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# A Comparative Study of Antimicrobial Activity of *Curcuma amada* and *Alpinia galanga* of Zingiberaceae Family

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The antimicrobial activity of different solvent extracts of *Curcuma amada* and *Alpinia galanga* against gram positive and gram negative bacteria and some fungal strains was studied. The results showed that the broad spectrum of activity against all tested microorganisms. The total phenolics of *C. amada* are ranging from 29.0 to 59.5 and *A. galanga* from 28.4 to 56.5 mg GAE per g of dry weight.

**Key Words: Antimicrobial activity, *Curcuma amada*, *Alpinia galanga*, Total phenolics.**

## INTRODUCTION

The plants belonging to Zingiberaceae family are known for their preservative[1] and medicinal values[2]. Number of plants from this family are being used in traditional system of medicine[3]. *Curcuma amada* (Mangoginger) and *Alpinia galanga* (Greater galanga) are the members of the family Zingiberaceae. The rhizome of *C. amada* is traditionally used in the treatment of skin diseases, bronchitis, inflammations and healing of wounds[4]. The rhizome of *A. galanga* is used for the treatment of rheumatoid arthritis, inflammation, asthma and bronchitis[5]. The presence of multiple chemical constituents[6,7], antiinflammatory, antifungal and antimicrobial activities against some microorganisms were reported in the rhizome extracts of *C. amada*[8-10] and *A. galanga*[11].

Infectious diseases are major health hazards all over the world[12]. *Staphylococcus aureus* is the most common microorganism causing skin disease[13] and *E. coli* is the best known member of the normal microbiota of the human intestine and a versatile gastrointestinal pathogen[14]. In present study,

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the antimicrobial activities of *C. amada* and *A. galanga* were evaluated against a few clinically important microorganisms.

## EXPERIMENTAL

Fresh rhizomes of Mangoginger (*C. amada*) and greater galanga (*Alpinia galanga*) were collected locally. Media components were purchased from Hi Media, Mumbai, India. All other chemicals used were of analytical grade.

Gram negative *E. coli* and *K. pneumoniae*, *P. vulgaris* and gram positive *Staphylococcus aureus* and *Bacillus subtilis* were used for *in vitro* antimicrobial activity and *Candida albicans, Aspergillus niger* and *Rhizopus oryzae* were used for antifungal activity. These microbial strains were obtained from Biotechnology Division, Andhra University, Visakhapatnam and maintained on nutrient agar.

**Preparation of crude solvent extracts:** Fresh rhizomes (20 g) are cut into small pieces and crushed in a homogenizer. Each slurry material was extracted with 150 mL of each ethanol, acetone, ether, chloroform and carbon tetrachloride. The extracts were evaporated under reduced pressure and dried using a rotary evaporator at 55 ºC. The dried extracts were dissolved in the aforesaid solvents to get 0.1 %, labelled as CET for ethanol, CAC for acetone, CE for ether, CCF for chloroform and CCT for carbon tetrachloride extracts of *C. amada*, AET for ethanol, AAC for acetone, AE for ether, ACF for chloroform and ACT for carbon tetrachloride extracts of *A. galanga*, and used for determination of antimicrobial activity and determination of total phenolic content.

**Antibacterial assay:** The antibacterial activity was determined by agar well diffusion method[15]. 20 mL of molten Muller Hinton Agar No. 1 along with 0.2 mL of inoculum was poured into a petri plate. The plates were allowed to solidify, after which wells were made in the plates with the help of a cup borer and each well was filled with 20 µL of solvent extract. The wells loaded with the pure solvent served as control. In order to compare the activity of the test material, 10 µL of 0.1 % of streptomycin (S) and ampicillin (A) were used. The plates were incubated at 37 ºC for 24 h.

**Antifungal assay:** Antifungal activity was determined by the method described by Odds[16]. The fungal cultures studied were incubated in Saboraud dextrose broth for 24 h. 1 mL of inoculum containing $10^6$ CFU was spread on Saboraud dextrose agar plates. Wells were made in the plates with the help of a cup borer and each well was filled with 20 µL of solvent extract. The wells loaded with the pure solvent served as control. In order to compare the antifungal activity of the test material, 10 µL of 0.1 % of nystatin (Ny) was used. The plates were incubated at 25 ºC for 10 d. Growth inhibition was determined by measuring the diameter of the zone of inhibition.

**Determination of total phenolic content:** The total phenolics present in different extracts were determined using the Folin Cio-calteau reagent as described by Javanmardi *et al.*[17]. To 0.5 mL of the each sample 2.5 mL of diluted Folin Cio-calteau reagent and 2.0 mL of 7.5 % (w/v) sodium carbonate were added and incubated at 45 ºC for 15 min. The absorbance was measured in a spectrophotometer at 765 nm. Calibration curve was prepared using different concentrations (10-100 mg) of gallic acid. The results were expressed as mg of gallic acid equivalent (GAE) per g weight.

## RESULTS AND DISCUSSION

Antimicrobial activity of different solvent extracts of rhizome of *C. amada* and *A. galanga* against *E. coli* is depicted in the Fig. 1. The results indicate that the ethanol extract of *C. amada* and ether extract of *A. galanga* showed highest activities, whereas lowest activities are observed with carbon tetra-chloride extract of *C. amada* and chloroform extract of *A. galanga*. Further, the zone of inhibition of ethanol extract of *C. amada* and streptomycin are equal. All extracts of *C. amada* and *A. galanga* except chloroform extract exhibited higher antimicrobial activity than ampicillin. The ethanol extract of *C. amada* showed highest antimicrobial activity against *E. coli* compared to DMS extract[18].

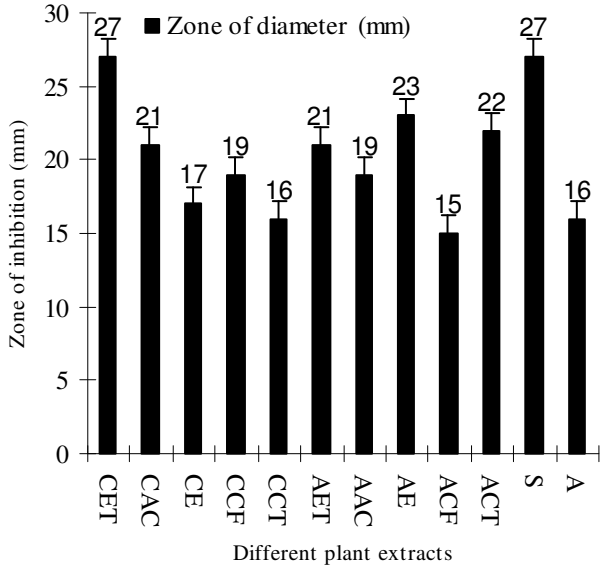


Fig. 1. Antimicrobial activity of *C. amada* and *A. galanga* extracts against *E. coli.* All the values are an average of four determinations and expressed as mean ± SD

Antimicrobial activities of rhizome extracts of *C. amada* and *A. galanga* against *S. aureus* are given in Fig. 2. The results indicate significant activity with ethanol, ether and chloroform extracts of *C. amada* and ethanol extract of *A. galanga*. The zone of inhibition of ethanol, ether and chloroform extracts of *C. amada* and ethanol, acetone extracts of *A. galanga* are higher than streptomycin. However, the zone of inhibition of ethanol and chloroform extracts of *C. amada* is similar to ampicillin. All the solvent extracts of *C. amada* of the present study showed higher antimicrobial activity than 1,4-dioxane extracts against *S. aureus*[18].

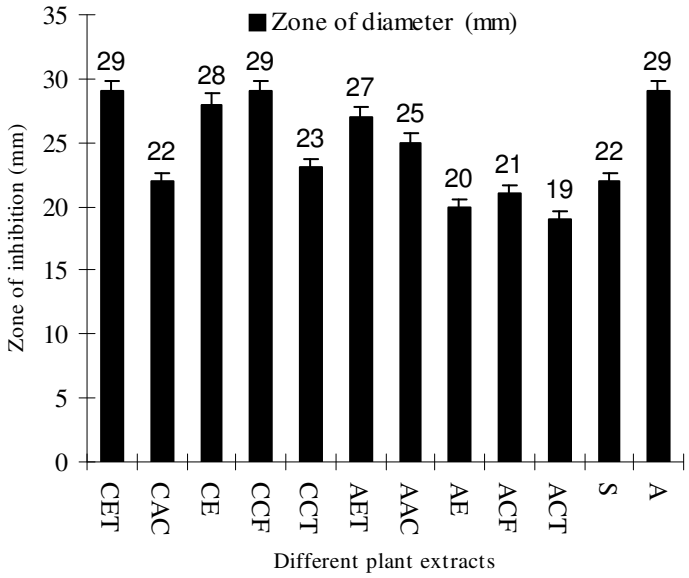


Fig. 2. Antimicrobial activity of *C. amada* and *A. galanga* extracts against *S. aureus.* All the values are an average of four determinations and expressed as mean ± SD

It is also clear from Fig. 3 that the rhizome extracts of *C. amada* and *A. galanga* have significant antibacterial activity against *K. pneumoniae*. The zone of inhibition of ethanol and carbon tetrachloride extracts of *C. amada* are higher than the other extracts studied but almost equal to streptomycin. Similarly chloroform extract of *A. galanga* exhibited highest antimicrobial activity. The zone of inhibition of all extracts of *C. amada* and acetone, chloroform extracts of *A. galanga* are higher than ampicillin. However, the zone of inhibition of ethanol extract of *A. galanga* and ampicillin are equal. Moreover, the zones of inhibition of ether, carbon tetrachloride extracts of *A. galanga* are less than ampicillin.

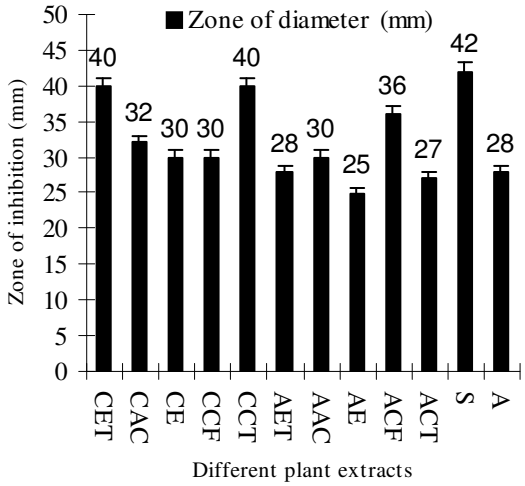


Fig. 3. Antimicrobial activity of *C. amada* and *A. galanga* extracts against *K. pneumoniae*. All the values are an average of four determinations and expressed as mean ± SD

The results obtained from the antimicrobial activities of different solvent extracts of rhizome of *C. amada* and *A. galanga* against *B. subtilis* (Fig. 4) showed that the chloroform extracts of both *C. amada* and *A. galanga* have produced the zone of inhibition of 40 mm which is also close to that of streptomycin.

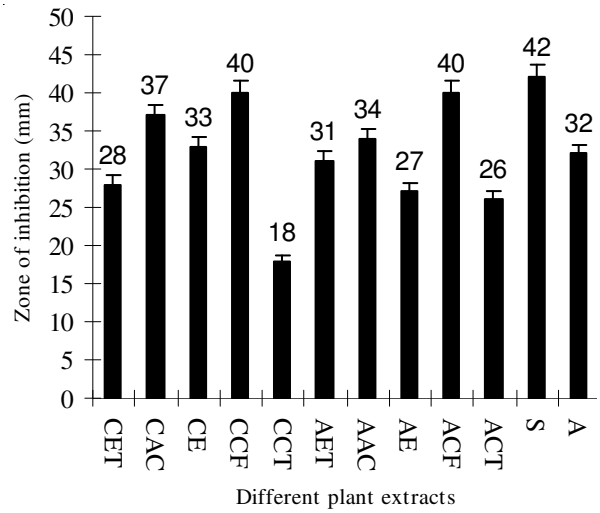


Fig. 4. Antimicrobial activity of *C. amada* and *A. galanga* extracts against *B. subtilis*. All the values are an average of four determinations and expressed as mean ± SD

Comparative antimicrobial activity of different solvent extracts of rhizome of *C. amada* and *A. galanga* against *P. vulgaris* are depicted in the Fig. 5. The results indicated that the ethanol and acetone extracts of *C. amada* and *A. galanga* have exhibited highest activity. Antimicrobial activity was not detected with the chloroform extract of *C. amada* and ether, chloroform and carbon tetrachloride extracts of *A. galanga*. The zones of inhibition of ethanol, acetone extracts of *C. amada* and acetone extract of *A. galanga* are higher than ampicillin but lower than streptomycin.

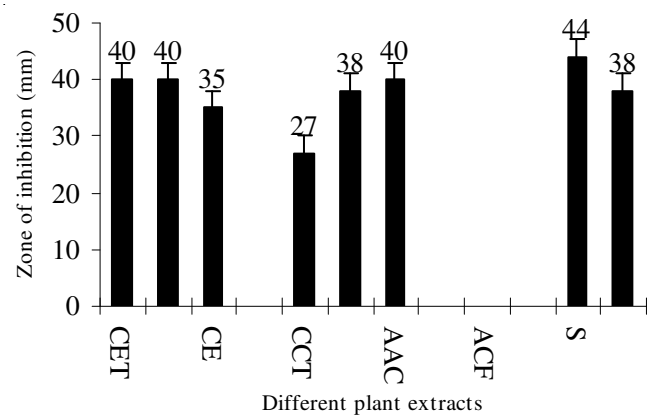


Fig. 5. Antimicrobial activity of *C. amada* and *A. galanga* against *P. vulgaris.* All the values are an average of four determinations and expressed as mean ± SD

Antifungal activity of different rhizome extracts of *C. amada* and *A. galanga* were determined against fungal pathogen strains *C. albicans, A. niger* and *O. sativum*. All the extracts were failed to inhibit the growth of *A. niger* and *O. sativum*. However ethanol, ether and carbon tetrachloride extracts of *C. amada* and ethanol, ether and chloroform extracts of *A. galanga* showed antifungal activity against *C. albicans* (Fig. 6). Maria *et al.*[19] reported that the chloroform extracts of *A. galanga* exhibited weak activity against *C. albicans*.

The total phenolic contents of different solvent extracts of *C. amada* and *A. galanga* are presented in Table-1. The results indicate that the total phenolics of *C. amada* range from 29.0 to 59.5 and *A. galanga* from 28.4 to 56.5 mg GAE per g of dry weight. Highest phenolic content was reported in ethanol extract and lowest in carbon tetrachloride extract of both *C. amada* and *A. galanga*.

From the present study, it can be concluded that several solvent extracts of examined plants were active against the tested microorganisms. The study further revealed that the phenolics may be responsible for the

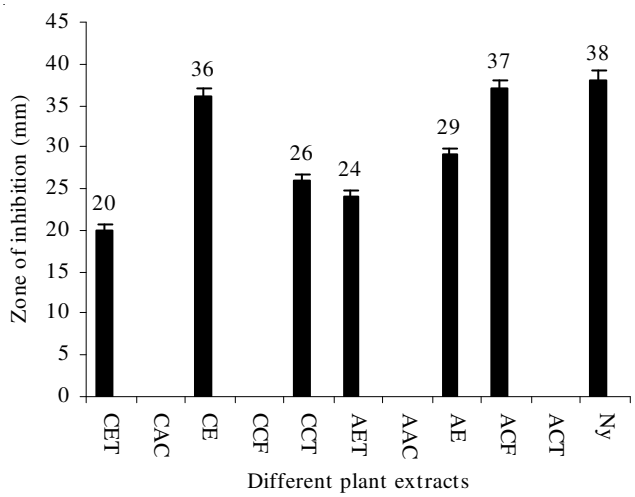


Fig. 6. Antimicrobial activity of *C. amada* and *A. galanga* extracts against *C. albicans.* All the values are an average of four determinations and expressed as mean ± SD

TABLE-1
TOTAL PHENOLIC CONTENT OF DIFFERENT EXTRACTS OF *C. amada* and *A. galanga*

| Name of the extract | Total phenolics (mg of GAE/g) | |
|---|---|---|
| | *C. amada* | *A. galanga* |
| Ethanol | 59.5 ± 0.02 | 56.5 ± 0.01 |
| Acetone | 41.4 ± 0.01 | 39.5 ± 0.02 |
| Ether | 42.3 ± 0.01 | 41.2 ± 0.02 |
| Chloroform | 59.9 ± 0.02 | 52.5 ± 0.02 |
| Carbon tetrachloride | 29.0 ± 0.01 | 28.4 ± 0.01 |

All the values are an average of four determinations and expressed as mean ± SD.

antimicrobial activity of tested plant extracts. Further, phytochemical studies are required to determine the type of phenolic compound responsible for the antimicrobial effect of these medicinal plants. In addition, the results support the uses of these plants in traditional medicine for the treatment of infectious diseases.

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