Sruthi Gurajala. *et al.* / *International Journal of Pharmacy & Therapeutics*, 3(3), 2012, 282-286.

e- ISSN 0976-0342
Print ISSN 2229-7456

International Journal of Pharmacy & Therapeutics

Journal homepage: www.ijptjournal.com

IJPT

EVALUATION OF ANTIMICROBIAL PROPERTY OF *ANOGEISSUS ACUMINATA* (COMBRETACEAE) LEAVES

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ABSTRACT

The present study was carried out to evaluate the antimicrobial property of *Anogeissus acuminata* (Combretaceae) leaves against certain bacterial and fungal stains causing microbial infection using cup plate method. The agar disc diffusion method is used to detect the antimicrobial activity of plant extract against gram negative bacteria (*Salmonella paratyphi, Klebsiella pneumoniae*) and gram positive bacteria (*Streptococcus pneumoniae, Bacillus cereus*) as well as in Fungi (*Candida albicans*). All the different concentration of 70% methanolic and aqueous leaf extracts of *Anogeissus acuminata* exhibited the concentration dependent significant anti-microbial activity comparable with Ciprofloxacin (5µg/ml) and Fluconazole (5µg/ml) were used respectively as standard drug for bacteria and fungi stains.

Key Words: *Anogeissus acuminata*, Agar disc diffusion method, Antimicrobial activity, Ciprofloxacin, Fluconazole.

INTRODUCTION

India is a land of rich biodiversity. The total number of lower and higher plants in India is about 45,000 species. The plants are potential source of medicines since ancient times (Cragg *et al.*, 2001). The history of antimicrobials begins with the observations of Pasteur and Joubert, who discovered that one type of bacteria could prevent the growth of another (Jahir alam khan and Saurabh Tewari, 2011). Plant materials remain an important resource to combat serious diseases in the world. The pharmacological investigations of plants were carried out to find novel drugs or templates for the development of new therapeutic agents (Iwu *et al.*, 1999). During the last two decades, the development of drug resistance as well as the appearance of undesirable side effects of certain antibiotics has lead to the search of new antimicrobial agents (Okemo, 2003). Diseases that have been managed traditionally using medicinal plant include malaria, epilepsy, infantile convulsion, diarrhea, dysentery, fungal and bacterial infections (Sofowora, 1996).

Anogeissus acuminata (Combretaceae) is a moderate size tree with small leaves, growing in South Africa and Arabian Peninsula. It is commonly known as Button tree (Wealth of India, 1950). In telugu it is known as Pasichettu, Peddmanu and Buchakram. Leaves of the plant are used in traditional and tribal medicine of Andhra Pradesh to treat painful inflammatory conditions (Hemamalini *et al.*, 2010). *Anogeissus acuminata* is popular all over the world for its medicinal uses in skin diseases like eczema, dermatitis, skin ulcers and also used as anti-inflammatory, analgesic etc (Corley *et al.*, 2003). Methanolic extract and crude extracts of leaves of the plant are used in traditional and tribal medicine to treat painful inflammatory conditions and to heal wounds (Hemamalini *et al.*, 2011). Therefore, the present study is an attempt to investigate antimicrobial properties of *Anogeissus acuminata* leaves.

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MATERIALS AND METHODS

Plant material

Anogeissus acuminata (Combretaceae) leaves were collected from Tirunelveli District of Tamilnadu, India, in the month of February, 2012. It was authenticated by Botanist Dr. Chelladurai, Professor, Department of Botany, University of Tamilnadu, India. The voucher specimen of the plant was deposited in the college for further reference.

Preparation of Extracts

70% Methanol Extract: The leaves of *Anogeissus acuminata* plant were dried under shade and powdered mechanically. The dried powder material (100gm) was subjected to continuous hot extraction with 70% methanol in Soxhlet apparatus for 8hrs. The solvent was removed from extract by vacuum distillation. Then it was concentrated, dried, cooled and weighed. The percentage yield of 70% methanol extract of *Anogeissus acuminata* was found to be 23% w/w.

Aqueous Extract: The dried powder of *Anogeissus acuminata* was subjected for cold maceration. About 100gm of powdered drug was immersed in distilled water (500ml) in flat bottom flask and kept for cold maceration for 7 days with occasional shaking. At the end of seventh day it was filtered through Buchner funnel. The filtrate was dried in freeze drier until free from moisture. It was than weighed and kept in vacuum desiccator to free from moisture. The percentage yield of aqueous extract of *Anogeissus acuminata* was found to be 17% w/w.

Microorganisms used

Microorganisms were identified and obtained from National Centre for Industrial Microorganisms (NCIM), Pune, India. The bacteria studied were three strains gram positive (*Streptococcus pneumoniae, Bacillus cereus* and *Staphylococci aureus*) and three strains gram negative (*Escherichia coli, Salmonella paratyphi* and *Klebsiella pneumoniae*). Fungal strain namely *Candida albicans*. The bacterial strains were cultured on nutrient agar slants. The cultures were maintained by sub culturing periodically and preserved at 4ºC until further use.

Standard drugs: Ciprofloxacin and Fluconazole were procured from Hi-media laboratories, Mumbai, India.

Media and Chemicals: Nutrient broth (NB), Nutrient Agar (NA), Sabouraud dextrose agar (SDA), Sabouraud dextrose broth (SDB), Peptone water were procured from Hi-media laboratories, Mumbai, India. Dimethyl sulfoxide (DMSO) was procured from Sd fine Ltd., Mumbai, India.

Media Used: Nutrient Agar Media.

Evaluation of Antimicrobial Activity

The antibacterial study was carried out by agar diffusion technique in particular cup plate method against gram +ve and gram –ve organisms. The antibacterial activity of the *Anogeissus acuminata* extracts were systematically performed against four different strains of bacteria (two gram positive and two gram negative) *Streptococcus pneumoniae* G^{+ve}, *Bacillus cereus* G^{+ve}, *Salmonella paratyphi* G^{-ve}, *Klebsiella pneumoniae* G^{-ve} by agar cup plate method. The bacteria used were reference standard solution 5µg/ml was prepared by dissolving ciprofloxacin in water for injection (5µg/ml). The medium was sterilized by autoclaving at 121^0C (15lb/inch^2).

About 30ml of molten nutrient agar medium inoculated with the respective strain of bacteria (6ml of inoculums to 300ml of nutrient agar medium) was transferred aseptically into each sterilized petriplate (10cm diameter). The plates were left at room temperature to allow solidification. In each plate 5 wells of 8mm diameter were made with a sterile borer. Accurately 0.2ml of the test solution was added to the cups aseptically and labeled accordingly. After incubation of the plates at 37±1 for 24h, the diameter of the zone of inhibition surrounding each of the well was noted (Dhanabal *et al.,* 1999).

Determination of Minimal Inhibitory Concentration (MIC) for Bacteria

Plates were prepared under aseptic conditions. A sterile 96 well plate was labeled. A volume of 100µl of test material in DMSO (usually a stock concentration of 0.2mg/ml for purified compounds, and 2mg/ml for crude extracts) was pipetted into the first row of the plate. To all other wells 50µl of sterile broth was added. Serial dilutions were performed using a multichannel pipette. Tips were discarded after use such that each well had 50µl of the test material in serially descending concentrations. To each well 10µl of resazurin indicator solution was added. Using a pipette 30µl of sterile broth was added. Finally, 10µl of microbial suspension (0.5 McFarland) was added to each well. Each plate was wrapped loosely with cling film to ensure that cultures did not become dehydrated. Each Plate has a set of Positive, negative and a standard **(**Satyajit *et al.,* 2007**).**

The plates were prepared and placed in an incubator set at 37°C for 18–24h or 28°C for 48h. The color change was then assessed visually. Any color changes from purple to pink or colorless were recorded as positive. The lowest concentration at which color change occurred was taken as the MIC value.

Determination of Minimal Inhibitory Concentration (MIC) for Fungi

The Minimum Inhibitory Concentration (MIC) of the test substances against *Candida albicans* was determined by liquid broth method of two fold serial dilution technique. In this assay, the minimum concentration of each test substance required to inhibit the growth of microorganism was determined **(**Gibbons *et al.*, 2002)**.**

For this assay, a series of assay tubes were prepared containing uniform volume (1ml) of sterile SD broth and equal volume of known concentration of test substance was added. The test substance in the first tube was serially diluted in two fold decreasing concentrations through the sixth tube and seventh tube was left without test substance as positive control. The tubes with the test substance i.e. from one to seventh were inoculated with 1ml of inoculum ($1x10^6$ CFU/ml). The final concentration of test substance ranged from 1000 to 31.25µg/ml. Solvent control and sterility controls were maintained in the experiment. The tubes were incubated at 28^0C for 48h. Standard antibiotic, fluconazole was tested as standard drug at concentrations ranging from 100 to 3.12µg/ml. The tubes were inspected visually to determine the growth of the organism as indicated by turbidity (In fact, turbidity of the culture medium is indicative of the presence of a large number of cells), the tubes in which the antibiotic is present in concentration sufficient to inhibit fungal growth remain clear. In experimental terms the MIC is the concentration of the drug present in the last clear tube, i.e. in the tube having the lowest concentration in which growth is not observed.

RESULTS AND DISCUSSION

The disc diffusion method is used to detect the antimicrobial activity of plant extract against gram +ve and gram -ve. The solidified Muller Hinton agar plates were swapped with the test organism and the standard sample and blank discs were impregnated. After the incubation the zone was measured. The antimicrobial activity of plant extracts was detected by the indication of zone around the disc.

Both aqueous and 70% methanol extract of *Anogeissus acuminata* showed good antibacterial activity against all the specific organisms tested *Streptococcus pneumoniae*, *Bacillus cereus* (gram positive) and *Salmonella paratyphi*, *Klebsiella pneumoniae* (gram negative) at 400µg. The antimicrobial activity of the 70% methanolic and aqueous extracts compared with standard drug ciprofloxacin (5µg/ml). The 70% methanolic extract (zone of inhibition 14 - 23mm) was found to be more effective than the aqueous extract (zone of inhibition 11- 22mm) against all the organisms. The results were recorded in Table.3.2.

The samples showed Minimum Inhibitory Concentration (MIC) against *Staphylococci aureus* and *Escherichia coli* strains by micro-titter method and *Candida albicans* strains by tube dilution method. Various solvents extract of leaf of *Anogeissus acuminata* were examined. The standard drug used for fungi is Fluconazole and for bacteria is Ciprofloxacin. MIC values shows positive response against bacteria whereas negative response against fungi. Therefore, the MIC identified 500µgm for leaf extracts of *Anogeissus acuminata* (Combretaceae) MIC values for the standard drug ciprofloxacin is 7.8mgm/ml. The antimicrobial activity of aqueous and 70% methanolic extract showed positive results against entire organism. The results were tabulated in tab-3.8.

The obtained results indicate both aqueous (200µl) and 70% methanolic extracts has antimicrobial activity against organisms especially in 70% methanol extract than aqueous extract. The results show that the methanol extract of *Anogeissus acuminata* showed more inhibitory effect than the other plant extracts. This tends to show that the active ingredients of the plant parts are better extracted with methanol than aqueous solution. The methanol extracts contain alkaloids, coumarins and tannins (Okemo, 1996). Coumarins and tannins have antibacterial properties. The present study justifies the claimed uses of *Anogeissus acuminata* leaves in the traditional system of medicine to treat various diseases caused by the microorganisms.

Table 1. Antimicrobial activity profile of extracts of *Anogeissus acuminata* leaves

Bacteria	Zone of inhibition Aqueous extract				Zone of inhibition 70% Methanolic extract				Ciprofloxacin
	100 µg	200 µg	300 µg	400 µg	100 µg	200 µg	300 µg	400 µg	5µg/ml
Streptococcus pneumoniae	19	20	20	20	20	20	20	20	24
Bacillus cereus	14	10	17	20	14	18	21	22	25
Salmonella paratyphi	17	17	22	22	21	21	22	23	23
Klebsiella pneumoniae	11	11	14	15	18	20	20	20	25

Table 2. Minimal Inhibitory Concentration (MIC) of the extracts of *Anogeissus acuminata* leaves

Micro organisms	MIC of Standard drugs in µg	MIC of Extracts in µg	
		Aqueous Extract	Methanol Extract
Staphylococci aureus	7.8	500	500
Escherichia coli	7.8	500	500
Candida albicans	12.5	>1000	1000

Fig 1. Antimicrobial activity of Aqueous extract of *Anogeissus acuminate*

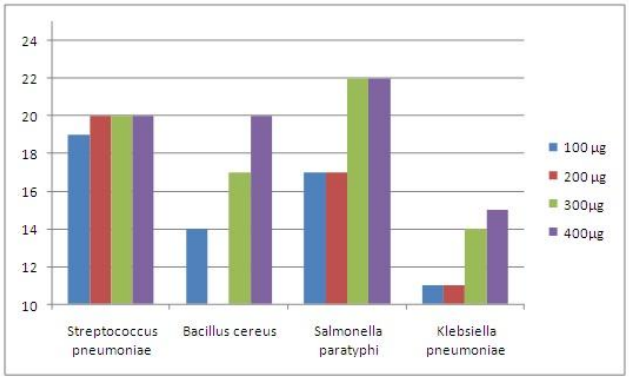
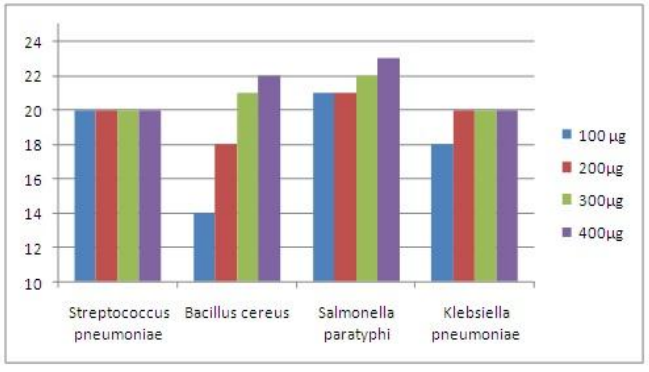


Fig 2. Antimicrobial activity of 70% Methanol extract of *Anogeissus acuminate*



CONCLUSION

It can be concluded from this study, that *Anogeissus acuminata* (Combretaceae) leaf extracts (Aqueous and 70% Methanolic) have antimicrobial activity against certain bacteria and fungi. It is expected that using natural products as therapeutic agents will probably not elicit resistance in microorganisms. This can explain the rationale for the use of the plant in treating infections in traditional medicine. It is essential that research should continue to isolate and purify the active components responsible for the medicinal uses of this plant.

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