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# Secondary metabolites assessment: *In vitro* Antiarthritic and antihemolytic potential of various extracts of ginger

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### Abstract

Herbal medicines are one of the dietary supplements to maintain or improve health. They are used as prophylactic and therapeutic agents. *Zingiber officinale* is widely used around the world in food as the spice. The primary pungent agents (phenylalkylketones or vanillyl ketones) of ginger are gingerol with other general analogues such as the shogaols, paradole and zingerone. Present study is of primary and secondary metabolite assessment based in which main focus is on anti-arthritic and antihemolytic activity of ginger extracts. Preliminary screening shows the presence of carbohydrate and protein, alkaloids saponins, steroids and tannins. *In vitro* anti-arthritic study showed the inhibition of protein denaturation which indicated that the ginger extracts are capable of controlling the production of auto antigens. Another study of antihemolytic activity indicate the capacity of Ginger extract to reduce hemolysis of RBC. Further the FTIR- spectra of *Zingiber* has also been performed for rapid determination and identification of various functional groups responsible for the medicinal properties such as phenolics, ether, aromatics, carboxylic acid and alcohol.

**Keywords:** Herbal drugs, secondary metabolites, *zingiber officinales*, antiarthritis, antihemolytic

### 1. Introduction

Herbal medicines are one of dietary supplements to maintain or improve health. They are used as prophylactic and therapeutic agents against hepatic, renal, cardiovascular as well as inflammatory diseases, by modulating risk factors such as hypertension, high blood cholesterol, thrombosis, and preventing other chronic diseases associated with ageing (Rahman 2001, Tanaka *et al.*, 2006, Rahman, 2003, Neil *et al.*, 1994)<sup>[16, 17]</sup>.

World Health Organisation (WHO) reported that about 80% of the world's population depend mainly on traditional medicine and the traditional treatment involve mainly the use of plant extracts. Secondary plant metabolites play a very important role and have bio applications. Natural antioxidants are the secondary metabolites of the plant and do not have side effects when taken *in vivo* (Chen *et al.*, 1992; Walton and Brown, 1999)<sup>[4]</sup>. Many plants contain wide variety of free radicals scavenging molecule such as phenolic compounds, nitrogen compounds, vitamins and terpenoids. Some other endogenous metabolites phenolic compounds are also present which are important secondary metabolites and having redox properties which neutralizes the free radicals there by quenching singlet and triplet oxygen decomposing peroxides as an important antioxidant (Osawa, 1994)<sup>[12]</sup>. Tannins also found to be as a potential agent in variety of disease states (Packer *et al.*, 1999)<sup>[14]</sup>.

Ginger (*Zingiber officinale*, Zingiberaceae) is widely used around the world in foods as a spice. Ginger is a perennial rhizome and cultivated in the tropical climates of Australia, Brazil, China, India, Jamaica, West Africa, and parts of the United States (Langner *et al.*, 1998)<sup>[20]</sup>. Ginger rhizome was traditionally used in Chinese and Ayurvedic medicine as an antiemetic, antipyretic, and anti-inflammatory agent (Leung AY. Chinese Herbal Remedies, Universe Books, New York, 124). The constituents of ginger are numerous and vary depending on the place of origin and whether the rhizomes are fresh or dry. The primary pungent agents (phenylalkylketones or vanillyl ketones) of ginger are gingerol, with other gingerol analogues such as the shogaols, paradol and zingerone also found in high levels in rhizome extracts. The major pharmacological activity of ginger appears to be due to gingerol and shogaol (Duke and Beckstrom 1999). Phenylalkyl ketones or vanillyl ketones of ginger

include 6-gingerol 8-gingerol and 10-gingerol, 6-shogaol, 8-shogaol, 10-shogaol and zingerone. 6-paradol, 6- and 10-dehydrogingerdione and 6- and 10-gingerdione have also been identified. (Chrubasik *et al.*, 2007)<sup>[5]</sup>.

## 2. Materials and Methods

### 2.1. Collection of Material

For the present study, fresh ginger rhizome were collected from nearby crop field of ITM University campus. Collected ginger rhizome was weighed to 1000gm and cleaned properly.

### 2.2. Preparation of Methanolic Extract

Cleaned ginger was peeled and cut in to small pieces and shade dried. Dried rhizomes were powdered using mixer-grinder and subjected to Soxhlet extraction. Fifty grams of powder has been sequentially extracted with methanol and water respectively in soxhlet apparatus for 72 h. The obtained crude extract was dried in rotatory evaporator and stored in air tight container in refrigerator. These condensed extracts were used for the presence of various bioactive compounds by following standard methods. (Kumar *et al.*, 2012)<sup>[8]</sup>.

### 2.3. Preparation of aqueous extract

Fifty grams of powder has been extracted with water in soxhlet apparatus for 72 hrs. The obtained crude extract was air dried in Rotatory evaporator. After evaporation the obtained powdered extract was used for various biochemical analysis (Kumar *et al.*, 2012)<sup>[8]</sup>.

### 2.4. Phytochemical analysis of primary metabolites

The prepared extracts were used for Preliminary phytochemical screening for the presence of basic primary metabolites like carbohydrates, proteins and lipids (Plummer, 1987)<sup>[15]</sup>.

#### 2.4.1. Carbohydrates

For carbohydrate screening, dried extracts were dissolved individually in 5 ml distilled water and filtered through whattman filter paper 1. Molisch's test and Fehling's test was performed by using this filtrate for the presence of carbohydrates and reducing sugars respectively.

#### 2.4.2. Proteins

Presence of protein in the sample was tested by performing most suitable and appropriate test i.e. Biuret and Ninhydrin. Protocol of these tests are as follows:

**2.4.3. Biuret's test:** For this screening 1 ml of test extract, was added with 4% of sodium hydroxide solution and few drops of 1% copper sulphate solution. After few minutes, mixture becomes of violet and red colour which indicated the presence of peptide bond.

**2.4.4. Ninhydrin test:** For this laboratory assay a few drops of 0.25% of ninhydrin in acetone was added in 2 ml of test extract. Then the mixture has been heated in boiling water bath for 10 min. The formation of bluish purple colour in the tube indicated the presence of amino acid. (Kumar *et al.*, 2012)<sup>[8]</sup>

### 2.5. Phytochemical analysis of secondary metabolites

Secondary metabolites were screened in the prepared test extract for the presence of alkaloids, reducing sugars,

proteins, flavonoids, tannins, phenols, phytosterol and saponins by using the standard procedures. (Kumar *et al.*, 2012)<sup>[8]</sup>.

#### 2.5.1. Alkaloids

Alkaloids were detected by using Wagner's test. For this 2ml of extracts was mixed with 2ml of Wagner's reagent (1.27gm of Iodine and 2gm of KCl in 100ml distilled water). Formation of brownish- red precipitate indicates the presence of alkaloids.

#### 2.5.2. Saponins by Froth test

Saponins was tested by Froth formation test. For this 2ml extract was diluted with distilled water to 20ml and shaken vigorously in a graduated cylinder for 15 seconds. Formation of persistent foam layer of approximately 1cm at the surface indicate the presence of saponins in the extract. This froth become stable for 1 minute.

#### 2.5.3. Steroids by Salkowski test

Five milligram of extract was added with 2 ml of chloroform and 2 ml of conc. Sulphuric acid. Tubes were shaken and vigorously and allowed to kept at normal temperature for settling down. A golden yellow red colour indicated the presence of phytosterols.

#### 2.5.4. Tannins (ferric chloride test)

For tannins ferric chloride test was conducted by using solvent extract. Crude extract (1ml) was taken in a test tube and added with 500microlitre of FeCl<sub>3</sub> solution (2%); an intense green, purple, blue or black colour developed was taken as an evidence for the presence of tannins in foliar extract.

### 2.6. In-vitro anti-arthritis activity by inhibition of protein denaturation method

- The test experimental (0.5ml) consist of 0.45ml of Bovine serum albumin (5%w/v aqueous solution) and 0.05ml of test solution (250 µg/ml).
- Test control solution (0.5ml) consist of 0.45ml of bovine serum albumin (5%w/v aqueous solution) and 0.05ml of distilled water.
- Product control (0.5ml) consists of 0.45ml of distilled water and 0.05 ml of test solution (250 µg/ml).
- Standard solution (0.5ml) consists of 0.45ml of Bovine serum albumin (5%w/v aqueous solution) and 0.05ml of diclofenac sodium (250 µg/ml).

All the above solutions were adjusted to pH 6.3 using HCl (1N). The samples were incubated at 37°C for 20 minutes and the temperature was increased to keep the samples at 57°C for 3 minutes. After cooling, 2.5 ml of phosphate buffer was added to the above solutions. The absorbance was measured using UV- Visible spectrophotometer at 416nm (Venkataraman *et al.*, 2013)<sup>[19]</sup>. The percentage inhibition of protein denaturation can be calculated as:

Percentage Inhibition =  $[100 - (\text{optical density of test solution} - \text{optical density of product control}) / (\text{optical density of test control})] \times 100$ .

The control represents 100% protein denaturation. The results were compared with standard diclofenac sodium. The percentage inhibition of protein denaturation of different concentration was tabulated.

## 2.7. Heat induced haemolysis

Reaction mixture (2 ml) consisted of 1 ml test solution and 1 ml of 10% RBC suspension. Saline was replaced with drug in test solution. Aspirin was taken as a standard drug. The tubes were incubated in a water bath at 56 °C for 30 min. At the end of the incubation, the tubes were cooled under running tap water. Reaction was centrifuged at 2500 rpm for 5 minutes and OD of supernatant taken at 560 nm with UV-visible spectrophotometer (Okoli *et al.*, 2008)<sup>[13]</sup>.

## 2.8. FTIR Spectroscopy

The FTIR from 4000 to 400 cm<sup>-1</sup> was recorded on a PerkinElmer (spectrum 2) spectrometer. FTIR is used as a tool for the characterization and identification of compounds or functional groups (chemical bonds) present in an unknown mixture of plants extract (Eberhardt *et al.*, 2007; Hazra *et al.*, 2007)<sup>[6]</sup>.

## 3. Results and Discussion

Phytochemicals, also known as secondary metabolites, are biologically active, which have many health benefits. Results of the phytochemical screening of the extracts showed the presence of flavonoids, steroids, tannins and reducing sugars in both the aqueous and ethanolic extracts. Alkaloids and saponins can be traced only in the ethanolic and aqueous

extracts respectively (Table 1). Flavonoids have strong history in ayurvedic medicine and also have various uses in skin protection, normal brain function, maintaining blood sugar level and blood pressure regulation. Flavonoid is one of the main group of phenolic compound and widely distributed flavonoid, flavones and flavonols. Flavonoids and phenolics are the effective scavengers of the free radicals and chain breaking agents due to presence of hydroxyl group. Their presence indicates high analgesic and anti-inflammatory effects as a result of their membrane stabilizing ability against free radicals produced as a result of lipid peroxides and superoxides which causes cell membrane destabilization. It also possess anticancer, anti-diabetic, anti-aging properties and prevention of cardiovascular diseases. Tannins possess antiviral, antibacterial and antiparasitic effects and have the potential to fight against cancer.

In the present study the antioxidant activity of the sample remain very high which is possibly due to its high phenolic and flavonoid content. Polyphenolic and flavonoid compounds contain conjugate ring structures and hydroxyl groups; which aids in providing an antioxidant status in cell free systems by scavenging singlet oxygen, superoxide anion, lipid peroxy radicals, hydroxyl ions, nitric oxide ions, and stabilizing free radicals involved in oxidative processes (Aliyu, B.S. 2006)<sup>[11]</sup>

**Table 1:** Phytochemical Characteristics of Aqueous and Ethanolic Extracts of *Z. Officinale*

Extract	Alkaloids	Saponins	Flavonoids	Steroids	Tannins	Reducing sugar
Ethanolic	+	-	+	+	+	+
Aqueous	-	+	+	+	+	+

KEY: + = present  
- = absent

Table 2 depicts the *in-vitro* antiarthritic activity by inhibition of protein denaturation. The inhibition of protein denaturation was observed in the methanolic extract of ginger significantly at 800 µg/ml (65%) as compared to standard drug i.e. diclofenac sodium. The aqueous extract of ginger powder showed significant activity at 800 µg/ml (40%) by inhibition of protein denaturation as compared to the standard drug diclofenac sodium.

The production of auto antigen (like Rheumatoid Factor) in certain arthritic disease is because of protein denaturation. Hence we can conclude that the methanolic extract of ginger are capable of controlling the production of auto antigen or functional groups (chemical bonds) present in an unknown mixture of plant extracts. (Londhe *et al.*, 2020)<sup>[11]</sup>.

**Table 2:** Effect of aqueous and methanolic extract of ginger on protein denaturation

Treatment	Concentration (µg/ml)	Inhibition (%)
Diclofenac sodium	200	89.20
	300	92.46
	500	92.50
	800	98.67
Methanolic Extract	200	36.00
	300	44.00
	500	52.00
	800	65.00
Aqueous Extract	200	17.00
	300	20.00
	500	24.00
	800	40.00

Table 3 explains the antihaemolytic activity of the extract in the presence of, where ascorbic acid was taken as a positive control. It was observed that when RBC cell were treated with H<sub>2</sub>O<sub>2</sub> along with two different extract, there was a marked reduction in the haemolysis whereas the cells treated with the toxicant alone remain as such. When cells were treated with the extract alone, no haemolysis was resulted which explain the non-toxic behaviour of the extracts on human RBC. Out of all extract, methanolic extract was superior than others. Both methanolic and aqueous extracts demonstrated a significant inhibition of hemolysis in *in vitro* studies. The inhibition occur in crude extracts of ginger at comparatively lower concentrations (37.5 µg/ml) was comparable with that of standard anti-hemolysis compounds such as aspirin. This experimental evidence indicates that aqueous and methanolic extracts could have a potential therapeutic efficacy in disease processes causing destabilization of biological membrane. (Anwar *et al.* 2020)<sup>[2]</sup>

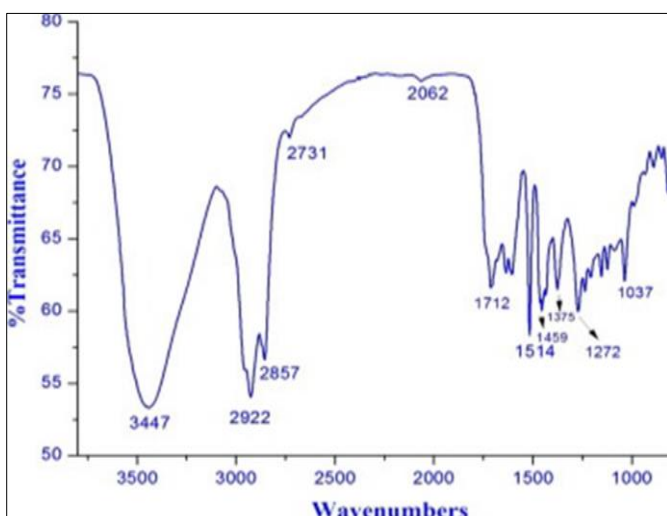
Tissue proteins are denatured causing production of auto antigens an arthritic diseases leading to inflammatory and arthritic diseases. Proteins denaturation sometimes causes alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding and observation of this study indicate such type of mechanism of action by ginger constituents. From the result of the present study it can be concluded that *Zingiber officinale* is capable of controlling the production of auto antigens due to *in-vivo* denaturation of proteins in rheumatic diseases. Protective effect on heat and hytonic saline-induced erythrocyte lysis is known to be a very good index of anti-arthritic activity of any agent. Such type of results are

also observed by Brown and Mackey, 1968 [3], since the membrane of RBC is structurally similar to the lysosomal membrane, the effect of any substance on stabilization of RBC membrane may be extrapolated to the stabilization of lysosomal membrane.

**Table 3:** Effect of ginger on heat induced hemolysis

Treatment	Concentration ( $\mu\text{g/ml}$ )	Inhibition (%)
Aspirin	200	89.20
	300	98.67
	500	92.46
	800	92.50
Methonolic Extract	200	51.00
	300	48.00
	500	42.00
	800	31.00
Aqueous Extract	200	44.00
	300	26.00
	500	21.00
	800	13.00

Figure 1 Showing the FTIR spectra for the Zingiber officinale. The present study of FTIR spectroscopy was performed for easy and rapid determination and identification of various functional groups responsible for medicinal properties. IR spectroscopy is basically a vibrational spectrum. The results indicated the presence of several functional groups such as phenolic, ether, aromatic, carboxylic acid, and alcohols are responsible for various medicinal properties of ginger



**Fig 1:** FTIR spectra for the *Zingiber officinale* extracts

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