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Research Article

Evaluation of antimicrobial activity of crude extracts of *Lepidagathis keralensis* against pathogenic organisms

Leena P*, Zeinul Hukuman N.H, Jisha M

Department of Chemistry, Sir Syed College, Taliparamba, Kannur, Kerala 670142, India

ABSTRACT

Lepidagathis keralensis (Acanthaceae) is a plant endemic to Kerala. The leaves and stem of the plant were extracted continuously using acetone, methanol and water. Antimicrobial efficiency of the different extracts was tested using agar well diffusion method against selected human pathogens (*Pseudomonas aeroginosa*, *Klebsiella pneumonia*, *Streptococcus mutans*, *Staphylococcus aureus* and *Candida albicans*). Total alkaloids and saponins of the plant were also quantitatively determined. The total alkaloid content was found to be higher in the leaves while the stem showed higher amount of saponins. Acetone extract of stem was found effective against *Klebsiella pneumonia* while the methanol extract of leaf inhibited effectively the growth of *Pseudomonas aeroginosa* and *Streptococcus mutans. Staphylococcus aureus* was inhibited efficiently by the aqueous extract of stem. The methanol extracts of the leaf and stem showed a maximum zone of inhibition against the fungal strain *Candida albicans*. The results highlight the importance of the plant as potent natural antimicrobials.

Keywords: Alkaloids; Antibacterial; Antifungal; *Lepidagathis keralensis*; Saponins.

INTRODUCTION

Antimicrobials are important class of compounds which has benefited the health of human beings by fighting against microbial infections. But the continuous use of certain antibiotics has resulted in the emergence of drug resistant bacteria which has reduced the effectiveness of certain antibiotics. Thus it is essential to investigate newer drugs with lesser resistance. (Gowri & Vasantha, 2010). There is an increasing attention in the world towards using plants for the treatments of several diseases. A major percentage of the population in the world mainly depends on traditional medicines for health related needs. (Kapoor et al. 2015). Plants are rich source of bioactive secondary metabolites which are produced to protect the plants against insect predators and diseases causing organisms. These secondary metabolites have different structural arrangement and hence different properties. Plant based antimicrobials effectively treats infectious diseases and reduces many of the side effects associated with synthetic antimicrobial drugs(Nithya et al., 2015; Ananthi & Ranjitha Kumari, 2013).

Saponins and Alkaloids are important class of secondary metabolites in plants found to possess various pharmacological properties. Saponins are non-volatile high molecular weight compounds consisting of sugar units linked to either a triterpene or a steroid aglycone. They possess haemolytic, antifungal and antibacterial properties, lower blood cholesterol and inhibit cancer cell growth. They are also used for hormone synthesis in pharmaceutical industry (Shi et al., 2009; Singh & Mendhulkar, 2015). Alkaloids are low-molecular-weight, nitrogen containing compounds having potent biological activities. They are found to possess antibacterial, anticancer, antihypertensive, muscle relaxant, analgesic properties etc (John et al. 2014; Fester 2010).

Lepidagathis keralensis (Family: Acanthaceae), an endemic plant of Kerala is found in lateritic hills near seacoast. It is a rigid prostrate under shrub with woody rootstock commonly occurring in exposed lateritic rocks (Madhusoodanan & Singh, 1992). The plant possesses several medicinal properties. The spines of the plant tied in a cloth are cooked with rice and resulting gruel is given to children as a preventive medicine for digestive disorders. Whole plant decoction is recommended for kidney stone. It is a blood purifier and increases blood (Prasad, 2012). The antimicrobial properties of the petroleum ether extract of the leaf and stem of the plant has been reported earlier (Leena et al. 2016).

The present study was done for the preliminary investigation of the various phytochemicals present in the plant. The total alkaloids and saponins of the plant were also quantitatively estimated. The study also evaluates the antimicrobial potency of the acetone, methanol and aqueous extracts of the plant against the

* Corresponding Author
Email: leenasajithp@gmail.com
Contact: +91-9497606146
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fungal strain, *Candida albicans* and two gram positive (*Streptococcus mutans* & Staphylococcus *aureus*) and two gram negative (*Pseudomonas aeroginosa* & *Klebsiella pneumonia*) pathogenic strains of bacteria.

MATERIALS AND METHODS

Collection of plant material

The plant *Lepidagathis keralensis* was collected from Madayipara, Kannur, Kerala and authenticated from the Department of Botany, Govt. Brennen College, Dharmadam, Kerala. The leaves and stem of the plant were separated, washed well with water and shade dried for two weeks. The dried plant parts were powdered well and kept in an air tight container for further use.

Preparation of the extract

The powdered plant parts were defatted using petroleum ether and extracted with acetone, methanol and water sequentially in a soxhlet apparatus. The extracts obtained were dried well and kept under refrigeration for future use.

Preliminary phytochemical analysis

Preliminary phytochemical screening for the presence of various secondary metabolites in the extracts was conducted by standard methods (Tiwari et al. 2011; Prakash et al. 2013; Evans, 1996).

Determination of total alkaloids

5 g of the powdered leaf and stem of the plant was weighed in an analytical balance and taken in a 250 ml beaker. To this, 200 ml of 10% acetic acid in ethanol was added. The beaker was covered and the solution was allowed to stand for 4 h. It was then filtered and the extract obtained was concentrated to one-quarter of the initial volume by evaporating on a water bath. To the extract so obtained, Conc. Ammonium hydroxide was added drop by drop till the precipitation was complete. The precipitate formed was collected after washing with dilute ammonium hydroxide solution. This precipitate which is the alkaloid was dried well and the yield noted (Gracelin et al. 2013).

Determination of total saponins

20 g Powdered leaf and stem of the plant was weighed in an analytical balance and put into a conical flask. To this, 100ml of 20% aqueous ethanol was added. The solution was then heated for 4 h with continuous stirring in a hot water bath maintained at about 55°C. The hot solution was then cooled and filtered. The filtrate was collected and the residue obtained was re-extracted again with 200 ml of 20% ethanol. The filtrates were combined and the volume was reduced to 40 ml by evaporating on a hot water bath maintained at 90^0C. The concentrated extract was then taken in a 250 ml separatory funnel. 20 ml of diethyl ether was added to this and shaken well. The aqueous layer was collected while the ether layer was discarded. This aqueous layer was re-extracted several times with 60 ml of n-butanol. The n-butanol extracts were then combined together and washed twice with 10 ml of 5% aqueous sodium chloride. The solution was then allowed to evaporate in a water bath. The residue obtained (saponin content) was then dried in an oven to a constant weight (Obdoni & Ochuko, 2001).

Evaluation of Antimicrobial activity

Test Organisms

Two gram negative bacterial strains (*Pseudomonas aeroginosa* ATCC 27853 & *Klebsiella pneumonia* ATCC 13883), two gram positive bacterial strains (*Streptococcus mutans* MTCC 890 & *Staphylococcus aureus* ATCC 25923) and one fungal strain (*Candida albicans* ATCC 10231) were used for the investigation of antimicrobial activity of the extracts.

Antibacterial activity: Agar well diffusion method

Antibacterial activities of the extracts were determined using agar well diffusion method(NCCL, 1993).1L Muller Hinton Agar Medium (prepared by dissolving 33.8 g of the commercially available Muller Hinton Agar Medium in 1L distilled water) was autoclaved for 15 minutes at 15 lbs pressure and 121°C. 20ml of the prepared medium was added to petriplates while hot and allowed to settle. 1L of nutrient broth (prepared by dissolving 13 g of commercially available nutrient broth in 1L of distilled water) was dispensed as required and then autoclaved for 15 minutes at 15 lbs pressure and 121°C. The plates were then seeded with bacterial culture .The growth of the culture was adjusted according to McFards Standard, 0.5%. Using a well cutter the plates were bored to make wells of approximately 10mm dimension. To this well, different concentrations of the sample (25µg, 50µg and 100µg) were added from a stock solution of concentration 1g/ml. The petriplates were then incubated at a temperature of 37°C for 24 hours. The assessment of antibacterial activity was done by determining the diameter of the zone of inhibition formed around the well. Streptomycin (20µg) was used as a positive control.

Antifungal assay: Agar well diffusion method

Agar well diffusion method was used to evaluate the antifungal activity of the extracts. The selected fungus *Candida albicans* was swabbed on previously prepared Potato Dextrose agar plates. Using a well cutter the plates were bored to make wells of approximately 10mm dimension and samples of different concentrations (25µg, 50µg and 100µg) were added to the plates. The plates were incubated overnight and the zone of inhibition was determined. The antifungal activity of the extracts were compared with standard Clotrimazole (20µg).

Statistical Analysis

Triplicate measurements were taken for all the analyses and the results were expressed as mean (n=3) ±

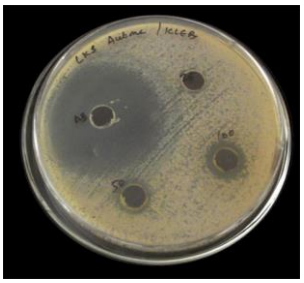

Figure 1: Stem acetone extract Streptococcus mutans

Figure 2: Stem methanol extract Klebsiella pneumonia

Figure 3: Stem methanol extract Candida albicans

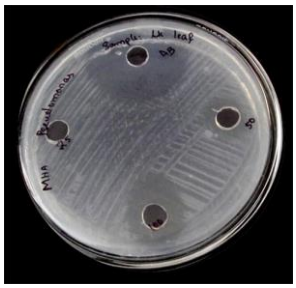

Figure 4: Leaf methanol extract Pseudomonas aeroginosa

Table 1: Preliminary Phytochemical analysis of the leaf and stem of Lepidagathis keralensis

Phytochemicals	Leaf extract			Stem extract		
	Acetone	Methanol	Aqueous	Acetone	Methanol	Aqueous
Alkaloids	+	+	+	+	+	+
Phenols	+	+	+	+	+	-
Flavonoids	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Glycosides	-	+	-	-	+	-
Terpenes	+	+	+	+	+	+
Phytosterols	+	-	-	+	-	-
Saponins	+	+	+	+	+	+
Carbohydrates	+	+	+	+	+	+
Amino acids	-	+	-	-	+	-
Proteins	-	+	+	-	+	+

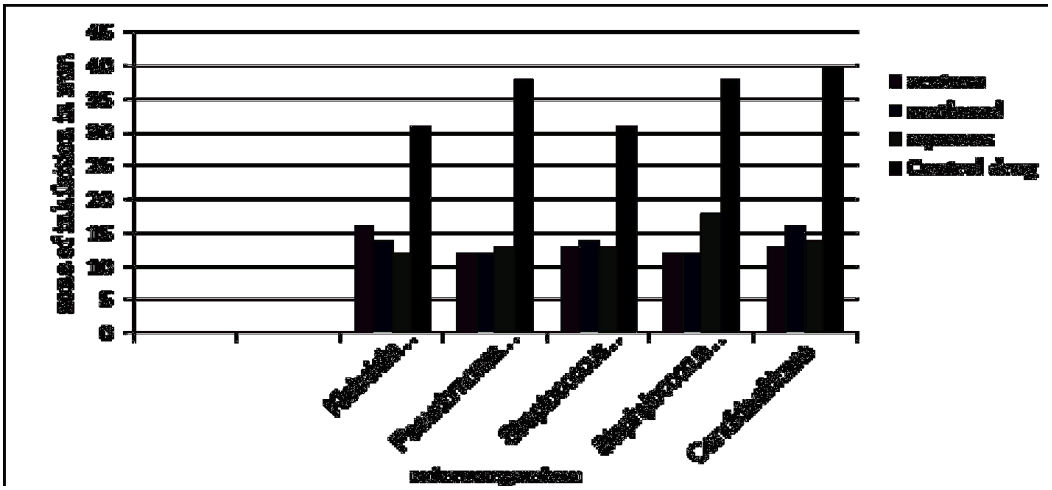

Figure 5: Comparative antimicrobial activity of the stem extracts

Table 2: Quantitative analysis of saponins and alkaloids (mg/g) in the powdered sample of leaf and stem of Lepidagathis keralensis

Phytochemicals	Stem	Leaf
Total alkaloids	48.16±0.86	56.93±0.69
Total saponins	17.13±0.47	14.6±0.35

Table 3: Antimicrobial activity of the acetone extracts of Lepidagathis keralensis

Organism	Diameter of zone of inhibition (mm)							
	Leaf extract				Stem extract			
	Concentration in µg/ml			Control drug in µg/ml	Concentration in µg/ml			Control drug in µg/ml
	25	20	100	20	25	50	100	20
Klebsiella pneumonia	Nil	Nil	10	37	11	13	16	39
Pseudomonas aeroginosa	Nil	12	17	40	Nil	Nil	12	38
Streptococcus mutans	Nil	Nil	13	40	10	11	13	35
Staphylococcus aureus	Nil	10	11	40	Nil	10	12	39
Candida albicans	Nil	10	12	39	10	11	13	38

Table 4: Antimicrobial activity of the methanol extracts of Lepidagathis keralensis

Organism	Diameter of zone of inhibition (mm)							
	Leaf extract				Stem extract			
	Concentration in µg/ml			Control drug in µg/ml	Concentration in µg/ml			Control drug in µg/ml
	25	20	100	20	25	50	100	20
Klebsiella pneumonia	Nil	12	15	31	Nil	11	14	31
Pseudomonas aeroginosa	10	13	20	38	Nil	10	12	38
Streptococcus mutans	Nil	12	15	31	Nil	11	14	31
Staphylococcus aureus	10	11	13	38	Nil	10	12	38
Candida albicans	10	11	15	40	10	12	16	40

Table 5: Antimicrobial activity of the aqueous extracts of Lepidagathis keralensis

Organism	Diameter of zone of inhibition (mm)							
	Leaf extract				Stem extract			
	Concentration in µg/ml			Control drug in µg/ml	Concentration in µg/ml			Control drug in µg/ml
	25	50	100	20	25	50	100	20
Klebsiella pneumonia	10	10	12	35	Nil	10	12	38
Pseudomonas aeroginosa	Nil	Nil	13	38	Nil	10	13	38
Streptococcus mutans	Nil	Nil	11	40	10	10	13	37
Staphylococcus aureus	Nil	Nil	12	39	10	11	18	39
Candida albicans	10	11	13	39	Nil	10	14	39

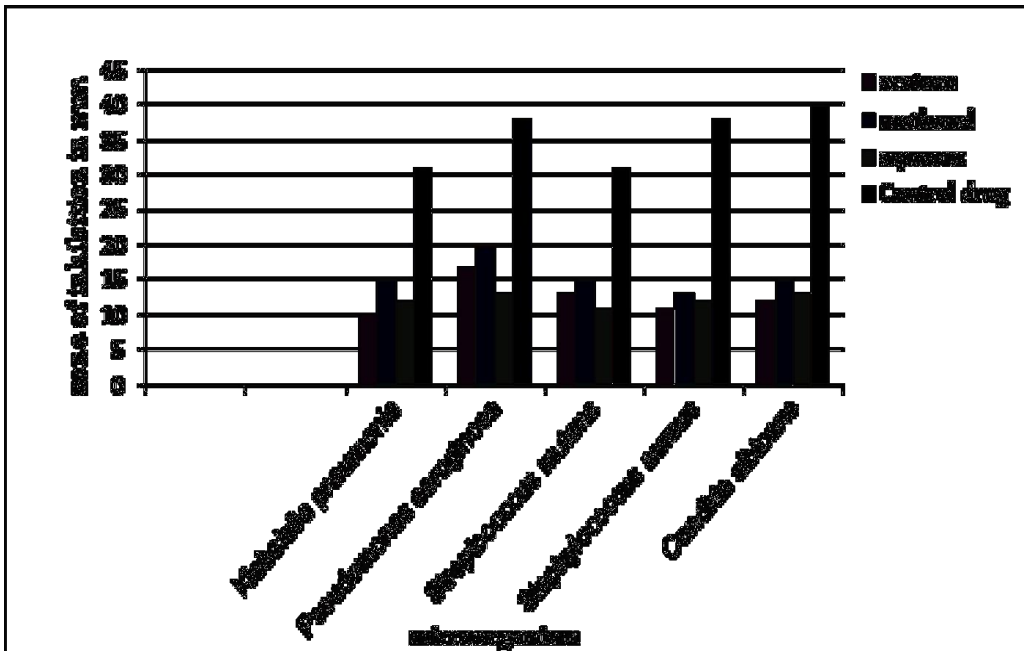

Figure 6: Comparative antimicrobial activity of the leaf extracts

standard deviation (SD).

RESULTS AND DISCUSSIONS

The results of preliminary phytochemical investigation of the different extracts of leaf and stem of *Lepidagathis keralensis* are tabulated in Table 1. The extracts were analyzed for the presence of 11 phytochemicals such as alkaloids, phenols, flavonoids, tannins, saponins, terpenes, phytosterols, glycosides, carbohydrates, amino acids and proteins. The methanol extracts of both the leaf and stem showed positive result for all the phytochemicals tested except for phytosterols. The acetone and aqueous extracts also showed the presence of major phytochemicals responsible for various biological activities in plants.

The dried and powdered leaf and stem of the plant were analyzed for the quantitative determination of total alkaloids and saponins. Both the plant parts showed appreciable amounts of alkaloids and saponins as shown in Table 2 .Alkaloids were found to be more in the leaf than in stem while saponins were found to be more in the stem. The presence of the estimated phytochemicals in large quantities suggests the medicinal importance of the plant as both alkaloids and saponins possess wide biological activities (Chaturvedi et al. 2012; Cheok et al. 2014; Saxena et al., 2013).

The different extracts tested for antimicrobial activity showed good results against the selected pathogens. The zone of inhibition obtained for acetone, methanol and aqueous extracts of the plant are shown in tables (Tables 3-5) and Fig. 1-4 depicts the maximum zone of inhibition against certain microbes. Comparative study of antimicrobial efficiency of the stem and leaf against selected microbes are shown in Fig. 5 and 6 respectively.

Klebsiella pneumonia, a gram negative bacterium is known to cause respiratory tract infections, urinary tract infections, wound and soft tissue infections etc. Infections due to *klebsiella* species are leading cause of mortality (Brisse et al., 2006; Sikarwar & Batra, 2011). Growth of *Klebsiella pneumonia* is effectively inhibited by the acetone extract of the stem while the methanol extract of leaf is found effective against this pathogen with zones of inhibition of 16mm and 15mm respectively. The methanol and acetone extract of leaf shows higher efficacy against *Pseudomonas aeroginosa* with the methanol extract showing a maximum zone of inhibition of 20mm. The extracts of stem were less effective against this gram negative bacterium causing pneumonia, bacteremia, meningitis, urinary tract infections etc in critically ill patients (Gomez et al., 2012).

Among the two gram positive bacteria, *Streptococcus mutans* is considered to be the primary cause of dental caries and infective endocarditis (Banas, 2004). *Staphylococcus aureus* is the major cause of food borne diseases (Hakim et al., 2016). All the extracts of leaf and stem were effective against *Streptococcus mutans* with the methanol extract of leaf showing maximum zone of inhibition of 15mm. Growth of *Staphylococcus aureus* was inhibited efficiently by the aqueous extract of stem with an inhibition zone of 18mm. All other extracts showed almost similar activity.

The fungus selected for study, *Candida albicans* causes candidiasis in oral and vaginal cavities and the digestive tract, the severity of which depends on the health of the individual (Molero et al., 1998). The methanol extracts of leaf and stem was found effective against this fungus with zones of inhibition of 15mm and 16mm respectively.

The results obtained for the present investigation highlight the importance of the plant, *Lepidagathis keralensis* against microbial infections. Preliminary screening suggests the presence of various bioactive secondary metabolites in the leaf and stem of the plant. Quantitative estimation also reveals the presence of high amounts of alkaloids and saponins. The presence of these phytochemicals could be attributed for the observed antimicrobial activity of the plant.

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