AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Phytochemical Screening and Evaluation of Antimicrobial Activity of *Andrographis Nallamalayana* Ellis, a rare and Endangered species.

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ABSTRACT

The present article is designed for screening and evaluation of biomolecules, antimicrobial potential from extracts of *A. nallamalayana* (root), belongs to the family Acanthaceae. *A. nallamalayana* is the rare and endemic to the forests of Nallamalais, the nucleus of Eastern Ghats, India. It has been used in the folklore system of medicine for the treatment of mouth ulcers, leucorrhoea and abortion / sterility. Phytochemical screening and antimicrobial activity of the test species is hither to not reported. The phytochemical investigation on all such extracts revealed the presence of flavonoids, alkaloids, phenols, steroids and triterpenoids. The antimicrobial activity of various extracts (petroleum ether, ethyl acetate and methanol) of *A. nallamalayana* revealed that the methanolic extract (root) exhibited maximum inhibition on all test pathogens followed by ethyl acetate while petroleum ether failed to show inhibition. *Klebsiella pneumoniae, Salmonella typhimurium, Staphylococcus aureus* were proved as most sensitive while *Bacillus subtilis* as resistant strain to the test extracts.

Keywords: *Andrographis nallamalayana*, Phytochemical screening, Antimicrobial activity.

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Received 27 December 2012, Accepted 8 January 2013

Please cite this article in press as Raju RRV *et al.*, Phytochemical Screening and Evaluation of Antimicrobial Activity of *Andrographis Nallamalayana* Ellis, a rare and Endangered species. American Journal of PharmTech Research 2013.

INTRODUCTION

Medicinal plants are an important sources for the therapeutic remedies of various ailments. India is known for its rich diversity of medicinal world [1]. Nearly 70 per cent of the world population is dependent on the traditional medicines for primary health care. The knowledge of medicinal plants has been accumulated during the course of many centuries based on different medicinal systems such as Ayurveda, Unani and Siddha [2].

Plants with their wide variety of chemical constituents offer a promising source of new antimicrobial agents with general as well as specific activity [3]. There are many reports on the presence of antimicrobial compounds in various plants [4, 5] but there are no reports on antimicrobial potential on *Andrographis nallamalayana*. The secondary metabolites of medicinal plants showing antimicrobial properties have the potential of filling this need, because their structural composition is different from those of the popular microbial sources and therefore their mode of action may too very likely differ [6]. There is growing interest in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity [7]. The useful medicinal effects of plant materials typically effect from the combinations of secondary products present.

MATERIALS & METHODS:

Collection of plant material

The plant parts of *A. nallamalayana* were collected from the forests of Nallamalais. The specimen was identified with the help of the regional floras [8, 9] and the voucher specimen was deposited at Sri Krishnadevaraya University Herbarium (SKU), Anantapur.

Preparation of plant extract

The collected roots were shade dried, powdered and extracted with petroleum ether, ethyl acetate and methanol using Soxhlet apparatus for 6 hours. The extracts were filtered and the filtrates were concentrated under reduced pressure at 40º C using a rotoflash evaporator. The crude samples were subjected to antimicrobial screening against the pathogenic bacteria and fungi.

Known weight of crude extracts (50, 75 and 100 mg/ml) were dissolved in dimethyl sulphoxide (DMSO). Sterilized whatmann No.1 filter paper discs of 5mm diameter were saturated with 20µl of the extract and allowed to dry at room temperature in laminar air flow bench.

Microorganism used

The microbial strains viz., *Bacillus cereus* MTCC 4079, *Bacillus subtilis* MTCC 1133, *Staphylococcus aureus* MTCC 7443, *Micrococcus luteus* MTCC 7256, *Escherichia coli* MTCC 1668, *Klebsiella pneumoniae* MTCC 7028, *Pseudomonas aeruginosa* MTCC 7296, *Salmonella typhimurium* MTCC 98, *Candida albicans* MTCC 7315, were used. The organisms were obtained from the Microbial Type Culture Collection Centre, Institute of Microbial Technology (IMTECH), Chandigarh, India.

Phytochemical Screening

Phytochemical analysis was carried out for all the extracts using standard methods [10- 12].

Detection of alkaloids:

Extracts were dissolved individually in dilute hydrochloric acid. The resulting acidic solution was tested for alkaloids by adding Mayer's reagent and Wagner's reagent. The formation of a faint turbidity or precipitation on the addition of the above reagents indicates the presence of alkaloids.

Anthracene glycosides:

One ml of ethanol was tested for the presence of anthracene glycosides. Ethereal solutions of ethanol and water extracts were treated with 2.5% ammonium hydroxide. Formation of red colour indicates the presence of anthracene glycosides.

Shinoda test for flavonoids:

To 2-3 ml of extract dissolved in 50% methanol separately on sand bath with a piece of magnesium ribbon and 1 ml of concentrated hydrochloric acid were added. Red or yellow coloration of the solution indicates the presence of flavonoids.

Ferric Chloride test for Phenols:

extract were dissolved in alcohol or water and treated with a few ml of ferric chloride solution, any change in colour indicates the presence of phenols.

Steroids & Triterpenoids:

One ml of extract dissolved in 1ml of acetic anhydride, 1ml of chloroform and 1ml Conc. HCl separately. Formation of green colour indicates the presence of steroids, while red-violet colour indicates triterpenoids.

Iridoids:

Fresh sample was tested for the presence of iridoids. The plant material was chapped and treated with 5ml of 1% aqueous hydrochloric acid. After 3-6 hours, the extract was treated with 1ml of Trim-Hill reagent (10ml of acetic acid, 1ml of 0.2% copper sulphate in water and 0.5ml of concentrated hydrochloric acid) and heated on a water bath. The appearance of green colour indicates the presence of iridoids (monoterpenoids).

Antimicrobial activity

The antimicrobial activity of extract was evaluated by disc diffusion method [13]. Standard antibiotics viz., ampicillin, kanamycin, tetracycline and vancomycin (30µg/disc) obtained from Hi-Media, Mumbai, were used as positive controls. The discs containing petroleum ether, ethyl acetate and methanol served as negative controls. The assessment of antimicrobial activity was based on measurement of inhibition zones formed around the discs.

The minimum inhibitory concentration (MIC) was determined by the broth –micro – dilutions [14] using 96 well microtiter plates. The lowest concentration of each extract showing growth was taken as its minimum inhibitory concentration (MIC). The solution DMSO (100µl/ml) served as negative control. All samples were tested in triplicates to confirm the activity.

Minimum bacterial/fungal concentrations (MBC/MFC) were determined by adopting standard methods [15, 16]. To determine MBC10µl of broth medium from each well of MIC tested plate was taken and incubated in nutrient agar at 37° C for 24 h for bacteria or in Sabouraud's Dextrose agar at 30° C for 48 h for the yeasts. The least concentration showing no visible growth on agar subculture was taken as MBC/MFC value. This is the lowest concentration, expressed in mg/ml. Each test was performed in three replicates and repeated twice to confirm the activity and results were tabulated.

RESULTS & DISCUSSION:

In the present study, the phytochemical screening and antimicrobial activities were performed with petroleum ether, ethyl acetate and methanol extract of *Andrographis nallamalayana*. Preliminary phytochemical screening of root extract revealed the presence of alkaloids, flavonoids, triterpenoids, steroids and phenols (Table-1).

Table 1: Preliminary phytochemical screening of different extracts of *A. nallamalayana* (Root)

Components	Pet. Ether	Ethyl acetate	Methanol
Alkaloids	+	+	++
Anthracen glycosides	--	--	+
Flavonoids	--	+	+
Iridoids	--	--	+
Steroids	+	--	--
Triterpenoids	--	+	+
Phenols	--	--	+

'--' Absence, '+' Presence.

Flavonoids have been referred to as nature's biological response modifiers, because of their inherent ability to modify the body's reaction to allergies and they showed their anti- allergic, anti-inflammatory, anti-microbial and anti-cancer activities [17]. Alkaloids are formed as metabolic by-products and have been reported to be responsible for the antibacterial activity [18] while steroids and triterpenoids are known for anti-inflammatory, lipolytic and anti-cholesteremic activities [19].

The methanolic extract was more effective on test pathogens when compare to that of ethyl acetate. The methanol extract showed significant inhibition zones ranged from 6 – 10 mm against all the organisms except *Bacillus subtilis*. In the present study *Klebsiella pneumoniae* and *Salmonella typhimurium* were proved as more susceptible to the extracts than other test pathogens. The ethyl acetate extract exhibited inhibition zones between 6 – 9 mm with the respective MIC values, 312 – 625 µg/ml of both extracts against the all test pathogens. The results of the MBC values revealed that *Klebsiella pneumoniae, Micrococcus luteus, Salmonella typhimurium* and *Staphylococcus aureus* were completely inhibited at 8mg/ml concentration and *E. coli* was the least sensitive organism to ethyl acetate extract (12 mg/ml)

(Table-2). The petroleum ether extract fail to inhibit the growth of microorganisms.

Table -2: Antimicrobial activity of *A. nallamalayana* – Root

Organism	Inhibition zone (mm^{-1})														STD µg/disc
	Pet. ether mg/ml				Ethyl acetate mg/ml					Methanol mg/ml					
	50	75	100	MIC µg/ml	50	75	100	MIC µg/ml	MBC	50	75	100	MIC µg/ml	MBC	
Bc	-	-	-	-	-	-	-	-	-	6	6	7	625	10	22^{A}
Bs	-	-	-	-	-	-	-	-	-	-	-	-	-	-	25^{V}
Ca	-	-	-	-	-	-	-	-	-	-	6	7	625	10	25^{V}
Ec	-	-	-	-	6	7	10	1250	12	-	7	8	625	10	22^{T}
Kb	-	-	-	-	-	-	-	-	-	9	10	10	312	8	23^{T}
Ml	-	-	-	-	-	-	-	-	-	-	7	8	312	8	23^{K}
Pa	-	-	-	-	-	7	7	625	10	6	6	9	312	8	28^{T}
Sal	-	-	-	-	-	-	-	-	-	7	8	10	312	8	22^{A}
St	-	-	-	-	-	6	7	625	10	-	7	9	312	8	23^{K}

Bacillus cereus, Bs: *B. subtilis,* Ca: *Candida albicans,* Ec: *Escherichia coli,* Kb: *Klebsiella pneumoniae,* Ml: *Micrococcus luteus,* Pa: *Pseudomonas aeruginosa,* Sal: *Salmonella typhimurium,* St: *Staphylococcus aureus,* **A – *Ampicillin,* K – *Kanamycin,* T – *Tetracycline,***

V – *Vancomycin,* MIC: Minimum inhibitory concentration, **MBC:** Minimum bacterial concentration

CONCLUSION:

The *A. nallamalayana* root methanol extracts showed significant antibacterial activity against the gram positive and negative organisms. The phytochemical analysis confirms the presence of alkaloids, flavonoids, steroids and triterpenoids. The observations confirm the folk uses of the crude drugs and justify the ethnobotanical approach in the search for novel bioactive compounds. The isolation and structural elucidation of bioactive molecules is in progress.

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