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Evaluation of active components from ethanolic extract of *Zingiber officinale* Rhizomes

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Abstract

The present study was carried out to evaluate the presence of active phytochemicals in ethanolic extract of rhizomes of *Zingiber officinale* following GC-MS method. The qualitative phytochemical screening of ethanolic extract of *Zingiber officinale* rhizomes revealed the presence of alkaloids, glycosides, saponins, Tannin and flavonoids, reducing sugars. Gas chromatography and mass spectroscopy analysis of ethanolic extract of *Zingiber officinale* showed the presence of 12 major components as Isopropenyl dimethyl, 3-Allyl 6 methoxyphenol, Thujaketone, 2 Butanoic acid, 2 methoxy methyl, Hexahydro farnesol, Gingerol, Tetradecanoic acid, 1 Phenyl 3-6 diazohomoadamantan-9-hydrozone, 7-methyl-2-tetradecen-1-ol-acetate, Eicosane, Androstan and Squalene. From the study, it was concluded that *Zingiber officinale* possesses significant bioactive compounds having therapeutic effects, hence can be considered as a potential of medicinal herb.

Keywords: medicinal plants; *Zingiber officinale*; phytochemicals; gc-ms

Introduction

Indigenous medicine has an important role in traditional system of medicine in India which contains various medicinal plants used for treatment in the field of Veterinary as well as in human being. It is known that, medicinal plants are the part and parcel of society to combat diseases from the dawn of civilization in world [7] and also continued in providing valuable therapeutic agents, both in modern and in traditional medicine [6]. As, most of the side effects are associated with modern medicine, the traditional medicines are gaining importance and are now being studied to find the scientific root of their therapeutic properties [3]. Therefore, there is an increased attention among phyto therapist to make use of herbs with antioxidant property for protection against various toxicities [4]. Identifications of the phyto constituents having pharmacological properties is most valuable work amongst the research worker.

Ginger, the rhizomes of the plant *Zingiber officinale* (Family *Zingiberaceae*), is arguably one of the most widely used culinary agent and spice in the world [1]. It is popularly known as 'Aale' in Marathi and 'Adrak' in Hindi. It is a creeping herb with thick, branching rhizomes and sturdy upright stem with pointed lance like leaves. The ginger plant has long history of cultivation originating from Asia and widely grown in India, Southeast Asia, West Africa and Caribbean. The unique culinary and medicinal properties of ginger are due to the presence of various bioactive components [9]. Preclinical studies carried out on laboratory animals have shown that ginger possess hepatoprotective property against the toxicity produced by heavy metals [5]. An active constituents present in the ginger has promising antioxidant, anti-inflammatory, and anti-gout properties and can be used as potential natural drug against oxidative stress and inflammation [8].

Ginger is widely employed in Chinese, Ayurvedic, Unani medicines and home remedies since antiquity for many ailments including pain, inflammation, and gastrointestinal disorders and the nutraceutical attributes of ginger are its positive influence on gastrointestinal tract including digestive stimulant action, anti-inflammatory influence, and anticancer effect [14]. The present study was planned to investigate the presence of phytochemical constituents in 50% ethanolic extract of dried rhizomes of plant *Zingiber officinale*.

Materials and Methods

Collection of Plant material

The plant material i.e. roots of plant *Zingiber officinale* was collected from the local market of Nagpur and were dried at room temperature. The dried rhizome of the plant were powdered and sieved through muslin cloth. The finely powdered material was stored in glass-stopper bottle in a cool and dry place, away from direct sunlight and moisture.

Preparation of 50% Ethanol cold extract

500 gram of powder of dried rhizome of *Zingiber officinale* was mixed with 2000ml of 50% Ethanol in stoppered flask and allowed to stand at room temperature for 72 hrs with frequent agitation until the soluble matter get dissolved. After 72 hrs, the mixture was filtered through muslin cloth, so as to remove the insoluble material. The filtrates were again filtered through filter paper and then poured in clean and already weighed Petri plate and allowed for complete evaporation at room temperature and finally stored in desiccators in cool and dry place.

Qualitative Phyto chemical analysis of certain active principles

After the preparation of 50% ethanolic extract of *Z. officinale*, the phyto chemical investigation was undertaken as per the standard procedures [12].

A. Test for sterols

a. Salkowskis's reaction

2ml of chloroform and 1mL of concentrated sulfuric acid were added to 10 drops of the extract dissolved in isopropyl alcohol, slowly until double phase formation. The test tube was shaken for a few minutes; red colour development in the chloroform layer and greenish yellow fluorescence in the lower layer indicates the presence of sterols in the extract.

b. Liebermann Burchard Reaction

1ml of anhydrous acetic acid and 3 drops of concentrated sulfuric acid were added to 2ml of the extract dissolved in isopropyl alcohol. Transient colour development from red to blue and finally green colour indicates the presence of sterols.

B. Test for alkaloids

About 50mg was taken in 5 ml of 5 % hydrochloric acid (V/V) and filtered; the filtrate was used for testing alkaloids.

a Dragendorff's reagent

It was prepared by mixing solution A (17 gm of Bismuth sub nitrate +200 gm of tartaric acid+ 800 ml of distilled water) and solution B (160 gm potassium iodide + 400 ml of distilled water) in 1:1 proportion (V/V). From this solution a working standard was prepared by taking 50 ml of this solution and adding 100 gm of tartaric acid and making its volume up to 500 ml with distilled water. This reagent was sprayed on a filter paper and allowed for drying. The 1ml filtrate was applied on the paper using a capillary tube. Development of an orange-red color indicates the presence of alkaloids.

b. Wagner's reagent

1.27 gm iodine and 2 gm potassium iodide were dissolved in 5 ml of distilled water and this was further diluted in to 100 ml distilled water. To this 2ml of filtrate was added. Appearance of brown flocculent precipitate indicates the presence of alkaloids.

C. Test for amino acids

Ninhydrin Test

3 drops of 0.1 % solution of ninhydrin in alcohol, when added to the 3 ml of diluted extract, violate or purple colour development indicates the presence of amino acids.

D. Test for proteins

a. Xanthoprotein Test: About 5mg of extract was taken in 2 ml of water and to it 0.5 ml of concentrated nitric acid was

added. The appearance of (white or yellow) precipitate indicates the presence of proteins.

b. Biuret Test: About 5 mg of extract was taken in water and 1 ml of 1 % solution of sodium hydroxide was added. Followed by a drop of 1% solution of copper sulphate. Violate–pink colour development indicates the presence of proteins.

E. Test for reducing sugars

The 50mg extract was dissolved in warm distilled water and tested with Benedict's and Fehling's reagent.

a. Benedict's reagent: 5ml of diluted extract was taken and equal quantity of Benedict's reagent was added to it and heated. The appearance of brownish red precipitate (reduction) indicated the presence of reducing sugars.

b. Fehling's reagent: 2ml of diluted extract was added to 0.5 ml of Fehling's reagent (Fehling's solution A and B mixed immediately before use) and 2 ml of 10.5% sodium hydroxide solution. The mixture was then heated on a water bath for 10 minutes. The appearance of red precipitate indicated the presence of reducing sugars.

F. Test for glycosides

a. Benedict's reagent: The above tests for reducing sugars were repeated with extract. The solution obtained in Benedict's test was filtered and dilute hydrochloric acid was added for hydrolyzing the glycosides. The pH of the solution was alkaline. Equal quantity of Benedict's reagent was added and boiled. The appearance of brownish precipitate indicated the presence of glycosides.

b. Fehling's reagent: This test was performed with the solution obtained in Fehling's test and to this few drops of dilute HCL were added and boiled for 5 minutes for hydrolyzing the glycosides. Fehling's reagent was again added to note any further reduction, which indicates the presence of glycosides.

G. Test for saponins

Foam test: 1 drop of extract (20mg/ml in isopropyl alcohol) and 5 drops of sodium bicarbonate were added to 2ml of distilled water in test tube, then it was shaken vigorously. Formation of froth about 2cm indicates the presence of saponins.

H. Test for tannins

About 100mg of extract diluted in 10ml of ethanol and 25ml of distilled water. Then it was warmed and filtered. Tests were carried out with this filtrate using following reagents.

a. Lead acetate test

A few drops of lead acetate solution were added to above filtrate. The formation of precipitate indicates the presence of tannins.

b. Ferric chloride Test

4 drops of 10% ferric chloride solution added to 10 ml of above filtrate. A green coloration in the filtrate of extract indicates the presence of tannins.

I. Test for anthraquinones

Bontrager's Test: 1ml of benzene was added to 10ml of

extract dissolved in isopropyl alcohol, followed by stirring and filtration. 0.5mL of 10% ammonia solution was added to the filtrate. This mixture was shaken. Formation of pink, colour in the ammonical (lower) phase indicated the presence of anthraquinones.

J. Test for flavonoids

10 drops of diluted extract were dissolved in 5 ml ethanol (95%) and treated with 3 drops of concentrated hydrochloric acid and 0.5 gm of magnesium turnings. Development of either pink or magnet colour indicates the presence of flavonoids.

K. Test for resins

1ml of diluted extract was dissolved in alcohol. To this, a few drops of distilled water was added. The appearance of turbidity considered as a positive test for resins.

Gas chromatography-mass spectroscopy (GC-MS) analysis

GC-MS analysis of ethanolic extract of *Zingiber officinale* was performed by using Bruker Model Scion TQ MH-System and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with a column DB-5MS Agilent (30m × 0.25mm ID, composed of 100% dimethyl polysiloxane). For GC-MS detection, electron ionization system with ionization energy of 70eV was used. Helium gas (99.99%) was used as the carrier gas at constant flow rate 1.0 ml/min with a split ratio of 10:1. The oven temperature was operated according to the following oven temperatures: 40°C held for 1 min, raising at the rate of 20°C min⁻¹ up to 150°C then, raising at the rate of 3°C min⁻¹, raising at the rate of 20°C min⁻¹ up to 300°C with 10 min held, injector temperature and volume 250°C and 2 µl, respectively. The total GC running time was about 40 min. The MS operating conditions were ionization voltage 70 eV, source temperature of 250°C, inlet line temperature 280°C, mass scan (m/z)-30-500, solvent delay: 3.0 min, total MS running time 36 min. Compounds were identified in terms of Rt values and mass spectra with those obtained from the NIST library.

Results and Discussions

Table 1 shows the results of qualitative phytochemical screening of 50% ethanolic extract of *Zingiber officinale* rhizomes. The results obtained from the investigation revealed the presence of alkaloids, reducing sugars, glycosides, saponins, tannin and flavonoids, reducing sugars in the extract. Saponins are the heterogeneous groups of natural products which have strong expectorant property and also help in the absorption of nutrients [10]. Flavonoids which is most potent antioxidant and enhance the healthy circulation of

blood and sometimes referred to phytoestrogens as it associates with release of menopausal systems, decrease of osteoporosis, improvement of blood cholesterol levels and help in decreasing the threat of occurring certain hormone-related cancers and coronary heart disease [10]. Tannins present in the extract of *Zingiber officinale* are astringent in nature and are used for treating intestinal disorders such as diarrhea, colitis and dysentery [13]. The alkaloids contain in plants are used for therapeutic purpose and ginger rhizome extract and its phyto-components can be used as alternative and effective source to therapeutic strategy [13]. The results of the present investigation for the qualitative screening of phytochemicals are mostly consistence with the previous findings.

Gas chromatography and mass spectroscopy analysis of compounds was carried out in ethanolic extract of *Zingiber officinale*, shown in Table 2. The GC-MS chromatogram peaks of the compounds detected is shown in Figure 1. Chromatogram GC-MS analysis of the ethanolic extract of *Zingiber officinale* showed the presence of 12 major peaks and the components corresponding to the peaks were determined as Isopropenyl dimethyl, 3-Allyl 6 methoxyphenol, Thujaketone, 2 Butanoic acid, 2 methoxy methyl, Hexahydro farnesol, Gingerol, Tetradecanoic acid, 1 Phenyl 3-6 diazohomoadamantan-9-hydrozone, 7-methyl-2-tetradecen-1-ol-acetate, Eicosane, Androstan and Squalene at retention time 15.22, 16.65, 17.16, 17.33, 18.92, 20.15, 20.26, 21.34, 21.82, 28.80, 31.09 and 32.20, respectively. Out of these compounds, four compounds 2 Butanoic acid, 2 methoxy methyl, Hexahydro farnesol, Gingerol and Tetradecanoic acid showed higher per cent of area than other compounds as 1.072, 1.692, 3.15 and 1.59, respectively. Among this, gingerol was found in more quantity which is the main chemical present in the *Z. officinale* responsible for the pungent smell. Pungency of the ginger slowly decreases when the concentration of gingerol get reduced [16]. The components like Gingerol, Isopropenyl dimethyl proved to have anti-inflammatory, antioxidant and anticancer properties [2, 15]. Volatile oils, shogaols, Gingerols and diarylheptanoids in chloroform extract of ginger has antimicrobial efficacy by modulating the genetic or metabolic activities [11]. The GC-MS analysis findings are correlated with the previous studies.

Conclusion

Today, looking towards the side effects of modern medicine, the invention of novel active compounds against new targets is a matter of urgent priority in drug discovery. From the results obtained in the present investigation, it could be concluded that *Zingiber officinale* possesses remarkable bioactive compounds having therapeutic effects and can be considered a potential source of medicinal herb.

Table 1: Qualitative Phytochemical Analysis of 50% ethanolic Extract of *Zingiber Officinale*.

Sr. No.	Active principle	Test Applied	Observation	Result
1	Sterols	Salkowskis reaction	No Development of red colour in chloroform layer	Absent
		Liberman Buchard reaction	No Development of transient colour	Absent
2	Alkaloids	Dragondroffs reagent	Development of orange red colour	Present
		Wagners reaction	Appearance of brown flocculent precipitation	Present
3	Amino acids	Ninhydrin test	No Development of violet colour.	Absent
4	Proteins	Xanthoprotein test	White precipitate was not formed	Absent
		Biuret test	Violet pink colour not developed	Absent
5	Reducing sugars	Benedicts reagent	Development of brownish red precipitate	Present
		Fehlings reagent	Appearance of red precipitate	Present
6	Glycosides	Benedicts reagent	Formation of brownish precipitate	Present
		Fehlings reagent	Further reduction was observed	Present

7	Saponins	Foam test	Formation of froth	Present
8	Tannins	Lead acetate test	Formation of precipitate	Present
		Ferric chloride test	Occurrence of green colouration of filtrate	Present
9	Antraquinones	Bontragers test	Ammonical layer did not acquire any pink colour	Absent
10	Flavonoids	Test for flavonoids	Development of red or pink colour	Present
11	Resins	Test for resins	No turbidity was appeared	Absent

Table 2: List of compounds detected in 50% ethanolic extract of *Zingiber officinale* using GC-MS analysis

Sr. No	Retention time (min)	Identified compound	% of area
1	15.22	Isopropenyl dimethyl	0.47
2	16.65	3-Allyl 6 methoxyphenol	0.188
3	17.16	Thujaketone	0.602
4	17.33	2 Butanoic acid, 2 methoxy methyl	1.072
5	18.92	Hexahydro-farnesol	1.692
6	20.15	Gingerol	3.15
7	20.26	Tetradecanoic acid	1.59
8	21.34	1-Phenyl 3-6 diazohomoadamantan-9-hydrozone	0.07
9	21.82	7-methyl-2-tetradecen-1-ol-acetate	0.12
10	28.8	Eicosane	0.031
11	31.09	Androstan	0.47
12	32.20	Squalene	0.52

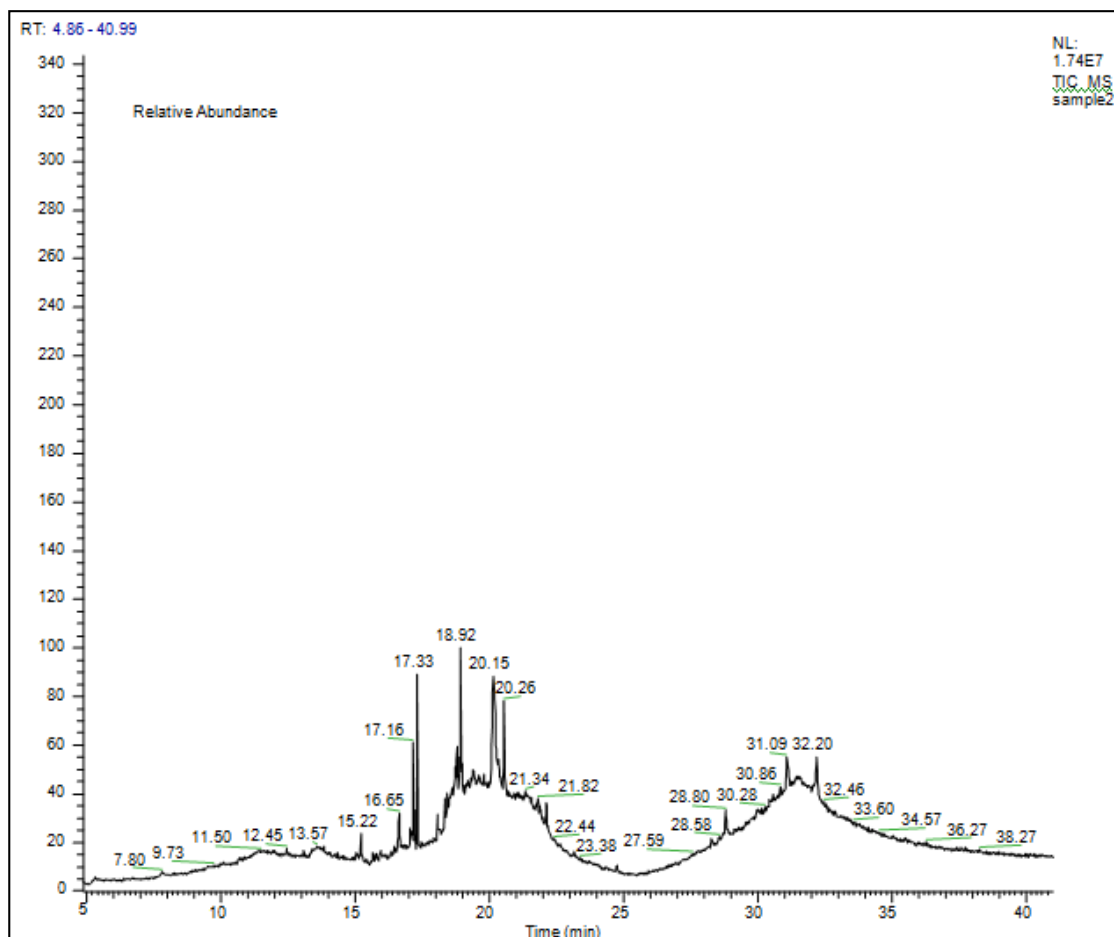


Fig 1: GC-MS spectra of 50 % ethanolic extract of *Zingiber officinale*

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