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Original Research Article

Antimicrobial Activity of *Plectranthus amboinicus* (Lour.) Against Gram Negative Bacteria *Klebsiella pneumoniae* and *Shigella flexneri* and their Phytochemical Tests

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ABSTRACT

Plectranthus amboinicus have remarkable properties, which has been investigated since ancient times. Scientific evidence stated that *P. amboinicus* is capable of cure from cough, chronic asthma, hiccup, bronchitis, helminthiasis, colic, convulsions, and epilepsy. This study was carried out with an objective to investigate the antibacterial potentials of leaves of *P. amboinicus*. The aim of the study is to assess the antimicrobial activity and to determine the zone of inhibition of extracts on bacterial strains. In the present study, the microbial activity of hydroalcohol extracts of leaves of *P. amboinicus* was evaluated for potential antimicrobial activity against medically important bacterial and fungal strains. The antimicrobial activity was determined in the extracts using agar well diffusion method. The antibacterial activities of extracts of *P. amboinicus* were tested against two Gram-negative bacteria- *Klebsiella* pneumoniae and *Shigella flexneri*. The findings revealed that *P. amboinicus* showed inhibition zone on the growth of *K. pneumoniae* and *S. flexneri*. It is therefore confirmed as a useful antimicrobial agent. The phytochemical analyses of the plants were carried out. The leaves of *P. amboinicus* rich in phytochemicals and secondary metabolites such as steroids, tannins, flavonoids, alkaloids etc. which are probably responsible for its medicinal properties. The microbial activity of the *P. amboinicus* was due to the presence of various secondary metabolites. It therefore suggests that constituent of plant extract could serve as a source of drugs useful in the chemotherapy of enteric diseases**.** Hence, these plants can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals research activities**.**

Key words: *Plectranthus amboinicus*, antibacterial activity, agar well diffusion, phytochemical, secondary metabolites.

INTRODUCTION

Plectranthus amboinicus is a tender fleshy perennial plant in the family *Lamiaceae* with an oregano-like flavor and odor, native to Southern and Eastern Africa, from South Africa and Swaziland to Angola and Mozambique and north to Kenya and Tanzania. [1] It is widely cultivated and naturalized elsewhere in the Old and New World tropics. It is used as a decorative plant in many houses in south India. The leaves have many traditional medicinal uses, especially for the treatment of cough and cold, sore throats and nasal congestion; but also for a range of other problems such as infections, rheumatism and flatulence. In Indonesia *P. amboinicus* is a traditional food used in soup to stimulate lactation for

the month or so following childbirth. This medicinal plant is used for abrasion, laceration, for burns, for conjunctivitis, etc. The leaves are strongly flavored and make an excellent addition to stuffing for meat and poultry. Finely chopped, they can also be used to flavour meat dishes, especially beef, lamb and game. The herb is also used as a substitute for oregano in the food trade and food labeled "oregano-flavoured" may well contain this herb.

An antimicrobial is an agent that kills microorganisms or inhibits their growth.] Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. For example, antibacterials are used against bacteria and antifungals are used against fungi. They can also be classified according to their function. [2] Agents that kill microbes are called microbicidal, while those that merely inhibit their growth are called microbiostatic. The use of antimicrobial medicines to treat infection is known as antimicrobial chemotherapy, while the use of antimicrobial medicines to prevent infection is known as antimicrobial prophylaxis.

The main classes of antimicrobial agents are disinfectants ("nonselective antimicrobials" such as bleach), which kill a wide range of microbes on non-living surfaces to prevent the spread of illness, antiseptics (which are applied to living tissue and help reduce infection during surgery), and antibiotics (which destroy microorganisms within the body). The term "antibiotic" originally described only those formulations derived from living organisms but is now also applied to synthetic antimicrobials, such as the sulphonamides, or fluoroquinolones. The term also used to be restricted to antibacterials (and is often used as a synonym for them by medical professionals and in medical literature), but its context has broadened to include all antimicrobials. [3] Antibacterial agents can be further subdivided into bactericidal agents, who kill bacteria, and bacteriostatic agents, which slow down or stall bacterial growth.

The study was conducted to investigate the antibacterial effect of *P. amboinicus* towards the growth of selected gram negative bacteria *Klebsiella pneumoniae* and *Shigella flexneri.*

MATERIALS AND METHODS

Collection of plant material

The leaves of *P. amboinicus* were collected from zoology department of St.Albert's College, Ernakulam. The materials were immediately brought to lab, where the experiments were conducted.

Collection of microorganisms

Bacterial strains were used for the antibacterial activity of *P. amboinicus* were obtained from stock cultures maintained in the pathology laboratory of Dept. of Zoology, St. Albert's College, Ernakulam.. All the strains were maintained on nutrient agar at 4°C and were subcultured every month.

Extraction of plant material using soxhlet extractor

A Soxhlet extractor is a piece of laboratory apparatus invented in 1879 by Franz von Soxhlet. It was originally designed for the extraction of a lipid from a solid material. Typically, a Soxhlet extraction is used when the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. It allows for unmonitored and unmanaged operation while efficiently recycling a small amount of solvent to dissolve a larger amount of material.

The source material containing the compound to be extracted is placed inside the thimble (5g). The thimble is loaded into the main chamber of the Soxhlet extractor. The extraction solvent to be used is placed in a distillation flask (100ml). The flask is placed on the heating element. The Soxhlet extractor is placed atop the flask. A reflux condenser is placed atop the extractor.

Phytochemical test of *P. amboinicus*

Test for carbohydrate

Qualitative determination of carbohydrates was performed by Benedicts test: to 0.5ml of the filtrate. 0.5ml Benedict's reagent was added. The mixture was heated on boiling water bath for 2 minutes. A characteristic red coloured precipitate indicates the presence of sugar.

Test for tannins

For tannins about 0.5mg of dried powdered samples was boiled in 20 ml of water in test tubes then filtered. Few drops of 0.1% ferric chloride was added and observed for brownish green or blue black colouration.

Test for terpenoids

Liebermann-Burchard test: Crude extract was mixed with few drops of acetic anhydride, boiled and cooled, conc. H2SO4 was then added from the sides of the test tube. A brown ring at the junction of two layers was formed. The upper layer turned green which showed the presence of steroids and formation of deep red colour indicated the presence of triterpenoids.

Salkowski test: 5ml of the extract was mixed with 2ml of chloroform and concentrated sulphuric acid to form a layer. A reddish brown colouration of the interface showed the presence of terpenoids.

Test for Saponins

Froth test, Crude extract was mixed in 1 ml water in a semi-micro tube, shake well and noted the stable froth. Stable froth indicated the presence of saponins.

Test for flavonoids

Alkaline reagent test: Crude extract was mixed with few drops of sodium hydroxide solution. An intense yellow colour was formed. Yellow colour turned to colorless on addition of few drops of diluted acid, marked the presence of flavonoids.

To 5ml of the dilute ammonia solution a portion of the aqueous extract was added, followed by addition of concentrated sulphuric acid. Appearance of yellow colouration indicates the presence of flavonoids. 152 Four milliliters of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red color was observed for flavonoids and orange color for flavones.

Test for alkaloids

Mayer's test: The alcoholic extract was evaporated to dryness and the residue was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Mayer's reagent. The samples were then observed for the presence of turbidity or yellow precipitation

Dragendorff's test: Crude extract was mixed with Dragendorff's reagent (potassium bismuth iodide solution). Reddish brown precipitate was formed which suggested the presence of alkaloids.

Screening of extracts for antimicrobial activity

Antibacterial activity was assessed by Agar well diffusion method of Kirby Bauer. Wells of 7mm diameter were bored using sterile borer. Wells were loaded with plant extract (antimicrobial), and were incubated at 37°C for 24 hours .The antimicrobial present in the plant extract are allowed to diffuse out into the medium and interact in a plate freshly seeded with test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimetres.

Preparation of culture

Bacterial cultures of *Klebsiella pneumoniae* and *Shigella flexneri* were obtained from the pathology laboratory of St. Albert's College, Ernakulam. These cultures were sub cultured into nutrient broth according to the standard protocols for sub culturing (Gilbert, 1987) and allowed to grow in an incubator at 37ºC for 24 hours and used for further experiments.

Preparation of growth curve

Nutrient broth was inoculated with extract and test organisms. One tube was kept as control (without extract) and one

tube as blank (nutrient broth and extract only). Inoculated samples were kept in a shaker at 120 rpm at room temperature. Starting at time 0, the O.D. was recorded at every 24 hours for a total of 4 time points. Growth curve was plotted using a standard linear graph.

Calculation of growth rate

For calculation of growth rate, O.D. was recorded at 0 hour of incubation and at 24 hour of incubation. The specific growth rate of the bacterial culture was calculated using the following equation:

$$\mu = (m2 - m1) / (t2 - t1)$$

Where μ is specific growth curve, m1 and m2 is the biomass produced at time t1 and t2 of the bacterial culture.

RESULT

Phytochemical test of *P. amboinicus*

Carbohydrates, terpenoids and flavonoids present in both ethanol and water extract of *P. amboinicus* (Table 1)**.** But tannins and alkaloids absent in ethanol extract and present in water extract. Saponins absent in both ethanol and water extract.

Table 1: Phytochemical analysis of extracts *Plectranthus amboinicus* young Leaves.

SL. NO.	COMPOUNDS	EXTRACT IN ETHANOL	EXTRACT IN WATER
1	Carbohydrate	+	+
2	Tannins	-	+
3	Terpenoids	+	+
4	Saponins	-	-
5	Flavonoids	+	+
6	Alkaloids	-	+

+ → Present
- → Absent

Agar well diffusion assay

Klebsiella pneumonia shows 18 mm zone of inhibition in alcohol control and 20 mm in zone of inhibition in water control. They show 16 mm zone of inhibition in alcohol extract of *P. amboinicus* and 31 mm zone of inhibition in water extract of *P. amboinicus. Klebsiella pneumonia* is more sensitive to water extract of *P. amboinicus and* less sensitive to alcohol extract of *P. amboinicus.*

Shigella flexneri shows 14 mm zone of inhibition in alcohol control and 11 mm in zone of inhibition in water control. They show 18 mm zone of inhibition in alcohol extract of *P. amboinicus* and 9 mm zone of inhibition in water extract of *P. amboinicus. Klebsiella pneumonia* is more sensitive to alcohol extract of *P. amboinicus and* less sensitive to water extract of *P. amboinicus.*

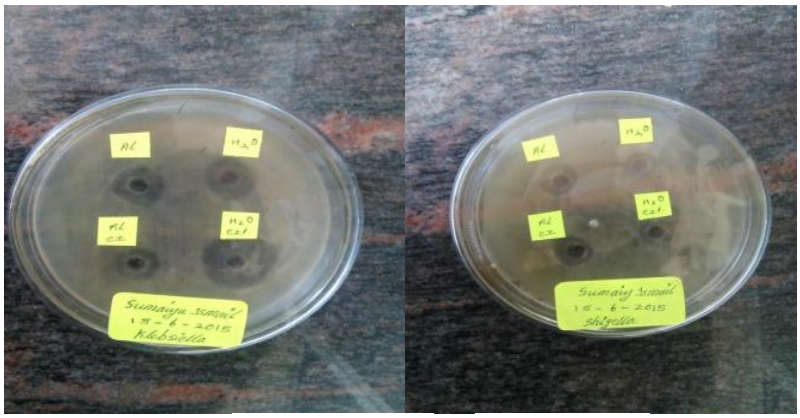

Figure 1: Zone of inhibition of *Klebsiella pneumoniae* and *Shigella flexneri* in *P. amboinicus* extract.

Table 2: Zone of inhibition of *Klebsiella pneumoniae* and *Shigella flexneri* in *P. amboinicus* extract

Sample	Zone of inhibition (in mm)	
	Klebsiella pneumoniae	*Shigella flexneri*
Alcohol	18	14
Water	20	11
Alcohol extract	16	18
Aqueous extract	31	9

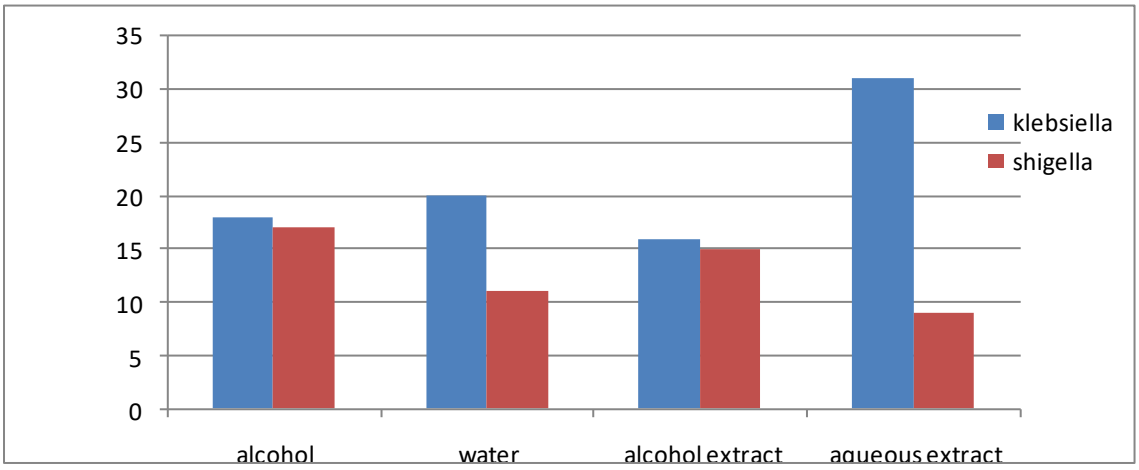

Figure 2: Graphical representation of Zone of inhibition of *Klebsiella pneumoniae* and *Shigella flexneri* in *P. amboinicus* extract

Growth curve of *Klebsiella pneumonia*

Klebsiella pneumonia shows maximum growth in alcohol extract of *P. amboinicus* and minimum growth in aqueous extract of *P. amboinicus.*

Table 3: O.D. value of *Klebsiella pneumonia* culture in extract of *P. amboinicus.*

TIME IN HOURS	CONTROL	ALCOHOL EXTRACT	AQUEOUS EXTRACT
24	0.72	0.92	0.62
48	0.63	0.73	0.44
72	0.41	0.49	0.32
96	0.13	0.32	0.20

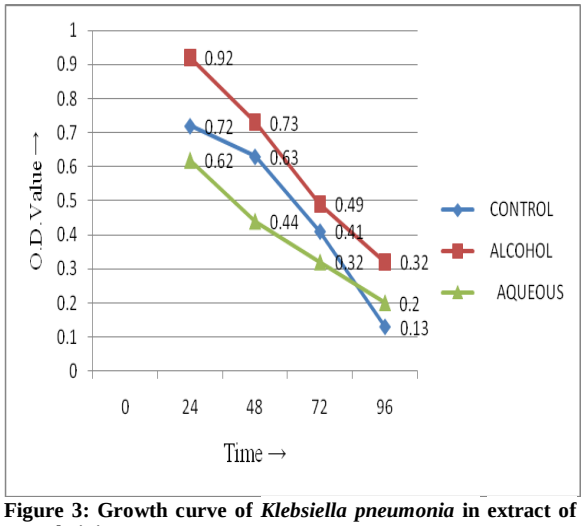

Figure 3: Growth curve of *Klebsiella pneumonia* in extract of *P. amboinicus*.

Growth curve of *Shigella flexneri*

Shigella flexneri shows maximum growth in aqueous extract of *P. amboinicus* and minimum growth in alcohol extract of *P. amboinicus.*

Table 4: O.D. value of *Shigella flexneri* culture in extract of *P. amboinicus.*

TIME IN HOURES	CONTROL	ALCOHOL	AQUEOUS
24	0.52	0.42	0.72
48	0.36	0.32	0.53
72	0.30	0.18	0.22
96	0.18	0.08	0.23

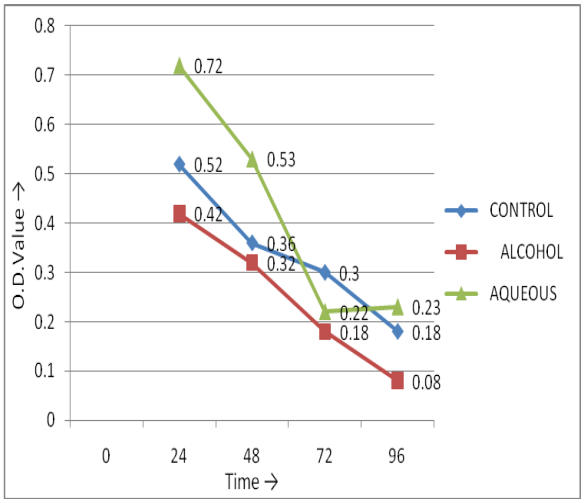

Figure 4: Growth curve of *Shigella flexneri* in extract of *P. amboinicus*.

DISCUSSION

Although the genus *Plectranthus* comprises many plants of medicinal interest and its chemistry is poorly known. [4] Such observed antibacterial activity occurs due to the presence of several phytocompounds with known antimicrobial properties. Several studies have reported that caracole and essentials oils, present in *P. amboinicus*, modify the constitution and increase the fluidity of the cell membrane, typifying a dose-dependent effect, as fully demonstrated in this study. [5] Other phytocompounds found in the HE of *P. amboinicus* also possess antibacterial activity. Some studies describe damage to the membrane and lyses cells caused by cinnamic derivatives and terpens. [6] The bactericidal action of the extract seems promising, once the speed of cell kill, in the first hours of exposition of the HE of *P. amboinicus*, is similar to fluoroquinolone, pefloxacin, ofloxacin and norfloxacin (wide spectrum antimicrobials very used in Human and Veterinary medicine). These results are consistent with others in vitro experimental findings. [7] Antimicrobial effects of leaves from *P. amboinicus* in the form of essential oil and crude extracts, respectively. Moreover, some studies demonstrated absence of toxicity in vitro and in vivo, [8] which makes the use of this species very safe in the treatment of multi-resistant infections. The results indicated a promising antimicrobial action of HE of leaves of *P. amboinicus* in methicillin-resistant strains. The time-kill curve have shown that the extract possesses bactericidal or bacteriostatic action in the first hours of treatment, varying according to the concentration. [9] Such results are very important once MRSA strains possess a great resistance spectrum. The preliminary results of this study have corroborated with the use of *P. amboinicus* in folk medicine for the treatment of infections caused by *S. aureus* pathogen. However, future studies should investigate whether the use of this medicinal specie interferes with drugs in the antimicrobial therapy.

The result of the whole plant extracts of *P. Amboinicus* showed that ethanol extracts contain a greater proportion by mass of the component compounds. [10] The medicinal properties of the plant could be attributed to the presence of one or more of the detected plant natural products. Ethyl acetate extract of *Plectranthus glandulous* contain flavonoids such a quercetin which has antioxidant properties. [11] These findings give credence to the traditional medicinal application of the plant as remedies for measles, internal and external wounds and infections. Ethanol extracts were positive for saponin and alkaloids; class of compounds that are known to be effective for the treatment of syphilis and other venereal disease. [12] Hexane and ethyl acetate extracts were positive for steroids. It should be noted that steroidal compounds are of importance and interest in pharmacy due to sex hormones. The practice of using plants as source of medicine dates back to about 5000 B.C. Early men gained knowledge about medicinal plants through trial and error only.

The Chinese were the first to use plants as source of medicine. The present investigation reports that Antibacterial activity was carried out using clinical isolates of pathogenic microorganisms such as *E.coli*, *S. aureus*, *P. vulgaris* and *K. pneumonia*. All organisms were highly resistant to methanol extract when compared with acetone and chloroform extract. [13] The different solvent extract of the leaves of *P. amboinicus* were evaluated for their anti oxidant and antibacterial properties. The leaf extract exhibited good antioxidant potency and showed significant reducing power, *P. amboinicus* leaves owing to lower extraction of total phenotics and total flavonoids in its ethanolic extract. Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the in vitro antibacterial activity assay. As resistance towards prevailing antibiotics has become wide spread among bacteria and fungi, new class

of antimicrobial substances are required immediately. From the experimental data the dry leaf methanol extract showed the formation of white precipitate followed by a colour change of yellow by further heating indicates the presence of protein in the entire conformation test. While none of other solvent extract showed the presence of protein. [14] Similar result was obtained for methanolic extract of leaves against *Erythrina senegalensis* was found to have protein the active principle responsible for antibacterial activities was a protein used in the development of drugs for therapeutic use in human. In carbohydrate test the formation of rapid colour change from blue to green and then to orange indicates the presence of carbohydrates.[15] Glucosides are carbohydrates occur in plants of *Brassicaceae*, *Rutaceae*, *Rosaceae* families. Malaria can be treated by this glucoside.

Rutin Glucosides are obtained from Ruta graveolens. Similarly the presence of carbohydrates, proteins, amino acids, glycosides, terpenoids, flavonoids, phenols, alkaloids and tannins in the methanolic extract of *P. amboinicus* have also been reported earlier. The phytochemical constituents are separated using TLC technique Chloroform benzene in the ratio 1:1 was used as mobile solvent and slurry prepared with silica and water in the ratio 1:2 was identified as suitable stationary and mobile phase for TLC separation of methanol dry leaf extract then the plates were kept under iodine vapour for staining the stained band was visualized after 24 hours incubation. Methanolic extract of the leaves of this herb has been found to contain chlorogenic acid, caffeic acid, coumaric acid polyphenolic compounds with strong antioxidant property the compounds with therapeutic antimicrobial activity can be purified in future development of drugs for therapeutic use in human. Higher plants as sources of medicinal compounds have continued to play a dominant role in the maintenance of human health since ancient times. [16]

CONCLUSION

Extract of *Plectranthus amboinicus* possesses antimicrobial potentials against *Klebsiella pneumoniae* and *Shigella flexneri*. It is therefore confirmed as a useful antimicrobial agent. The leaves of *P. amboinicus* rich in phytochemicals and secondary metabolites such as steroids, tannins, flavonoids, alkaloids etc. which are probably responsible for its medicinal properties. It therefore suggests that constituent of plant extract could serve as a source of drugs useful in the chemotherapy of enteric diseases.

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