

IN VITRO EVALUATION OF ANTIOXIDANT ACTIVITY OF GINGER (*Zingiber Officinale*)

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Abstract:-

Find out the scientifically evidence of Ginger (*Zingiber Officinale*) for it's antioxidant property. Preliminary phytochemical screening and in-vitro antioxidant activity of Ginger (*Zingiber Officinale*) extract were investigated but the extraction was done at different temperature(35°C,60°C,100°C) by decoction process. The antioxidant activity was studied in some in-vitro antioxidant models like DPPH radical scavenging activity, superoxide radical scavenging activity, ferric reducing power and hydrogen peroxide scavenging activity. Total antioxidant capacity was also determined. The Ginger (*Zingiber Officinale*) extract showed antioxidant activity by inhibiting DPPH, scavenging superoxide and hydrogen peroxide. It also showed reducing power ability in ferric reducing model. Total antioxidant capacity was found to be 18.32 mg/gm expressed as L-Ascorbic acid. Significant antioxidant activity of Water extract of Ginger (*Zingiber Officinale*) was found which might be due to the presence of Acidic compounds, Flavonoids, Phenols, Saponins, Tannins (Phenolic compounds) and Triterpenoids found in the preliminary Phytochemical screening.

Key words: Antioxidant, Ginger, Ferric Reducing, Zingiberaceae

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Introduction:-

Living cells needs Oxygen, Oxygen is essential for the survival of all on this earth. During the process of oxygen utilization in a normal physiological and metabolic process, approximately 5% of oxygen gets univalently reduced to oxygen derived free radicals like superoxide, hydrogen peroxide, hydroxyl and nitric oxide radicals. All these radicals known as reactive oxygen species (ROS) exert oxidative stress towards the cells of human body rendering each cell to face about 10,000 oxidative hits per second [1].

Antioxidants are added as red-ox systems possessing higher oxidative potential than the drug that they are designed to protect or as chain inhibitors of radical inducted decomposition. In general, the effect of antioxidants is to break up the chains formed during the propagation process by providing a hydrogen atom or an electron to the free radical and receiving the excess energy possessed by the activated molecule[2].It has been suggested that fruits, vegetables, natural plants contain a large variety of substance called phytochemicals which are present in plants and are the main source of antioxidant in the diet, which could decrease the potential stress caused by reactive oxygen species. The natural

antioxidants may have free-radical scavengers, reducing agents, potential complexers of prooxidant metals, quenches of singlet oxygen etc [3].The antioxidants can interfere with the oxidation process by reacting with free radicals [4].Recently interest has increased considerably in finding natural occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants which are being restricted due to their side effects such as carcinogenicity[5].Antioxidants principles from natural resources possess multifacetedness in their multitude and magnitude of activity and provide enormous scope in correcting imbalance[6].Food industry uses natural antioxidants as a replacement of conventional synthetic antioxidants [7]

Ginger (*Zingiber officinale* Roscoe, Zingiberaceae) is widely used around the world in foods as a spice. Native to tropical Asia, ginger is a perennial cultivated in the tropical climates of Australia, Brazil, China, India, Jamaica, West Africa, and parts of the United States [8].Ginger rhizome has a long history of use in Chinese and Ayurvedic medicine as an antiemetic, antipyretic, and anti-inflammatory agent, Here, the aim was to summarize the more recent and common actions and therapeutic application of ginger and its active constituents [9].

Literature Review:-

Ginger is a medicinal plant that has been widely used in Chinese, Ayurvedic and Tibb-Unani herbal medicines all over the world and has a long history of use in traditional systems of medicine. The primary pungent agents are due to the presence of phenylalkylketones or vanillyl ketones. Gingerol and shogaol are two most active constituents of ginger based preparations. They are reported to demonstrate antiemetic, antipyretic, analgesic, antiarthritic, and anti-inflammatory activities. Ginger, the rhizome of *Zingiber officinale*, is one of the most widely used species of the Ginger family (*Zingiberaceae*) and is a common condiment for various foods and

beverages. Ginger has a long history of medicinal use dating back 2,500 years in China and India for conditions such as headaches, nausea, rheumatism, and colds. Characterized in traditional Chinese medicine as spicy and hot, ginger is claimed to warm the body and treat cold extremities, improve a weak and tardy pulse, address a pale complexion, and strengthen the body after blood loss.

Botanical Description of Ginger:-

Ginger is herbaceous rhizomatous perennial, reaching up to 90 cm in height under cultivation. Rhizomes are aromatic, thick lobed, pale yellowish, bearing simple alternate distichous narrow oblong lanceolate leaves.

The herb develops several lateral shoots in clumps, which begin to dry when the plant matures. Leaves are long and 2 - 3 cm broad with sheathing bases, the blade gradually tapering to a point. Inflorescence solitary, lateral radical pedunculate oblong cylindrical spikes. Flowers are rare, rather small, calyx superior, gamosepalous, three toothed; open splitting on one side, corolla of three sub equaloblong to lanceolate connate greenish segments.

Phytoconstituents are present in Ginger:-

The constituents of ginger are numerous and vary depending on the place of origin and whether the rhizomes are fresh or dry but to summarize the major components that have been implicated in the pharmacological activities of the crude drug. The primary pungent agents (phenyl-alkyl-ketones 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. Phenylalkylketones 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 [10].

Material & Methods:-

Chemicals:-

L-Ascorbic acid, Rutin, Gallic acid, Hydrogen peroxide, Potassium ferricyanide, Trichloroacetic acid, Ferric chloride, Folin-ciocalteu reagent, Indigosulphonic acid, α - α diphenyl β picryl hydrazyl (DPPH), Riboflavin, Nitro Blue Tetrazolium (NBT) and Dimethyl Sulphoxide (DMSO) were all purchased from Merch chemicals, India, all other reagents used were of analytical grade.

Instruments:-

UV spectrophotometer (LABINDIA-Model-UV-3000⁺), Laboratory Centrifuge Machine (LABY, Instrument Industry).

Plant Material:-

The rhizome of *Zingiber officinale* were collected locally from the market of Nadaun, Dist;- Hamirpur, Himachal Pradesh(India) and were authenticated by Central National Herbarium, Botanical Survey Of India, Botanical Garden, Howrah-711103, West Bengal.



Fig.1 Ginger (*Zingiber officinale*, Family :- Zingiberaceae) rhizome

Extraction of Plant Material:-

The Ginger rhizomes are graded, cleaned and disintegrated to required mesh (20-60). It is then weighed and equally divided into 3 parts, which were then extracted separately with distilled water at different temperature conditions by the method of Decoction viz. prevailing room temperature and more 35°C, 60°C and 100°C for 10-15 minutes and were kept overnight. Next day the extracts were filtered off and the resulting filtrates were used for the study.(Due to that processing of extraction at different temperature, different chemical constituents presents in different extracts or degrades some constituents at higher temperatures also possible by this study that will be establish also).

Preliminary Phytochemical Screening:-

Preliminary phytochemical screening of the Ginger rhizome aqueous extract was carried out for the detection of the various constituents present in it. [11].

Preparation of Ginger Stock Solutions:-

Aqueous extracts (was done at three different temperature respectively 35° C,60° C,100° C for *Ferric Reducing Power Determination for other activity use only aqueous extract was done at 35° C*) of Ginger was prepared at the concentration of 1,000 μ g/ml in distilled water. From the stock solution different concentration viz. 10, 20, 40, 60, 80 and 100 μ g/ml were prepared in same solvent and used for antioxidant studies.

Preparation of Standard Stock Solution of L-Ascorbic Acid:-

L-Ascorbic acid used as standard for the study and its stock solution was prepared in the concentration of 1,000 μ g/ml in distilled water. It was prepared freshly and used immediately for the study. From the stock solution different concentration viz. 10, 20, 40, 60, 80 and 100 μ g/ml were prepared in distilled water and used for antioxidant studies.

Total Antioxidant Capacity:-

For total antioxidant capacity assay, 0.3 ml of the Ginger extract (at 35° C) (10 mg/ml) dissolved in water and mixed with 3ml of reagent solution (0.6 M sulfuric acid, 28mM sodium phosphate and 4 mM ammonium molybdate) in Eppendorf tube. The tubes were capped and incubated in a thermal block at 95°C for 90 min. After 90 min, the mixture was cooled to room temperature; the absorbance was measured at 695 nm against reagent blank. Methanol (0.3 ml) in the place of extract is used as the blank. L-Ascorbic acid was used as the standard and the total antioxidant capacity is expressed as equivalents of L-Ascorbic acid [5].

(For the purpose of determination of Total Antioxidant Capacity, using only aqueous extract of Ginger which was done at 35° C, because in that extract most of the constituents are present and at that temperature no one constituents are degraded e.g. Flavonoids etc)

DPPH Radical Scavenging Activity:-

Ginger extract (only for 35° C) and standard ascorbic acid solution (0.1 ml) of different concentrations viz. 10, 20, 40, 60, 80 and 100 µg/ml was added to 3 ml of a 0.004% methanol solution of DPPH. An equal amount of methanol and DPPH served as control. After 30 minutes incubation in the dark, absorbance was recorded at 517 nm, and the percentage inhibition activity was calculated from $[(A_0-A_1)/A_0] \times 100$, where A_0 is the absorbance of the control, and A_1 is the absorbance of the extract/standard. The antioxidant activity of the extract was expressed as IC_{50} . The IC_{50} value was defined as the concentration (in µg/ml) of extracts that inhibits the formation of DPPH radicals by 50%. All the tests were performed in triplicate and the graph was plotted with the average of three observations [5].

Superoxide Radical Scavenging Activity:-

Each 3ml reaction mixture contained 50 mM sodium phosphate buffer (pH 7.6), 20 µg riboflavin, and 12 mM EDTA and 0.1 mg NBT

and 1ml of sample solution. Reaction was started by illuminating the reaction mixture with different concentrations of Ginger extract (only for 35° C) and standard ascorbic acid solution viz. 10, 20, 40, 60, 80 and 100 µg/ml for 5min. Immediately after illumination, the absorbance was measured at 590 nm. Identical tubes with reaction mixture and 1ml of methanol were kept in the dark along and served as control. The percentage inhibition of superoxide anion generation was calculated from $[(A_0-A_1)/A_0] \times 100$, where A_0 is the absorbance of the control, and A_1 is the absorbance of the extract/standard. The Antioxidant activity of the extract was expressed as IC_{50} . All the tests were performed in triplicate and the graph was plotted with the average of three observations [5].

Scavenging Of Hydrogen Peroxide:-

A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffer saline (pH 7.4), different concentrations of Ginger extract (only for 35° C) and standard L-Ascorbic acid solution viz. 10, 20, 40, 60, 80 and 100 µg/ml in methanol (1 ml) where added to hydrogen peroxide solution (2 ml). Absorbance of hydrogen peroxide at 230nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. For each concentration, a separate blank sample was used for back ground subtraction. The percentage inhibition activity was calculated from $[(A_0-A_1)/A_0] \times 100$, where A_0 is the absorbance of the control and A_1 is the absorbance of extract/standard. The antioxidant activity of the extract was expressed as IC_{50} . All the tests were performed in triplicate and the graph was plotted with the average of three observations [5].

Ferric Reducing Power Determination:-

Different concentrations of Ginger extract (which was done at three different temperature respectively 35° C, 60° C,100° C) and standard L-Ascorbic acid solution viz. 10, 20, 40, 60, 80 and 100 µg/ml in 1ml of methanol were mixed with phosphate buffer (2.5 ml, 0.2 M pH 6.6) and

potassium Ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50° C for 20 min. A portion (2.5 ml) of Trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3,000 g (rpm) for 10 min at room temperature. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride ($FeCl_3$) (0.5 ml, 0.1%) and the absorbance of the reaction mixture indicated increased reducing power. The absorbance was measured at 700 nm. All the tests were performed in triplicate and the graph was plotted with the average of three observations [5] [12].

(For the purpose of other determinations except Ferric Reducing Power Determination using only aqueous extract of Ginger which was done at 35° C, because in that extract most of the constituents are present and at that temperature no one constituents

Table.1:- Preliminary Phytochemical Screening.

Name of Herb	Part used	Scientific name	Alkaloids	Acids	Carbohydrates	Fixed Oils	Flavonoids	Glycosides	Gums	Resins	Saponins	Sterols	Tannins	Terpenes
Ginger	Rhizomes	<i>Zingiber officinale</i> Roscoe, Family: - <i>Zingiberaceae</i>	+	+	+	+	+				+	+	+	+

The phytochemical screening results showed the presence of Alkaloids, Carbohydrates, Proteins, Saponin glycosides and Tannins in the Ginger aqueous extracts.

are degrades e.g. Flavonoids etc. But in *Ferric Reducing Power Determination comparing with all Aqueous extract of Ginger* which was done at three different temperature respectively 35° C, 60° C, 100° C with a Standard)

Statistical Evaluation:-

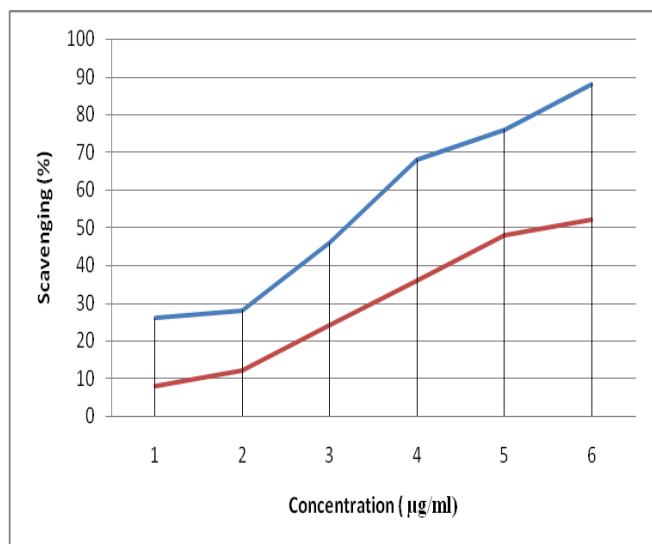
Experimental results were Mean±SEM of three parallel measurements. Linear regression analysis was used to calculate the IC₅₀ value. Student’s t-test was used for the comparison between two means for the possible significant interrelation. Data were considered statistically significant only when *p* value < 0.05.

Results and Discussion :-

Total Antioxidant Capacity. The total antioxidant capacity in the Ginger extract (at 35°C)

measured spectrophotometrically was 18.32 mg/gm expressed as L-Ascorbic acid.

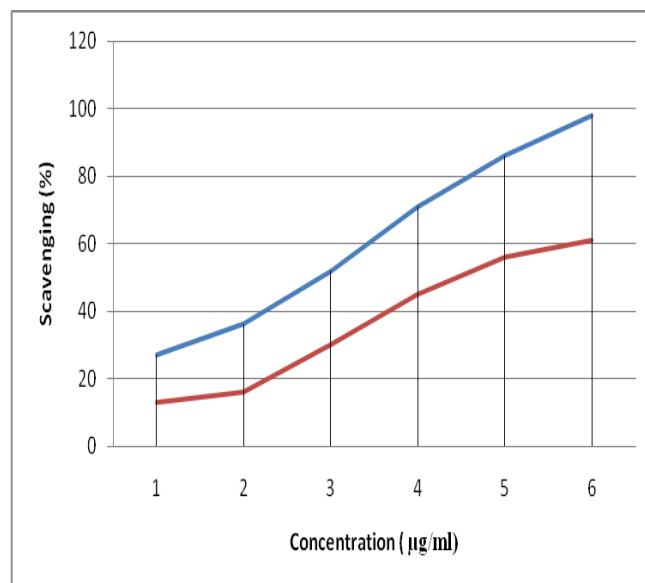
DPPH Radical Scavenging Activity:- Fig. 2 illustrates a significant ($p < 0.05$) decrease in the concentration of DPPH radicals due to the scavenging ability of Ginger extract. This activity was dose dependent. Maximum scavenging activity (52.50%) was observed at 100 $\mu\text{g/ml}$ concentration and the IC_{50} value of Ginger extract and L-Ascorbic acid were found to be 87.80 $\mu\text{g/ml}$ and 44.60 $\mu\text{g/ml}$ respectively.



- Blue line represent L-Ascorbic acid curve.
- Red line represent Aqueous extract of Ginger curve.

Fig. 2. DPPH radical scavenging activity of Ginger extract.

Super Oxide Radical Scavenging Activity: - Fig.3. Reveals that a significant ($p < 0.05$) dose response relationship is found in the superoxide free radical scavenging activity in Ginger extract. Maximum scavenging activity (61.29%) was observed at 100 $\mu\text{g/ml}$ concentration of and the IC_{50} value of Ginger extract and L-Ascorbic acid were found to be 82.20 $\mu\text{g/ml}$ and 44.99 $\mu\text{g/ml}$ respectively.

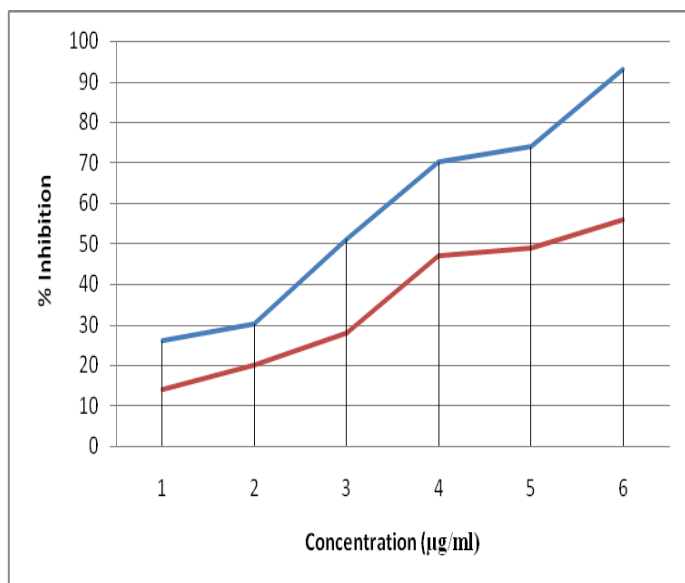


- Blue line represent L-Ascorbic acid curve.
- Red line represent Aqueous extract of Ginger curve.

Fig. 3. Superoxide radical scavenging activity of Ginger extract.

Scavenging of Hydrogen Peroxide:-

Fig. 4 reveals that a significant ($p < 0.05$) dose dependent response was found in the hydrogen peroxide scavenging activity in Ginger extract. Maximum scavenging activity (56.62%) was observed at 100 $\mu\text{g/ml}$ concentration and the IC_{50} value of Ginger extract and L-Ascorbic acid were found to be 82.55 $\mu\text{g/ml}$ and 39.84 $\mu\text{g/ml}$ respectively.

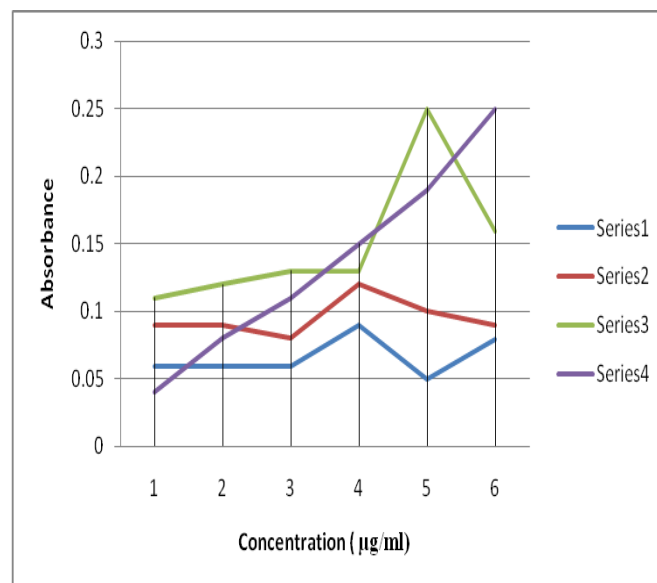


- Blue line represent L-Ascorbic acid curve.
- Red line represent Aqueous extract of Ginger curve.

Fig. 4. Hydrogen peroxide radical scavenging activity of Ginger extract.

Ferric Reducing Power Determination:-

Fig. 5 reveals that reducing power of Ginger extract was statistically significant ($p < 0.05$). The result clearly indicates that the reducing power of the Ginger extract increased with increasing the concentration and is comparable with the standard L-Ascorbic acid, hence it is having the antioxidant activity.



- Blue line represent at 35°C
- Red line Represent at 60°C
- Green line represent at 100°C
- Violet line represent Standard curve for L-Ascorbic Acid.

Fig. 5. Reducing power of Ginger extract (Represent a Comparison Study of Antioxidant Activity, between different extraction was done at different temperatures 35°C, 60°C, 100°C respectively of Ginger)

CONCLUSION

The Ginger aqueous extract showed antioxidant activity by inhibiting DPPH, scavenging super oxide as well as hydrogen peroxide and reducing power ability which may be due to presence of Tannis, Phenolic compounds, alkaloids and saponin, glycosides, Triterpenoids, Flavonoids etc found in the preliminary phytochemical screening. Thus, the radical scavenging activity suggests that Aqueous extract of **Ginger (Zingiber Officinale)** *In-vitro* antioxidant activities. Further studies are needed to evaluate the *in-vivo* antioxidant potential of Ginger extract in various animal models.

ACKNOWLEDGMENT:-

The authors gratefully acknowledge the supports of Prof.(Dr.) R. Parthasarathy Sir, Department of Pharmacognosy ,as well as Mr.Ashok Sharma Sir,Himachal Institute Of Pharmaceutical Education & Research,Nadaun,Hamirpur,Himachal Preadesh, and Special thanks to Mr.Sambhu Nath Ghosh,Mrs.Kalayani Ghosh and Mrs.Sarita Devi For providing all facility to complete this work successfully .

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