Antioxidant Potential of Zingiber Officinale Rhizome Extracts

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Abstract

Ginger is one of the plants that are rich in phenolic compounds. Presence of volatile oil in the crude drug is responsible for the aromatic odour along with pungency of the drug is due to the yellowish oily component known as gingerol. Ginger has various pharmacological applications and used as anti-oxidant, anti-inflammatory, anti-microbial, anti-cancer, cardiovascular protective, anti-obesity, anti-diabetic, anti-emetics, spasmolytic, carminative, condiment etc. It has been used in treatment of dyspepsia, vomiting spasms, asthma, sore throat, and loss of voice is treated by chewing a piece of ginger.

This study aimed to determine the total phenolic content, antioxidant potential, analysis, and comparison of the antioxidant potential of the extracts obtained from rhizomes of zingiber officinale using different methods of extraction. Extraction of phytoconstituents were carried out by the continuous hot extraction (Soxhlet extraction) and maceration. Phytochemical screening of the extracts was done for the presence of various phytoconstituents, while the antioxidant activity of the different extracts was determined by using the DPPH method. Ginger has sufficient antioxidant activity on extraction by continuous hot extraction (Soxhlet Extraction) & maceration while anti-oxidant potential was found to be greater in the continuous hot extract.

Keywords - Ginger, Phenols and flavonoids, Antioxidant activity, DPPH method.

I. Introduction

Ginger consists of the dried rhizomes of the Zingiber officinale Roscoe, belonging to the family Zingiberaceae. Ginger contains 1-2% volatile oil, 5-8% pungent resinous mass, and starch. In fresh ginger gingerols are major polyphenolic components present in form of 6-gingerol, 8-gingerol and 10-gingerol. Gingerol due to heat and long term storage can be get convert into shogals, which is less pungent component and is not present in fresh ginger. While on hydrogenation shogals converted into paradols. The volatile oil is responsible for the aromatic odor and the pungency of the drug is due to the yellowish oily components called gingerol which is
odorless. The volatile oil is composed of sesquiterpene hydrocarbons like zingiber, sesquiterpene alcohol α-bisabolene, α-farnesene, and β-sesquiphellandrene.

Ginger has reported pharmacological applications and is used as an antiemetic, positive inotropic effect, spasmyloytic, aromatic stimulant, carminative, condiment, and flavouring agent. It is also prescribed for dyspepsia, flatulent colic, vomiting spasms, as an adjunct to many tonic and stimulating remedies, for painful affections of the stomach, cold, cough, and asthma etc. Sore throat, hoarseness, and loss of voice can be treated by chewing a piece of ginger. Accumulated investigations have demonstrated that ginger possesses multiple biological activities, including antioxidant, anti-inflammatory, anti-microbial, anti-cancer, neuroprotective, cardiovascular protective, respiratory protective, anti-obesity, anti-diabetic, anti-nausea, and antiemetic activities.

**Antioxidant**

A substance that protects cells from the damage caused by free radicals (unstable molecules made by the process of oxidation during normal metabolism). Free radicals may play a part in cancer, heart disease, stroke, and other diseases of aging. Antioxidants include beta-carotene, lycopene, vitamins A, C, and E, and other natural and manufactured substances. Gingerol is the main bioactive compound in ginger. It is responsible for much of ginger's medicinal properties. According to research gingerol has powerful anti-inflammatory and antioxidant effects. A diet high in antioxidants may reduce the risk of many diseases (including heart disease and certain cancers). Antioxidants scavenge free radicals from the body cells and prevent or reduce the damage caused by oxidation. The protective effect of antioxidants continues to be studied around the world these are some of the marketed preparations available in the market Lemony Ginger, Ginger Juice, Krishna’s Herbal & Ayurveda, Organic Ginger Tablets, Nadia Ginger powder, Doctor’s Best, High Potency Ginger Root Extract, and all has good medicinal property. These components are useful to stimulate several antioxidant enzymes and reduced the generation of reactive oxygen species and lipid peroxidation

II. **Experimental Work**

2.1 Extraction

2.1.1 Continuous Hot Extraction (Soxhlet Extraction)

For extraction of phytoconstituents from the crude drug, operational conditions include optimized sample, temperature, extraction time, and the ratio of crude drug to solvent. The operating temperature for experiments was from 70-80°C. Here the term extraction time is used for the duration of time it took for an experiment to run. In this research, the experiments were carried out from 1-1.5 hrs. of extraction time. The experiments were carried out using the equipment set up. The extraction was proceeded for 50gm of crude drug with 200ml of solvent (Ethanol)
2.1.2 Maceration

100 g of the fresh crude drug was soaked in 500 ml of absolute ethanol in a beaker for 72 hours. After 72 hours, the whole mixture was filtered to remove residue.

2.2 Preliminary Phytochemical Investigation of Extract

Preliminary Phytochemical Investigations of both the extracts obtained from continuous hot extraction and maceration process were carried out for different phytoconstituents and further evaluated for anti-oxidant activity. Both extracts shows positive test for following phytoconstituents.

Table no. 1: Test for Alkaloids

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Test</th>
<th>Observation</th>
<th>Inference</th>
<th>Image</th>
</tr>
</thead>
<tbody>
<tr>
<td>01.</td>
<td>Dragendroff's test: Few mL filtrate + 1-2 ml Dragendroff's reagents.</td>
<td>Formation of an orange-brown color precipitate.</td>
<td>Alkaloids are present.</td>
<td></td>
</tr>
<tr>
<td>02.</td>
<td>Mayer's test: Add a few drops of Mayer's reagent to the 3 ml of extract.</td>
<td>Formation of a cream color precipitate.</td>
<td>Alkaloids are present.</td>
<td></td>
</tr>
</tbody>
</table>

Table no.2: Test for Flavonoids & Phenolic compound

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Test</th>
<th>Observation</th>
<th>Inference</th>
<th>Image</th>
</tr>
</thead>
<tbody>
<tr>
<td>01.</td>
<td>Lead acetate test: To a small quantity of extract, add lead acetate solution.</td>
<td>Yellow-colored precipitate formation.</td>
<td>Flavonoids &amp; Phenolic compounds are present.</td>
<td></td>
</tr>
<tr>
<td>02.</td>
<td>Sodium Hydroxide test: 1 mL extract added in 2 mL of 2% NaOH solution (+ few drops dilute HCl)</td>
<td>An intense yellow color becomes colorless on addition of diluted acid.</td>
<td>Flavonoids &amp; Phenolic compounds are present.</td>
<td></td>
</tr>
<tr>
<td>03.</td>
<td>Ferric chloride test: A green precipitate.</td>
<td>A green precipitate.</td>
<td>Flavonoids &amp; Phenolic</td>
<td></td>
</tr>
</tbody>
</table>
Extract aqueous solution followed by addition of few drops of 10% ferric chloride solution.

Table no.3: Test for saponins

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Test</th>
<th>Observation</th>
<th>Inference</th>
<th>Image</th>
</tr>
</thead>
<tbody>
<tr>
<td>01.</td>
<td>Foam test:</td>
<td>The appearance of creamy miss of small bubbles</td>
<td>Saponins are present</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.2gm plant extract added in 5mL distilled water, shaken well and heated to boiling</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>02.</td>
<td>NaHCO3 test:</td>
<td>Stable honeycomb like froth</td>
<td>Saponins are present</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Plant extract added in few mL sodium bicarbonate solution and distilled water</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table no.4: Test for Tannins

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Test</th>
<th>Observation</th>
<th>Inference</th>
<th>Image</th>
</tr>
</thead>
<tbody>
<tr>
<td>01.</td>
<td>Braymer's test: Extract added in 3mL distilled water and 1 drop of 10% Ferric chloride solution.</td>
<td>Blue-green color.</td>
<td>Tannins are present.</td>
<td></td>
</tr>
<tr>
<td>02.</td>
<td>10% NaOH test: 0.4mL plant extract mixed with 4mL 10% NaOH and shaken well</td>
<td>Formation of emulsion (Hydrolysable tannins)</td>
<td>Tannins are present.</td>
<td></td>
</tr>
<tr>
<td>03.</td>
<td>Bromine water test: 10 ml of bromine water</td>
<td>Decoloration of bromine</td>
<td>Tannins are present.</td>
<td></td>
</tr>
</tbody>
</table>
reacted with 0.5gm plant extract

2.3 Determination of Antioxidant Activity

Antioxidant activity in the sample compounds was estimated for their free radical scavenging activity by using DPPH (1, 1-Diphenyl-2, Picryl-Hydrazyl) free radicals (George et al., 1996). 100μL of test compounds water was taken in the microtiter plate. 100μL of 0.1% methanolic DPPH was added over the samples and incubated for 30 minutes in dark conditions. The samples were then observed for discoloration; from purple to yellow and pale pink were considered as strong and weak positive respectively and read the plate on Elisa plate reader at 490nm.

Radical scavenging activity was calculated by the following equation:

DPPH radical scavenging activity (%) =

\[
\frac{[(\text{Absorbance of control} - \text{Absorbance of test sample}) / (\text{Absorbance of control})] \times 100}{1}
\]

III. RESULT AND DISCUSSION

Extracts by using different extraction methods were prepared & evaluated for their antioxidant potential using a DPPH scavenging assay. The following table gives the result of the antioxidant potential of prepared extracts using different methods.

* **Sample SEEG:** Extract obtained from continuous hot extraction
  (Crude drug: *Zingiber officinale rhizome*, Solvent: Ethanol)

* **Sample MEEG:** Extract obtained from maceration
  (Crude drug: *Zingiber officinale rhizome*, Solvent: Ethanol)
Table no. 5: Antioxidant potential of different extracts

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Concentration</th>
<th>Absorbance</th>
<th>Mean</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>--</td>
<td>1.842</td>
<td>1.563</td>
<td>1.555</td>
</tr>
<tr>
<td>Standard Ascorbic acid</td>
<td>1mg/ml</td>
<td>0.664</td>
<td>0.578</td>
<td>0.642</td>
</tr>
<tr>
<td>Sample: SEEG</td>
<td>100µl</td>
<td>0.789</td>
<td>0.852</td>
<td>0.765</td>
</tr>
<tr>
<td>Sample: MEEG</td>
<td>100µl</td>
<td>0.845</td>
<td>0.789</td>
<td>0.895</td>
</tr>
</tbody>
</table>

The delocalization of electrons also gives rise to the deep violet colour, characterized by an absorption band in ethanol solution centered at about 517 nm. When a solution of DPPH is mixed with that of a substrate that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet color. The direct scavenging activity of samples was evaluated against DPPH scavenging assays. In this, all compounds exhibited good inhibition against standard ascorbic acid.

IV. Conclusion

Extraction methods have been used for decades to extract active components with pharmacological activity. Various extraction methods are reported like maceration, continuous hot extraction, decoction, digestion, percolation, etc. for extraction of the phytoconstituents, and the selection of all these methods depends on various factors like phytoconstituents to be extracted, type of crude drug, time of extraction, solvent required for extraction, etc.
In the present study, the antioxidant potential of *Zingiber officinale* was determined by using different extracts prepared by different extraction methods like continuous hot extraction & maceration. A phytochemical investigation of each extract was carried out which indicates the presence of different phytoconstituents like alkaloids, flavonoids, phenolic compounds, saponins, tannins, etc.

Each extract was further evaluated for antioxidant potential & after comparing both the extract for antioxidant potential, it was found that the percent inhibition was 51.48% & 49.00% for sample SEEG & MEEG respectively. Both samples possess antioxidant potential but sample SEEG has the highest percent of inhibition as compared to sample MEEG. Further scientific validation is needed for quality formulation from extracts.

V. Conflict of Interest
The authors have no conflicts of interest regarding this investigation.

VI. Acknowledgment
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