Research Article

IN VITRO EVALUATION OF ANTI BACTERIAL ACTIVITY OF LEAF EXTRACT OF *AGATHOSMA BETULINA* ON URINARY TRACT PATHOGENS

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ABSTRACT

The aim of the present study was to evaluate the anti bacterial activity of leaf extract of *Agthosma betulina* on selected Urinary tract pathogens. *Agathosma betulina* commonly known as buchu is one of the oldest known herb famous for its vast variety of therapeutic properties. The leaves of this herb have been used as an herbal remedy for ailments of the gastrointestinal and urinary tracts, as it has diuretic and antiseptic properties due to various phenolic compounds. Antibacterial activity of ethanolic leaf extract of *Agathosma betulina* was screened against *E.coli, Klebsiella pneumonia, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus saprophyticus and Enterococcus faecalis* using disc diffusion technique. The results of this study showed that the extracts at different concentrations exhibited anti bacterial activity against the bacterial species tested compared with standards.

Keywords: *Agathosma betulina,* disc diffusion technique, Mac Farland's standard, zone of inhibition.

INTRODUCTION

Urinary tract infection (UTI) is a condition where one or more parts of the urinary system (the kidneys, ureters, bladder, and urethra) become infected. UTIs are the most common of all bacterial infections and can occur at any time in the life of an individual. Almost 95% of cases of UTIs are caused by bacteria that typically multiply at the opening of the urethra and travel up to the bladder. Much less often, bacteria spread to the kidney from the bloodstream.[1-3] Urinary tract infection is commonly treated with prescription antibiotics however this approach has some drawbacks. Continued allopathic treatment with various antibiotics may cause side effects. It is also known that the bacteria causing infection can develop resistance to the existing antibiotics that have been prescribed, if the medication is used for a long time. These issues have led to a continuous exploration of different modes of treatment and alternate therapies. Herbs have a long history and proven to be very effective in preventing and treating urinary tract infections. Herbal remedies may relieve urinary tract infections by combating the bacteria, decreasing irritation and healing urinary tract tissues.[4-5] Some herbs also help prevent future occurrences. *Agathosma betulina* commonly known as buchu is one of the oldest known herb famous for its vast variety of therapeutic properties.[6] The leaves of *Agathosma betulina* have traditionally been used as an herbal remedy for ailments of the gastrointestinal and urinary tracts, as it has diuretic and antiseptic properties due to various phenolic compounds.

***Agathosma Betulina* [Family: *Rutaceae*]**

Agathosma betulina [Buchu] is found in wet low elevations in the western portion of South Africa. It is a shrub that grows to nearly 2 meters tall, and has a red-brown to violet-brown bark. The leaves are of a pale green colour, leathery and glossy, with a blunt, strongly-curved tip and finely-toothed margin, with round oil glands scattered through the leaf that give them a oily, wet appearance. The leaves have a strongly aromatic taste and a peppermint-like odour. Flowers are white or pale pink, quite small, and have a distinctive star shape.[7-10]

Agathosma betulina contains flavonoids (mainly diosmin), mucilage and resins. The main compounds of interest, however, are the essential oil components. These include limonene, isomenthone, diosphenol (buchu camphor) and terpinen-4-ol. Sulphur-containing compounds, including 8-mercapto-p-methan-3-one, which are responsible for the characteristic black current flavor.[11-13] The leaf of this herb is widely used in western herbal medicine and its primary use is in the treatment of chronic diseases of the genitourinary tract like chronic inflammation of the mucous membranes of the bladder and urethra, conditions where there is urinary discharges and unusually acidic urine, and incontinence linked to prostate disease. Buchu leaf is a diuretic and urinary tract antiseptic, the latter activity is considered to be due to its essential oil content.[14-17] The underside of Buchu leaves have oil glands containing an essential oil which consists mainly of the monoterpene, diosphenol. The oils that give buchu its very pleasing black currant taste are responsible for its ability to kill bacteria in the urinary tract. The oils are absorbed by the stomach and excreted by the kidneys into the bladder. As the oils pass through the bladder and urethra, they kill bacteria as they go. Science has revealed that Buchu is a urinary tract disinfectant of the truest sort.[18-20]

MATERIALS AND METHODS

Plant material

The ethanolic leaf extract of *Agathosma betulina* was obtained from Green Chem Herbal Extract & Formulations, Bangalore.

Test microorganisms

Bacterial strains used were *E.coli [Gram negative bacilli-GNB], Klebsiella pneumonia [GNB], Proteus mirabilis [GNB], Pseudomonas aeruginosa [GNB], Staphylococcus aureus [Gram positive cocci-GPC], Staphylococcus saprophticus [GPC] and Enterococcus faecalis [GPC]*. The organisms were obtained from department of Microbiology, Saveetha Dental College and maintained in nutrient agar slope at 4°C.

Methodology

The extracts were prepared in the following concentrations in sterile water. 2mg/ml, 4mg/ml and 6mg/ml. 50µl of extract of different concentrations were loaded on sterile filter paper discs measuring 6mm in diameter, so that the concentration of the extract on each disc was 100ug, 200µg, 300 µg respectively. The discs were dried and kept aseptically.

Screening of antibacterial activity [disc diffusion technique]

The extracts at different concentrations were tested against the bacterial strains using disc diffusion technique [Kirby Bauer method]. Broth culture of the bacterial strains compared to Mac Farland's standard 0.5 were prepared.[21,22] Lawn culture of the test organisms were made on the Muller Hinton agar [MHA-Hi media M1084] plates using sterile cotton swab and the plates were dried for 15 minutes. Filter paper discs loaded with different concentrations of the extract were placed on the plates. The plates were incubated at 37°C overnight and the zone of inhibition of growth was measured in millimeter diameter. Standard antibiotic discs of Amoxicillin (30mcg/disc) and Ciprofloxacin (30mcg/disc) were used as positive control. All the tests were done in triplicate to minimize the test error.

Determination of minimum inhibitory concentration

Macro broth dilution or tube dilution method was done to determine the Minimum inhibitory concentration (MIC) of the extracts.[23,24] A series of two fold dilution of each extract ranging from 8mg/ml to 0.125mg/ml was made in Muller Hinton broth as specified by National Committee for Clinical Laboratory Standards (NCCLS, 1998). 100µl of standard inoculum of the bacterial strains matched to 0.5 Mc Farland's standards were seeded into each dilution. Two control tubes were maintained for each test batch. These included antibiotic control (tube containing extract and growth media without inoculum) and organism control (tube containing the growth medium and the inoculum). The tubes were incubated at 37°C for 24 hours and checked for turbidity. MIC was determined as the highest dilution (that is, lowest concentration) of the extract that showed no visible growth.

Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration (MBC) of the extracts was carried by pipetting 100ul of broth from the tube that showed no growth in MIC determination and streaked on to MHA plates and incubated for 24 h at 37°C. The least concentration of the extract with no visible growth after incubation was taken as the minimum bactericidal concentration.

RESULTS AND DISCUSSION

The antibacterial activity of the extracts at different concentrations was screened by disc diffusion technique and the zone of inhibition was measured in mm diameter. The results are given in the table 1 and Fig: 1. The minimum inhibitory concentration [MIC] and minimum bactericidal concentration [MBC] were also determined for the extracts and the results are given in table 2 and Fig: 2.

The extracts at different concentration exhibited antibacterial activity against all bacterial strains tested. The ethanolic extract was more effective against *Staphylococcus saprophyticus Proteus mirabilis and E.coli,* with a zone of inhibition of 25mm, 24mm and 23 mm diameter (at conc 300µg) respectively and was less effective against *Pseudomonas aeruginosa* and *Staphylococcus aureus* with zone of inhibition of 14mm each (at conc. 300 µg). Among the other bacterial species studied *Klebsiella pneumoniae* and *Enterococcus faecalis* showed a zone of inhibition of 19mm and 18mm (at conc. 1000 µg.) respectively.

Table 1: Anti bacterial activity of ethanolic leaf extract of *Agathosma betulin*

Extract	Conc [µg]	Zone of inhibition [in mm diameter]						
		B1	B2	B3	B4	B	B6	B7
Ethanolic	100	14 ± 0.4	13 ±0.4	9 ±0.3	7 ±0	9 ±0	12 ±0.3	10 ±0.3
	200	18 ± 0.5	17 ±0.4	13 ±0.3	10 ±0.4	11 ±0.4	20 ±0.4	14 ±0
	300	23± 0.2	24 ±0.3	19 ±0.4	14 ±0.4	14 ±0.4	25 ±0	18 ±0.4
Ciprofloxacin	30mcg/disc	24 ±0	21± 0.2	22±0	22±0	23±0.3	23±0.3	24±0.4
Amoxycillin	30mcg/disc	25±0.2	23± 0.4	20± 0.3	24±0	25± 0	25±0	22±0

The observations are expressed as mean ± Standard deviation, n = 3; B1- *E.coli* , B2- *Proteus mirabilis*, B3- *Klebsiella pneumonia*, B4- *Pseudomonas aeruginosa,* B5 *Staphylococcus aureus,* B6- *Staphylococcus saprophyticus*, B7- *Enterococcus faecalis.*

Table 2: MIC and MBC values of Ethanolic extract of *Agathosma betulina*

Extract		MIC/MBC values [mg/ml]						
		B1	B2	B3	B4	B5	B6	B7
Ethanolic	MIC	2	2	4	4	4	2	4
	MBC	2	2	4	8	8	2	4

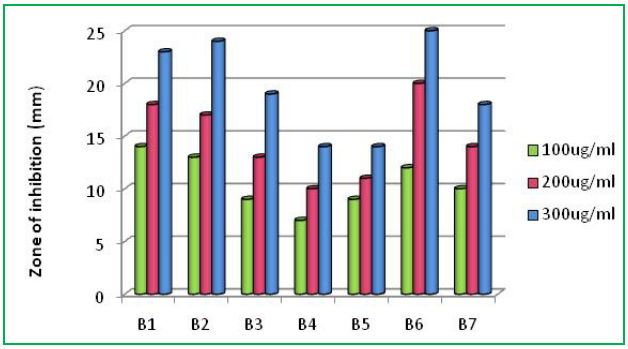

Figure 1: Anti bacterial activity of ethanolic leaf extract of *Agathosma betulina*

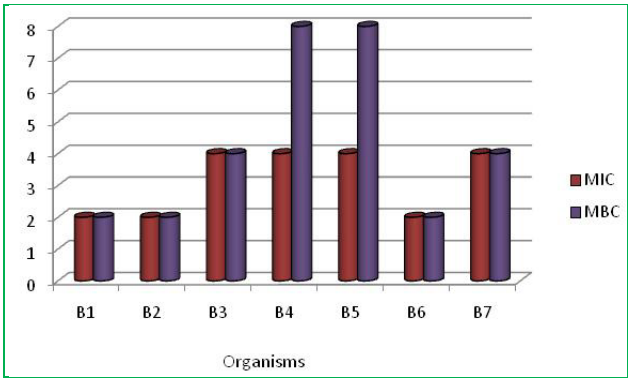

Figure 2: MIC and MBC values of Ethanolic extract of *Agathosma betulina*

The ethanolic extract was found to have Low MIC and MBC values of 2mg/ml & 2mg/ml for the three bacterial species *E.coli, Proteus mirabilis, and Staphylococcus saprophyticus.* With *Klebsiella pneumoniae* and Enterococcus feacalis it was 4mg/ml and 4mg/ml for both. Ethanolic extract showed a MIC and MBC value of 4mg/ml & 8mg/ml for both *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The lower MIC and MBC value is an indication of high effectiveness of the extract whereas higher MIC and MBC indicates the less effectiveness of the extract.

In the modern world, as people are becoming aware of the potency and side effect of synthetic drugs, there is an increasing interest in the natural product remedies. Throughout the history of mankind, many infectious diseases have been treated with herbals. This plant-based, traditional medicine system continues to play an essential role in health care, with about 80% of the world's population relying mainly on traditional medicines for their primary health care. Hence it is necessary to evaluate, in a scientific base, the potential use of folk medicine for the treatment of infectious diseases produced by common pathogens.[25] The present study was to evaluate the antibacterial activity of ethanolic leaf extract of *Agathosma betulina* against urinary tract pathogens. All the extracts showed varying degrees of antimicrobial activity on the microorganisms tested.

CONCLUSION

The present results therefore offer a scientific basis for traditional use of *Agathosma betulina* against urinary tract pathogens. The use of herbs in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases. These herbs should be investigated to better understand their properties, safety and efficacy. It is clear from the results that, the extract acts as a good source of antimicrobial agent against various bacterial pathogens tested and exhibited broad spectrum of antibacterial activity. The anti-bacterial activities could be enhanced if active components are purified and adequate dosage determined for proper administration.

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