



ANTIMICROBIAL ACTIVITY OF *BUCHANANIA ANGUSTIFOLIA*, (ANACARDIACEAE)

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ABSTRACT

Medicinal plants have been used as traditional treatments for numerous human diseases causing by microorganisms from thousands of years and in many parts of the world. Screening of plants for biologically active compounds against human pathogens is a renewed interested research field. In this study *Buchanania Angustifolia* Anacardiaceae screened for its potential antimicrobial activity against two gram positive (*Streptococcus pneumoniae*, *Bacillus cereus*) and two gram negative (*Salmonella paratyphi*, *Klebsiella pneumoniae*) and fungi *Candida albicans* by agar cup-plate method. The antimicrobial activity of methanolic and aqueous extract of stem of *Buchanania Angustifolia*, showed significant inhibitory action against gram positive and gram negative bacterias and fungi. In this study the aqueous and methanolic extracts of *Buchanania Angustifolia* was compared to standard drug Ciprofloxacin for bacteria and Fluconazole for fungi.

Key words: Human diseases, *Buchanania Angustifolia*, Inhibitory action, Bacteria, Fungi.

INTRODUCTION

An antimicrobial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi or protozoan's, as well as destroying viruses. Antimicrobial drugs either kill microbes (microbicidal) or prevent the growth of microbes (microbistatic). Disinfectants are antimicrobial substances used on non-living objects (Frobisher *et al.*, 1974). Infectious diseases are disorders caused by pathogenic microorganisms like bacteria, viruses, fungi, protozoa and multicellular parasites. These diseases are also called as communicable or transmissible diseases since they can be transmitted from one person to another via a vector or replicating agent. Infectious diseases account for about half of the deaths in tropical countries. Bacterial diseases are a type of infectious diseases caused by pathogenic bacteria. It is notable that majority of bacteria are non pathogenic and are not harmful to human health. Some bacteria are even

helpful and necessary for the good health. Millions of bacteria normally live in the intestine, on the skin and the genitalia. Bacterial diseases results when the harmful bacteria get into a body area, multiply their and thrash the body's defensive mechanism (Khosravi *et al.*, 2006).

Pathogenic bacteria can invade in the body through various routes like inhalation into nose and lungs, ingestion in food or through sexual contact. Once bacteria enter the body, the immune system of the body recognizes the bacteria as foreign intruder and tries to kill or stop them from multiplying. However, even a healthy immune system is not always able to stop the bacteria from reproducing and spreading. As a result bacteria thrive in the body and emit toxins which damage cells and tissues that consequently results in the symptoms of bacterial disease (Khosravi *et al.*, 2006).

The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in immune compromised patients in developing countries and many infectious microorganisms are resistant to synthetic drugs; hence an

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alternative therapy is very much needed (Al-Bari *et al.*, 2006). Since ages, man has been dependent on nature for curing various body diseases. From ancient civilization various parts of different plants were used to eliminate pain, control suffering and counteract disease. Most of the drugs used in primitive medicine were obtained from plants and are the earliest and principal natural source of medicines (Vats Manisha *et al.*, 2009). Technically, antibiotics are only those substances that are produced by one microorganism that kill, or prevent the growth, of another microorganism. Of course, in today's common usage, the term antibiotic is used to refer to almost any drug that cures a bacterial infection. Antimicrobials include not just antibiotics, but synthetically formed compounds as well (Jahir alam khan and Saurabh Tewari, 2011).

The discovery of antimicrobials like Penicillin and Tetracycline paved the way for better health for millions around the world. Before 1941, the year penicillin was discovered; no true cure for gonorrhoea, strep throat or pneumonia existed. Patients with infected wounds often had to have a wounded limb removed, or face death from infection. Now, most of these infections can be cured easily with a short course of antimicrobials.

Recently, the global consumer started to demand high quality foods that are minimally processed and sustain more naturally occurring bioactive ingredients. Moreover, the consumer perception that the use of chemical antimicrobials could have further toxicological consequences has attracted the attention of national food agencies and the food industry. During the last decade, concerns over allergies and carcinogenic compounds developed from chemical additives resulted in an increase in the demand of more naturally processed foods. All these trends are forcing food processors to find new means to extend shelf life and maintain the product safety while decreasing the use of food additives or severe treatments, such as heat treatments (Gould and Russell, 2003; Naidu, 2000; Roller, 2003).

The plant *Buchanania Angustifolia* belongs to family Anacardiaceae it is very common plant in dried parts of India. This plant grows in deciduous forests in north western India. This tree is upto 10 metre tall, hairless branches stout, bark rough, deeply fissured, leaves are linear oblong, elliptic, lanceolate rounded or narrow at base, entire at margin, blunt, rounded or notched at tip, flowers are 3-6 mm across in axillary and terminal hairless branched panicles, sepals are 5 semicircular. Petals are 5, oblong or ovate. Fruits are obliquely globose, slightly compressed. Wound healing, Cardio tonic, CNS depressant, Diarrhea Dysuria, poly urea, Aphrodisiac, Skin disease, The plant seed and bark is used in the form of decoction to treat intrinsic haemorrhage, diarrhoea with blood and tonic (Madhavachetty *et al.*, 2008).

MATERIALS AND METHODS

Microorganisms:

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 4°C until further use.

Standard drug: 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.

Preparation and Standardization of Stock cultures:

Cultures on receipt were sub cultured in NA/SDA (Bacteria/Fungi) plates and further stored in slants as stock cultures. For the experiments, stock culture was prepared by inoculating each culture from slants to flask in sterile NB/ SDB and incubated at 37°C/28°C for 24h/48h (Bacteria/Fungi). The stock culture was adjusted to 0.5McFarland standard turbidity and used for assay.

Preparation of Agar Plates:

Nutrient agar of 20ml was mixed thoroughly with 0.2ml of overnight culture at 45-50°C and plated. After the agar is set and solidified four cups of 8mm diameter bores were made in each petridish with the help of sterile borer in the aseptic area created for the purpose.

Preparation of resazurin solution:

The resazurin solution was prepared by dissolving a 270mg of tablet in 40ml of sterile distilled water. A vortex mixer was used to ensure that it was a well-dissolved and homogenous solution.

Methodology

Evaluation of antimicrobial activity:

The antibacterial activity of the extracts were systematically performed against four different strains of bacteria (two gram positive and two gram negative) *Streptococcus pneumoniae* G⁺, *Bacillus cereus* G⁺, *Salmonella paratyphi* G⁻, *Klebsiella pneumoniae* G⁻ by agar cup plate method. The bacteria used were reference standard solution 5µg/ml was prepared by dissolving ciprofloxacin tablet in water for injection (5µg/ml). The medium was sterilized by autoclaving at 121°C (15lb/inch²). (Hugo and Russel, 1987).

About 30ml of molten nutrient agar medium inoculated with the respective strain of bacteria (6ml of inoculum 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 24hrs, 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 (Fig.1). A volume of 100 μ l 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 50 μ l of sterile broth was added. Serial dilutions were performed using a multichannel pipette. Tips were discarded after use such that each well had 50 μ l of the test material in serially descending concentrations. To each well 10 μ l of resazurin indicator solution was added. Using a pipette 30 μ l of sterile broth was added. Finally, 10 μ l 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.

The plates were prepared and placed in an incubator set at 37°C for 18–24h/ 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 (Satyajit *et al.*, 2007).

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.

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 twofold 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 (1×10^6 CFU per ml). The final

concentration of test substance ranged from 1000 to 31.25 μ g per ml. Solvent control and sterility controls were maintained in the experiment. The tubes were incubated at 28°C for 48h. Standard antibiotic, fluconazole was tested as standard drug at concentrations ranging from 100 to 3.12 μ g per 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 (Gibbons *et al.*, 2002).

RESULTS

Antimicrobial study:

The antibacterial study was carried out by agar diffusion technique in particular cup plate method against gram positive and gram negative organisms. Both aqueous and 70% methanolic extract of *Buchanania Angustifolia*, Anacardiaceae show good antibacterial activity against all the specific organisms tested *Streptococcus pneumoniae*, *Bacillus cereus* (gram positive) and *Salmonella paratyphi*, *Klebsiella pneumoniae* (gram negative).

Qualitative phytochemical analysis of aqueous and 70% methanolic extract of *Buchanania Angustifolia*, Anacardiaceae is carried out to ensure that the type of active constituent present in the plant and the result are tabulated in tab-1.

The samples shown Minimum Inhibitory Concentration (MIC) against *Staphylococci aureus* and *Escherichia coli* strains by micro titer method and *Candida albicans* strains by tube dilution method. Various solvents extract of stem of *Buchanania Angustifolia*, Anacardiaceae were examined. The standard drug used for fungi is Fluconazole and for bacteria is Ciprofloxacin. The results are tabulated in tab-2.

DISCUSSION

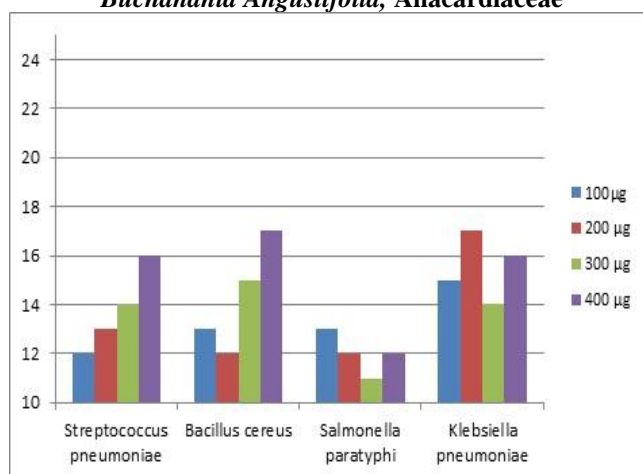
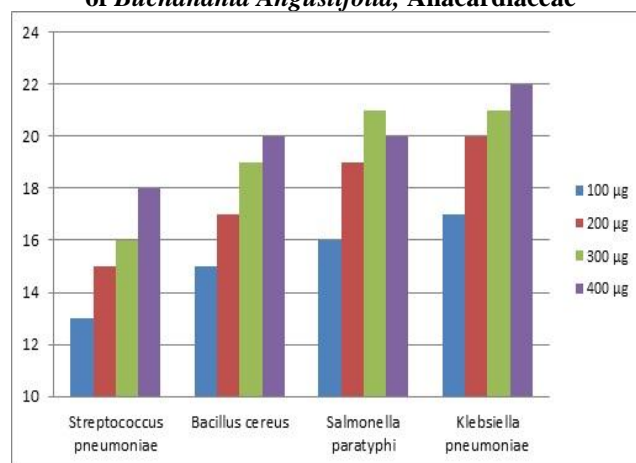
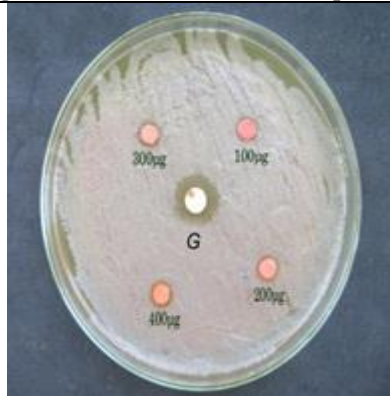
An antimicrobial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, or protozoan's, as well as destroying viruses. Antimicrobial drugs either kill microbes (microbicidal) or prevent the growth of microbes (microbistatic). Disinfectants are antimicrobial substances used on non-living objects (Frobisher *et al.*, 1974). *Buchanania Angustifolia*, Anacardiaceae is a plant. Traditionally it is used to treat viral infections (Yoganarasimhan, 2000). The antimicrobial activity of 70% methanolic and aqueous extract of *Buchanania Angustifolia*, Anacardiaceae was evaluated.

Table 1. Antimicrobial activity profile of 70% methanolic and aqueous extracts from the stem of *Buchanania Angustifolia*, Anacardiaceae

Bacteria	Zone of inhibition Aqueous extract				Zone of inhibition 70% Methanolic extract				With Ciprofloxacin standard 5µg/ml
	100 µg	200 µg	300 µg	400 µg	100 µg	200 µg	300 µg	400 µg	
<i>Streptococcus pneumoniae</i>	12	13	14	16	13	15	16	18	24
<i>Bacillus cereus</i>	13	12	15	17	15	17	19	20	25
<i>Salmonella paratyphi</i>	13	12	11	12	16	19	21	20	23
<i>Klebsiella pneumoniae</i>	15	17	14	16	17	20	21	22	25

Table 2. MIC of the extracts of the stem of *Buchanania Angustifolia*, Anacardiaceae.

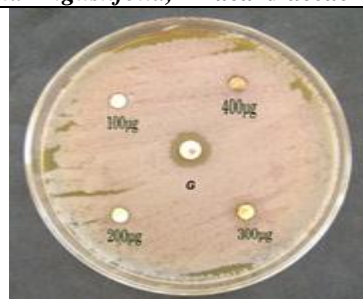
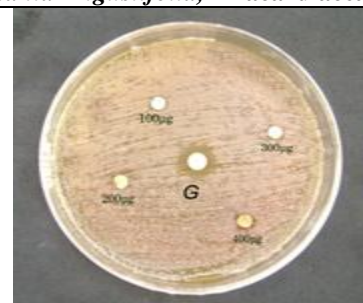
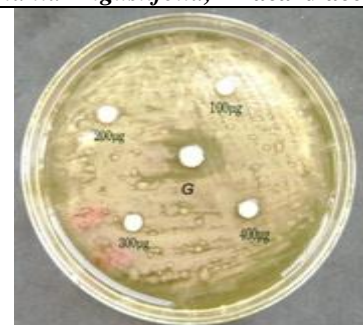
Micro organisms	MIC of Standard Ciprofloxacin/ Fluconazole (mg/ml)	Minimal inhibitory concentration in µgm	
		Aqueous extract	70% Methanol extract
<i>Staphylococci aureus</i>	7.8	500	500
<i>Escherichia coli</i>	7.8	500	500
<i>Candida albicans</i>	12.5	>1000	>1000

Fig. 1. Antimicrobial activity of Aqueous extract of *Buchanania Angustifolia*, Anacardiaceae**Fig. 2. Antimicrobial activity of 70% Methanolic extract of *Buchanania Angustifolia*, Anacardiaceae****Fig. 3. Zone of inhibition of *Streptococcus pneumoniae* of *Buchanania Angustifolia*, Anacardiaceae**

Aqueous extract



70% Methanolic extract

Fig. 4. Zone of inhibition of *Bacillus cereus* of *Buchanania Angustifolia*, Anacardiaceae*Aqueous extract**70% Methanolic extract***Fig. 5. Zone of inhibition of *Salmonella paratyphi* of *Buchanania Angustifolia*, Anacardiaceae***Aqueous extract**70% Methanolic extract***Fig. 6. Zone of inhibition of *Klebsiella pneumoniae* of *Buchanania Angustifolia*, Anacardiaceae***Aqueous extract**70% Methanolic extract*

The antibacterial activities of medicinal plants are attributed due to the presence of Flavonoids, Tannins and Steroidal Alkaloids (Fewell and Roddick, 1993; Burapedjo and Bunchoo, 1995). These reports and presence of Alkaloids, Carbohydrates, Glycosides, Saponins, Tannins, Phenolic, Proteins, Amino acids, Steroids and Triterpenoids compounds in the crude methanolic and aqueous extract of *Buchanania Angustifolia*, Anacardiaceae, confirm its potential against all selected pathogens.

The present work reports the anti microbial activity of the 70% methanolic and aqueous extracts of the *Buchanania Angustifolia*, Anacardiaceae against two gram positive (*Streptococcus pneumoniae*, *Bacillus cereus*) and two gram negative (*Salmonella paratyphi*, *Klebsiella pneumoniae*) by agar cup-plate method. The antimicrobial activity of the 70% methanolic and aqueous extracts compared with standard drug

Ciprofloxacin (5µg/ml). The 70% methanolic extract (zone of inhibition 13 - 22mm) was found to be more effective than the aqueous extract (zone of inhibition 11 - 17mm) against all the organisms. In general antimicrobial activity increases with increasing in concentration of extract as evident by the zone of inhibition.

The samples had shown Minimum Inhibitory Concentration (MIC) against *Staphylococci aureus* and *Escherichia coli* strains by micro titer method and *Candida albicans* strains by tube dilution method. MIC values shows positive response against bacteria whereas negative response against fungi. Therefore, the MIC identified 500µgm for extract of *Buchanania Angustifolia*, Anacardiaceae. MIC values for the standard drug ciprofloxacin is 7.8 mgm/ml.

The increase of antibiotic resistance of microorganisms to conventional drugs has necessitated

the search for new, efficient and cost effective ways for the control of infectious diseases. The results of different studies provide evidence that some medicinal plants might indeed be potential sources of new antimicrobial agents (Kone *et al.*, 2004). The use of medicinal plants is part of the Indian tradition. Many local regions all over India have a great variety of vegetation used by the local population to treat and prevent diseases.

CONCLUSION

It is concluded from the data, that the 70% methanolic and aqueous extract of stem of

Buchanania Angustifolia, Anacardiaceae possess significant antimicrobial activity. From this study we can concluded that, this medicinal plant has a wide range of antimicrobial activity and supports the traditional use of these plants as medicines. This study demonstrated that herbal medicine can be as effective as modern medicine to combat pathogenic microorganisms. Using different purification methods the activity of antimicrobial compounds can be improved for further pharmaceutical uses.

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