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Research Article

ANTIMICROBIAL ACTIVITY OF SOLVENT EXTRACTED *KAEMPFERIA GALANGA* RHIZOMES

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ABSTRACT

Kaempferia galanga L. is one of the valuable medicinal plant in Zingiberaceae family, an ingredient of many ayurvedic drug preparations. Antimicrobial efficiency of *K. galanga* (rhizome) were examined using methanol and chloroform, as solvents and tested against four human pathogenic bacteria: *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae*. Plant extract using both the solvent showed a significant activity against all pathogens, but the chloroform extract of *K. galanga* showed maximum zone of inhibition and minimum inhibitory concentration against all the microorganisms. The minimum zone of inhibition and comparatively greater inhibitory concentration were determined in methanolic extract. Rhizome extracts of *K. galanga* with its antimicrobial activity assures it to be a possible and effective source of herbal medicines to treat infections, and thereby justifying its ethnic use in various human ailments.

INTRODUCTION

Medicinal plants have been used to cure a number of diseases thereby playing a crucial role in overcoming the disadvantages of using synthetic antibiotics (Srivastava *et al*., 2013). Many strains of pathogenic species are now resistant to widely available antibiotics and their resistance have become a cause of concern (Theuretzbacher and Mouton, 2011; Adwan and Mhanna, 2008). The effectiveness of current drugs has been reduced due to the emergence of multidrug resistant bacteria (Hancock, 2005). In the search of developing better drugs researchers are considering herbal products as an effective agent against multidrug resistant bacteria. Plant based antimicrobials are therapeutically potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials (Hussain *et al*., 2004). Continued exploration of plant derived antimicrobials is needed today.

Kaempferia galanga, a perennial that grows in most of the Asian countries, contains essential oils that have been used in a powdered form for indigestion, cold, asthma, pectoral and abdominal pains, and headache. Maceration has been applied as liniment for rheumatism (Kirtikar & Basu 1997). Workers have reported its larvicidal, nematicidal, vasorelaxant and anti neoplastic effects (Choochote *et al*., 2007; Kim *et.al*., 2008). Since the plant has great medicinal relevance (Sadimann, J. 1992), the objectives of this work was to study and compare the antimicrobial activity of chloroform and methanol extracts from the rhizome of *Kaempferia galanga* against common pathogen bacteria, including *Escherichia coli*, *Staphylococcus aureus, Pseudomonas aeruginosa and Klebsiella pneumoniae* and also evaluating minimal inhibitory concentrations and thereby comprehend the role of the plant in microbial control.

MATERIALS AND METHODS

Plant Sample Collection

The Plants of *Keaempferia galanga*, an aromatic herb, is widely distributed in tropical regions. The plant material for the present investigation was collected from Vennikulam, Koodal and Chittar regions in Kerala. Their botanical identity was determined. The rhizome of the plant was used for the further investigation.

Preparation of extract

The rhizome of the plant collected were cleaned and washed with distilled water. Further it was shade dried at room temperature. The dried materials were crushed with grinding machine and sieved for obtaining fine powder. Ground material was extracted with soxhlet using methanol and chloroform solvent. Weighed 15gm sample added to 300ml solvent and

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extraction done by Soxhlet method. After extraction the solvent evaporated by vacuum method and concentrated extract dissolved in distilled water for further analysis.

Test organisms

Four different bacterial strains viz. *E.coli*, *Staphylococcus aureus, Pseudomonas aeruginosa and Klebsiella pneumoniae* was taken into consideration for the present study. The strains were the clinical isolates and were maintained by periodic subculture in nutrient agar slants.

Antimicrobial activity

In vitro antibacterial activity was determined by the agar well diffusion method. For the preparation of anti-bacterial sensitivity test sterile Muller Hinton Agar plates were prepared and the bacterial inoculums were uniformly swabbed in each plate. Wells of 8 mm size were made in the growth media with sterile borer. Nearly 50 ul extracts were added to the wells of growth media. The MHA (Muller-Hintor Agar) plates of bacteria were incubated at 37 °C for 24 hours, after incubation diameter of zones of inhibition was measured. amoxicillin disc was used as positive control.

Minimum inhibitory concentration

The resazurin assay utilizing microtitre-plate (described by Drummond and Waigh, 2000), has been modified to achieve more accuracy in the determination of the minimum inhibitory concentration (MIC) values of natural products against various bacterial strains

Procedure

The bacterial (indicator) strains were suspended in peptone water obtained a turbidity, and then was diluted 200 fold ($1x10^6$ CFU/ml) in sterile peptone water. 96 welled microtitre plate was used for this method. The wells are arranged in 12 rows and 8 columns. The columns were labeled with names of bacterial strain added and columns were labeled with multiples of 8 (from 8 to 80) which indicated the microlitres of *K. galanga* extracts added. 50 microlitres of indicator strains were added to all marked wells in the microtitre plate. Sterile peptone water was added to each well so as to make up to 200microlitres in each well, thus several fold dilution of sample is obtained in each column. Suspension in the microtitre plate was incubated at 35^{o}C for 18 hours. After that, the 10 microlitres of 0.18% resazurin were added to each well and incubated for 2 hours in order to determine viable cells of indicator micro organisms. If the extract has the ability of pathogens inhibition, the colour of resazurin does not change (blue or purple). If it cannot inhibit pathogens, the pathogen will oxidize the resazurin (pink). The lowest concentration of crude extract showed inhibition of pathogens, i.e., the lowest concentration at which colour change occurred was taken as the MIC value. In the present study different concentration of liquid extract such as 8 µl,16 µl, 24 µl, 32 µl, 40 µl, 48 µl,56 µl, 64 µl,72 µl and 80µl (multiples of 8) used for MIC.

Another well was used as control positive, in which the crude extract was replaced by an antbiotic (amoxicillin) and another well was used as control negative which was devoid of bacterial strains. Both the controls are treated with 10 microlitres of 0.18% resazurin. The confirmatory test for the bacterial growth and mortality was performed by inoculating the culture from random wells of MIC plate on to an agar plate and incubated overnight.

RESULT AND DISCUSSION

Much attention has been diverted in the search for antimicrobial from natural source and efforts are put to identify compound that can act as suitable antimicrobials agents to replace synthetic one. Several Zingiberaceae family members show active principles in treatment of various human ailments. Well known examples for the use as spices are the rhizome of the *Zingiber officianals (Ginger)* ***or*** *Curcuma longa (Syn. C.domestica), Kaempferia pandurota* and *K. galanga* are on the other hand important medicinal plants used in the folk medicine of Southest Asia for the treatment of stomach discomforts as expectorant or antiseptic for wound (Pandji *et al*., 1993).

The constituents of the rhizome of *Kaempferia galanga*, hitherto reported, have included cineol, boreneol,3-carene, camphene, kaempferide, cinnamaldehyde, P-methoxycinnamate (Nakao and Shibu, 1924). The methanolic extract of *Kaempferia galanga*, which identifies as ethyl cinnamate, ethyl P-methoxycinnamate and P - methyoxzycinnamic acid, showed larvicidal activity against the second stage larva of dog roundworm, *Toxocara canis* (Kiuchi *et al*., 1988). Kiuchi *et al*., (1988) found that the rhizome extract exhibited Epstein-Bar virus (EBV) activation inhibitory activity when screened for anti-tumour promoter activity. Chu *et al*., (1998) found that *Kaempferia galanga* extract exhibited amebicidal activity in vitro against three species of Acanthomoeda; A. *culbertsoni,* A. *Castellanii*, and A. Polyphaga that were not lytic for normal macrophage culture.

However the present study confirms the role of *K. galanga* in antibacterial activity. The antibacterial activity of the methanol and chloroform extract of *Kaempferia galanga* was studied against both gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas*) bacterial strains

The strongest antibacterial activity was observed for the chloroform extracts as compared to that of methanol extract. The chloroform extract showed higher degree of inhibition zone in *E.coli* (25 mm), *Pseudomonas* (22 mm) followed by *Staphylococcus aureus* (21 mm). The Zone of inhibition for chloroform extract ranged from 17 mm to 25 mm whereas the ethanolic extract had zone of inhibition ranging from 13 mm to 20 mm. Negative control (disc containing only solvent *i.e.* chloroform and methanol) exhibited no zone against four different organisms (*E. coli*, *S. aureus, K. pneumoniae*. and *P. aeruginosa*). All the positive control (Standard antibiotic-amoxicillin) showed antibacterial activity against tested bacteria (Table-1). Our result agree with the findings of other workers, which reported the antibacterial activity of *Kaempferia galanga* against gram positive bacteria *Staphylococcus aureus* and gram negative *Escherichia coli* (Kochuthressia *et. al*., 2012; Arambewela, 1999). However it was observed that *E.coli* was most sensitive to the chloroform extract whereas *S. aureus* was more sensitive to the methanol extract.

Determination of minimum inhibitory concentration is important in diagnostic laboratories because it helps in

conforming resistance of microorganism to an antimicrobial agent and it monitors the activity of new antimicrobial agents. For MIC Different extracts were studied under a wide range of concentrations ranging from 0.8 µg/ml to 8.0 µg/ml. The extracts were active against all the pathogens with average MICs ranging from 2.4 to 5.6 µg /ml. The chloroform extract was excellent in showing very low values of MIC, in the range of 2.4 -4.0 µg /ml. The methanol extracts also had significant MIC values in the range of 2.4-5.6 µg/ml (Table-2). As expected *S. aureus* was relatively sensitive, while *K. pneumoniae* was more resistant to both chloroform and methanol extracts (4.0 µg/ml and 5.6 µg/ml respectively).

Antibiotics used for prevention and killing of microbes have many side effects. The naturally occurring plant materials that are abundant in our state also have the same capacity of killing or preventing the growth of microbes. Compared to synthetic antibiotics, the plant extracts have no side effects. However our study confirms the role of *K. galanga* in antibacterial activity and is almost equivalent to chemical antibiotics and thus can be compared to wide spectrum of antibiotics.

Table 1 Evaluation of In-vitro Antibacterial Activity by Zone of Inhibition of four different bacterial strain using the methanol and chloroform extract of *Kaempferia galanga*

Sl,no	Microorganism	Zone diameter of different solvent		
		Methanol	Chloroform	Antibiotics
1.	*Staphylococcus aureus*	19mm	21mm	37mm
2.	*E.coli*	14mm	25mm	21mm
3.	*K. pneumoniae*	13mm	17mm	15mm
4.	*P. aeruginosa*	20mm	22mm	24mm

Table 2 The minimum inhibitory concentration of the formulation was determined and the value is tabulated below

Microorganism	Minimum inhibitory concentration(µg) of sample	
	Chloroform	Methanol
E.coli	3.2 µg /ml	4.8 µg /ml
Klebsiella	4.0 µg /ml	5.6 µg /ml
Staphylococcus aureus	2.4 µg /ml	2.4 µg /ml
P. aeruginosa	3.2 µg /ml	2.4 µg /ml

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