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Evaluation on phytochemical and antimicrobial activity of *Zingiber Officinale* L

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Abstract
Zingiber officinale is a regular food for many countries. *Zingiber officinale* (ginger) is a well-known tropical and sub-tropical shrub medical plant. In the present study to evaluate the phytochemical analysis and antimicrobial activity of *Z.officinale* extract. Qualitative and quantitative analysis of phytochemical screening results showed that the presence of alkaloids, aminoacid, carbohydrate, flavonoids, protein, saponins, steroids, tannins and terpenoids compounds were recorded in aqueous and methanol extracts of *Z.officinale*. Antimicrobial activity of properties of *Z.officinale* extracts with solvents (aqueous, methanol, hexane and diethyl ether) against bacteria and fungi. The maximum zone of inhibition were recorded in 100µl concentration of aqueous and methanolic extracts of *Z.officinale* against *Bacillus* sp., *Escherichia coli, Klebisella penumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Aspergillus flavus, A.niger, A.terreus, Fusarium* sp. and *Penicillium* sp. than compared to other concentration. The screening this study revealed that the plant has good metabolites which justify that it has therapeutic utility and to take day to day life.

Keywords: phytochemical, antimicrobial, *Zingiber officinale* L

Introduction
Medicinal plants are cheap and renewable sources of pharmacologically active substances are known to produce certain chemicals that are naturally toxic to bacteria (Basile *et al.*, 1999) [5]. India is one of the countries that extensively use herbal medicines to meet their healthcare needs. The plants belonging to Zingiberaceae family are known for their preservative (Nielsen and Rios, 2000) [16] and medicinal values (Fisher, 1992) [6]. Number of plants from this family is being used in traditional system of medicine (Hussain *et al*., 1992) [10]. The rhizome (underground stem) of *G.officinale* is used as a spice and also as a medicine. It can be used fresh, dried and powdered, or as a juice or oil. *G.officinale* has been used as a medicinal plant in Asia, India, Jamaica and Nigeria. In China, ginger has been used to aid digestion, treat stomach upset, diarrhea and nausea for 2000 years (Azu and Onyeagba, 2007) [2].

Ginger has a wide range of action on the human body and has been found effective in the treatment of cataract, heart disease, migraines, struck amenorrhea, athlete's foot, bursitis, chronic fatigue, cold, coughs, depression, dizziness, fever, erectile difficulties, kidney stones, renal disease and viral infection. It is a valued remedy for coughs and bronchitis and also serves as a soporific in fever its natural diuretic stimulates the kidney to flush o toxins faster. The rhizomes of *Zingiber officinale* substances with several properties of interest, including bactericidal, fungicidal, antiviral, antiulcerative and antioxidant activity; they also contain enzymes with proteolytic activity (Millar, 1998; Kim *et al*., 2008; Takara *et al*., 2005) [15, 11, 24].

Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoid, alkaloids and phenolic compounds (Krishnaiah *et al*., 2007) [12]. Terpenoids exhibit various important pharmacological activities i.e., anti-inflammatory, anticancer, anti-malarial, inhibition of cholesterol synthesis, anti-viral and anti-bacterial activities (Mahato and Sen, 1997) [13]. Alkaloids are used as anaesthetic agents and are found in medicinal plants (Herouart *et al*., 1988) [9]. Ginger can be available in different commercial products like cookies, candy, teas, tinctures, sodas, jam, beer, capsule and syrup (Maxwell, 2008) [14]. The chief active constituents of ginger are Volatile oil (zingiberene, zingiberol, D-camphor), Shogaols, Diarylheptanoids, Gingerols, Paradol, Zerumbone, 1 Dehydro- (10) gingerdione Terpenoids and Ginger flavonoids (Baliga *et al*., 2012) [4].

Materials and methods
Plant material and preparation
Ginger rhizomes were collected from green house of triazole treated pot. The rhizomes were washed to remove soil, peeled and washed again in clean water. After washing, the rhizomes were dried, powdered and submitted to

successive extraction by aqueous, methanol, hexane and diethyl ether in separating funnel at room temperature. Collect the lower layer of aqueous, methanol, hexane and diethyl ether in conical flask and heat the solution for a while to evaporate the solvents form flask and the extracts further sterilized by filtration (0.22µm).

Qualitative phytochemcial analysis of *G.officinale*

Phytochemical tests were done to find the presence of the active chemical constituents such as alkaloid, aminoacid, carbohydrate, flavonoids, phlobotannins, protein, glycosides, saponins, steroids, tannin and triterpenoids by the following procedure (Harbone, 1999).

Table 1

Tests	Description	Inference
Alkaloids test	5ml of the ginger extracts take into flask and stirred with 5ml of 1% aqueous hydrochloric acid on a steam bath. Then 1ml of that filtrate was treated with few drops of Dragendorff's reagent.	Appear blue black colure
Aminoacid test	The ginger extracts and to added 1% ninhydrin solution. The reduction product obtained from ninhydrin then reacts with NH_3.	Appear blue color
Anthroquinones	About five ml of the extract was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of $CHCl_3$ was added to the filtrate. Few drops of 10% NH_3 were added to the mixture and heated.	Formation of pink colour
Carbohydrate test	Take 1 ml of ginger extract, add few drops of Molisch's reagent and then add 1 ml of concentrated sulphuric acid at the side of the tubes. The mixture was then allowed to stand for 2 to 3 minutes.	Formation of red or dull violet colour
Cardiac glycosides	To 2 ml of ginger extract with dilute HCl and 2 ml sodium nitrate in pyridine and sodium hydroxide solution were added.	Formation of pink to blood red color
Flavonoids test	When 5ml of diluted ammonia solution was added to aqueous filtrate of the test sample (ginger extract) followed by the addition of concentrated H_2SO_4.	Appear yellow colour
Phlobotannins test	When an aqueous extract of the ginger extract was boiled with 1% hydrochloric acid, disposition	Appear red precipitate
Protein test	The ginger extract to added equal volume of 40% NaOH solution and two drops of one percent copper sulphate solution.	Appear violet colour
Saponins test	5ml of the ginger extracts and 5ml distilled water in a test tube.	Appear of Frothing on tube
Steroids test	2 ml of acetic anhydride was added to 0.5 ml of ginger extract and 2 ml of sulphuric acid was added by the sides of the test tube	Appear violet or blue-green
Tannins test	5ml of the ginger extracts along with 100ml distilled water and filtered, then ferric chloride reagents was added	Appear blue-black or blue green precipitate
Terpenoids test	2 ml of chloroform was added to 1ml of the extract and Conc. H_2SO_4 (3 ml) was added to form a layer.	Appear reddish brown colour

Quantitative phytochemcial analysis of *G.officinale* (Harborne, 2001)

Alkaloids

Five gram of the sample was weighed into a 250 ml beaker. 200 ml of 10% acetic acid in ethanol was added and allowed to stand for 4 hours. This mixture was filtered and extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop by drop to the extract to get precipitation. The whole solution was allowed to settle down and the precipitate was collected and washed with diluted ammonium hydroxide and filtered. The residue was may be alkaloid that was dried and weighed. From this alkaloid content was determined.

$$\text{Alkaloids Content (\%)} = B - A \times 100 / S$$

Flavonoids

One grams of ginger sample was repeatedly extracted with 100ml of 80% aqueous methanol at room temperature. The mixture was filtered through a Whatman No. 1 filter paper into a pre-weighed 250ml beaker. The filtrate was transferred into a water bath and allowed to evaporate to dryness and weighed.

Carbohydrate

100 mg of sample was hydrolyzed in a boiling tube with 5 ml of 2.5 N HCl in a boiling water bath for a period of 3 hours. It was cooled at room temperature and solid sodium carbonate was added until effervescence ceases. The contents were centrifuged and the supernatant was made to 100 ml by using distilled water. From this 0.2 ml of sample was pipetted out and made up the volume to one ml with distilled water. Then one ml of phenol reagent was added and followed by 5.0 ml of sulphuric acid. The tubes were kept at 25-30˚C for 20 min. The absorbance was read at 490 nm.

Tannins

Powdered ginger sample weighted 0.5 gm was transferred to 250 ml conical flask and 75 ml water was added. The flask was heated gently and boiled for 30 min. The samples run at 2,000 rpm for 20 min. The supernatant was collected in 100 ml volumetric flask and made up the volume. One ml of the sample was added to 100 ml volumetric flask containing 75 ml water. 5 ml of folin's reagent and 10 ml of sodium carbonate solution was added and diluted to 100 ml with water. The mixture was shaking well. Absorbance was read at 700 nm after 30 min.

Proteins

0.2 ml of ginger extract was made to 1.0 ml with distilled water. 3.0 ml of alkaline copper reagent was added and allowed it to stand for 10 minutes. Then 0.5 ml of Folin's Ciocalteau reagent was added and incubated in dark for 30 minutes. The intensity of the colour developed was read at 660 nm.

Amino acids

One ml of the ginger extract was pipetted out into a test tube. A drop of methyl red indicator was added. The sample was neutralized with 1 ml of 0.1 N sodium hydroxide. To this, 1 ml of ninhydrin reagent was added and mixed

thoroughly. The content of the test tube was heated for 20 minutes in a boiling water bath. Five ml of the diluents solution was added and heated in water bath for 10 minutes. The tubes were cooled under the running water and the contents were mixed thoroughly. Blank was prepared with 1 ml of distilled water or ethanol. The absorbance was read at 570 nm in a UV-spectrophotometer.

Cardiac Glycosides

10 gram of dried plant powder was extracted with 250 ml methyl alcohol in soxhlet extractor. Another wash was also carried out with same solvent, filtered and Alcoholic extract was then treated with lead acetate solution to precipitate tannins, proteins, coloring matter and other non-glycosidal parts. The precipitate formed was filtered and to the filtrate H_2S gas (HCl + ferrous sulphide) was passed to precipitate excess lead as lead sulphide and removed by filtration and Filtrate was evaporated to dryness on water bath. The residue was dried, collected and weighed to get total glycoside content.

Saponins

1 ml of test samples were mixed with 80% methanol in 2ml, then added 2ml of 72% sulphuric acid solution was added, mixed well and heated on a water bath at 60˚c for 10minutes, absorbance was measured at 544nm against reagent blank.

Steroids

1ml of extract of different solvents acetone, ethanol, was transferred into 10 ml volumetric flasks. Sulphuric acid (4N, 2ml) and iron (III) chloride (0.5% w/v, 2 ml), were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated in a water-bath maintained at 70±20°C for 30minutes with occasional shaking and made up to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank.

Terpenoids

Dried plant extract 100mg (wi) was taken and soaked in 9mL of ethanol for 24 hour. The extract after filtration, was extracted with 10mL of petroleum ether using separating funnel. The ether extract was separated in pre-weighed glass vials and waited for its complete drying (wf). Ether was evaporated and the yield (%) of total terpenoids contents was measured by the formula (wi-wf/wi×100).

Test microorganisms

The clinical microbes are *Bacillus* sp., *Escherichia coli*, *Klebisella penumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Aspergillus flavus*, *A.niger*, *A.terreus*, *Fusarium* sp. and *Penicillium* sp. were procured Indian Biotrack Research Institute, Thanjavur and used for antimicrobial studies.

Screening for antimicrobial activity by well diffusion method (Srinivasan *et al*., 2001)

The antimicrobial activity was carried out with 24hrs bacterial cultures of *Bacillus* sp., *Escherichia coli*, *Klebisella penumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and 48 hrs fungal cultures (*Aspergillus flavus*, *A. niger*, *A. terreus*, *Fusarium* sp. and *Penicillium* sp.) with different solvents of methanol and aqueous extracts of *G. officinale* was tested separately using Agar well diffusion method. The nutrient agar and potato dextrose agar plates were swabbed with bacterial and fungal strain individually inoculated and maintained. A well 6mm diameter was made using a sterile cork borer. The different concentration (25, 50, 75 and 100µl) of various solvents individually with the extracts was introduced in the well. The plates were incubated at 37± 2°C for 24hrs and antifungal assay plates were incubated at 28 ± 2°C for 48 hrs and every 24 hrs the results were recorded.

Results and discussion

In the present study four different solvents (aqueous, methanol, hexane and diethyl ether) were used for the analysis of phytochemical analysis of *G. officinale.* In qualitative phytochemical analysis alakaloids, aminoacids, carbohydrate, flavonoids, protein, saponins, steroids, tannins and terpenoids was present in aqueous and methanolic ginger extracts than compared to hexane and diethyl ether extracts (Table 1). Aziz *et al*. (2015) [1] reported that the phytochemical screening of *Z. officinale* extracts contained maximum in alkaloids followed by flavonoids and then saponins. Riaz *et al*. (2015) [19] evaluated that the phytochemical screening of chloroform ginger extract showed presence of alkaloid, phlobotannins, flavanoids, glycosides, saponins, tannin and terpenoids and absence of steroids recorded respectively.

Current investigation reported that the maximum quantity of phytocompounds recorded in methanolic extract as alkaloids (20.0±0.20mg/g), aminoacids (30.9±2.65mg/g), carbohydrate (26.2±1.69mg/g), flavonoids (33.0±1.47mg/g), protein (29.0±1.32mg/g), saponins (24.0±2.19mg/g), steroids (34.2±2.60mg/g), tannins (27.0±2.64mg/g) and terpenoids (20.0±2.33mg/g) respectively (Table 2). Osabor *et al*. (2015) determined that the phytochemical screening of *Zingiber officinale* rhizome revealed the presence of alkaloids, saponins, flavonoids, polyphenols and reducing sugars in the aqueous extracts while cardiac glycosides, saponins, flavonoids, polyphenols and reducing sugars were present in the petroleum ether extracts (PEE). Phytochemical analysis for ethanol extracts of leaves, stem and root of *Phyllanthus niruri* are flavonoids and phenols are present in ethanol extract of *Phyllanthus niruri* leaves and root. Amino acids and proteins showed positive test with leaves and stem extract. Saponins were also present in stem and root extracts of the plant (Nigam and Arnold, 2021) [17].

Effect of antibacterial activity of aqueous and methanolic extracts of *Z. officinale* with different concentration of 25, 50, 75 and 100µl against *Bacillus* sp., *Escherichia coli*, *Klebisella penumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus.* The maximum zone of inhibition were recorded in 100µl concentration of methanolic extract of *Z. officinale* was 10.3±0.76, 13.5±0.89, 11.7±0.55, 14.3±0.78 and 12.3±0.44mm diameter in *Bacillus* sp., *Escherichia coli*, *Klebisella penumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and aqueous extract in 13.0±0.00, 11.0±0.67, 7.6±0.47, 06.3±0.11 and 14.0±0.30mm diameter measured in *Bacillus* sp., *Escherichia coli*, *Klebisella penumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* recorded respectively. Minimum zone of inhibition were recorded in 25µl concentration of both aqueous and methanolic extracts of *Z. officinale* (Table 3). Plants with an average zone of

inhibition in diameter of ≥ 2.8 mm was considered as those recording a significant antimicrobial activity. It indicates that *Z. officinale* has strong antimicrobial activity against *Staphylococcus aureus*, *Klebsiella* sp., *Escherichia coli* and *Streptococcus* sp. (Aziz *et al*., 2015) [1].

Evidently, these studies justify that the *C. procumbens* methanolic extract of 100µl concentration was more suitable for this investigation (Babu *et al*., 2018) [3]. The maximum concentration of 100µl was excellent antibacterial properties in bacteria like *Pseudomonas* sp. (20.5±0.06mm), *Escherichia coli* (15.5±0.03mm) and *Staphylococcus aureus* (15.5±0.07mm) and fungi are *Penicillium* sp. (20.0±0.07mm) and *Fusarium* sp. (19.0±0.07mm) were observed in *Aerva lanata* ethanolic extract than compared to methanol extract (Suresh *et al*., 2019) [23]. Sarda *et al*. (2017) [20] reported that the ethanol extract shows highest 17mm zone against *E. coli* and 13 mm zone against *S. aureus*, followed by acetone extract shows 17mm against *E. coli* and 15 mm zone against *S. aureus*, whereas aqueous extract shows 9mm zone diameter against *E. coli* and 12 mm zone against *S. aureus* recorded respectively.

In the present study, the results indicated that all the aqueous and methanolic extracts exhibited strong *in-vitro* antifungal activities of *Z.officiale* rhizome against *Aspergillus flavus*, *A.niger*, *A.terreus*, *Fusarium* sp. and *Penicillium* sp. These both extracts were more resistance to selective fungal pathogens regarding 25, 50, 75 and 100µl concentration. The concentration is increasing the growth inhibition also increased (Table 4). The findings of the present study revealed that *Zingiber officinale* contain potent antimicrobial property against tested microbes. The antimicrobial activity of the ginger extracts (chloroform extract) was initially evaluated by agar diffusion method using four strains of pathogenic bacteria *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus faecalis* and two stains of fungi *Candida albicans* and *Aspergillus niger* (Riaz *et al*., 2015) [19]. In the same way, *A. raperi*, *A. terreus* and *Fusarium* sp. also detected the highest inhibition in methanolic extract of *C. procumbens*. *Aspergillus sydowi* was more sensitive in 12, 13, 14 and 15 mm diameter at 25, 50, 75 and 100µl concentration of methanolic extract followed by ethanol, acetone and water extract (Babu *et al*., 2018) [3].

Table 2: Qualitative Phytochemical analysis of *Zingiber officinale* extract

Name of the Compounds	Aqueous	Methanol	Hexane	Diethyl ether
Alkaloids	+	+	-	-
Aminoacid	+	+	-	-
Anthroquinones	-	-	-	-
Carbohydrate	+	+	-	-
Flavonoids	++	+++	+	+
Phlobotannins	-	-	-	-
Protein	+	+	+	-
Saponins	+	++	+	-
Steroids	+	++	-	+
Tannins	+	++	+	+
Terpenoids	+	++	+	+

(+++) - Strongly present, (+) - present, (-) – absent

Table 3: Quantitative Phytochemical analysis of *Zingiber officinale* extract

Name of the compounds	Quantity (mg/g)			
	Treated			
	Aqueous	Methanol	Hexane	Diethyl ether
Alkaloids	24.0±1.10	20.0±0.20	-	-
Aminoacid	25.7±1.64	30.9±2.05	-	-
Carbohydrate	25.4±2.89	26.2±1.69	-	-
Flavonoids	22.0±2.67	33.0±1.47	18.0±0.27	11.0±0.33
Protein	24.2±1.28	29.0±0.32	8.0±0.30	-
Saponins	18.0±3.16	24.0±2.19	14.0±0.23	-
Steroids	22.5±3.29	34.2±2.60	-	14.0±0.83
Tannins	18.0±2.67	27.0±2.64	12.0±0.65	10.0±0.65
Terpenoids	13.0±2.16	20.0±2.33	16.0±0.32	16.0±0.33

The values are expressed in terms of (Mean ± Standard deviation), (-) – absent

Table 4: Effect of antibacterial activity of *Zingiber officinale* extract against bacteria

Name of the bacteria	Zone of inhibition (mm)							
	Aqueous				Methanol			
	25µl	50 µl	75 µl	100 µl	25µl	50 µl	75 µl	100 µl
Bacillus sp.	08.0±0.10	12.0±0.09	14.0±0.00	13.0±0.00	12.3±0.78	12.0±0.10	14.3±0.11	10.3±0.76
E. coli	05.2±0.89	07.3±0.11	12.3±0.39	11.0±0.67	14.6±0.55	11.3±0.24	12.7±0.22	13.5±0.89
K. pneumoniae	04.0±0.67	06.7±0.67	07.6±0.47	08.7±0.67	13.7±0.89	14.0±0.10	13.3±0.11	11.7±0.55
Pseudomonas aeroginosa	05.0±0.16	06.3±0.11	06.3±0.11	06.0±0.10	08.3±0.11	10.0±0.37	15.0±0.67	14.3±0.78
Staph. Aureus	11.0±0.33	13.3±0.11	14.0±0.30	15.4±0.73	15.3±0.68	14.7±0.25	15.3±0.11	12.3±0.44

The values are expressed in terms of (Mean ± Standard deviation)

Table 5: Effect of antifungal activity of *Zingiber officinale* extract against fungi

Name of the fungi	Zone of inhibition (mm)							
	Aqueous				Methanol			
	25µl	50 µl	75 µl	100 µl	25µl	50 µl	75 µl	100 µl
Aspergillus flavus	06.3±0.11	07.6±0.55	07.3±0.44	12.0±0.10	05.0±0.14	08.0±0.67	06.7±0.29	13.5±0.10
A.niger	05.3±0.78	08.0±0.67	09.3±0.39	06.6±0.89	05.6±0.89	06.0±0.10	10.5±0.35	14.3±0.26
A.terreus	05.0±0.67	05.7±0.89	08.6±0.89	09.0±0.12	06.7±0.22	08.7±0.65	09.7±0.23	10.6±0.18
Fusarium sp.	09.0±0.11	10.6±0.89	11.7±0.89	11.3±0.76	08.3±0.78	11.4±0.43	08.0±0.13	11.7±0.39
Penicillium sp.	08.6±0.22	11.0±0.67	12.3±0.11	13.7±0.55	04.4±0.33	05.6±0.26	11.3±0.24	12.2±0.22

The values are expressed in terms of (Mean ± Standard deviation)

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