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Antimicrobial and radical scavenging activity of leaf and rhizome extract of *Alpinia galanga* (L.) Willd (Zingiberaceae)

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Abstract:
Alpinia galanga (L.) Willd belonging to the family Zingiberaceae is widely distributed in tropical areas. The plant is used in food preparation and as medicine. The present study was carried out to determine antimicrobial and radical scavenging effect of leaf and rhizome extract of *A. galanga*. The powdered leaf and rhizome were extracted by soxhlet extraction using methanol. Antimicrobial activity of extracts was determined by Agar well diffusion assay against 15 clinical isolates of bacteria (from burn, dental caries and urinary tract infection) and two fungi (*Candida albicans* and *Cryptococcus neoformans*). Radical scavenging activity of extracts was determined by DPPH free radical scavenging assay. Total phenolic and flavonoid contents were estimated by Folin-Ciocalteau reagent and Aluminium chloride colorimetric estimation method respectively. Rhizome extract was found to possess high inhibitory activity against fungi and clinical isolates of bacteria. Inhibitory activity was marked against burn and dental caries isolates when compared to urinary tract isolates. Overall, Gram positive bacteria showed higher susceptibility to extracts. Among fungi, *C. neoformans* was inhibited to higher extent. The extracts have shown dose dependent scavenging of free radicals. The rhizome extract (IC_{50} 32.34µg/ml) was more efficient in scavenging free radicals than leaf extract as revealed by low IC_{50} value. The content of total phenolics and flavonoids were high in rhizome extract when compared to leaf extract. Marked antimicrobial and radical scavenging potential of rhizome extract can be ascribed to high phenolic and flavonoid content. The plant can be used for the development of agents active against pathogenic microbes and radical induced damage.

Key words: *Alpinia galanga*, DPPH, Agar well diffusion, Total phenolic, flavonoid

Introduction

Alpinia galanga (L.) Willd (Zingiberaceae) is a rhizomatous plant having distinctive aroma (due to essential oils and phenolic compounds), distributed widely in tropical areas and used as a medicine in many countries. The rhizomes act as carminative, used to treat rheumatism, spleen pain, bronchitis, diabetes mellitus and loss of appetite. The plant is used to treat stomachaches in China and Thailand. The fresh rhizome has a characteristic fragrance and pungency. The rhizome is used to flavor cooked food. The rhizome is used as an essential component in Thai curries paste[1,2,3]. The plant has got traditional uses. The rhizome is sold for stimulant property by local herbal vendors in Tamil Nadu, India[4]. To relieve asthma, the root powder, along with other plants, is mixed with natural honey and administered[5]. The plant has been traditionally used as offerings to god in kani tribal community[6]. In Malaysia, the juice from the pounded leaves is applied topically on skin diseases[7]. From the rhizomes, compounds *viz.*, β-Sitosterol Diarabinoside[8], β-sitosterol diglucosyl caprate[9], Galangoflavonoside[10] and 1′-Acetoxychavicol acetate[11] have been isolated.

Different parts of the plant have shown to possess various bioactivities. Rhizome extracts showed analgesic[12], anthelmintic[13], anti-

mycobacterial[14], antioxidant[15], anti-inflammatory[16], gastric antisecretory[17], antiulcer[17], cytoprotective[17], CNS stimulant[18], antidiabetic[19], hepatoprotective[20], hypolipidemic[21] activity and protective effect against diabetic nephropathy[22]. The essential oil of rhizome showed antimicrobial activity[1,23,24], antioxidant activity[23]. 4'-hydroxycinnamaldehyde isolated from rhizome was cytotoxic to cell lines human leukemic HL-60 and U937[25]. Leaf extract was shown to exhibit antibacterial[26] and wound healing[27] property. Seed essential oil was found to possess antibacterial activity[26]. The flower extract was shown to possess inhibitory effect against *Listeria monocytogenes* and *Staphylococcus aureus* in a ready-to-eat Turkey ham product[28]. The root extract possess anti-inflammatory activity in rat paw edema[29]. 1'-Acetoxychavicol acetate isolated from rhizomes ameliorates ovalbumin-induced asthma in mice[11]. The leaves and pseudostem were shown to possess phytoremediation activity in terms of potential to absorb zinc and lead[3]. The present study was performed to estimate total phenolic and flavonoid content and to determine antimicrobial and radical scavenging effect of leaf and rhizome extract of *A. galanga*.

Materials and Methods

Collection of plant material

The leaves and rhizomes of *A. galanga* were collected at Maragalale, Thirthahalli (taluk), Shivamogga (district), Karnataka. The plant material was authenticated by Dr. Vinayaka K.S. Voucher specimen (SSC/PK-Ag-97) was deposited in the department.

Extraction

The plant materials were shade dried and powdered mechanically. For extraction, 25g each of leaf and rhizome powders were extracted using methanol (HiMedia, Mumbai) in a soxhlet apparatus. The extracts were filtered through Whatman No. 1 filter paper and concentrated in vacuum under reduced pressure[30]. The leaf and bark extracts were stored in amber colored containers until use.

Antibacterial activity of leaf and rhizome extracts

Inhibitory effect of leaf and rhizome extract was assessed by Agar well diffusion assay[30] against a panel of 15 bacteria which included 5 isolates of *Streptococcus mutans* (from dental caries), 5 isolates of *Staphylococcus aureus* (from burn) and 5 bacteria *viz., Staphylococcus aureus, Enterococcus faecalis, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Escherichia coli* isolated from urinary tract infection. 24 hours old Nutrient broth (HiMedia, Mumbai) cultures of test bacteria were inoculated on sterile Nutrient agar (HiMedia, Mumbai) using sterile cotton swabs. Wells of 6mm diameter were punched in the inoculated plates using sterile cork borer. 100µl leaf and rhizome extract (20mg/ml of 25% Dimethyl sulfoxide (DMSO; HiMedia, Mumbai)), Chloramphenicol (1mg/ml of sterile water) and DMSO (25% in sterile water) were transferred into labeled wells. The plates were incubated at 37◦C for 24 hours in upright position. Zones of inhibition were measured using a ruler.

Antifungal activity of leaf and rhizome extracts

Antifungal effect of extracts was evaluated against *Candida albicans* and *Cryptococcus neoformans* by Agar well diffusion assay[30]. In brief, 48 hours old Sabouraud Dextrose broth (HiMedia, Mumbai) cultures of test fungi were

swabbed on sterile Sabouraud Dextrose agar (HiMedia, Mumbai) using sterile cotton swabs followed by punching wells of 6mm diameter using sterile cork borer. 100µl leaf and rhizome extract (20mg/ml of 25% Dimethyl sulfoxide (DMSO; HiMedia, Mumbai)), Fluconazole (1mg/ml of sterile water) and DMSO (25% in sterile water) were transferred into labeled wells. The plates were incubated at 37°C for 48 hours in upright position and the zones of inhibition were measured using a ruler.

Radical scavenging activity of leaf and rhizome extracts

In order to determine radical scavenging effect of extracts, we employed DPPH free radical scavenging assay[31]. Here, 2ml of different concentrations of leaf and rhizome extracts (0-100 µg/ml) were mixed with 2ml of DPPH solution (0.002% in methanol) in separate tubes. The tubes were incubated in dark for 30 minutes at room temperature. The absorbance of each tube was measured at 517nm in a UV-Vis spectrophotometer. The absorbance of control (2ml of DPPH+2ml of methanol) was also noted. Ascorbic acid was used as reference standard. The radical scavenging activity of each concentration of leaf and rhizome extract was calculated using the formula:

Radical scavenging activity (%) = (Ac-At/Ac) x 100, where Ac is absorbance of control and At is the absorbance of extract/standard. The concentration of extract required to scavenge 50% of free radicals (IC_{50}) was calculated.

Total phenolic content of leaf and rhizome extracts

The content of total phenolics in leaf and rhizome extracts was estimated using Folin-Ciocalteau reagent (FCR) method[32]. A dilute concentration of each extract (0.5ml) was mixed with 0.5ml of F-C reagent (1:1) and 2ml of sodium carbonate (7%) in clean and labeled tubes. The tubes were allowed to stand for 30 minutes and the optical density was measured at 765nm using a UV-Vis spectrophotometer. A standard curve was plotted using different concentrations of Gallic acid (standard, 0-1000µg/ml) and the TPC of extracts was expressed as µg Gallic acid equivalents (GAE) from the graph.

Total flavonoid content of leaf and rhizome extracts

Aluminium chloride colorimetric method was employed to estimate total flavonoid content of extracts. Here, a dilute concentration of extract (0.5ml) was mixed with 0.5ml of methanol, 4ml of water and 0.3ml of $NaNO_2$ (5%) and incubated for 5 minutes at room temperature. After incubation, 0.3ml of $AlCl_3$ (10%) was added and the tubes were again incubated at room temperature for 6 minutes. Later, 2ml of 1M NaOH and 2.4ml of distilled water were added and the absorbance was read at 510nm using UV-Vis spectrophotometer. A calibration curve was constructed using different concentrations of Catechin (0-120µg/ml) and the flavonoid content was expressed as µg Catechin equivalents (CE) from the graph[33].

Results

The result of inhibitory effect of leaf and rhizome extract against clinical isolates of burn (Sa-01 to Sa-05), dental caries (Sm-01 to Sm-05) and urinary tract infection is shown in table 1. The extracts were effective against all test bacteria. The inhibitory effect was marked in case of rhizome

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extract when compared to leaf extract. Overall, inhibitory effect of rhizome extract was marked against burn (zone of inhibition 1.6-1.9cm) and dental caries (zone of inhibition 1.7-2.0cm) isolates when compared to urinary tract isolates (zone of inhibition 1.4-1.7cm). In case of urinary tract isolates, inhibitory effect was marked against Gram positive bacteria than Gram negative bacteria. Reference antibiotic caused higher inhibition of test bacteria when compared to extracts. DMSO did not cause inhibition of any test bacteria.

Table 1: Antibacterial activity of leaf and rhizome extract

Test bacteria	Zone of inhibition in cm		
	Rhizome extract	Leaf extract	Antibiotic
Sm-01	1.7±0.1	1.2±0.1	2.9±0.2
Sm-02	2.0±0.2	1.5±0.1	3.5±0.2
Sm-03	1.7±0.1	1.3±0.0	2.8±0.2
Sm-04	2.0±0.1	1.2±0.0	3.6±0.1
Sm-05	1.9±0.2	1.4±0.1	3.3±0.0
Sa-01	1.9±0.1	1.3±0.0	3.3±0.1
Sa-02	1.6±0.1	1.0±0.0	2.6±0.2
Sa-03	1.6±0.1	1.3±0.1	2.4±0.1
Sa-04	1.7±0.2	1.4±0.0	2.7±0.1
Sa-05	1.7±0.0	1.1±0.1	3.1±0.2
E. coli	1.5±0.1	1.1±0.0	2.5±0.0
S. aureus	1.7±0.1	1.3±0.1	3.5±0.2
E. faecalis	1.6±0.0	1.2±0.0	3.5±0.2
P. aeruginosa	1.4±0.0	1.2±0.0	2.5±0.0
K. pneumoniae	1.4±0.0	1.0±0.0	2.3±0.0

Table 2 shows the result of antifungal effect of extracts. The extracts were found inhibitory against both fungi. Susceptibility was higher in case of *C. neoformans* when compared to *C. albicans*. Rhizome extract was more inhibitory than leaf extract. Reference antibiotic caused higher inhibition of test fungi than extracts. DMSO did not cause inhibition of fungi.

Table 2: Antifungal activity of leaf and rhizome extract

Test fungi	Zone of inhibition in cm		
	Rhizome extract	Leaf extract	Antibiotic
C. albicans	1.9±0.1	1.4±0.2	3.6±0.1
C. neoformans	2.1±0.1	1.7±0.1	3.9±0.1

The radical scavenging nature of leaf and rhizome extract of *A. galanga* was assessed by DPPH radical scavenging assay. The extracts exhibited dose dependent scavenging of free radicals. The rhizome extract (IC_{50} 32.34µg/ml) scavenged free radicals more effectively when compared to leaf extract (IC_{50} 62.09µg/ml). Reference antioxidant ascorbic acid scavenged radicals to higher extent with an IC_{50} value of 2.56µg/ml when compared to leaf and rhizome extract (Figure 1).

Figure 1: DPPH radical scavenging activity of leaf and rhizome extract

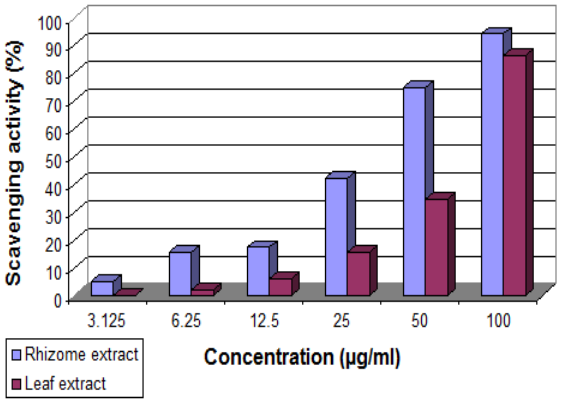

Table 3 shows the content of phenolics and flavonoids in leaf and rhizome extracts. Content of total phenolics as well as total flavonoids were high in rhizome extract when compared to leaf extract.

Table 3: Total phenolic and flavonoid content in leaf and rhizome extracts

Extract	TPC (µg GAE/mg)	TFC (µg CE/mg)
Leaf	41.67±2.98	18.06±2.08
Rhizome	75.14±3.41	32.26±3.12

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Discussion

Infectious diseases, caused by bacteria, fungi, viruses and parasites, are due to a complex interaction between pathogen, host and the environment. These diseases have devastated mankind in early periods. The developments in the field of chemotherapy especially the discovery of antibiotics is considered as one of the important milestones in medical field. The use of these wonder drugs resulted in eradication of infections that once challenged mankind. However, the therapy of infections using these antibiotics is going through a crisis because of development of resistance in pathogens against antibiotics. A number of pathogens such as *S. aureus*, *E. coli*, *P. aeruginosa*, vancomycin resistant enterococci, *M. tuberculosis*, *C. albicans*, *C. neoformans* etc have developed resistance against widely used antibiotics. Above all, these organisms have the ability to transmit or acquire the resistance. The infections caused by these antibiotic resistant bacteria are difficult to treat[34-38]. More than 80% of world's population depends on traditional medicine for primary healthcare needs. Plants have been used to treat infections long before the discovery of antibiotics. They contain a number of secondary metabolites, in particular phenolic compounds, having therapeutic value. Traditional healers (herbal healers) from different parts of the world use many plants as anti-infective agents. Plant based medicines are cost effective and generally lack side effects which are associated with the use of antibiotics[39,40]. In this study, the rhizome extract of *A. galanga* exhibited stronger inhibitory potential when compared to leaf extract against clinical isolates of bacteria. The essential oil of rhizome showed inhibitory activity against food borne bacteria[1,24]. The ethanolic extract of rhizome was shown to possess anti-mycobacterial activity[14]. The flower extract was shown to possess inhibitory effect against *Listeria monocytogenes* and *S. aureus* in a ready-to-eat Turkey ham product[28].

The free radicals are produced in the cells through aerobic respiration and on exposure to external factors. These free radicals exist in different forms such as superoxide, hydroxyl, hydroperoxyl, peroxyl and alkoxyl radicals. Normally, the natural antioxidant defense system of the body i.e., antioxidant enzymes such as catalase, superoxide dismutase and peroxidase and small molecules such as ascorbic acid, vitamin E etc., in healthy persons remove these free radicals. However, when the balance between free radical production and the antioxidant defense is disturbed, overproduction of free radicals occurs and it leads to a situation called oxidative stress which is implicated in hundreds of disorders such as cancer, aging, cardiovascular diseases, neurological disorders etc. In oxidative stress, damage to biomolecules such as DNA and proteins occurs mainly because of overproduction of free radicals. In such situations, antioxidants in particular dietary antioxidants are helpful. Hence, it is important to consume food rich in antioxidants, such as fruits and vegetables[41-43].

A number of *in vitro* assays are used to determine radical scavenging nature of various kinds of samples including plant extracts. One of the most popular assays is DPPH free radical scavenging assay. This assay is simple, sensitive, rapid and easy to perform and hence used widely to determine radical scavenging effect of samples. DPPH is a stable, organic, nitrogen centred free radical having absorption maxima at around 515-520nm in alcoholic solution. On reduction, the purple color of the radical is converted to yellow color

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according to the number of electrons paired. The result is usually expressed as IC_{50} value which is defined as the concentration of sample that causes a 50% decrease in the DPPH absorbance[44-47]. In the present study, we determined radical scavenging nature of leaf and rhizome extract by DPPH assay and found a dose dependent scavenging activity by the extracts. Rhizome extract scavenged DPPH radicals to higher extent as revealed by low IC_{50} value. Although radical scavenging potential of extracts was lesser than that of ascorbic acid, it is evident that the extracts possess hydrogen donating ability and therefore the extracts could serve as free radical scavengers, acting possibly as primary antioxidants[48]. The essential oil of rhizome showed marked antioxidant activity[23]. The ethanol extract of rhizome was shown to possess DPPH radical scavenging activity[15].

The term 'phenolic' refers to substances possessing one or more hydroxyl groups bonded onto an aromatic ring. The compounds containing several or many aromatic rings and hydroxyl substituents are often referred to as polyphenols. The polyphenolic compounds including flavonoids are widely distributed in plant kingdom. They are responsible for UV protection, protection against pathogens, color, taste etc. They are present in almost all parts of both edible and non-edible plants and are known to exert a variety of biological activities including antioxidant activity. The antioxidant action of the phenolic constituents is strongly associated with their structure, such as conjugated double bonds and number of hydroxyls in the aromatic ring structure[49,50]. FCR and aluminium chloride colorimetric estimation methods are commonly used for estimation of total phenolic compounds and total flavonoids respectively[33,41,45,51]. In the present study, the total phenolic and flavonoid contents were highest in rhizome extract than leaf extract. Many studies revealed a direct correlation between the content of total phenolics and flavonoids and the antioxidant activity[31-33,52-54]. In our study also, the rhizome extract possessing high total phenolic and flavonoid content exhibited marked radical scavenging activity when compared with leaf extract.

Conclusion

A marked antimicrobial and radical scavenging potential of leaf and rhizome extract of *A. galanga* was observed in this study. The high potential of rhizome extract could be ascribed to the presence of high phenolic and flavonoid content in it. The plant can be a potential source for the development of bioactive agents having therapeutic applications.

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