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Antimicrobial and antioxidant activity of the methanolic extract of *Alpinia purpurata* rhizomes

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Abstract

The present study was undertaken to determine antimicrobial and antioxidant activities of the methanolic extract of rhizomes of *Alpinia purpurata*. Antimicrobial activity was tested against *Escherichia coli* (MTCC 25922), *Enterococcus aerogenes* (MTCC 29212), *Pseudomonas aeruginosa* (MTCC 27853), *Staphylococcus aureus* (MTCC 25923) and *Proteus vulgaris* (MTCC 7299) by disc diffusion assay method. Antioxidant activity was determined by DPPH free radical scavenging assay. The rhizomes of *Alpinia purpurata* exhibited significant antioxidant inhibitory activities with an 93.82% and 88.56% respectively at the concentration 80 µg/ml and 100 µg/ml and well compared with standard ascorbic acid drug. As the concentration of rhizomes of *Alpinia purpurata* increased from 20-100 µg/ml, the inhibitory actions of the *Alpinia purpurata* increased towards all the strains used in this study. At concentration 100 µg/ml, the rhizomes of *Alpinia purpurata* exhibited the antimicrobial activity all the five bacteria and five fungal pathogens, but was more susceptible against *Escherichia coli* (20 mm), *Staphylococcus aureus* (19 mm) at concentration 100µg/ml., followed by the highest activity against *Aspergillus flavus* and *Candida albicans* (10 mm zone of inhibition) at 100 µg/ml, followed by the highest activity against *Aspergillus niger*, *Candida vulgaris* and *Candida tropicalis* (9 mm zone of inhibition). The result confirms that the rhizomes of *Alpinia purpurata* antibacterial and antifungal activity against the tested bacteria.

Keywords: Rhizomes, *Alpinia purpurata*, antioxidant, DPPH free radical scavenging assay, antimicrobial

Introduction

Medicinal plants are the backbone of traditional medicine and the antibacterial activity of plant extract is due to different chemical agent in the extract, which was classified as active antimicrobial compound (Kumar *et al.*, 2009; Ignacimuthu *et al.*, 2009; Doughari *et al.*, 2007; Adegoke and Adebayo-tayo, 2009) [1-4]. In recent years, pharmaceutical companies have been doing phytochemical research and investing billions of dollars in developing natural remedies to produce drugs in affordable price to general population (Doughari, 2006) [5]. The rising incidence in multidrug resistance amongst pathogenic microbes has further necessitated the need to search for fewer antibiotic sources from plants (Mohamed Khadeer Ahamed *et al.*, 2007) [6]. *Alpinia purpurata* (Vieill.) K. Schum (red ginger) is a herbaceous perennial plant, internationally known in the ornamental plant market as potted plant, landscape accent and cut flower (Moron, 1987) [7]. The rhizome has sharp odour, which could improve appetite, taste and voice. It is also used for headache, rheumatism, sore throat and renal disease (Prajapathi *et al.*, 2003) [8]. The plant possesses moderate antibacterial and anticancer activities, which may be due to the presence of secondary metabolites in the leaves of *A. purpurate* (Villaflores *et al.*, 2010) [9]. In addition to the proposed anti-inflammatory activity, its phytomedicinal potential to treat tuberculosis is also described (Raj *et al.*, 2012) [10]. The chemical constituents shows that the presence of volatile oil, chiefly sesquiterpene, hydrocarbons, sesquiterpene alcohols, gingerole., starch, tannins flavonoids like galangin (Cheah and Gan, 2010; Bisset and Wichtl, 2001; Altman and Marcussen, 2001; Deepti *et al.*, 2012) [11-13]. Therefore, in the present study, the antimicrobial and antioxidant activities of the methanolic extract of rhizomes of *Alpinia purpurata* were evaluated employing *in vitro* assay methods.

Materials and methods**Collection of plant material**

The rhizomes of *Alpinia purpurata* were collected in the month of May from the mullipatti, pudukkottai, Tamil Nadu, India. The plant was identified and rhizomes of *Alpinia purpurata* were authenticated and confirmed from Dr. S. John Britto, Director, Rapinat herbarium, St. Joseph College, Tiruchirapalli, and Tamil Nadu for identifying the plants. The voucher specimen number SGP001 (7.07.2019).

Chemicals and reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid and acarbose were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Soluble starch, sodium potassium tartarate, sodium dihydrogen phosphate (NaH₂PO₄), Di-sodium hydrogen phosphate (Na₂HPO₄) sodium chloride, sodium hydroxide, potassium ferricyanide, ferric chloride (FeCl₃) were from Merck Chemical Supplies (Damstadt, Germany). All the chemicals used including the solvents, were of analytical grade.

Collection of test organisms

To examine the antimicrobial activity of isolated compound, five strains [*Escherichia coli* (MTCC 25922), *Enterococcus aerogenes* (MTCC 29212), *Pseudomonas aeruginosa* (MTCC 27853), *Staphylococcus aureus* (MTCC 25923) and *Proteus vulgaris* (MTCC 7299)] were prepared as test organisms. The clinical fungal test organisms used for study are *Candida albicans* (MTCC 282), *Candida tropicalis* (MTCC No.184) *Aspergillus niger*, (MTCC 227), *Aspergillus clavatus* (MTCC 1323) and *Aspergillus flavus* (MTCC-3396). All the strains were procured from the Microbial Type Culture and Collection (MTCC) at Chandigarh, India.

Preparation of methanol extracts

The methanolic extract of rhizomes of *Alpinia purpurata* were washed in running water, cut into small pieces and then shade dried for a week at 35-40°C, after which it was grinded to a uniform powder of 40 mesh size. The methanol extracts were prepared by soaking 100 g each of the dried powder plant materials in 1 L of methanol using a soxhlet extractor continuously for 10 hr. The extracts were filtered through whatmann filter paper No. 42 (125mm) to remove all unextractable matter, including cellular materials and other constitutions that are insoluble in the extraction solvent. The entire extracts were concentrated to dryness using a rotary evaporator under reduced pressure. The final dried samples were stored in labeled sterile bottles and kept at -20°C. The filtrate obtained was used as sample solution for the further isolation (Deepti *et al.*, 2015) [14].

Antioxidant activity (DPPH free radical scavenging activity) determination

The antioxidant activity of the methanolic extract of rhizomes of *Alpinia purpurata* was examined on the basis of the scavenging effect on the stable DPPH free radical activity (Braca *et al.*, 2002) [15]. Ethanolic solution of DPPH (0.05 mM) (300 µl) was added to 40 µl of methanolic extract of rhizomes of *Alpinia purpurata* with different concentrations (20 - 100 µg/ml). DPPH solution was freshly prepared and kept in the dark at 4°C. Ethanol 96% (2.7 ml) was added and the mixture was shaken vigorously. The mixture was left to stand for 5 min and absorbance was measured spectrophotometrically at 540 nm. Ethanol was used to set the absorbance zero. A blank sample containing the same amount of ethanol and DPPH was also prepared. All determinations were performed in triplicate. The radical scavenging activities of the tested samples, expressed as percentage of inhibition were calculated according to the following equation (McCue and Shetty, 2004) [16].

Percent (%) inhibition of DPPH activity = [(A - B) / A] x 100
Where B and A are the absorbance values of the test and of the blank sample, respectively. A percent inhibition versus concentration curve was plotted and the concentration of sample required for 50% inhibition was determined.

Antibacterial activity of methanolic extract of rhizomes of *Alpinia purpurata* (disc diffusion method)

Antibacterial activity of crude methanolic extract was determined using the disc diffusion method. The petridishes (diameter 60 mm) was prepared with Muller Hinton Agar and inoculated with test organisms. Sterile disc of six millimeter width were impregnated with 10 µl of crude methanolic extract at various concentrations of 20-100 µg/ml respectively. Prepared discs were placed onto the top layer of the agar plates and left for 30 minute at room temperature for compound diffusion. Negative control was prepared using the respective solvent. The dishes were incubated for 24 h at 37°C and the zone of inhibition was recorded in millimeters and the experiment was repeated twice (Karumaran *et al.*, 2016) [17].

Determination of antifungal activity of methanolic extract of rhizomes of *Alpinia purpurata*

Antifungal activity of crude extracts was determined using the disc diffusion method The petridishes (diameter 60 mm) was prepared with Sabouraud's dextrose agar (SDA) and inoculated with test organisms. Sterile disc of six millimeter width were impregnated with 10 µl of crude extract at various concentrations of 20-100 µg/ml respectively. Prepared discs were placed onto the top layer of the agar plates and left for 30 minute at room temperature for compound diffusion. The dishes were incubated for 24 h at 37°C and the zone of inhibition was recorded in millimetres (Vivek *et al.*, 2013) [18].

Statistical analysis

All assays were conducted in triplicate. Statistical analyses were performed with SPSS 16.0 for an analysis of variance (ANOVA) followed by Duncan's test. Differences at P < 0.05 were considered to be significant.

Results and Discussion

Antioxidant activity of methanolic extract of rhizomes of *Alpinia purpurata* by DPPH method

The result showed that the compound had better percentage antioxidant activities at high concentrations when compared with ascorbic acid (Table 1). The compound showed 93.82 % activity at 100 µg/ml while ascorbic acid gave 95.79 % at the same concentration (fig. 1). The previous study suggested that the *Alpinia galanga* has antioxidant properties by scavenging free radicals, decreasing lipid peroxidation and increasing the endogenous blood antioxidant enzymes levels (Michel K Tchimine *et al.*, 2016) [19].

Table 1: Antioxidant activity of methanolic extract of rhizomes of *Alpinia purpurata* by DPPH method and comparison with standard drug ascorbic acid.

S. No	Concentrations	Scavenging Effect (%)	
		methanolic extract of rhizomes of <i>Alpinia purpurata</i>	Ascorbic acid
1	20 (µg/ml)	64.87±1.49	70.50±1.33
2	40 (µg/ml)	71.01±1.26	74.35±1.37
3	60 (µg/ml)	85.05±1.42	86.73±1.42
4	80 (µg/ml)	88.56±1.44	85.24±1.47
5	100 (µg/ml)	93.82±1.28	95.79±1.50

Note: Each value was obtained by calculating the average of three experiments and data are presented as mean± SEM

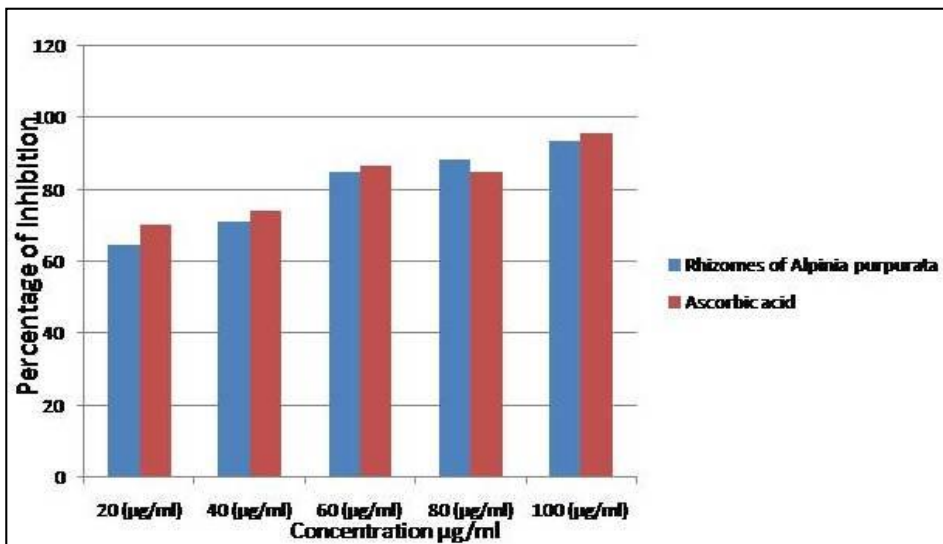


Fig 1: Antioxidant activity of methanolic extract of rhizomes of *Alpinia purpurata* by DPPH activity

Antibacterial activity of methanolic extract of rhizomes of *Alpinia purpurata* by disc diffusion assay method

The results of the antibacterial activity of methanolic extract of rhizomes of *Alpinia purpurata* were tested against pathogens by disk diffusion method are shown in (Table 2). The rhizomes of *Alpinia purpurata* showed growth inhibitory activity against *Escherichia coli* (20 mm), *Staphylococcus aureus* (19 mm) at concentration 100µg/ml. At concentration 80 µg/ml, the extracts exhibited the antibacterial activity all the five bacteria, but was more susceptible against *Escherichia coli* (18 mm),

Pseudomonas aeruginosa and *Proteus vulgaris* (17 mm). However, the extracts showed better inhibitory actions against pathogens at a concentration 60, 80 and 100 µg/ml than at lower concentration (fig 2). As the concentration of extracts increased from 20-100 µg/ml, the inhibitory actions of the plant extracts increased towards all the strains used in this study. Previous study suggested that the quercetin inhibited *S. aureus*, *P. aeruginosa* at concentration 20 mcg/mL while *P. vulgaris* and *E. coli* were inhibited at concentration 300 mcg/mL and 400 mcg/mL respectively (Renu Narendra *et al.*, 2017) [20].

Table 2: Antibacterial activity of methanolic extract of rhizomes of *Alpinia purpurata*

Plant extracts	Concentrations (µg/ml)	Organisms/Zone of inhibition (mm)				
		methanolic extract of rhizomes of <i>Alpinia purpurata</i>				
		<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Enterococcus aerogenes</i>	<i>Pseudomonas aeruginosa</i>	<i>Proteus vulgaris</i>
Extracts	20	10	11	10	12	11
	40	12	12	11	13	15
	60	13	13	13	13	17
	80	18	15	15	17	17
	100	20	19	17	18	18
Methanol	10 µl/disc	0	0	0	0	0

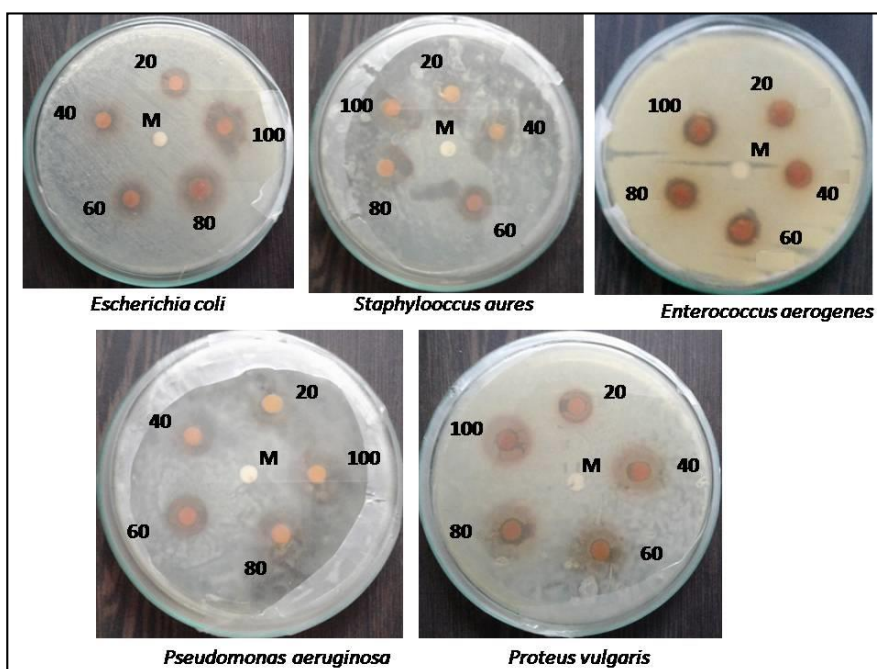


Fig 2: Antibacterial activity of methanolic extract of rhizomes of *Alpinia purpurata*

The antifungal susceptibility test of the different concentration of methanolic extract of rhizomes of *Alpinia purpurata* against the test organisms (table 3). From the result, the rhizomes of *Alpinia purpurata* were the most effective and the highest activity was demonstrated against *Aspergillus flavus* and *Candida albicans* (10 mm zone of inhibition) at 100 µg/ml, followed by the highest activity against *Aspergillus niger*, *Candida vulgaris* and *Candida tropicalis* (9 mm zone of inhibition) at 100 µg/ml (fig 3). At concentration 80 µg/ml, the

extracts exhibited the antifungal activity all the five bacteria, but was more susceptible against *Candida albicans*, *Candida vulgaris* and *Aspergillus flavus* (9 mm). However, the methanolic extract of rhizomes of *Alpinia purpurata* showed better inhibitory actions against pathogens at a concentration 60, 80 and 100 µg/ml than at lower concentration. As the concentration of extracts increased from 20-100 µg/ml, the inhibitory actions of the rhizomes of *Alpinia purpurata* increased towards all the strains used in this study.

Table 2: Antifungal activity of methanolic extract of rhizomes of *Alpinia purpurata*

Plant extracts	Concentrations (µg/ml)	Organisms/Zone of inhibition (mm)				
		methanolic extract of rhizomes of <i>Alpinia purpurata</i>				
		<i>Candida albicans</i>	<i>Candida vulgaris</i>	<i>Aspergillus flavus</i>	<i>Aspergillus niger</i>	<i>Candida tropicalis</i>
Extracts	20	5	6	6	6	6
	40	6	7	7	7	7
	60	8	8	8	7	7
	80	9	9	9	8	8
	100	10	9	10	9	9
Methanol	10 µl/disc	0	0	0	0	0

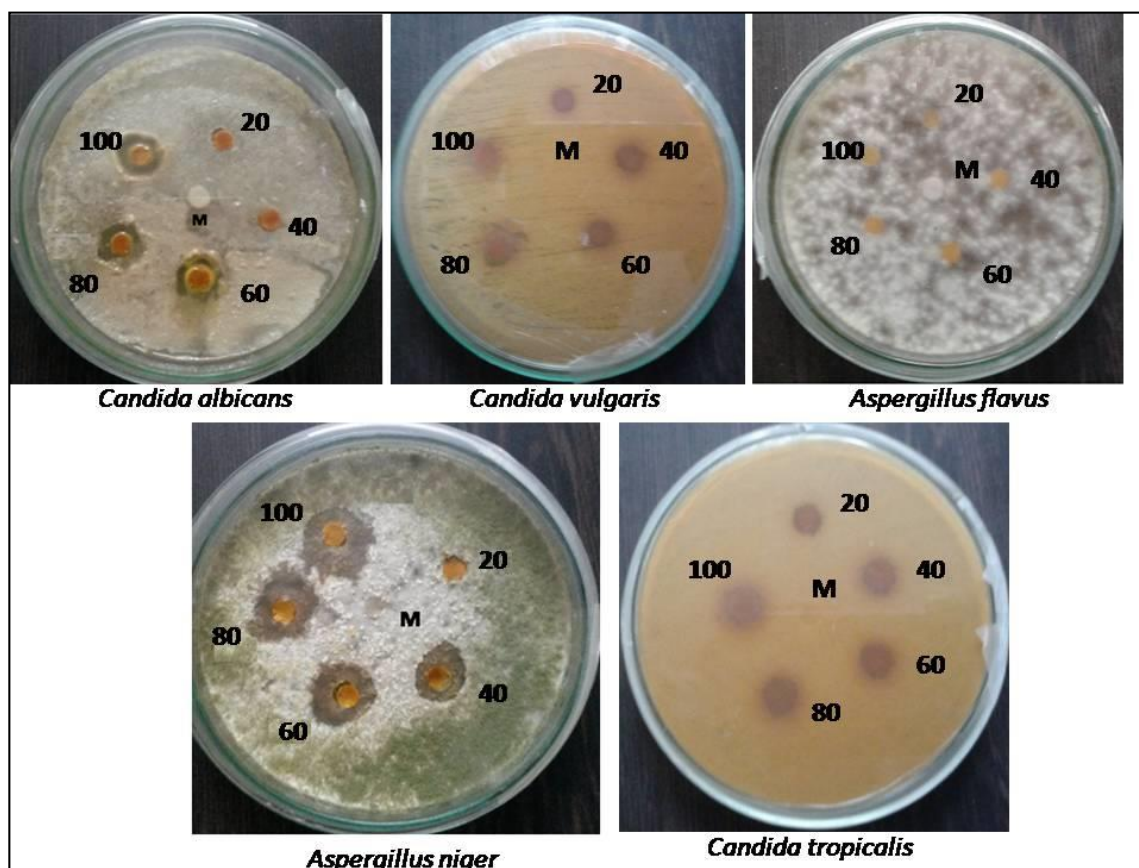


Fig 3: Antifungal activity of methanolic extract of rhizomes of *Alpinia purpurata*

Conclusion

These results suggest that the methanolic extract of rhizomes of *Alpinia purpurata* have good antibacterial and antifungal activity against selected pathogens. The rhizomes of *Alpinia purpurata* showed increased antioxidant activity with an increase in the treated concentrations. The plants can be used as potential source for the development of antimicrobial and antioxidant agents.

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Conflicts of Interests

The authors declare that they have no conflict of interest. It has not been published elsewhere. That it has not been simultaneously submitted for publication elsewhere. All authors agree to the submission to the journal.

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