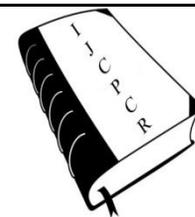




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EVALUATION OF ANTIMICROBIAL ACTIVITY OF HEXANE EXTRACTS OF MEDICINAL PLANT: *KAEMPFERIA GALANGA*

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ABSTRACT

Medicinal plants have been used to cure a number of diseases. The use of medicinal plants helps to overcome the disadvantages of using synthetic antibiotics. *Kaempferia galanga* is an acquiescent perennial that grows in most of the Asian countries, which contains essential oils that have been used in a powdered form for indigestion, cold, pectoral and abdominal pains, and headache. Maceration has been applied as liniment for rheumatism. *Kaempferia galanga* rhizomes are considered stimulating expectorant, carminative and diuretic. To further understand its role in combating microorganisms like bacteria, the present study was designed to evaluate its antimicrobial activity. Dried rhizome of the plant was used for the assay. The dried rhizome part of *Kaempferia galanga* was extracted with hexane as solvent and was screened for antibacterial activities, by using Agar well diffusion assay against three strains of bacteria *Escherichia coli*, *Klebsiella pneumonia* and *Staphylococcus aureus*. The microbial strains were subjected to different volume of concentration at 0.3mg/ml. Minimum Inhibitory Concentration was also studied. The study revealed that *Kaempferia galanga* have antibacterial activity against two strains of bacteria i.e. *Escherichia coli* and *Staphylococcus aureus*. However, *Klebsiella pneumonia* was resistant to the plant extract. Further, it was observed that with increase in volume of the extract there was an increase in the zone of inhibition. The maximum zone of inhibition was seen at 40 μ l. The minimum inhibitory concentration, for both *E.Coli* and *S. aureus* was determined at 1.5 mg of the sample.

Key words: *Kaempferia galanga*, Hexane, Bacteria.

INTRODUCTION

The use of antibiotics has revolutionized the treatment of various bacterial infections. However, the consequence of such indiscriminate use has lead to antibiotic resistance among microorganisms [1], apart from their side effect such as soft stools, diarrhea or mild stomach upset and nausea. Less commonly, some peoples have allergic reactions to an antibiotic, and some have died from a severe allergic reaction. Recent years have witnessed a renewed interest in exploring natural recourse for developing such compounds. [2]. There is a paradigm shift to the use of medicinal plants and their extracts to treat diseases as well as alleviate symptoms [3]. Usage of herbs, in the traditional medicine and their curative potentials are widely exploited besides well documented

[4]. New drugs developed between 1981 and 2002 were based on natural products and they have been very successful, especially in the areas of infectious disease and cancer [5]. The secondary metabolites in plants such as tannins, terpenoids, flavonoids etc., have been found to have antimicrobial properties [6]. The biologically active compounds, in the medicinal plants, after possible chemical manipulation provide new and improved drugs to treat the infectious diseases [7]. Plant based products/ extracts are cheaper alternatives to the development of synthetic drugs. *Kaempferia galanga*, an aromatic rhizomatus herbal medicinal plant, belongs to the family Zingiberaceae family. It's a mordent of many ayurvedic drug preparations [8].

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In some countries the root and leaves are put into curries as a flavoring, and the plant is used as a medicine, as well [9]. Rhizomes of this plant have been traditionally used as stomachic and incense [10] and for relieving toothache, abdominal pain, muscular swelling and the rheumatism [11]. Larvicidal and anticancer principles have also been reported from the rhizome extract [12].

The rhizome of *Kaempferia galanga* find important place in indigenous medicine as carminative, expectorant, diuretic and stimulant. The most common indication for its uses besides hypertension include asthma, headaches, cough, toothaches and as a poultice for applying to bruises and wounds [13]. *Kaempferia galanga* extract exhibited amebicidal activity in vitro against three species of Acanthamoeba; *A. culbertsoni*, *A. Castellanii*, and *A. Polyphaga* that were not lytic for normal macrophage culture [14].

In the current investigation carried out, a screening of the hexane extracts of *Kaempferia galanga* rhizome against pathogenic bacteria is done in order to evaluate its antimicrobial property.

MATERIALS AND METHODS

Collection of plant materials

Kaempferia galanga plants were collected from Aryavaidyasala of Kottakkal Medicinal Garden, Malappuram district, Kerala during May, 2014. The plant material was identified and shade dried for the present 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 hexane. The concentration of the sample was 0.3g/ml.

Test organism

The bacterial strains were the clinical isolates obtained from Poyanil hospital, Kozhencherry, Kerala. The bacteria used were *Klebsiella pneumonia*, *Escherichia coli* and *Staphylococcus aureus*.

Test for antibacterial assay and Determination of MIC

In vitro antibacterial activity was determined by the agar well diffusion method. For the preparation of antibacterial sensitivity test sterile Muller Hinton Agar plates were prepared and the bacterial inoculums (*E. coli*, *K. pneumonia*, *S. aureus*) were uniformly swabbed in each plate. The wells were punched over the agar plates using sterile gel puncher and various concentrations (5, 10, 20 and 40µl) of extract were added to the wells. The plates were incubated at 35-37°C for 18-48 hours, a period of time. The disc with solvent alone with which the extraction was carried out was used as negative control. After incubation the diameter of inhibitory zones formed around each discs were measured in mm and recorded. Ciprofloxacin disc was used as positive control. The assay was repeated thrice and mean \pm SD was calculated. MIC was calculated from the fully grown plates.

Table 1. Antibacterial Activity of hexane extract of *Kaempferia galanga*

Microorganism	Volume of sample (µl)	Zone of inhibition of Test sample (mm)	Zone of inhibition of Positive sample (mm)
<i>E.coli</i>	5	-	17
	10	11.5 \pm 0.50	
	20	17.3 \pm 0.29	
	40	22.2 \pm 1.26	
<i>Klebsiella pneumonia</i>	5	-	18
	10	-	
	20	-	
	40	-	
<i>Staphylococcus aureus</i>	5	10.2 \pm 1.26	13
	10	11.47 \pm 0.55	
	20	23.1 \pm 0.85	
	40	26.7 \pm 0.58	

Mean of three replicate determination \pm S D ; Standard antibiotic- Ciprofloxacin.

Table 2. Determination of MIC of the Sample preparation

Microorganism	Minimum Inhibitory Concentration (mg) of Sample
<i>E.coli</i>	1.5
<i>Klebsiella</i>	-
<i>Staphylococcus</i>	1.5

RESULT AND DISCUSSION

The antibacterial activity of the hexane extract of *Kaempferia galanga* was studied against both gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli*, *Klebsiella pneumonia*) organism at the concentration of 0.3 g/ml. The antibacterial activity was carried out at four different volumes (5 µl, 10 µl, 20 µl, and 40 µl). All test strains of bacteria were found to be sensitive to Ciprofloxacin, which was used as positive control. Hexane, which was used as the negative control, did not show any zone of inhibition against tested bacteria.

The result of the antimicrobial screening of the plant extract in hexane is summarized in Table.1. It was observed that with the increase in volume of the test sample there is an increase in the zone of inhibition for *Escherichia coli* and *Staphylococcus aureus*. It ranged from 11.5 ± 0.50 to 22.2 ± 1.26 mm and 10.2 ± 1.26 to 26.7 ± 0.58 mm respectively. Rhizome hexane extract showed maximum inhibition in *Staphylococcus aureus* (26.7 ± 0.58 mm). However, it was also observed that zone of inhibition of the positive sample (antibiotic), which was used as standard, was less than the test sample at 40 µl. No zone was

exhibited for the negative control (solvent only). The MIC values of hexane extract against *Escherichia coli* and *Staphylococcus aureus* were found to be 1.5 mg. Strong antibacterial activities of ethanol, methanol and chloroform extracts of *Kaempferia galanga* rhizome have been reported by earlier workers [15]. However the zone of inhibition with hexane as solvent was observed to be more (26.7 ± 0.58 mm). The constituents of this rhizome, hitherto reported, have included cineol, borneol, 3-carene, camphene, kaempferol, kaempferide, cinnamaldehyde, p-methoxycinnamic acid, ethyl cinnamate, and ethyl p-methoxy cinnamate is reported to inhibit monoamine oxidase [16].

From the present study it can be concluded that the rhizomes of *K. galanga* possesses antibacterial activity, and therefore, suggest that the traditional use of this plant for the treatment various ailments.

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