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ANTIOXIDANT ACTIVITY, TLC AND PHYTOCHEMICAL ANALYSIS OF GINGER (*ZINGIBER OFFICINALE* L.) RHIZOME

Smrati Sharma and Ramesh Kumar*

Department of Biochemistry, Bundelkhand University, Jhansi - 284 128 (U.P.), India.

Abstract

Zingiber officinale belongs to the Zingiberaceae family is a common condiment for various foods and beverages and widely utelized in the traditional Ayurvedic medicines from a long years ago.The unique fragrances and flavor of come from its natural oils, gingerol. It produced in many countries but it does best in moist, tropical conditions. Raw ginger contains approx 79% water and rest are carbohydrates, proteins and fat. Ginger has been found effective in multiple studies for tearing nausea caused by sea sickness, morning sickness, chemotherapy and also help in reduce muscle pain, soreness, lower blood sugars, improve heart disease risk factors, chronic indigestion and reduce menstrual pain etc. The plant is reported to have antibacterial, anti-oxidant, antiprotozoal, anti-fungal, anti-inflammatory and anti-insecticidal activity. Therefore, the aim of this study was to carry out the antioxidant potential ,thin layer chromatography(TLC) and phytochemical analysis of ginger *Z. officinale*rhizome. Phytochemical analysis of the aqueous and methanolic extracts of the rhizomes revealed the presence of alkaloids, reducing sugars, flavonoids, glycosides, tannins, saponins etc, in them. The present study revealed that ginger has antioxidant potential. TLC of the methanolic extract showed the presence of three compounds in the rhizomes of *Z. officinale* Linn.

Key words : *Z. officinale*, antioxidant potential, phytochemicals, TLC, ascorbic acid.

Introduction

Ginger rhizome is the root part of the *Zingiber officinale*a very usefulherb plant belongs to the family Zingiberaceae.Itwidely utilized as a pleasant condiments and as in the traditional Ayurvedic medicines.It is a perennial aromatic herb approx 2 feet of height produces clusters of white and pink flower buds which blooms into greenish yellow flowers like orchid plants. The unique fragrances and flavor of come from its natural oils, gingerol.

India has one of world's richest medicinal plant heritages (Sharma *et al*., 2017). Nutritional plants have been used in medicine by having application in treating many diseases since ancient times. Ginger (*Zingiber officinale* Rosc.) is not only one of the frequently used species to enhance the taste and flavour of the food in many parts of the world (Peter, 2006), but also contains a numerous number of potential bioactive compounds that have biological and pharmaceutical effects. The rhizomes of ginger are one of the most widely used spice and condiment (Baliga *et al*., 2011). Plants are considered as a rich source of bioactive chemicals and they may be an alternative source of mosquito control agents (Narendiran *et al*., 2016). The medicinal value of plants lies in some chemical active substances that produce define physiological action on the human body (Yadav *et al*., 2017). Today according to the world Health organization (WHO), as many as 80% of the world's people depend on traditional medicine for their primary healthcare needs. There are considerable economic benefits in the development of indigenous medicines and in the use of medicinal plants for the treatment of various diseases (Sarad *et al*., 2017). Over the past decade, antioxidants have achieved prominence in the food industry due to their ability to eliminate free radicals interaction (Harini and Nithyalakshmi, 2017).

The consumption of ginger have been claimed to be useful in many oxidative stress related medical conditions, some of which include hypertension (Akinyemin *et al*., 2013), diabetes-induced pancreatic and renal

Author for correspondence

derangements (Akinyemi *et al*., 2013), tumour progression (Kazeem *et al*., 2015) and Alzheimer diseases (Surh *et al*., 1999). In traditional medicine, it has been used for treating headaches, nausea, febrile conditions, colds, arthritis, rheumatic disorders and muscular discomfort (Mathew and Subramanian, 2014). It has been also suggested that ginger has anti-inflammatory, anticancer, hepatoprotective (Kazeem *et al*., 2012) renoprotective (Mohamed *et al*., 2015) effects, androgenic properties (Mahmoud *et al*., 2012), antiglycation potential (Ghlissi *et al*., 2013) and antioxidant effects (Nagendra *et al*., 2013; Tohmal *et al*., 2017). Ginger has been used as a refrigerant, astringent and flavouring agent, and as a digestive in medicine (Silvia *et al*., 2015). Rhizome paste has been traditionally applied for improving the healing of wounds, cuts and antipruritic (Srivastava *et al*., 2006).

Materials and Methods

Plant material

Ginger rhizome was collected from local market of Jhansi (U.P) in the month of December. Firstly the collected rhizomes were washed to remove soil with tap water for 3-4 times and then with de-ionized water for two times. After that, it was chopped into pieces and kept in the dark for drying at room temperature. And finally crushed with the help of electric grinder, stored for further use.

Preparation of extract

The powdered root of ginger was percolated using 80% of methanol in the soxhlet apparatus at 60-65°C. This percolate was filtered and then it was evaporated to dryness in a water bath at temperature lower than 40^0C. Obtained extract was stored in air tight bottles at temperature lower than 5^0C.

Phytochemical analysis

The study of the phytochemicals for the presence of alkaloids, saponins, flavonoids, triterpenoids &Steroids, carbohydrates, protein & A.A., hydrolysable tannin, reducing sugars, glycosides, tannin and phenolic were carried out by Kokate (2000), Harbone (1999), Prashanth *et al*. (2011) with some modifications (table I).

Total antioxidant capacity (TAC) by Phosphomolybdenum assay

The total antioxidant capacity of the methanolic extract of ginger rhizome was done by the phosphomolybdenum assay carried out by Prieto *et al*. (1999) with some modifications.

The assay is based on the subsequent formation of green phosphate/Mo (V) complex at acidic pH by the reduction of Mo (VI) to Mo (V) in the presence of methanolic extract. A 0.2 µl of extract was combined with 2 ml of reagent solution (0.6M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Tubes of reaction mixture were incubated for 90 min. at 90°C. After cooling to room temperature the absorbance was measured at 695 nm by using a multi plate reader. Methanol was used in the place of extract for the blank tube with same ratio. The antioxidant activity is expressed as the number of gram equivalent of ascorbic acid. The calibration curve was prepared with the respect of ascorbic acid (1000, 500, 250, 125, 62.5 and 31.25 µg/ml) in methanol as a standard.

Thin layer chromatography

The extracts were tested using TLC analytical plates coated with silica gel-G of 0.2 mm thickness. The solvent system used a mixture of Butanol- acetic acid-water (4:1:1 v/v) as described by somewhere else. This mixture migrates on the silica coated plates by the capillary action. Fully developed coated plate was air dried followed by heating for 20-25 minutes. The plate was sprayed with 0.2% freshly prepared ninhydrin solution to detect the bands.

These spots were expressed by its retention factor (Rf).

$$Rf = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

Results

Analysis of aqueous & methanolic extract of ginger rhizome reveals the presence of various secondary metabolites (table 1). For the qualitative detection of secondary metabolites different types of phytochemical tests were performed. The presence or absence of these phytochemical constituents depends on the test applied. Further, we also observed the antioxidant activities in all the extracts. Phosphomolybdenum assay is based on the reduction process of Phosphate-Mo (VI) to Phosphate Mo (V) by the methanolic extract and subsequent formation of a bluish green colored phosphate/Mo (V) complex (fig. 2). The thin layer chromatography of sample shows results for the methanolic extract. 3 spots were present having Rf values 0.63, 0.71, 0.79 respectively (table 2 and fig. 1).

Discussion

These secondary metabolites contribute significantly towards the biological activities of medicinal plants such as hypoglycemic, antidiabetic, antioxidant, antimicrobial,

Table I :

S no.	Test	Description	Appearance
1.	**Test for Alkaloids**		
	Mayer's	500µl extract + few drops of dil. HCL + Few drops of mayer's reagent	Creamy precipitate
	Wagner's	500µl extract + few drops of dil. HCL + Few drops of mayer's reagent	Reddish brown precipitate
	Hager's	500µl extract + few drops of dil. HCL + Few drops of mayer's reagent	Yellow precipitate
2.	**Test for carbohydrates**		
	Molisch	500µl extract + 2 drops of α- naphthol + 100 µl $H2SO_4$ (conc.)	Violet ring at junction
	Barfoed's	500µl extract + equal Barfoed's reagent + heat	Red colour
3.	**Test for Reducing sugar**		
	Fehling's	500µl extract + 500µl Fehling's A & B+ 10 min. heat	Red precipitate
	Benedict's	Equal volume of Benedict and extract+ 5-10 min. heat	Green, Yellow or Red precipitate
4.	**Test for Flavonoids**		
	Alkaline reagent	Extract treated with NaOH sol. Add dilute acid	Intense yellow Becomes colourless
	Lead acetate	Extract + few drops lead acetate	Yellow precipitate
5.	**Test for Glycosides**		
	Borntrager's	500µl extract + H2SO4 (dilute)+ 5 min. boiling + cool and equal volume of benzene + Ammonia	Pink to red colour
	Legal's	500µl extract+pyridine+500µl nitropruside sol.+10% NaOH	Pink to blood red colour
	Keller- killiani	500µl extract + 300µl glacial acetic acid + 1 drop $FeCl_3$ (5%) + 100 µl H_2SO_4 (conc.)	Blue colour
6.	**Test for Tannin & Phenolic**		
	Ferric chloride	500µl extract +H_2O + 500µl $FeCl_3$	Blue, green or violet colour
	Lead acetate	500µl extract +H2O + few drops lead acetate	White precipitate
	Dil. Iodine	500µl extract +H2O + few drops dil. Iodine	Transient red colour
7.	**Test for Saponin**		
	Foam Test	2ml extract + 2ml H2O + heat	Froth appears
8.	**Test for Protein & A.A.**		
	Ninhydrin	500µl extract + heat + 3 drops of 5% ninhydrin	Blue colour
	Biuret	500µl extract + 10% NaOH + heat + 1 drop 0.7% $CuSO_4$	Violet or pink
9.	**Test for Steroids**		
	Salkowski Test	500 µl extract + H_2SO_4 (conc.)	Lower layer turns red

antiinflammatory, anticarcinogenic, antimalarial, anticholinergic, antileprosy activities etc. (Negi *et al.,* 2011). Traditionally, medicinal plants play a vital role in developing countries for basic health needs (Gochukw, 2009). Ginger is used as a herbal medicine for various types of diseases and also used in flavour.

TAC of the phosphomolybdenum assay evaluates both water and fat soluble antioxidant capacity. PM assay measures the reduction degree of Mo (VI) to Mo (V). This is a quantitative method to investigate the reduction reaction rate among antioxidant, oxidant and molybdenum ligand. It involves in thermally generating auto-oxidation

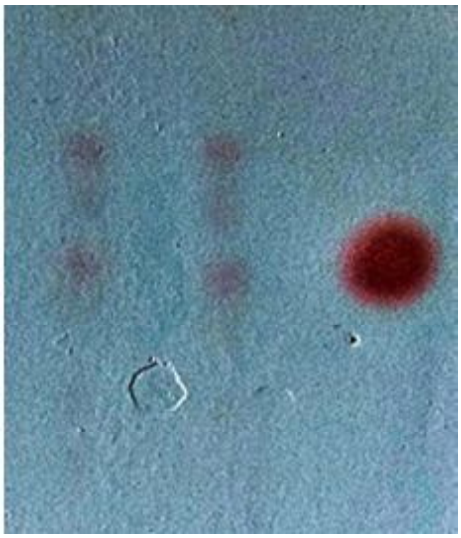

Fig. 1 : TLC plate.

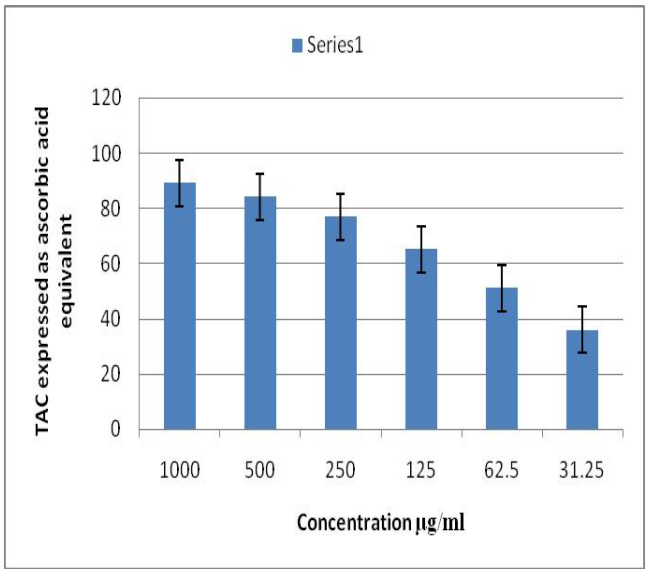

Fig. 2 : Total antioxidant capacity of *Z. officinale*.

during prolonged incubation period at higher temperature. And gives a direct estimation of reducing capacity of antioxidant (Phatak and Hendre, 2014).

Conclusion

Medicinal plants are play a role like boon for all the living beings. Plants are known to have beneficial therapeutic effects documented in Traditional Indian System of Medicine. Numerous phytochemical and pharmacological studies have been conducted on rhizome of ginger. *Zingiber officinale* rhizome contains saponin, alkaloids, carbohydrates etc. The present study shows the potential of ginger rhizome as a medicinal plant.Many phytochemicals present in rhizome are medicinally active. Here we conclude that presence of the phytochemicals are also depends on the used solvent, as we can see that

Table 1 : Phytochemical screening of *Zingiber officinale* rhizome.

S. no.	Phytochemical Test	Aq. 10 gm	Aq. 5 gm gm	Methanolic
1.	Alkaloids			
	Mayer's	+ ve	+ ve	+ ve
	Wagner's	- ve	- ve	+ ve
	Hager's	+ ve	- ve	+ ve
2.	Carbohydrates			
	Molisch	+ ve	+ ve	- ve
	Barfoed's	- ve	- ve	- ve
3.	Reducing Sugars			
	Fehling's	+ ve	+ ve	+ ve
	Benedict's	+ ve	+ ve	+ ve
4.	Flavonoids			
	Alkaline Reagent	+ ve	+ ve	- ve
	Lead Acetate	- ve	- ve	+ ve
5.	Glycosides			
	Borntrager's	- ve	- ve	- ve
	Legal's	+ ve	+ ve	+ ve
	Keller-killiani	- ve	- ve	- ve
6.	Tannin & phenolic			
	Ferric Chloride	- ve	- ve	- ve
	Lead Acetate	+ ve	+ ve	+ ve
	Dilute iodine solution	- ve	- ve	+ ve
7.	Test for Saponin			
	Froth	+ ve	+ ve	+ ve
8.	Protein & A.A.			
	Ninhydrin	+ ve	+ ve	+ ve
	Biuret	+ ve	- ve	- ve
9.	Triterpenoids & Steroids			
	Salkowski's	+ ve	+ ve	+ ve
10.	Hydrolysable tannin	- ve	- ve	+ ve
11.	Glycosides	+ ve	+ ve	+ ve

"+" shows presence and "-" shows absence.

Table 2 : Rf value of rhizomes.

S. no.	Extract	Spots no.	RF Value
1.	Std.	1	0.69
2.	Ginger Rhizome	3	0.63, 0.71, 0.79

some are present in methanol but not in aqueous and vise-versa. Phosphomolybdenum shows the potential of antioxidant.

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