International Standard Serial Number (ISSN): 2249-6807

International Journal of Institutional Pharmacy and Life Sciences 4(6): November-December 2014

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Life Sciences **Research Article……!!!**

Received: 02-12-2014; Revised: 25-12-2014; Accepted: 26-12-2014

PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF KING OF BITTER *ANDROGRAPHIS PANICULATA* AGAINST PATHOGENIC MICROBES

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Keywords:

Andrographis paniculata, Phytochemicals, Antibacterial activity

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ABSTRACT

In ancient times, traditional medicine used for various diseases. Traditional medicine involves the usage of different plant extracts due to the presence of bioactive compounds against various diseases. The study represents that a comparative study of phytochemicals was made on three different solvent derived leaf extract of *Andrographis paniculata*. The leaf extracts were prepared by using three solvents are aqueous, ethanol and acetone. The qualitative analysis of phytochemicals concludes that presence of steroids, alkaloids, flavonoids, tannins, glycocides, saponins and triterpenoids. Screening of antibacterial activity of three types of solvent derived extracts was assessed by Agar well diffusion method. It was observed that, ethanol extract of A. paniculata showed the significant antibacterial activity against *Klebsiella pneumonia* and *candida sp* compared with aqueous and acetone extract. The minimum inhibitory concentration is 50 µ L. Present results interestingly concluded that the crude ethanolic extracts of *A.paniculata* herbal plant leaves are promising medicinal value for harmful diseases.

INTRODUCTION

Currently, focus on use of herbal medicine approaches to treat diseases has been invigorated worldwide for human wellbeing and healthcare(Salka et al 2011; Negi et al 2008). Medicinal plants are considerably useful and economically essential. Usage of herbal medicine to treat various diseases was accepted among people due to its less or no side effects (Akbar 2011).Herbal drugs have become increasingly popular and their use is widespread (Dharmadasa et al 2013).Traditionally, plants have many active compounds like alkaloids, steroids, tannins, and phenolic compounds, flavonoids, resins fatty acids gums, so they have provided a source of inspiration for novel drug compounds have made large contributions to human health.Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modernmedicines, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Suparna et al 2014). In recent years, several new diseases are established in population rich countries and also the existed diseases are resistance to commercial antimicrobial drugs (Shah 2005). Therefore there is a need to develop new medicine for the treatment of infectious diseases from medicinal plants (Cordell, 2000; Roy et al 2010).

Worldwide, infectious disease is the number one cause of death accounting for approximately one-half of all deaths in tropical countries. Perhaps it is not surprising to see these statistics in developing nations, but what may be remarkable is that infectious disease mortality rates are actually increasing in developed countries, such as the United States (Pinner et al, 1996). Nosocomial infections prevalence has been on the continuous rising of methicillin resistant *Staphylococcus aureus*. Resistance to available therapeutic agents and thelimited development of new agents are threatening to worsen the burden of infections and cancers that are already theleading cause of morbidity and mortality(Hamill et al 2008; Mishra et al 2013).Nowadays, multiple drug resistance hasdeveloped due to the indiscriminate use ofcommercial antimicrobial drugs commonly usedin the treatment of infectious disease (Sevice, 1995).To overcome these problems, there is need for continuous search for other sources of new medicinal drug especially from natural plant.Herbal plants are utilized for their active compounds as therapeutic drugs are safe without side effects. *Andrographis paniculata* is herbaceous plant, classified under the family of Acanthaceae. It grows erect to a height of 30–110 cmin moist, shady places, locally it is known as Nilavembu, Sirunangai, Siriyanangai. It have lot of medicinal applications (Doss and

Kalaichelvan 2012). It is best antibacterial agent against various clinical pathogenic bacteria (Akbar 2011, (Niranjan et al 2010).

MATERIALS AND METHODS

Collection of plant leaves

The leaves were collected from Wandiwash, TN, India. The collected plant leaves were washed with running tape water and distilled water. Washed leaves were shade dried at room temperature for 3-5 days and grinded into fine powder. After they are kept in air tight container and used to solvents extraction. Powdered leaves were subjected for extraction with aqueous, ethanol and Acetone using soxhlet apparatus.

Preparation of aqueous extracts

About 10 g of dried leaves powder were mixed with 100 ml of sterile distilled water and kept on a water bath shaker for 12 h at 40°C. Thereafter, it was filtered through Whatman No 1 filter paper, then the filtrate was collected and used for preliminary chemical color reactions of phytochemical group.

Preparation of acetone and methanol extracts

The extraction procedure for acetone extracts is powder and the solvent acetone and water in the ratio of 4: 1. About 25 g of dried leaves powder was extracted using 100 ml of the extraction solvent acetone in soxhletat 55°C for 48 h. Methanol extract was prepared by using 80% of methanol solvent with 25 g of dried powder in soxhlet at 60°C for 48 h. The extracts were then concentrated by evaporation process under air dry. These extracts used to preliminary different phytochemical screening for the analysis of various phytochemical groups.

Phytochemical screening

The three extracts thus obtained were analyzed to preliminaryphytochemical screening following the standard protocols. Presence of alkaloids and flavonoids were estimated according to the method described by Harborne (1973) and Edeoga et al (2005) respectively.Saponins and tannins were performed using the method described by Farnsworth (1966). Glycosides were estimated using the method adopted by Haborne (1973) and other phytochemical constituents are Steroids, terpenoids, and Triterpenoids, followed by Siddiqui and Ali, (1997). Sugars- Benetic'stest, phenol-Ferric chloride test,protein estimation done by Lowry's method(Siddiqui and Ali, 1997).

Antimicrobial activity assay

Test bacterial strains

The bacterial strains *Bacillus subtilis. Klebsiella pneumonia, E. coli, Staphylococcus aureus and Candida sp*used in the present study were obtained from Microlabs, Tamilnadu, India. The *Bacillus subtilis* and *Klebsiella planticola* cultures were purchased from Microlabs, Chandigarh, India.

Agar well diffusion method

Qualitative assay of antibacterial activity of plant extracts was performed by standard methodology i.e. agar well diffusion method. The antibacterial medium Muller Hinton Agar was used in this study. Different volumes of crude plant extracts were dissolved in distilled water (10 mg/ml). Pathogenic bacteria were grown in Nutrient broth for 24 hr and swapped on the petriplates containing Muller Hinton Agar. In MHA agar plate, about 6 mm diameter well were made by gel puncture. Diluted extracts with different concentration (25, 50, and 75 µL) were applied into the well and the plates were incubated at 37°C for 24 hr. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well.

RESULTS AND DISCUSSION

Phytochemical constituents of *Andrographis paniculata*

The phytochemical analysis of the aqueous, ethanol, and acetone leaf extract of *Andrographis paniculata* were tested positive forthepresence of Triterpenoids, Reducing sugars, Tannins, Alkaloids, Flavonoids and Saponins (Table 1). *Andrographis paniculata* plants were traditional plants, used in herbal medicine. Chemical analysis and biochemical assays are important aspects in pharmacognostic evaluation of medicinal plants (Choudhury et al., 2009; Harborne, 1973).

Table 1 Phytochemical constituents of aqueous, ethanol and acetone extract of *A. paniculata* leaves

Compounds	Aqueous extract	Ethanol extract	Acetone Extract
Colour of the extract	Greenish brown	Yellowish green	Yellowish brown
Sugars	+	+	-
Proteins	+	+	+
Alkaloids	+/-	+	-
Steroids	-	+	-
Triterpenoids	-	-	+
Flavonoids	+	+	+
Glycosides	-	+	+
Terpenoids	-	-	+
Phenol	+	+	-
Tannins	+	+	-
Saponins	+	+	-

The aqueous extract of *A. paniculata* have positive result for the presence of sugars, proteins, alkaloids, flavonoids and tannins. Alkaloids present in low quantity. Steroids, triterpenoids, glycoside and phenol were absent in the aqueous extract. In ethanolic extract triterpenoids and phenol were absent, sugars and tannins are present in low quantity. Other phytochemical constituents were exhibited. Acetone extract confirmed that presence of proteins, triperpenoids, flavonoids, glycosides, and phenol. The various phytochemical compounds detected are known to have beneficial importance in medicinal science (Okeke*et al*, 2001).Preliminary phytochemical screening was showed that the ethanolic extract contain all the bioactive metabolites. Thus, it has higher activities.

Antibacterial activity

Aqueous, Ethanol and Acetone extract of *A.paniculata* leaves was also investigated for their antimicrobial activity against seven bacterial species including *E.coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, Klebsiellapneumonia, K. planticola,* and *Candida sp.* Of all the tested concentrations, direct antimicrobial activity of the ethanolic*A. paniculata*extracts were observed for human pathogens. Aqueous,Ethanol and Acetone extract of *A.paniculata*leaves showed potential antimicrobial activity against *K. pneumoniae* at the increased concentration (75µL). Antibacterial activity was increased while increasing the concentration of extracts.

Table 2 Antibacterial activity of aqueous extract of *A. paniculata* against pathogenic bacteria

Microorganisms	**Concentration of extract/ zone of inhibition**			
	25µL	**50µL**	**75µL**	***Ciprofloxacin***
E.coli	9	11	13	14
Pseudomonas aeruginosa	12	14	17	15
Staphylococcus aureus	10	13	15	14
Klebsiella pneumoniae	14	16	19	15
Bacillus subtilis	15	17	18	20
Candida sp	11	14	15	22
Klebsiella planticola	10	12	14	13

Table 3 Antibacterial activity of Ethanol Extract of *A. paniculata*

Microorganisms	Concentration of extract/ zone of inhibition			
	25µL	**50µL**	**75µL**	***Ciprofloxacin***
E.coli	10	12	14	15
Pseudomonas aeruginosa	12	15	18	17
Staphylococcus aureus	11	14	17	15
Klebsiellapneumoniae	15	18	21	20
Bacillus subtilis	13	16	18	22
Candida sp	11	15	16	19
Klebsiellaplanticola	9	11	13	12

Table 4 Antibacterial activity of Acetone Extract of *A. paniculata*

Microorganisms	Concentration of extract/ zone of inhibition			
	25µL	**50µL**	**75µL**	***Ciprofloxacin***
E.coli	8	10	11	14
Pseudomonas aeruginosa	10	11	13	17
Staphylococcus aureus	7	8	10	15
Klebsiellapneumoniae	11	13	15	20
Bacillus subtilis	13	14	16	18
Candida sp	9	10	13	15
Klebsiellaplanticola	10	12	14	22

Antimicrobial effects of Aqueous, ethanol and acetone extracts of A. paniculata shown in the Table 2, 3 and 4 and figure 1. Among these three extracts, ethanol extracts shows high antimicrobial efficiency. Minimum inhibitory concentration of extract is 25 µL. *Candida sp* are dermatophytes which they caused skin diseases. Ethanol extract of *A. paniculata* shows high inhibition activity around 16 mm diameter at 75 µL concentration. Highest inhibitory activity was observed against *K. pneumonia*, which they cause pneumonia fever. This results of antibacterial activity screening shows ethanol extract of *A. paniculata* leaves is alternative and effective herbal medicine against *K. pneumonia* and MRSA bacteria. Thisinhibitory activity may be due to the presence of Alkaloids, flavonoids and phenolic compounds present in the crude extracts.

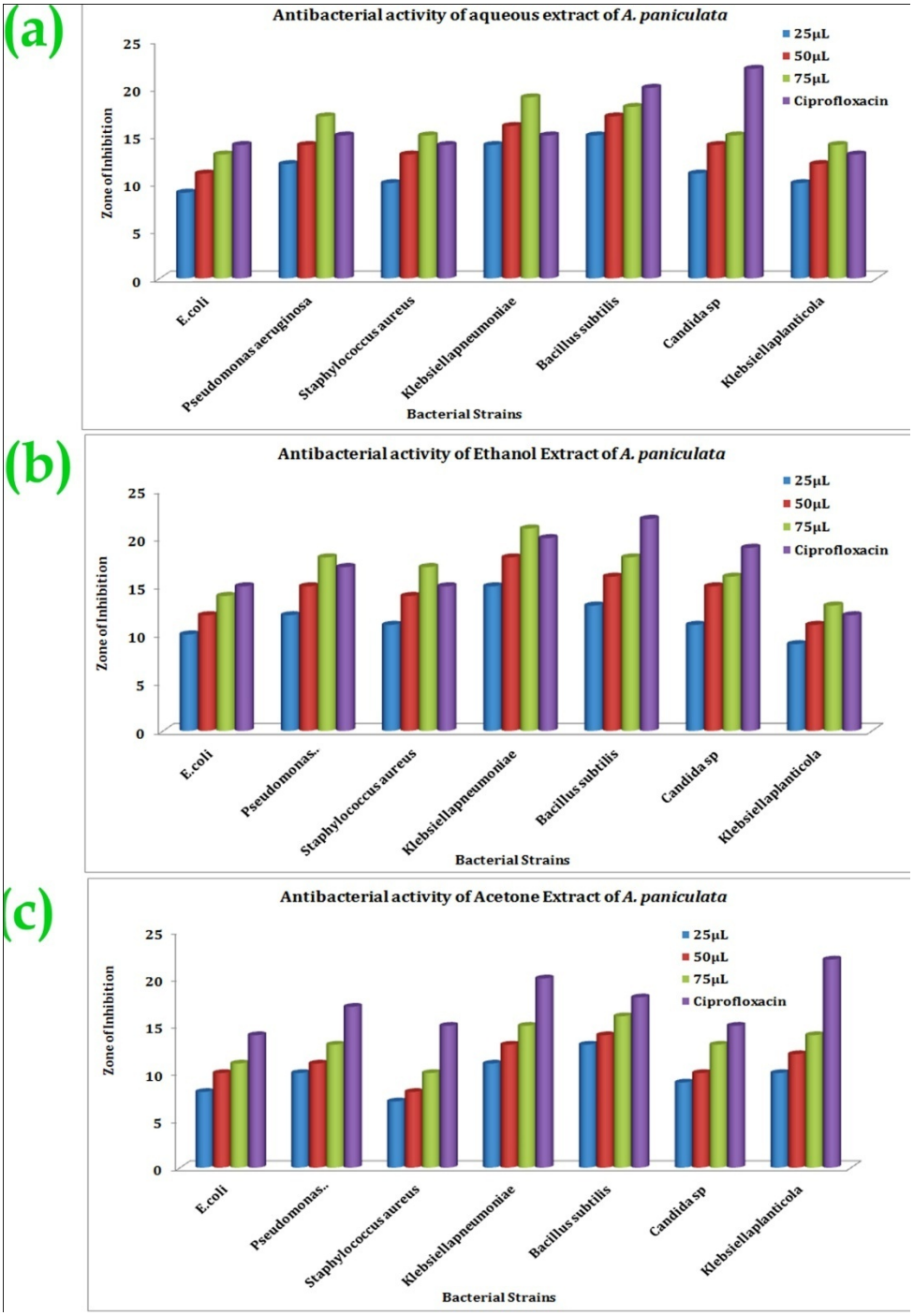

Figure 1: Antimicrobial activity of Aqueous, Ethanol and Acetone extract of *A.paniculata* leaves

The various phytochemicalcompounds detected are known to have beneficial importance in medicinal science. Phenolsare said to offer resistance to diseases and accounts for most of the anti-oxidant activity inplants (Aliyu et al., 2009). Flavonoids show anti-allergic, anti-inflammatory, anti-microbial and anti-canceractivities(Aiyelaagbe et al., 2009). Alkaloids have

been used to treat diseases like malaria and glycosides serve as defense mechanisms against many micro-organisms. Saponin protects the plant againstmicrobes and fungi. Extracts from this plant showed varying antimicrobial activities when compared to the standard antibiotic. The results suggest that the antimicrobial activity of this plant may contribute to its claimed activity as a therapeutic drug against human pathogens.

CONCLUSION

A. paniculata is the geat Indian medicinal plant having a lot of phytochemicals and vigorously involved in the killing of microorganisms. From the above results it can be concludedthat plant extracts have great potential as antibacterialcompounds against microorganisms and that they canbe used in the treatment of infectious diseases causedby resistant microorganisms.

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