Petri dishes, to make the total volume up to 20ml. On solidification the plates were wrapped with paraffin tape and incubated at room temperature for 7-14 days. Determination of MIC by tube dilution method is followed by plate dilution method, which serves as a conformatory test for the tube dilution method.

PRELIMINARY PHYTOCHEMICAL SCREENING

The compounds that are responsible for therapeutic effect are usually the secondary metabolites. The preliminary phytochemical analysis[10] was carried out by following procedures:

Test for Alkaloids: -A small portion of the extract is stirred with a few drops of 1% Hydrochloric acid and filtered. The filtrate is treated with Wagner's reagent. Reddish brown

precipitate indicates the presence of alkaloids[10].

Test for Saponins: -One ml of extract is diluted with 20ml of distilled water and shaken vigorously for 15 min, formation of stable foam indicates the presence of saponin[10].

Test for Tannins: -Development of blue green color in the extract when treated with ferric chloride indicates the presence of tannins[10].

Test for Phenols: -Phenol test Small quantity of extract is diluted with 5% ferric chloride solution. Development of intense color indicates the presence of phenols[10].

Test for Steroids and Triterpenes: -

Leibermann- Burchards test: - The extract is treated with 50% sulphuric acid and a few drops of acetic anhydride are added. The development of the reddish brown ring indicates the presence of steroids[10].

Salkowskis test: -A few drops of chloroform and a few drops of concentrated sulphuric acid were added to the extract. Appearance of yellow color in the lower portion indicates the presence of triterpenes[10].

Test for Flavonoids: -

Ferric chloride test: -The extract is treated with a few drops of 5% ferric chloride. The appearance of blackish green color indicates the presence of flavonoids[10].

TLC Technique

Each fractions of the column eluted sample was subjected to TLC to find out the separation of single compound. Thin Layer Chromatography was performed on the prepared plates with Silica gel F254 grade (Merck, Darmstadt, Germany) as stationary phase. A one-dimensional ascending development technique was used to detect the constituents of an extract on a TLC plate solvent system like Ethyl acetate, methanol (16:22). Visual detection was done in daylight and under UV light at a wave length of 254 and 344 nm depending on the nature of compounds separated.

Table 1

Phytoconstituents of Andrographispaniculata extract

Phytoconstituents	Ethanol
Alkaloids	-
Saponins	+
Tannins	-
Phenolic compounds	+
Steroids/Triterpenes	-
Flavonoids	+

Table 2

Anti-bacterial activity of ethanolic extract of *Andrographis panicualata* (Well Diffusion Method)

Name of the Organism	*Ethanolic extract Concentrations in µg/ml*					
	31.25	*62.5*	*125*	*250*	*500*	*1000*
S.aureus	--	--	++	++	++	++
K.pneumonia	--	--	--	++	++	++
P.vulgaris	--	--	--	++	++	++
P.aeruginosa	--	--	++	++	++	++
E.coli	--	--	--	++	++	++

Table 3
Anti-fungal activity of ethanolic extract of Andrographis panicualata (Plate and Tube Method)

Name of the Organism	Ethanolic extract Concentrations in µg/ml					
	31.25	62.5	125	250	500	1000
Aspegillusflavus	--	--	--	--	++	++
Aspergillusfumigatus	--	--	--	--	++	++
Mucorindicus	--	--	--	--	++	++
Rhizopus sp.,	--	--	--	--	++	++
Trichophyton Mentagrophytes	--	--	--	++	++	++
Trichophytonrubrum	--	--	--	++	++	++
Candida albicans	--	--	--	++	++	++

RESULTS AND DISCUSSION

It is growing interest in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity. Screening active compounds from plants has led to the discovery of new medicinal drugs which have efficient protecttion and roles against various disease[16]. *Andrographis panicualata* is an herbal medicine and has been used for therapy of respiratory tract infection as well as acute diarrhea with reported efficacy of 75-100% in Thailand[11]. To investigate whether anti-bacterial activity and anti-fungal activity was responsible for the reported therapeutic successes of *Andrographis panicualata* was determined by well diffusion tests and MIC. *Andrographis panicualata* has also been used clinically for symptomatic treatment of the common cold and uncomplicated sinusitis, pharyngotonsillitis, pneumonia and bronchitis[5,7]. Chinese clinical studies involving oral administration of *Andrographis paniculata* to patients suffering from bacterial and viral respiratory infections reported good effects. The phytochemical analysis of the leaf extract of *Andrographis paniculata* was tested positive for the presence of Saponins, Phenolic compounds and Flavonoids (Table 1). An ethanol extract of *A. paniculata* leaves showed potent antibacterial activity against *P. aueruginosa* (22mm) and *S. aureus* (21mm) at a concentration of 125 mg/ml and also possess activity against *K. pneumonia, P. vulgaris, E. coolant* concentration of 500mg/ml. The anti-fungal activity of *Andrographis panicualata* alcoholic extract tested against 7 fungal strains (*Aspergillus flavus, Aspergillus fumigatus, Mucorindicus, Rhizopus* sp, *Trichophyton rubrum, Trichophyton mentagrophytes and Candida albicans*) by MIC method (tube & plate dilution method) were used in this investigation. The result of screening the antifungal activity of ethanolic extract *A.paniculata* by MIC was displayed in table 3. The ethanolic extract of leaves *A.paniculata* exhibited significant antifungal activity against dermatophytes of at the concentration of 250µg/ml and above, filamentous fungi at the concentration of 500µg/ml and above and finally yeast, (*Candida albicans*) at the concentration of 250µg/ml. The antimicrobial activity may be due to the presence of Saponins, flavonoids and phenolic compounds present in the crude extracts. The present investigation explored the antimicrobial potential of the medicinal plant *A.paniculata* and against the clinical bacterial and fungal pathogens. However, remarkable activity was noticed against two bacterial strains *viz.*,

Klebsiella pneumonia and *S.aureus*. Three spots were obtained from the ethanol extracts of *A.paniculata* with the RF value range (0.43, 0.38 and 0.16) when eluted with the solvent system EA: Methanol (16:22) using TLC technique. These are the major harmful pathogens from the clinical background affecting the human beings. As the crude extracts of antimicrobial activity, separation of an individual compounds from the crude extract of this plant will be resulted in the development of a novel antibiotic.

ACKNOWLEDGEMENT

Sincere thanks for the co-operation of staff of the Department of Biotechnology Dr. MGR Educational and Research Institute University, Chennai for carrying out this work.

REFERENCES

1. AbhishekMathur, Ravinder Singh, SajadYousuf, AprajitaBhardwaj, Antifungal activity of some plant extracts against Clinical Pathogens, Advances in Applied Science Research, 2011, 2 (2): 260-264.
2. Aiyer KN and Kolammal M. *Pharmacognosy of Ayurvedic Drugs*, Dept of Pharcognosy, Uty. Of Kerala, Trivandrum, 1963, Nos 4-9.
3. Aniel Kumar O, Mutyala Naidu L and K G Raja Rao, *In vitro* antibacterial activity in the extracts of *Andrographispaniculata*Burm. F, International Journal of PharmTech Research, 2012, Vol.2, No.2, pp 1383-1385.
4. Burgos. R. A. and D.D.Caceres. A double blind study with a new monodrug: Kan-Jang: decrease of symptoms and enhancement of resistance in common colds. Research performed at the University of Chile, Departments of Pharmacology and School of Public Health, Santiago, Chile and funded by the Swedish Herbal Institute, 1994.
5. Chang, H.M. and P.P.H. Burt et al., 1986.Pharmacology and application of chinesemateriamedica. Singapore, World Scientific, 1:918-928.
6. Evans, W.c., Trease and evanspharmacognosy 14th edition, WB Sacender Company Ltd. 1996, pp 290
7. Hancke,J.,R.A. Burgos and D.D.Caceres, 1995.A double-blind study with a new monodrugkanjang: Decrease of symptoms and improvement in the recovery from common colds. Phytother Res., 9:559-562.
8. C. Perez, C. Anesini, *J. Ethnopharmacol*., 1993, 44, 41-46.
9. Gnanamani, A., ShanmugaPriya, K., Radhakrishnan N., Mary Babu., Antibacterial activity of two plant extracts on eight burn pathogens. J Ethnopharmacology, 2003,86,5961.
10. Kokate CK. 1993. Practical Pharmacognosy (4th Ed.) *Vallabh Prakashan*pp 107-111, 178-181.
11. Leelarasamee, A., S. Trakulsomboon and N. Sittisomwong, 1990.Undetectable anti-bacterial activity of Andrographispanicualata (Burma) wall. exes. J. MED. Assoc. Thai, 73:299-304.
12. Madav.H.C.T.Tripathi, and S.K.Mishra. Analgesic, antipyretic, and antiulcerogenic effects of andrographolide. *Indian J. Pharm. Sci.* 1995.57 (3):121-125.
13. Murry, P.R., Baron, E.J., Pfaller, M.A., Tenover, F.C., Yolken, H.R., Manual of clinical Microbiology, 6th Edition. ASM Press, Washington, DC, 1995, 15-18.
14. Olurinola, P.F., A Laboratory manual of Pharmaceutical microbiology. Idu, Abuja, Nigeria, 1996, 69-105.
15. Perez, C., Paul, M. and Bazerque, P., Antibiotic assay by agar-well diffusion method. ActaBiol Med Exp, 1990, 15, 113-115.
16. Soma Roy, KiranmayeeRao, CH Bhuvaneswari, Archanagiri, Lakshmi NarasuMangamoori. World J Microbial Biotechnol. 2010; 26: 85-91.
17. Warrier PK, Nambiar VPK and RamankuttyC... *Indian Medicinal Plants*. Vol. 1-5. Orient Longman Ltd., Madras.1993-1995.