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Phytochemical and antimicrobial evaluation of methanolic extracts of selected Zingiberaceae taxa from Peren district, Nagaland, Northeast India

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Abstract

The methanolic extract of ten Zingiberaceae taxa were evaluated for their phytochemical constituents, total phenolic and flavonoid contents along with *in-vitro* antioxidant and antimicrobial activities. Among the extracts, *Kaempferia parviflora* and *Zingiber montanum* were the most potent reducing agents, while *Zingiber officinale* exhibited strong free radical-scavenging activity. The high phenolic content observed in *Z. montanum*, *Z. officinale* and *Curcuma longa* may be attributed to the strong antioxidant activity. Furthermore, the extracts of *C. longa*, *K. parviflora* and *Z. montanum* effectively inhibite the growth of test pathogenic microbes. This study suggest that the ginger species are promising source of natural of antioxidant with strong antimicrobial properties.

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Introduction

Antioxidants are compounds which potentially neutralize the free radicals (1). Free radicals are produced by oxidation of food and chemical by a living system. Free radical-induced oxidative damage to biomolecules can cause cancer, aging, cardiovascular, inflammatory and neurodegenerative diseases (2). Phenolic constituents are ubiquitous in plants which act as antioxidants and effectively prevents oxidation process at the cellular and physiological levels (3). Spices and herbs are rich sources of phenolic compounds exhibiting high antioxidant activity (4). Synthetic antioxidants like butylated hydroxy toluene (BHT) and butylated hydroxy anisole (BHA) are the most commonly used, but their application in food products have been reduced due to and carcinogenic property. Hence, the naturally occurring antioxidant compound could be beneficial in the prevention and treatment of major diseases to promote human health (5).

Microbes are continuously evolving and become resistant to antibiotics used over a period. The resistant microorganisms cause infections that are more difficult to treat. Antibiotics resistance is a major threat faced by public health globally (6). Thus to tackle this problem, large numbers of broad-spectrum antibiotics need to be developed. With the inefficiency of the use of conventional antibiotics, the study of phytochemicals as antimicrobial agents has become popular (7). Use of traditional antibiotics is recognized because of their capacity to inhibit the growth through the inhibition of bacterial functions such as synthesis of the cell wall, DNA replication, RNA transcription and protein synthesis responsible for cell growth (8). According to WHO, medicinal plants will play a major role in obtaining a broad and new range of drugs with antimicrobial properties. The medicinal properties of plants are evaluated in the light of recent scientific developments due to their efficient pharmacological activities, low toxicity and economic viability (9).

Zingiberaceae belongs to one of the ten largest monocotyledonous families found in India. Of its 52 genera and 1400 species, the highest concentration is in the Indo-Malay-

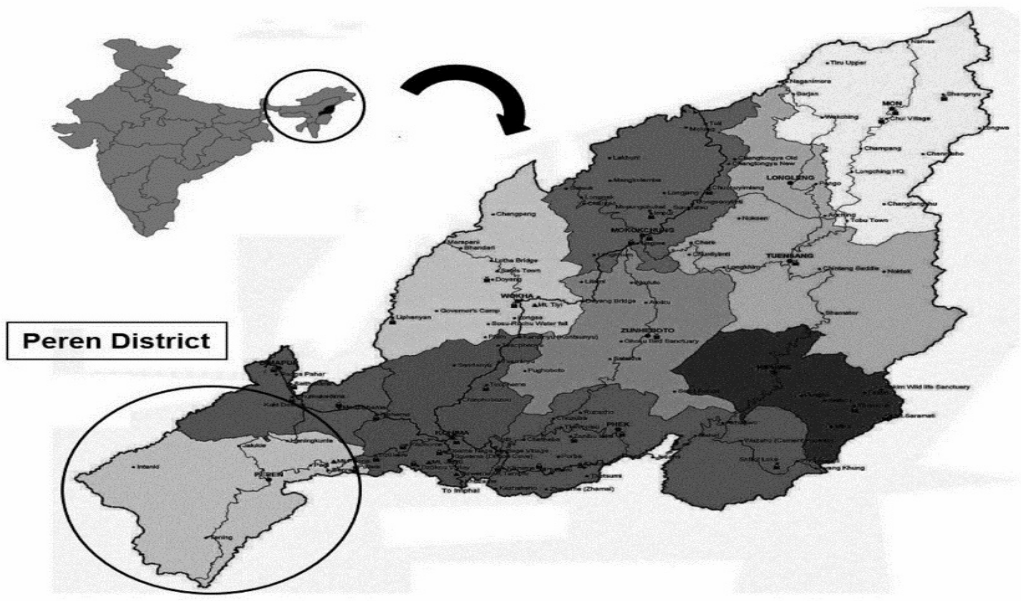

Figure 1. Geographical location of the study area.

an region of Asia; among these, 178 species of 22 genera are found alone in India (10). It is an important family with high medicinal properties and economic value. Although the members of Zingiberaceae have been used in traditional medicine, scientific evidence related to active principle largely needs to be evaluated. It is important to build the scientific basis for their therapeutic properties as these may serve as the source for the development of the potent drugs. The present study attempts to investigate the phytochemical and pharmacological properties of the selected Zingiberaceae members. The total phenolic and flavonoid content of the methanolic extracts of the rhizomes were determined and furthermore evaluated for their potential *in-vitro* antioxidant and antimicrobial activities.

Materials and Methods

Study area

Peren is also known as the green district of Nagaland, located at 25°31'00-26°52'00N latitude and 93°39'00-93°66'00E longitude (**Fig. 1**), and mainly inhabited by Zeliang tribe. The temperature ranges from 2-35°C, with an average rainfall of 2000 mm and humidity ranging from 25-98%. Despite its small geographic area (2300 sq km), the forest biome stretches from temperate to evergreen. The terrain is mountainous with altitude varying from 800-2500 m. The region is adorned with a rich diversity of flora and fauna, most of which are endemic and falls under one of the 35 biodiversity hotspots.

Plant materials collection and extraction

Plant specimen were obtained from Peren district, Nagaland, Northeast India during 2013-2015, with the help of local people and assigned voucher numbers. The plant materials were identified correctly using recent monographs and e-floras and deposited at the University of Hyderabad herbarium (UH). These plant taxa selected for detailed study are with the voucher numbers as of *Curcuma aeruginosa* (KJ-2025), *C. amada* (KJ-2022), *C. angustifolia* (KJ-2028), *C. aromatica* (KJ-2020), *C. caesia* (KJ-2026), *C. longa* (KJ-2030), *C. zedoaria* (KJ-2033), *Kaempferia parviflora* (KJ-2035), *Zingiber montanum* (KJ-202) and *Z. officinale* (KJ-2021). The rhizomes collected were washed thoroughly, sliced and shade dried at 25 °C followed by grinding using F5 anamill. The powder obtained was sieved with a 20-mesh sieve and stored at 4 °C for downstream experiments.

Air dried powdered samples (200 g) were extracted using Soxhlet's apparatus with n-hexane followed by methanol for 48 h. The methanol extract was concentrated under vacuum at 37 °C. The yield ranged between 6-20% of the dry weight. The methanol extracts were subjected to qualitative tests for the identification of various phytoconstituents and further screened for potential antioxidant and antimicrobial activities.

Preliminary phytochemical screening and physicochemical studies

Qualitative screening of phytochemical constituent of the plant extracts was performed using standard procedures as described by Harborne (11). Physicochemical properties such as moisture content, total ash, acid insoluble ash, water and alcohol soluble percentage were determined from the shade-dried powdered material based on Indian Pharmacopoeia and AOAC criteria (12,13).

Determination of total flavonoid and phenolic contents

The total flavonoid contents (TFC) of the extracts were evaluated using slightly modified aluminium chloride colorimetric method by Zhishen (14). Briefly, 0.10 ml of the methanolic extracts were incubated with 4 ml of deionized water and 0.3 ml of 5% $NaNO_2$ for 5 min followed by adding 3 ml of 1% $AlCl_3$ and 2 ml of 1.0 M NaOH solution. The final volume was made

up to 10 ml with deionized water and the absorbance was measured at 510 nm. The calibration curve was plotted with catechin as standard.

Total phenolic content (TPC) of the extract was estimated by Folin-Ciocalteu method with gallic acid as the reference compound (15). The reaction consisted of 0.10 ml of the extract, 0.5 ml of Folin-Ciocalteu reagent, 6 ml of deionized water and incubated for 1 min. To this, 2 ml of 15% $NaCO_3$ was added and made up to 10 ml with deionized water. The mixture was incubated for 2 h and the absorbance was taken at 750 nm.

DPPH (1,1- Diphenyl-2-Picryl Hydrazyl Radical) free radical scavenging activity

The methanol extracts were screened for their free radical scavenging activity following Shimada (16) with minor modifications. An aliquot of 1 ml of 0.1mM DPPH solution was added to 3 ml of the extract at different concentrations and incubated for 30 min at room temperature in the dark followed by recording the absorbance at 517 nm. Butylated hydroxytoluene (BHT) was used as a standard. Percentage inhibition was determined using the following formula:

% Inhibition = $[(A_{control}-A_{sample})/A_{control}]$ X 100, where $A_{control}$ is the absorption of the control and A_{sample} is the absorption of the test sample.

In-vitro anti-lipid peroxidation assay (ALP) in rat liver tissue homogenate

The *In-vitro* ALP of plant extracts were evaluated based on Ohkawa (17) with minor modifications. The rat liver homogenate was prepared in 0.15M KCl. The reaction mixture made up of 60 µl of liver homogenate, 100 µl of plant extract of different concentrations, 740 µl of 0.15M KCl and 100 µl of 1mM $FeCl_2$ was incubated at 37 °C for 30 min. The reaction was stopped by adding ice-cold 750µl TBARS (15% TCA, 0.25M HCl, 0.38% TBA in 1N NaOH) followed by boiling at 80 °C for 1 h and reading the absorbance at 532 nm. BHT was used as a standard. The percentage of ALP was calculated using the following formula:

ALP% = $[(A_{induced}-A_{sample})/A_{induced}- A_{control}]$ X 100, where $A_{induced}$ is the absorption of $FeCl_2$ induced, $A_{control}$ is the absorption of the control and A_{sample} is the absorption of the test sample.

Reducing power assay by ferricyanide method

The reducing activity of the extracts was screened according to Yen (18). The reaction mixture consisting of 2.5 ml of the extracts, 2.5 ml of 0.2M phosphate buffer (pH-6.6) and 2.5 ml of 1% potassium ferricyanide was incubated at 50 °C for 20 min. After incubation, 2.5 ml of 10% trichloroacetic acid (TCA) was added and spin at 3000 rpm for 10 min. The supernatant was mixed with deionized water and 0.1% ferric chloride in 1:1:1 ratio and the absorbance was measured at 700 nm. Ascorbic acid was used as the reference material. A higher absorbance indicates higher reducing power.

Nitric oxide scavenging activity

NO production from sodium nitroprusside was measured as per Sreejayan (19). Briefly, sodium nitroprusside 5mM in phosphate buffered saline (pH 7.4) was mixed with the extracts and incubated at 35 °C for 2 h followed by 0.5 ml of Griess reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride). The amount of NO produced by sodium nitroprusside was estimated by measuring nitrite accumulation at 546 nm. Alpha-tocopherol was used as reference compound.

Antimicrobial activity of the methanolic extracts

The microorganisms used included three ATCC bacterial strains of *Escherichia coli* (NA114) and multi-drug resistant *Staphylococcus aureus* (ATCC-29213), methicillin-resistant *Staphylococcus aureus* (ATCC-33591), clinical isolates of *Bacillus subtilis*, and fungal strains of *Candida tropicalis, Candida glabrata* and *Saccharomyces cerevisiae* were used for the susceptibility test. Mueller Hinton agar and Yeast extract peptone dextrose (YPD) agar were used for the study.

The *in-vitro* antimicrobial activity of the extracts was performed using Kirby-Bauer's disk diffusion technique that meets the standard national committee for clinical laboratory standards institute. An inoculum of each microbial culture to be tested was spread on agar plates and a sterilized disc of 8 mm diameter was impregnated into the plates. Different concentration of the extracts was pipetted to the discs and allowed to diffuse at room temperature. Discs with milliQ water served as negative controls while fosfomycin, imipenem and fluconazole were used as positive controls. The plates were incubated at 37 °C for 24 h for bacterial strains and 48 h at 35 °C for the fungal strain, and the activity was determined by measuring the diameter of inhibition zones (mm).

Determination of minimum inhibitory concentration (MIC)

MIC was performed in 96 well plates using MTT assay according to Andrews (20). Briefly, 100µl of the culture in LB broth for bacteria and YPD broth for fungal strains were seeded in a 96 well plate followed by 50 µl of different concentration of extracts and incubated for 24 h at 37 °C for bacterial strains and 72 h at room temperature for fungal strains. A 20 µl of MTT (3-[5,5-dimethylthyazol-2-yl-2,5-diphenyltetrazolium bromide) solution was added per well and incubated at 37 °C for 1 h. The medium was removed and 4 µl of DMSO per well was added. The plate was gently shaken and absorbance was measured at 600 nm. The lowest concentration of the extract with no turbidity was determined as the MIC value. Imipenem was used as reference antibiotic for the validation of results.

Statistical analysis

The data reported are an average of triplicate observations. Statistical comparison of means was conducted using the student t-test (GraphPad Prism 6).

Table 1. Physicochemical properties of the selected Zingiberaceae taxa (%) w/w [a]

Parameter	Moisture content	Total ash	Acid insoluble ash	Water soluble ash	Alcohol soluble extractive	Water soluble extractive
C. aeruginosa	10.05±0.05	15.45±0.02	2.67±0.08	11±0.08	5.53±0.02	19.68±0.02
C. amada	13.9±0.03	6.42±0.02	0.77±0.01	14.2±0.03	7.7±0.02	19.37±0.04
C. angustifolia	11.71±0.04	9.29±0.01	3.33±0.03	9.74±0.02	5.15±0.04	14.74±0.01
C. aromatica	10.66±0.04	11.08±0.02	1.96±0.06	9.39±0.04	4.93±0.07	15.90±0.02
C. caesia	5.47±0.01	11.77±0.04	4.37±0.01	10.38±0.04	6.07±0.01	12.32±0.03
C. longa	9.02±0.02	10.13±0.04	4.27±0.02	9.72±0.02	4.85±0.02	18.78±0.02
C. zedoaria	7.15±0.01	17.48±0.4	4.23±0.02	10.41±0.03	7.72±0.02	19.40±0.02
K. parviflora	7.75± 0.02	12.32±0.1	0.55±0.01	9.12±0.05	4.59±0.04	17.40±0.03
Z. montanum	8.02± 0.03	13.53±0.4	2.61±0.05	13.73±0.02	7.31±0.02	16.8±0.03
Z. officinale	9.31±0.05	11.82±0.7	1.96±0.02	11.92±0.03	6.09±0.01	18.07±0.02

[a]All values represents the mean ± SD (n=3)

Results

Preliminary phytochemical and physicochemical screening of the extracts

The results of preliminary phytochemical screening showed presences of secondary metabolites such as flavonoids, saponins, terpenoids, tannins, phenolic and reducing sugars in all the extracts. The physicochemical properties such as moisture content, total ash, acid insoluble ash, water soluble ash, alcohol soluble and water extractives were determined and the values were represented in **Table 1** where no significant difference was observed among the species.

Total phenolic and flavonoid contents

TPC and TFC of the methanolic extracts of ten species of Zingiberaceae are presented in **Fig. 2**.

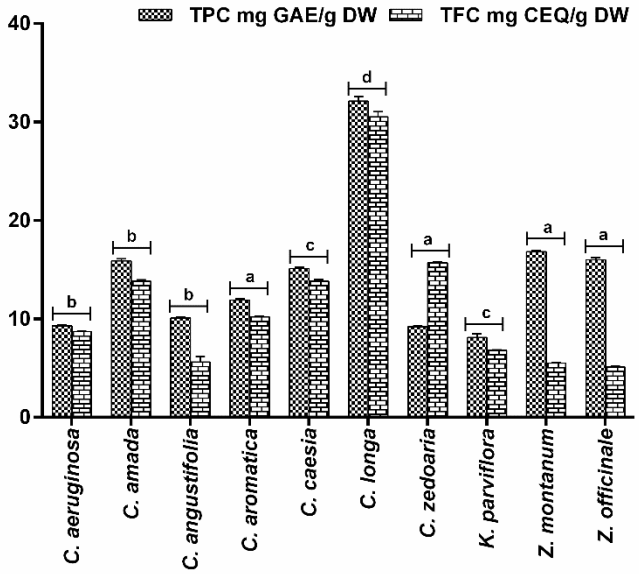

Figure 2. Total phenolic and flavonoid contents of rhizome. Different letter among different samples indicates significant differences ($p < 0.05$).

The total phenolic content estimated in terms of mg GAE/g dry weight while the total flavonoid content of the extracts was measured in terms of catechin equivalent as mg CEQ/g dry weight. The high content of TPC and TFC were reported in all the methanolic extracts.

Free radical scavenging by DPPH

The IC_{50} values of DPPH radical scavenging activity of the methanolic extract of the ginger species determine the hydrogen donating capabilities (**Fig. 3a**). All the extract exhibited strong free radical scavenging ability. The anti-lipid peroxidation ability of the extracts is in the order *Z. officinale* > *C. longa* > *C. aeruginosa* > *Z. montanum* > *C. amada* > *C. zedoaria* > *C. angustifolia* > *C. caesia* > *C. aromatica* > *K. parviflora* with an IC_{50} (µg/ml) of 16.44, 17.26, 19.04, 21.05, 21.20, 24.61, 25.59, 26.68, 30.56 and 35.15 respectively. BHT exhibited an IC_{50} value of 16.13 µg/ml. A positive correlation was observed between the free radical scavenging activity and the phenolic contents of extracts. The results of Student's t-test showed $p < 0.05$ indicated that readings are significant.

Anti-lipid peroxidation activity

The anti-lipid peroxidation property of the extracts was presented in **Fig. 3b**. The result showed that all the samples are dose-dependent. The anti-lipid peroxidation ability of the extracts is in the order *C. aeruginosa* > *Z. montanum* > *C. aromatica* > *C. longa* > *C. amada* > *Z. officinale* > *C. angustifolia* > *C. caesia* > *K. parviflora* > *C. zedoaria* with an IC_{50} (µg/ml) of 32.01, 34.89, 37.92, 38.24, 41.01, 41.86, 48.72, 49.89, 53.25 and 56.77 respectively. The reference compound BHT exhibited an IC_{50} value of 33.01 µg/ml. The results of Student's t-test showed $p < 0.03$ indicated that readings are significant.

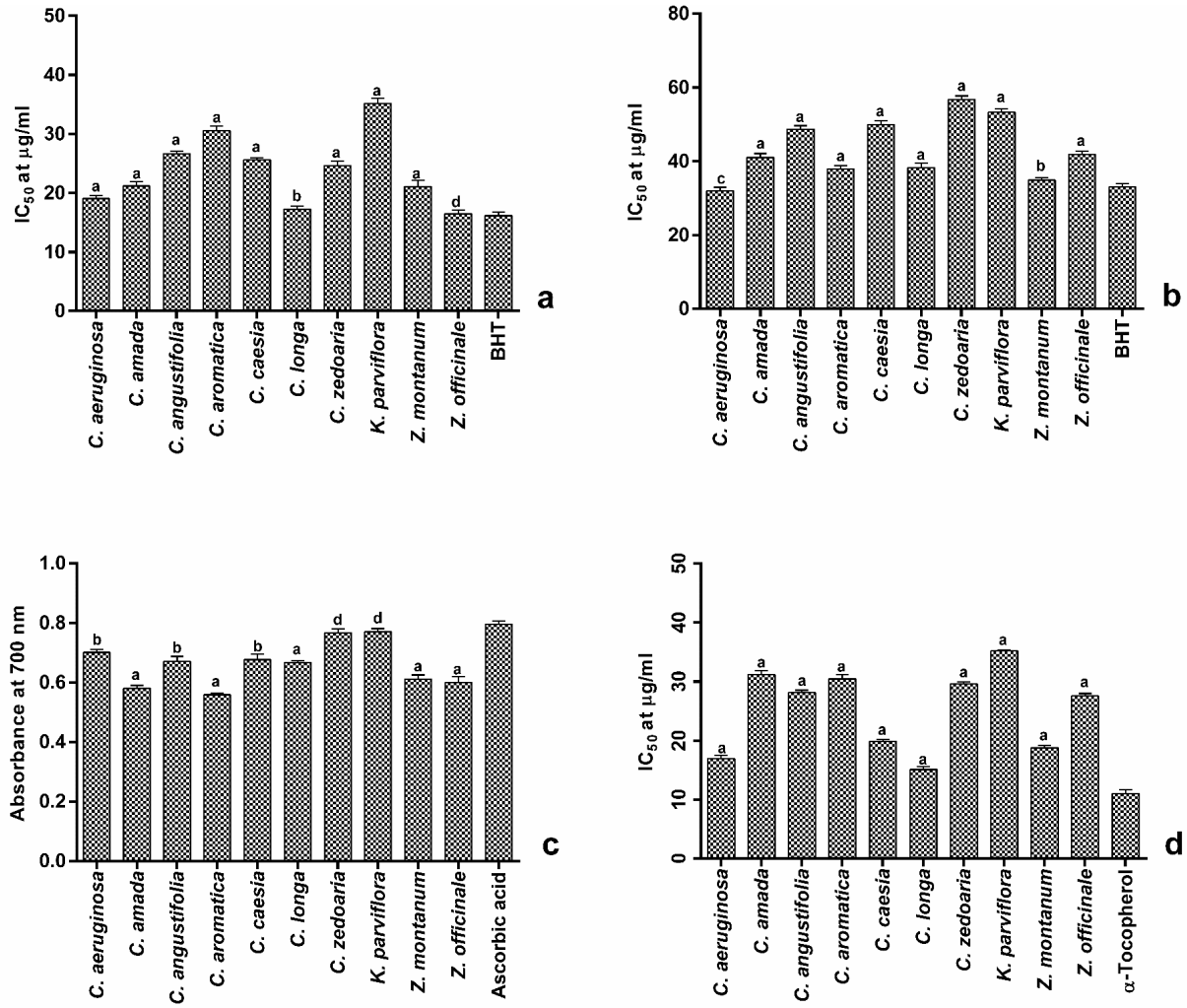

Figure 3. Antioxidant assays of the methanolic extract of the rhizomes. a- DPPH scavenging activity; b- Anti-lipid peroxidation activity; c- Reducing power assay; and d- Nitric oxide scavenging ability. All values are Mean ± SD (n=3) and different letter among different samples indicates significant differences ($p < 0.05$).

Reducing power ability

The reducing power of sample extracts and ascorbic acid at 100µg/ml as indicated in **Fig. 3c**. The extracts have shown dose-dependent reducing power. The reducing power ability of the extracts is in the order *C. aromatica* > *C. amada* > *Z. officinale* > *Z. montanum* > *C. longa* > *C. angustifolia* > *C. caesia* > *C. aeruginosa* > *C. zedoaria* > *K. parviflora* with an IC_{50} (µg/ml) of 0.558, 0.580, 0.599, 0.611, 0.667, 0.671, 0.676, 0.701, 0.767 and 0.770 respectively. Ascorbic acid reported an IC_{50} of 0.796 µg/ml. The results of Student's t-test showed $p < 0.04$ indicated that readings are significant.

Nitric oxide scavenging activity

The nitric oxide scavenging ability by various extracts and standard alpha-tocopherol are presented in **Fig. 3d**. The scavenging ability of the nitric oxide radical by the extracts is in the order *C. longa* > *C. aeruginosa* > *Z. montanum* > *C. caesia* > *Z. officinale* > *C. angustifolia* > *C. zedoaria* > *C. aromatica* > *C. amada* > *K. parviflora* with an IC_{50} (µg/ml) of 15.06, 16.92, 18.77, 19.86, 27.52, 28.12, 29.61, 30.42, 31.20 and 35.15 respectively. Alpha-tocopherol exhibited an IC_{50} of 11.02 µg/ml. The results of Student's t-test showed $p < 0.05$ indicated that readings are significant.

Antimicrobial activity and minimum inhibitory concentration

The result of antimicrobial efficacy of the extracts against four bacterial and three fungal strains was determined by the diameter of inhibition zones and MIC values (**Table 2** & **Table 3**). The extracts of *C. caesia*, *K. parviflora* and *Z. montanum* showed potent antimicrobial activity against all the test pathogenic microbes screened, viz. *E. coli,* two strains of *S. aureus*, *B. subtilis, C. glabrata*, *C. tropicalis* and *S.cerevisiae*. *C. aeruginosa* exhibited the least inhibitory efficacy. The extracts showed good inhibitory activity towards *B. subtilis, C. tropicalis* and *S.cerevisiae*. *C. aeruginosa* and *C. angustifolia* were susceptible towards the fungal strains.

Discussion

Phytochemicals have been an interesting area as the supply of antioxidants from natural sources for promoting health, food preservation and flavoring and in cosmetics is safer when com-

Table 2. Antimicrobial susceptibility test of the methanolic extracts[a]

Sample	*E. coli* NA114	*B. subtilis* WT	*B. subtilis* WT	*S. aureus* ATCC-33591	*C. glabrata* WT	*C. tropicalis* WT	*S. cerevisiae* WT
C. aeruginosa	--	--	14 ± 0.4	--	--	--	--
C. amada	--	16 ± 0.5	--	16 ± 0.4	--	--	15 ± 0.4
C. angustifolia	--	16 ± 0.4	17 ± 0.4	--	--	--	--
C. aromatica	--	15 ± 0.5	--	--	--	17 ± 0.6	--
C. caesia	14 ± 0.5	14 ± 0.6	17 ± 0.8	20 ± 0.5	15 ± 0.3	20 ± 0.3	16 ± 0.5
C. longa	14 ± 0.6	15 ± 0.8	--	18 ± 0.6	14 ± 0.6	18 ± 0.6	19 ± 0.7
C. zedoaria	--	16 ± 0.8	15 ± 0.8	--	--	14 ± 0.8	15 ± 0.4
K. parviflora	15 ± 0.4	21 ± 0.3	17 ± 0.7	17 ± 0.9	22 ± 0.5	17 ± 0.6	21 ± 0.5
Z. montanum	16 ± 0.8	18 ± 0.5	16 ± 0.4	22 ± 0.5	18 ± 0.6	20 ± 0.4	22 ± 0.7
Z. officinale	15 ± 0.7	17 ± 0.7	--	16 ± 0.7	16 ± 0.9	18 ± 0.7	20 ± 0.6

[a]All values represents the mean ± SD (n=3); WT-Wild type strain. *Concentration of extract in 50 ppm. Inhibition zone in mm.

Table 3. MIC (ppm) for the methanolic extracts [a]

Sample	*E. coli* NA114	*B. subtilis* WT	*S. aureus* ATCC-29213	*S. aureus* ATCC-33591	*C. glabrata* WT	*C. tropicalis* WT	*S. cerevisiae* WT
C. caesia	180 ± 0.9	160 ± 0.2	--	--	130 ± 0.2	140 ± 0.3	110 ± 0.5
C. longa	160 ± 0.6	140 ± 0.1	--	--	90 ± 0.6	70 ± 0.6	80 ± 0.1
K. parviflora	140 ± 0.4	60 ± 0.5	80 ± 0.5	90 ± 0.8	60 ± 0.3	120 ± 0.6	--
Z. montanum	140 ± 0.8	60 ± 0.7	100 ± 0.8	110 ± 0.6	100 ± 0.7	80 ± 0.4	100 ± 0.9
Z. officinale	200 ± 0.7	120 ± 0.4	--	100 ± 0.3	120 ± 0.4	100 ± 0.7	110 ± 0.7

[a]Each value represents mean of three observations; WT- Wild Type strain.

pared to synthetics compounds (21). Qualitative studies of the methanolic extracts of ginger species were useful for preliminary phytochemical screening and provide an empirical basis for the development of new drug. Preliminary phytochemical studies showed presences of flavonoids, saponins, terpenoids, tannins, phenolic and reducing sugars in all the methanolic extracts. Alkaloids and glycosides were absent in *Z. montanum, Z. officinale* and *C. amada*. Mucilage and gums were absent in all the extracts. Highest total ash value was observed in *C. zedoaria* (17.48±0.04) followed by *C. aeruginosa* (15.45±0.02) and *Z. montanum* (13.53±0.04). The percentage of moisture content of the rhizomes ranges from 5.47±0.01 to 11.71±0.04 with the highest value found in *C. angustifolia* followed by *C. aromatica* and *C. aeruginosa*. The acid insoluble ash value of the rhizomes lies between 0.55±0.01 and 4.37±0.01 percent while the water-soluble ash content varies from 9.12±0.05 to 14.2±0.03 percent. The percentage of alcohol and water-soluble extractive content lie between 4.59±0.04 to 7.31±0.02 and 12±0.03 to 19.40±0.02 respectively.

Phenolic compounds are one of the most effective hydrogen donors, which make them excellent antioxidants (22). In the present study, we estimated the total phenolic and flavonoid contents of the methanolic extracts by Folin-Ciocalteu and aluminium chloride colorimetric method respectively. All the species exhibited high phenolic content ranging from 32.1±0.2 to 8.1±0.1 mg GAE/g dry weight. The phenolic content was highest in *C. longa* followed by *Z. montanum* and *Z. officinale*. TFC of the extracts in terms of catechin equivalent was between 30.5±0.09 and 3.6±0.05 mg CEQ/g dry weight. *C. longa* exhibits the highest flavonoid content followed by *C. amada* and *C. caesia*. The presences of high phenolic and flavonoid contents in Zingiberaceae corroborate the earlier studies in this regard (23,24).

DPPH free radical scavenging activity is one of the most extensively used antioxidant assays to evaluate the antioxidant potential of medicinal plants (25). The antioxidative activity is due to the presence of an antioxidant that can donate hydrogen atoms or electron which in return scavenge the free radicals. In the present study, we estimated the decrease in the DPPH absorption at 517nm in the presence of the test samples. Lower

the IC_{50} value, higher is the scavenging activity. The IC_{50} value of the radical scavenging activity ranged between 16.45 to 35.15 µg/ml. *Z. officinale* exhibited significant radical scavenging activity exhibiting an IC_{50} of 16.45 µg/ml which is very proximate to that of BHT (16.13 µg/ml). Thus, the DPPH scavenging capacity of the extracts is probably due to the cumulative effect of its potential antioxidant compounds along with the phenolic compound. The antioxidant activity of the extracts was also evaluated by anti-lipid peroxidation TBARS assay. Lipid peroxidation causes the destruction of membrane lipids leading to damage of cells and even tissues which cause diseases such as atherosclerosis, Parkinson's disease, liver and kidney damage, aging, asthma, and even cancer (26). *C. aeruginosa* showed highest anti-lipid oxidation with IC_{50} (µg/ml) followed by *Z. montanum* and *C. aromatica*.

In reducing power ability, the reduction of ferric cyanide complex to ferrous form through the donation of an electron indicates the presence of reductants in the test samples. The reducing potential of the extracts was determined by measuring the absorbance at 700 nm resulting in a blue complex in presences of ferric ions. An increase in absorbance with increasing concentrations indicated reducing potential of the extracts. Among the tested extracts, *K. parviflora* showed highest absorbance (0.784±0.001) which is close to that of ascorbic acid (0.796±0.006) followed by *C. zedoaria* (0.767±0.005) and *C. aeruginosa* (0.701±0.008). *C. aromatica* showed the lowest absorbance (0.558±0.009) suggesting that it has comparatively lower reducing power. The reducing ability increased with the increased concentration of the test compounds. In the present study, the reducing power determination of the extracts demonstrated good reductive capabilities.

The nitric oxide scavenging method is one of the widely used antioxidant determination assays which is used to estimate the antioxidant potential of a compound. The scavenging of nitric oxide radical depends on the nitric oxide generation from sodium nitroprusside which produces nitrite ions in presences of oxygen that can be determined with Griess reagent at 546 nm. The extracts showed concentration-dependent nitric oxide scavenging activity. The IC_{50} values ranged from 15.069 to 34.656 µg/ml were, *C. longa* exhibited the highest scavenging capacity followed by *C. aeruginosa* and *Z. montanum*. Thus, the high antioxidant capacity of the extracts may be attributed to the high phenolic content.

Oxidative stress inducers lead to significant increase in ROS generation, such as the hydroxyl, superoxide and nitric oxide radical. The methanolic extracts efficiently scavenge free radicals such as DPPH and NO radicals at the lower concentrations and also significantly inhibited the oxidation of lipid in TBARS assay. The extracts also showed strong ferric ion reducing capacity. These results offer scientific evidence for the use of Zingiberaceae members in the traditional system of medicine.

Increasing antimicrobial resistances has led to a search for new plants products with potential antimicrobial activity (27). In the present study, the methanolic extracts were screened for their antimicrobial potential against *E. coli, S. aureus*, *B. subtilis, C. glabrata*, *C. tropicalis* and *S.cerevisiae*. Among all the extracts, *C. caesia*, *K. parviflora* and *Z. montanum* exhibited potent inhibitory efficacy against all the test pathogenic microbes. The extracts of *C. longa* and *Z. officinale* showed an intermediate zone of inhibition towards *E. coli, B. subtilis* and *C. glabrata* while *C. caesia* inhibited all the strains. Extracts of *C. amada* and *C. aromatica* inhibited the growth of *B. subtilis* similarly *C. aeruginosa* showed a zone of inhibition towards multi-drug resistant (MDR) strain of *S. aureus only*. *C. angustifolia* and *C. zedoaria* extracts inhibited the growth of *B. subtilis* and MDR *S. aureus* to some extent. The resistance in gram-negative bacteria to the extracts may be due to the presence of different outer polysaccharides membrane. This outer membrane does not allow certain antibiotics from penetrating the cell, a probable reason why they are more resistant to antibiotics than the gram-positive. Extracts of *C. caesia*, *C. longa, K. parviflora*, *Z. montanum* and *Z. officinale* showed intermediate antifungal activity. *C. amada* and *C. aromatica* respectively inhibited *S. cerevisiae* and *C. tropicalis* only. *C. aeruginosa* and *C. angustifolia* did not show anti-fungal activity towards the tested strains. *C. caesia*, *K. parviflora* and *Z. montanum* produced good inhibition zones against the test pathogenic gram-positive and gram-negative bacteria, including the fungal strains. *K. parviflora* extract exhibited the highest antimicrobial activity with a MIC value of 60 ppm for *B. subtilis* and *C. glabrata*, 80 ppm for MDR strain of *S. aureus* and 90 ppm for methicillin-resistant strains of *S. aureus*. Also, it completely inhibited the growth of *E. coli* at 140 ppm. The extract of *Z. montanum* also showed potent antimicrobial activity with a MIC of 60 ppm for *B. subtilis*, 100 ppm for *C. glabrata* and MDR strain of *S. aureus*, and 140 ppm for *E. coli.* The results indicate that despite the presence of an outer lipopolysaccharides membrane in gram-negative bacteria, extracts of *K. parviflora* and *Z. montanum* showed inhibitory activity active against *E. coli*. Thus, the antibacterial activities of these plants are noteworthy, considering the emergence of resistance to empirically used antibiotics.

Conclusion

The phytochemical studies determine the presences of reducing sugars, terpenoids, flavonoids, phenolics, saponins and tannins in all the members. *C. longa* exhibited the highest TPC and TFC followed by *Z. montanum* and *Z. officinale*. Extracts of *C. caesia*, *K. parviflora* and *Z. montanum* evinced potent antimicrobial activity against the pathogenic microbes followed by *C. longa*, *Z. officinale* and *C. zedoaria*. Furthermore, the free radical-scavenging activities revealed that all the ten ginger species showed significant antioxidant activity. With such promising antioxidant properties, these species have high potential to be developed into natural antioxidants for food and therapeutic use. Thus, laboratory assessments of the traditional medicine would help in developing new and more efficient natural antioxidants and antibiotics which would contribute to improving the health of humans.

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Conflict of interest statement

The authors declare there is no conflict of interest.

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