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# Phytochemical standardization and antimicrobial activity of methanolic extract of *Zingiber officinale*

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**Abstract**
Phytochemicals show increasing demand in the market with their potent antioxidant, immune stimulant, growth promoting, anti-pathogenic, and anti-stress activity. Phytochemical estimation and anti-bacterial activity tuber of *Zingiber officinale* methanolic extract was studied. The phytochemical constituents and their composition in solvent extracts of the rhizome from *Zingiber officinale* were quantitatively estimated. In phytochemical estimation, phenolic & flavonoid contents were found in methanolic extract (174±0.100 mg GAE/gm extract and 100.5 ± 0.6 mg rutin/gm extract) respectively. Methanolic extract exhibit maximum antibacterial activity against *E. coli* (15 mm zone of inhibition), and *Pseudomonas aeruginosa* (15 mm zone of inhibition) followed by *Bacillus subtilis* (11.66 mm zone of inhibition), and *Streptococus mutans* (8.33 mm zone of inhibition). Our findings show that the rhizome extract of *Zingiber officinale* contains natural preservative agents, which justifies its use in traditional remedies.

**Keywords:** *Zingiber officinale*, phytochemicals, antibacterial

## Introduction
Various plants and plant parts have been in use for a very long time as medicinal preparations. They are highly accepted due to their effectiveness in treating various ailments and are also considered as safe. Plant-derived products have been in use over centuries in almost every continent all over the world and enthusiasm for this ancient form of medicine has never been greater than it is today [1]. One kind of dietary supplement is herbal medicine, which can be obtained as capsules, tablets, extracts, fresh plants, dried plants or powders. A number of chemical compounds that have an effect on the body and aid in preserving and enhancing health are produced by and found in herbs [2, 3]. Herbal medicines are widely used in developing and even developed countries for their safety and lesser side effects; they are also extensively used particularly in many Asian and African countries [4]. India is one of the countries that extensively use herbal medicines to meet their healthcare needs. Here, the herbal drug market is around 1 billion U.S dollars, and the export of plant-based crude drugs is around 80 million U.S dollars. Despite their growing interest in herbal therapy, India, unlike China, has not been able to promote the use of its abundant herbal assets throughout the developed world [5]. Ginger (*Zingiber officinale*) is a perennial herb which grows from an underground rhizome widely used as herbal medicine. Numerous beneficial effects of ginger have been identified by modern scientific research, including its antioxidant qualities, capacity to prevent the synthesis of inflammatory compounds, & direct anti-inflammatory effects [6]. Fresh ginger was used in ancient India and China to cure asthma, heart palpitations, swelling, loss of appetite, coughing, rheumatism, fever, diarrhea, & sore throats [7]. It has been thought to stimulate circulation and enhance blood flow and has been used for centuries to aid in digestion, inhibit vomiting, and prevent motion sickness and seasickness, it is also believed to relieve the common cold, flu-like symptoms, headache, and even painful menstrual period [8]. Thus, with respect to the long history of ginger's therapeutic values, this research work was carried out with the aim to determine the antibacterial activities of the dried *Zingiber officinale* rhizome extracts using methanol, as solvents in order to help support evidence of its effectiveness in the treatment of various ailments and its use in food as additive.

## Materials and Methods
### Collection and Identification of Plant Material
Fresh rhizome of *Zingiber officinale* for this study was collected from Navjeevan vihar, Navanagar, Bilaunji and Khutar, District Singrauli, (M.P.). The collected rhizomes of *Zingiber officinale* were dried at room temperature in the shade and away from direct sunlight.

**Pharmacognostical evaluation**

**Total ash value:** Accurately weighed 5 gms of powdered rhizome of *Zingiber officinale* were taken in a dried silica crucible. It was incinerated at 600 °C temperature, until free from carbon and then cooled. With reference to the air-dried sample, the weight of the ash has been determined and its percentage was calculated. The percentage of total ash was calculated with reference to the air-dried powder [9].

$$\% \text{ Ash content} = \frac{\text{Weight of crucible + ash-Weight of crucible}}{\text{Weight of crucible + sample-Weight of crucible}} \times 100$$

**Loss on drying**

Accurately weighed 5 gms of powdered of Mikania micrantha leaves were taken in a crucible. It was kept in a hot air oven at 105-110 ˚C, until free from moisture. The air-dried sample was used to calculate the percentage of moisture content.

$$\text{LOD \%} = \frac{\text{Wt. of petridish + crude drug-After drying Wt. of petridish + sample}}{\text{Weight of crude drug}} \times 100$$

**Water soluble ash**

The total ash obtained was boiled with 25 ml of water for few minutes, filtered and the insoluble matter was collected on ashless filter paper. After being thoroughly rinsed with hot water, the material was ignited in a silica crucible for 15 minutes at a temperature no higher than 450 °C. After cooling, the residue was measured. Water-soluble ash is represented by the difference in weight. Finally, using the air-dried sample as a reference, the percentage of water-soluble ash was determined [9].

$$\% \text{ Water soluble ash} = \frac{\text{Weight of crucible + ash-Weight of crucible}}{\text{Crude drug weight}} \times 100$$

**Acid insoluble ash**

The obtained total ash was heated for 5 minutes with 25 ml of 2 N HCl, filtered, and the insoluble material was then collected on ashless filter paper. It was then thoroughly cleaned with hot water, igniting in a silica crucible for 15 minutes at a temperature no more than 450 °C, cooled, and the resulting residue was weighed. In order to calculate the percentage of acid insoluble ash, the air-dried sample was used as a reference [9].

$$\% \text{ Acid soluble ash} = \frac{\text{Weight of crucible + ash-Weight of crucible}}{\text{Crude drug weight}} \times 100$$

**Alcoholic extractive value**

5 gm of the coarsely powdered air-dried leaves were macerated with 100 ml of 95% ethanol for 24 hours, stirring regularly during the first 6 hours, and then left to stand for 18 hours. After that, it was quickly filtered while taking precautions to prevent solvent loss. 25 ml of the filtrate were evaporated to dryness in a shallow dish with a flat bottom and tarred bottom, dried at 105 °C, and weighed. Using the air-dried drugs as a reference; the percentage of ethanol soluble extractive was estimated [10].

Alcohol soluble extractive value = Weight of reside/Weight of the drug × 100

**Water soluble extract**

Macerated 5 gm of the air dried coarsely leaves powder with 100 ml of chloroform water in a closed flask for 24 hours. Shaking frequently during the first 6 hours and allowed to stand for 18 hours. Thereafter, it was filtered rapidly taking precautions against loss of the solvent. Evaporated 25 ml of the filtrate to dryness in a tarred bottom flat bottom shallow dish dried at 105 °C and weighed. The percentage of water-soluble extractive value was calculated with reference to the air-dried drug [10].

Water soluble extractive value = Weight of reside/Weight of the drug ×100

**Extraction**

**Soxhlet Extraction**

Dried and ground rhizome of ginger were placed in a Soxhlet apparatus and extracted with solvent in a round bottom flask containing glass beads for 24 h. After extraction, the solvent was removed from the extract in a vacuum rotary evaporator. Methanol was used as solvent. Extracts were collected in air tight container [11]. Extraction yield of all extracts were calculated using the following equation below:

$$\text{Formula of Percentage yield} = \frac{\text{Actual yield}}{\text{Theoretical yield}} \text{ x } 100$$

**Qualitative phytochemical analysis of plant extract**

Qualitative phytochemical investigation of *Zingiber officinale* plant extract for the existence of carbohydrates, alkaloids, saponins, terpenoids, anthraquinones, cardiac glycosides, flavonoids, phenolics and tannins was performed by using standard procedures [2].

**Quantitative Tests**

**Total Phenolic Content**

Plant extract from Zingiber officinal (0.2 ml) had been mixed with 2.5 mL of prediluted Folin-Ciocalteu reagent (10-fold with distilled water). The mixture was allowed to stand at room temperature for 5 minutes before 2 mL of sodium

bicarbonate (7.5%, w/v) was added. At 760 nm, absorbance was measured after standing for two hours at room temperature. For calibration, aqueous solutions with known gallic acid concentrations between 20 and 100 g/ml were utilized. mg Gallic acid equivalents (GAE) were used as a unit of measurement for the results [12].

**Total flavonoid content**

The determination of total flavonoids was performed by Aluminium chloride method [12]. Distilled water (1.5 mL) was added to 0.5 mL of the extract in a test tube. Then, 0.150 mL of 5% sodium nitrite solution was added, followed by 0.150 mL of 10% aluminum chloride solution. After 6 minutes of ambient temperature incubation, 2 mL of 4% sodium hydroxide was added to the solution contained in test tubes. In an instant, distilled water was used to dilute the reaction mixture to 5 mL. Using a test tube shaker, the mixture was fully mixed, and the absorbance of the produced yellow to orange color was measured at 510 nm. For calibration, aqueous solutions with rutin concentrations known to be between 20 and 100 g/ml were utilized. The results were represented as mg rutin equivalents (RE)/g sample.

**Antimicrobial Activity (Well Diffusion Assay)**

**Anti-bacterial Activity**

**Preparation of Dilutions of the Samples**

The dilutions of the *Zingiber officinale* plant extract were made for the concentration as 100µg/ml, 150µg/ml, 200µg/ml, and 250µg/ml respectively of the sample, after that volume makeup was done with distilled water upto 1 ml.

**Preparation of Nutrient-Agar Media**

Nutrient agar Media was prepared by suspending 28 g of the powder in 1000 ml of sterile distilled water and brought to boiling to dissolve the medium before sterilizing with autoclave at 121 °C at 15 lbs pressure for 15 minutes. pH of media was checked before sterilization.

**Well Diffusion Assay**

Inocula of the bacterial test organisms *E. coli* (MTCC 42), *S. mutans* (MTCC 389), *B. subtilis* (MTCC 736) and *P. aeroginosa* (MTCC 8076) were prepared from 24 hour old cultures. At 530 nm, the absorbance was measured, and it was subsequently adjusted using sterile distilled water to make it equal to the absorbance of a 0.5 Mac Farland standard solution. Other dilutions with sterile distilled water were prepared from this prepared bacterial solution to give a final concentration of approximately 107 colony forming units (Cfu) per milliliter. 100µl of the inoculum from the broth (containing $10^7$ CFU/ml) was taken with a micropipette and then transferred to fresh and sterile solidified Agar Media Plate. The plates were allowed to set for 1 hour. Wells of similar distance have been bored using a 6 mm sterilized cork borer. Each well was filled with different concentration *(*100µg/ml, 150 µg/ml, 200 µg/ml and 250 µg/ml*)* of samples *(Zingiber officinale)*. The plates were incubated at 37 °C for 24 hours. Inhibition indicated by clear halo around the wells were measured in mm. Zones were measured to a nearest millimeter using a ruler, which was held on the back of the inverted Petri plate [13].

## Results

**Pharmacognostical evaluation**

**Table 1:** Pharmacognostical evaluation of plant sample

| Parameters | Value in percentage (%) |
|---|---|
| | ***Zingiber officinale*** **(Ginger)** |
| Total ash value | 8.24 |
| Loss on drying | 7.04 |
| Water soluble ash | 23.24 |
| Acid insoluble ash | 2.13 |
| Water extractive value | 9.32 |
| Alcoholic extractive value | 23.49 |

**Plant Extraction**

The plant material was extracted by soxhlet extraction method and the percentage yield calculated by the following formula:

$$\% \text{ yield} = \frac{\text{Actual Yield}}{\text{Theoretical yield}} \times 100$$

**Table 2:** Percentage yield

| Solvent | Theoretical yield (in gm) | Actual Yield (in gm) | Percentage Yield (%) |
|---|---|---|---|
| *Zingiber officinale* methanolic extract | 73.39 | 20.31 | 27.67 |

**Solubility Determination**

**Table 3:** Solubility Determination of Extracts

| S. No. | Solvent | ***Zingiber officinale*** **(Ginger)** |
|---|---|---|
| 1. | Water | Soluble |
| 2. | Methanol | Soluble |
| 3. | Ethyl Acetate | Soluble |
| 4. | DMSO | Soluble |
| 5. | Petroleum Ether | Insoluble |

**Phytochemical Analysis**

**Table 4:** Qualitative Phytochemical Analysis of extracts

| S. No. | Experiment | ***Zingiber officinale*** **methanolic extract** |
|---|---|---|
| **Test for Carbohydrates** | | |
| 1. | Molisch's Test | + |
| 2. | Fehling's Test | + |

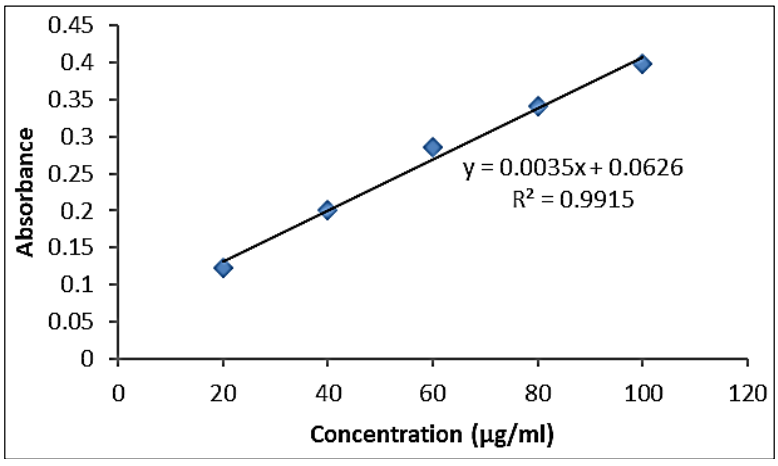
| | | |
|---|---|---|
| 3. | Benedict's Test | + |
| 4. | Bareford's Test | + |
| **Test for Alkaloids** | | |
| 1. | Mayer's Test | + |
| 2. | Hager's Test | + |
| 3. | Wagner's Test | + |
| 4. | Dragendroff's Test | + |
| **Test for Terpenoids** | | |
| 1. | Salkowski Test | + |
| 2. | Libermann-Burchard's Test | + |
| **Test for Flavonoids** | | |
| 1. | Lead Acetate Test | + |
| 2. | Alkaline Reagent Test | + |
| 3. | Shinoda Test | + |
| **Test for Tannins and Phenolic Compounds** | | |
| 1. | $FeCl_3$ Test | + |
| 2. | Lead Acetate Test | + |
| 3. | Gelatine Test | + |
| 4. | Dilute Iodine Solution Test | + |
| **Test for Saponins** | | |
| 1. | Froth Test | - |
| **Test for Protein and Amino acids** | | |
| 1. | Ninhydrin Test | + |
| 2. | Biuret's Test | + |
| 3. | Million's Test | + |
| **Test for Glycosides** | | |
| 1. | Legal's Test | + |
| 2. | Keller Killani Test | + |
| 3. | Borntrager's Test | + |

## Quantitative Phytochemical analysis

### Total Phenolic Content (TPC) Estimation

**Table 5:** Standard table for Gallic acid

| S. No. | Concentration (µg/ml) | Absorbance |
|---|---|---|
| 1. | 20 | 0.123 |
| 2. | 40 | 0.201 |
| 3. | 60 | 0.285 |
| 4. | 80 | 0.341 |
| 5. | 100 | 0.398 |




**Graph 1:** Graph represent standard curve of Gallic acid

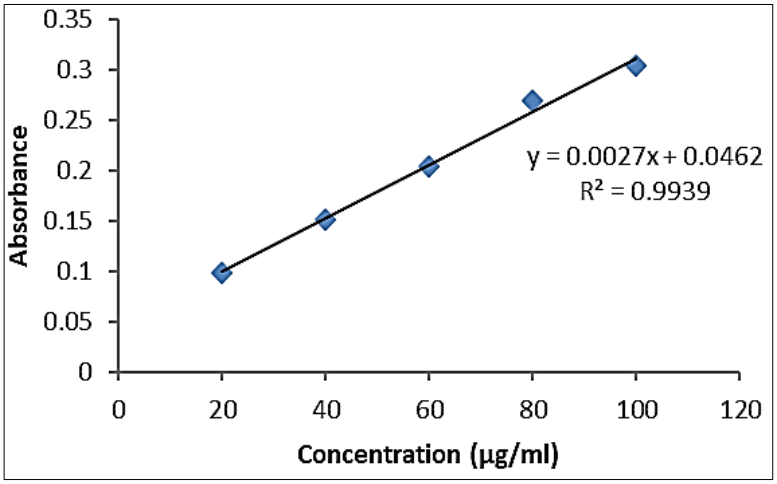
**Table 6:** Total Phenolic Content in extracts

| Extracts | Total phenolic content (mg/gm equivalent to Gallic acid) |
|---|---|
| | ***Zingiber officinale* methanolic extract** |
| Absorbance Mean ± SD | 0.584±0.012 |
| TPC (mg/gm) | 174 |

## Total Flavonoid Content (TFC) Estimation

**Table 7:** Standard table for Rutin

| S. No. | Concentration (µg/ml) | Absorbance |
|---|---|---|
| 1. | 20 | 0.098 |
| 2. | 40 | 0.151 |
| 3. | 60 | 0.204 |
| 4. | 80 | 0.269 |
| 5. | 100 | 0.304 |




**Graph 2:** Graph represent standard curve of Rutin

**Table 8:** Total Flavonoid Content in extracts

| Extracts | Total flavonoid content (mg/gm equivalent to rutin) |
|---|---|
| | ***Zingiber officinale*** **methanolic extract** |
| Absorbance Mean ± SD | 0.247±0.006 |
| TFC (mg/gm) | 100.5 |

## Antibacterial activity

**Table 9:** Antibacterial activity of *Zingiber officinale* against *E. coli*

| Concentration (µg/ml) | Plate 1 (mm) | Plate 2 (mm) | Plate 3 (mm) | Mean ± SD |
|---|---|---|---|---|
| 100 | 8 | 9 | 7 | 8±1 |
| 150 | 9 | 11 | 9 | 9.66±1.154 |
| 200 | 11 | 14 | 13 | 12.66±1.527 |
| 250 | 14 | 16 | 15 | 15±1.00 |

**Table 10:** Antibacterial activity of *Zingiber officinale* against *S. mutans*

| Concentration (µg/ml) | Plate 1 (mm) | Plate 2 (mm) | Plate 3 (mm) | Mean ± SD |
|---|---|---|---|---|
| 100 | 0 | 0 | 0 | 0±0 |
| 150 | 0 | 0 | 0 | 0±0 |
| 200 | 7 | 7 | 7 | 7±0 |
| 250 | 9 | 8 | 8 | 8.33±0.577 |

**Table 11:** Antimicrobial activity of *Zingiber officinale* extract against *B. subtilis*

| Concentration (µg/ml) | Plate 1 | Plate 2 | Plate 3 | Mean ± SD |
|---|---|---|---|---|
| 100 | 0 | 0 | 0 | 0±0 |
| 150 | 7 | 7 | 7 | 7±0 |
| 200 | 9 | 10 | 10 | 9.660±0.577 |
| 250 | 12 | 12 | 11 | 11.66±0.577 |

**Table 12:** Antimicrobial activity of *Zingiber officinale* extract against *P. aeruginosa*

| Concentration (µg/ml) | Plate 1 | Plate 2 | Plate 3 | Mean ± SD |
|---|---|---|---|---|
| 100 | 8 | 8 | 9 | 8.33±0.577 |
| 150 | 10 | 9 | 10 | 9.66±0.577 |
| 200 | 12 | 12 | 13 | 12.33±0.577 |
| 250 | 14 | 15 | 16 | 15±1.457 |

## Discussion

The current research deals with the studies on pharmacognostic, phytochemical and antibacterial activity on rhizome of *Zingiber officinale*. Raw materials were analyzed for identity, quality and purity as per the standards prescribed by WHO and Ayurvedic Pharmacopeia of India. The loss on drying of dry powder of *Zingiber officinale* was 7.04%. The

ash value was calculated using three different methods: total ash, acid-insoluble ash, and water-soluble ash. *Zingiber officinale's* crude powder was found to have 8.24% total ash, 23.24% water insoluble ash, and 2.13% acid insoluble ash. The water and alcoholic extractive value of crude powder of *Zingiber officinale* was found to be 9.32 and 23.49%.

The phytochemical analysis conducted on methanolic extract of *Zingiber officinale* contains alkaloids, carbohydrates, tannins, flavonoids, terpenoids, steroids, phenols, saponin and glycosides. Phytochemicals present in plant act as the source for the treatment of different health problem. Different phytochemical have different therapeutic value.

Total phenolic content (TPC) was measures by using Folin-ciocalteau's reagent method. And total flavonoid content (TFC) of *Zingiber officinale* was measured by Aluminum chloride method. The TPC and TFC of the extracts were expressed as milligram of Gallic Acid Equivalent per gram of extracts i. e. mg GAE/g extract and milligram of Rutin Equivalent per gram of extract i. e. mg RE/g extract respectively. The result of the present study revealed that the methanolic extract of *Zingiber officinale* has TPC (174mg GAE/g) and TFC value (100.5 mg RE/g).

Antibacterial activity was evaluated by well diffusion method against *E. coli*, *S. mutans*, *B. subtilis* and *Pseudomonas aeruginosa* with concentration ranging 100, 150, 200 and 250µg/ml. *Zingiber officinale* extract showed the maximum antibacterial activity against gram negative bacteria i.e. *E. coli* and *Pseudomonas aeruginosa*.

*Zingiber officinale* methanolic extract showed best zones of inhibition of 15mm in diameters at 250 µg/ml concentration against E. coli. Similarly, against *S. mutans*, *B. subtilis* and *P. aeruginosa*, *Zingiber officinale* showed zones of inhibition of 8.33, 11.66 and 15mm in diameters at 250 µg/ml concentration. It observe that, methanolic extract of plants contain phytochemicals including polyphenols and are reported to exhibit considerably high free radical scavenging and peroxide inhibition activity indicating its reducing character, which may in part explain the inhibition of bacterial growth.

## Conclusion

Thus, we can conclude that *Zingiber officinale* are the active material used against different diseases causing bacteria. It also shows that the rhizome extract of *Zingiber officinale* contains natural preservative agents, which justifies its use in traditional remedies. It will be helpful to phytochemists, pharmacologists and pharmaceutical industries.

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