In vitro antimicrobial evaluation of *Kaempferia galanga* L. rhizome extract

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ABSTRACT

In the present study, antimicrobial activity of ethanol, methanol, petroleum ether, chloroform and aqueous extracts of *Kaempferia galanga* rhizome were screened against ten human pathogenic bacteria such as *Staphylococcus aureus*, *Streptococcus faecalis*, *Bacillus cereus*, *Bacillus subtilis*, *Enterobacter aerogenes*, *Salmonella typhi*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Vibrio cholerae* and four fungal species: *Aspergillus niger*, *A. flavus*, *A. fumigatus* and *Candida albicans* using disc diffusion assay. All the extracts showed significant antibacterial and antifungal properties. Highest inhibition zone (21.3±0.08) was recorded for ethanolic extract against *Staphylococcus aureus*.

Key words: Rhizome, *Kaempferia galanga*, antimicrobial activity, disc diffusion assay.

INTRODUCTION

Herbal medicines are gaining priorities in treating various health ailments of diverse origins in man. Before the inventions of the modern synthetic medicines, man’s dependence was totally on plants. Traditional systems of plant based products have existed with the changes in culture, traditions and mode of life of man; except for a short period when the inventions of the modern synthetic medicines came into existence. Plant based antimicrobials have enormous therapeutical potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials. Continued exploration of plant derived antimicrobials is needed today (Hussain et al., 2004). The pathogenic microorganisms can be controlled with the antibiotics presently available; however the need of new antibiotics has increased due to current problems of resistance associated with them (Davies, 1994 and Prabhat et al., 2005). The drug resistant bacteria and fungal pathogens have complicated the treatment of infectious diseases. In the present scenario of emergence of multiple drug resistance to human pathogenic organisms this has necessitated a search for new antimicrobial substances from other sources including plants. Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties (Harbone and Baxter, 1995). The substances that can either inhibit the growth of pathogen or kill them and have no or least toxicity to host cells are considered candidates for developing new antimicrobial drugs. The antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world (Saxena, 1999). It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant microbial pathogens.

*Kaempferia galanga* L. is one of the valuable medicinal plants in Zingiberaceae family, an aromatic rhizomatous herbal spice, is an ingredient of many ayrvedic drug preparations (Sadimann 1992). The rhizome and root tubers are used for curing bronchitis, asthma, malaria, skin disease, wounds and spleenic disorders (Kirtikar & Basu 1997). The ethyl p-methoxy transcinnamate present in the rhizome extract is highly cytotoxic to He La cells (Kosuge et al., 1985). Larvicidal and anticancer principles have also been reported from the rhizome extracts (Kiuchi et al., 1988, Kosuge et al., 1985). Rhizome extract contain n-pentadecane, ethyl p-methoxy cinnamate, ethyl cinnamate, l-Δ3 carene, camphene, berneol, cineol, p- methoxy styrene, kaempferol, kaempferide (Anonymous, 1959). Leaves and flowers contain flavanoids (Ghani 1998). The 95% ethanol extract of this plant possessed antibacterial activity against *Staphylococcus aureus* and hot water extract against *Escherichia coli* (George and Pandalai, 1949). Recently, Arambewela et al (1999) reported that the essential oils of *K. galanga* root and rhizome showed antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. Parvez et al., 2005 reported that the petroleum ether and methanol extract of *K. galanga* showed significant activity against Gram positive and
Gram negative bacteria. The present study was undertaken to determine the antibacterial and antifungal sensitivity of the *Kaempferia galanga* rhizome extracts.

**MATERIALS AND METHODS**

**Plant sources:** *Kaempferia galanga* plants were collected from Kerala Agriculture University, Vellanikkara, Trissur, Kerala. They were identified at the Rapinat Herbarium, St. Josephs college (Autonomous) Tiruchirapalli, S. India and a voucher specimen was also deposited.

**Extract preparation:** 50 gms of shade dried powdered materials of rhizome were soaked in 300ml of each of the solvent viz ethanol, methanol, petroleum ether, chloroform and aqueous in a soxhlet apparatus for 72 hour at 31°C until complete exhaustion of the material. Each mixture was stirred at every 24 hour using a sterile glass rod. At the end of 72 hour each extract was passed through whatmann No.1 filter paper and filtrates were concentrated in vacuum rotary evaporator in order to reduce the volume into 50ml. The extracts were stored in labelled screw bottles and kept in refrigerator at 4°C.

**Microbial strains and Culture media:** Ten bacterial species: *Staphylococcus aureus* MTCC # 3163, *Streptococcus faecalis* MTCC# 459, *Bacillus cereus* MTCC # 1306, *Bacillus subtilis* MTCC # 121, *Enterobacter aerogenes* MTCC # 2990, *Salmonella typhi* MTCC #734, *Escherichia coli* MTCC # 119, *Klebsiella pneumoniae* MTCC # 3040, *Pseudomonas aeruginosa* MTCC # 2474 and *Vibrio cholera*. The fungal strains: *Asperillus niger* MTCC # 2612, *A. flavus* MTCC # 2813, A. *fumigatus* MTCC # 2584 and *Candida albicans* MTCC # 1637 were used for this study. Bacterial stock cultures were subcultured in nutrient broth for incubation at 37°C. The 24 hr culture was stirred with 0.9% saline to achieve 0.5 Mc farland (10 8 cells/ml for bacteria and 10⁶ for fungi) (Okusa et al., 2007). Kannamycin and nystatin were used as reference antibiotics (RA).

**Antimicrobial assay:**

**Disc diffusion assay:** The discs of 6 mm were prepared using a whatmann filter paper. 100 discs were obtained by punching and putting in vials bottles and sterilizing in an oven at 1500°C for 15 minutes. The discs were impregnated with 10μl of concentrated crude extracts. The discs were evaporated at 37°C for 24hrs. Prepared discs containing the various fractions were carefully placed on the inoculated plates using a sterilized forceps in each case (Fatope and Adoum, 1993). The disc with solvent alone with which the extraction was carried out was used as negative control. The plates were then turned upside down and incubated at 37°C for 24hr in an incubator. The results were taken by considering the zone of growth and inhibition of the organism by the test fractions. Antimicrobial activity was evaluated by measuring the diameter of the inhibition zone (IZ) around the disc. The assay was repeated thrice and mean ± SD was calculated (Kuete et al., 2007).

**RESULTS AND DISCUSSION**

**Antibacterial activity:** The antibacterial activity of ethanol, methanol, petroleum ether, chloroform and aqueous extracts of *Kaempferia galanga* rhizome is presented in Table 1. The rhizome ethanolic extract showed higher degree of inhibition zone in *Staphylococcus aureus* (21.3±0.08 mm), *Streptococcus faecalis* (19.7±0.37 mm), *Bacillus subtilis* (19.7±0.20 mm) and *B. cereus* (18.4±0.41 mm). The moderate inhibition was noted against *Escherichia coli* (17.8±0.55 mm) and *Enterobacter aerogens* (16.2±0.37 mm). The other bacteria showed inhibition zone such as *Salmonella typhi* (15.5±0.17 mm), *Klebsiella pneumoniae* (14.9±0.95 mm), *Vibrio cholerae* (12.3±0.16 mm) and *Pseudomonas aeruginosa* (12.1±0.40 mm). This observation is in agreement with the report of earlier workers Sathish Kumar (2008) and Bualeea (2007), who reported that the ethanol extract produced strong inhibitory activities against some of the microorganisms. The methanol extract showed good activity in gram-positive bacteria such as *S. aureus* (18.1±0.30 mm) and *S. faecalis* (16.4±0.34 mm) and the gram-negative organism *E. aerogenes* (15.6±0.26 mm and *Escherichia coli* (12.8±0.34 mm). These research findings give a scope to further screen the chemical constituents of the extracts which will be very useful to compact the common infections caused by *Staphylococcus aureus* (Oonmetta-aree et al., 2006). The petroleum ether and chloroform rhizome extract showed higher level of inhibition zone in *B. cereus* 14.3±0.29 mm and 14.5±0.32 mm respectively. These results are also in substantial agreement with many previous studies (Burt, 2004; Maillard, 2002).
Table 1. Antibacterial activity of rhizome extract of *Kaempferia galanga* - Disc diffusion method

| Microorganisms            | Ethanol extract 5mg/disc | Methanol extract 5mg/disc | Petroleum ether extract 5mg/disc | Chloroform extract 5mg/disc | Aqueous extract 5mg/disc | Standard antibiotic 30µg/disc |
|---------------------------|--------------------------|---------------------------|----------------------------------|-----------------------------|-------------------------|-------------------------------|
| **Bacteria**              |                          |                           |                                  |                             |                         |                               |
| *Staphylococcus aureus*   | 21.3±0.08                | 18.1±0.30                 | 12.3±0.36                        | 10.4±0.25                   | 9.2±0.91                | 23                             |
| *Streptococcus faecalis*  | 19.7±0.37                | 16.4±0.34                 | 13.4±0.62                        | 11.1±0.20                   | 10.1±0.29               | 22                             |
| *Bacillus cereus*         | 18.4±0.41                | 14.2±0.13                 | 14.3±0.29                        | 14.5±0.32                   | 9.3±0.26                | 23                             |
| *Bacillus subtilis*       | 19.7±0.20                | 15.1±0.57                 | 11.3±1.12                        | 12.6±0.26                   | 10.3±0.25               | 20                             |
| *Enterobacter aerogenes*  | 16.2±0.37                | 15.6±0.26                 | 10.9±0.67                        | 10.8±0.46                   | 9.5±1.20                | 20                             |
| *Klebsiella pneumoniae*   | 14.9±0.95                | 12.3±0.30                 | 10.1±0.24                        | 9.2±0.92                    | 9.3±0.5                 | 25                             |
| *Salmonella typhi*        | 15.5±0.17                | 11.4±0.50                 | 12.7±0.23                        | 10.7±0.56                   | 8.8±1.39                | 20                             |
| *Escherichia coli*        | 17.8±0.55                | 12.8±0.34                 | 12.4±0.25                        | 10.4±0.98                   | 8.2±0.92                | 20                             |
| *Pseudomonas aeruginosa*  | 12.1±0.40                | 12.4±0.40                 | 9.3±0.49                         | 9.2±0.19                    | 8.9±1.30                | 23                             |
| *Vibrio cholerae*         | 12.3±0.16                | 12.4±0.05                 | 9.3±0.25                         | 8.2±1.30                    | 8.2±1.30                | 24                             |

Mean of three replicate determination ±S D ; Standard antibiotic- Kanamycin

Table 2. Antifungal activity of rhizome extract of *Kaempferia galanga* - disc diffusion method

| Microorganisms            | Ethanol extract 5mg/disc | Methanol extract 5mg/disc | Petroleum ether extract 5mg/disc | Chloroform extract 5mg/disc | Aqueous extract 5mg/disc | Standard antibiotic 30µg/disc |
|---------------------------|--------------------------|---------------------------|----------------------------------|-----------------------------|-------------------------|-------------------------------|
| **Fungus**                |                          |                           |                                  |                             |                         |                               |
| *Aspergillus niger*       | 16.3±0.45                | 14.2±0.26                 | 12.4±0.39                        | 11.5±0.38                   | 9.3±0.27                | 24                             |
| *Aspergillus flavus*      | 15.3±0.36                | 13.2±1.20                 | 11.4±0.21                        | 9.24±0.91                   | 9.2±1.10                | 23                             |
| *Aspergillus fumigatus*   | 14.0±0.48                | 10.8±1.89                 | 10.3±0.46                        | 9.6±1.29                    | 9.2±1.5                 | 25                             |
| *Candida albicans*        | 12.2±0.45                | 11.3±1.39                 | 9.7±0.39                         | 9.5±0.49                    | 8.3±0.28                | 24                             |

Mean of three replicate determination ±S D ; Standard antibiotic- Nystatin
Antifungal activity: Rhizome ethanol extract showed maximum inhibition in *Aspergillus niger* (16.4±0.45 mm), *A. flavus* (15.3±0.36), *A. fumigatus* (14.0±0.48) and *Candida albicans* (12.2±0.45 mm). Methanolic rhizome extract showed high inhibition zone against *Aspergillus niger* (14.2±0.26 mm). Petroleum ether, chloroform and aqueous extracts exhibited moderate level of inhibition zone against all tested fungi (Table 2.). This observation is in agreement with the report of earlier workers Sathish Kumar (2008) and Bualeea (2007), who reported that the ethanol extract produced strong inhibitory activities against some of the microorganisms.

The antibacterial activity of plant extracts was not only due to one main active chemical but to the combined action of additional other compounds (Sunayana, 2003). Examples include Phenolic acids (Fernandez et al., 1996), alkaloids (Vanbeek et al., 1999), terpenoids (Watcher et al., 1999), flavanoids, (Chakraborty and Brantner, 1999) terpenes (Coveney et al., 1985), terpenoids (Habibi et al., 2000) and naphthoquinones (Cai et al., 2000). It is clear that the chemical structure of the antimicrobial agents found in higher plants belong to most commonly encountered classes of higher plant secondary metabolites.

In conclusion, *K. galanga* showed good *in vitro* antibacterial and antifungal effects. The result presented here may explain the traditional use of this plant.

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