

COMPARATIVE PHARMACOGNOSTICAL STUDY OF *ZINGIBER OFFICINALE* ROSCOE HARVESTED IN DIFFERENT SEASONS**Swapnil S. Tirmanwar^{*1}, Shiwani P. Wadichar², Gopal Pondhe³, Saurabh D. Kolaskar⁴, Kajal Prasad⁵ and Hitesh Bisen⁶**¹Research scholar, Department of Pharmacognosy, Priyadarshini J. L. College of Pharmacy, Nagpur, Maharashtra, India – 440016.²Associate professor, Department of Pharmacognosy, Priyadarshini J. L. College of Pharmacy, Nagpur, Maharashtra, India – 440016.³Research scholar, Department of Pharmaceutical Chemistry, Indira College of Pharmacy, Nanded, Maharashtra, India – 431601.⁴Research scholar, Department of Pharmaceutics, Indira College of Pharmacy, Nanded, Maharashtra, India – 431601.⁵Research scholar, Department of Pharmaceutics, Priyadarshini J. L. College of Pharmacy, Nagpur, Maharashtra, India – 440016.⁶Research scholar, Department of Pharmaceutics, Priyadarshini J. L. College of Pharmacy, Nagpur, Maharashtra, India – 440016.***Corresponding Author: Swapnil S. Tirmanwar**

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ABSTRACT

Zingiber officinale Roscoe, commonly known as ginger, is an important medicinal plant belonging to the Zingiberaceae family. The rhizome of this plant is utilized in traditional Indian medicine to address various health issues, including fertility problems, hypertension, cancer, and urinary tract disorders. Given its significant role in traditional medicine, standardizing the rhizome is crucial. Analyzing the variations among plants across different seasons can yield valuable insights related to optimal cultivation periods and geographic conditions. Various quality control parameters were assessed, including extractive values with ethanol, ash values, foreign organic matter, loss on drying, pH of the aqueous solution, and total content of phenolics and tannins. The results from the preliminary pharmacognostic standardization of *Zingiber officinale* rhizome are instrumental in determining the quality and purity of this crude drug. They also provide an understanding of the efficacy and comparative qualities of wild and cultivated plants.

KEYWORDS: Medicinal plant, comparative, quality control, standardization.**INTRODUCTION**

Ginger (*Zingiber officinale*) belongs to the Zingiberaceae family. The rhizome is the part of the plant that is used. This plant produces an orchid-like flower with greenish-yellow petals streaked with purple color. Ginger is cultivated in areas characterized by abundant rainfall. Although it is native to southern Asia, ginger is also cultivated in tropical areas such as Jamaica, China, Nigeria, and Haiti, and it is an important spice crop in India. Ginger, *Zingiber officinalis*, is a perennial herbaceous plant that is a part of the Zingiberaceae family. It is an important plant with several medicinal, ethnomedicinal, and nutritional values. Ginger is the underground rhizome of the ginger plant with a firm, striated texture. *Zingiber officinale* R., commonly known as ginger, belongs to the family Zingiberaceae.^[1,2]

Ginger extracts contain polyphenol compounds, such as 6-gingerol and its derivatives, which exhibit high antioxidant activity. This activity is attributed to the presence of phytochemicals including flavones, isoflavones, flavonoids, anthocyanin, coumarin, lignans, catechins, and isocatechins. The antioxidant property of ginger is an extremely significant activity that can be used as a preventive agent against a number of diseases. Phenolic compounds, mainly gingerols, shogaols, and paradols, account for the various bioactivities of ginger. Ginger is abundant in active constituents, such as phenolic and terpene compounds. In fresh ginger, the major polyphenols are gingerols, including 6-gingerol, 8-gingerol, and 10-gingerol. As the phytoconstituents in plants vary in quantity and quality due to changes in climate and surrounding natural factors, this study is based on the comparative evaluation of ginger grown in different seasons.^[2,3]

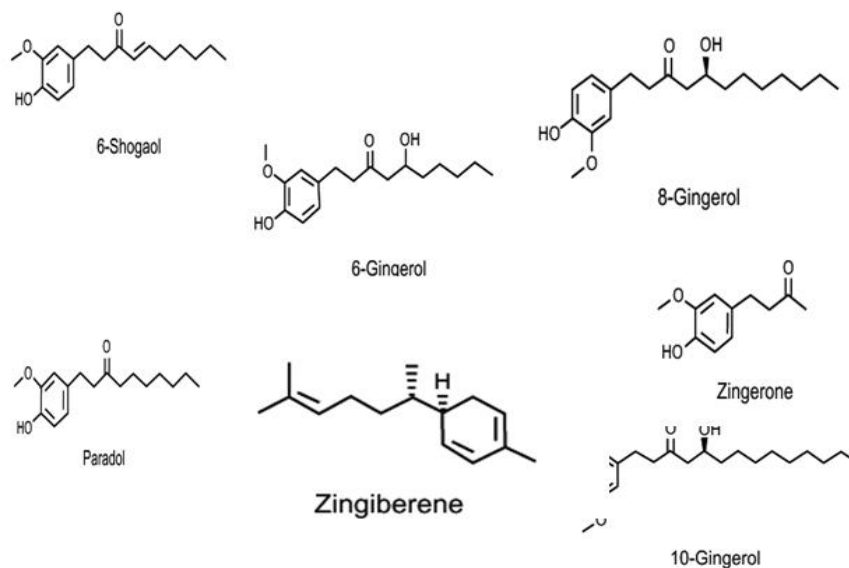


Figure 1: Chemical constituents of Ginger.

Plant Profile

Synonyms: Ginger root, Black Ginger, Zingiberic rhizome, Zingiber, Zingiberis.

Biological source- Ginger obtained from the dried rhizomes of *Zingiber officinale* Roscoe.

Kingdom: Plantae
Subkingdom: Tracheophytes
Division: Angiosperms
Subdivision: Monocots
Class: Commelinids
Order: Zingiberales
Family: Zingiberaceae
Genus: *Zingiber*
Species: *Z. officinale* Roscoe



Figure 2: Ginger plant and Rhizome.

MATERIALS AND METHOD

Plant material

The ginger rhizomes were procured from the local market of Nagpur, in November and April. The rhizomes were extracted with 70% Ethanol by the maceration method.

Determination of Ethanol soluble extractive value

4 grams of accurately weighed powdered material was placed in a glass stoppered conical flask. 100 ml of ethanol was added to the flask, and the total weight, including the flask, was measured. The flask was then shaken well and allowed to stand for 1 hour. A reflux condenser was attached to the flask, and it was gently boiled for 1 hour. Then, it was cooled and weighed. The weight was adjusted to the original total weight by adding the required amount of ethanol. The flask was shaken well and filtered rapidly through a dry filter paper. After that, 25 ml of the filtrate was transferred to a tarred flat-bottomed dish and evaporated to dryness in a water bath. Then, the dish was dried at 105 °C for 6 hours, cooled in a desiccator, and weighed.^[4,5,8]

The content of extractable matter (% w/w) air-dried material was calculated as follows.

% Methanol soluble extractive matter = $\frac{\text{weight of residue}}{\text{weight of sample}} \times 100$

Determination of total ash content

The powdered material (2 g) was accurately weighed and placed in a crucible. The material was spread evenly in a layer and ignited to a constant weight by gradually increasing the heat to 500-600 °C until it turned white, indicating the absence of carbon. The remaining ash was allowed to cool in a desiccator.^[4,5] The content of total ash (in mg/g) of air-dried material was calculated as follows.

% Total ash = $\frac{\text{weight ash}}{\text{weight of sample}} \times 100$

Determination of acid-insoluble ash

HCl (2 N; 25 mL) was added to the crucible containing the total ash, covered with a watch glass, and boiled gently for 5 min. The watch glass was rinsed with 5 mL of hot water and the rinsed contents were added to the crucible. The acid-insoluble matter was collected on an ashless filter paper and washed with hot water until the filtrate was neutral. The filter paper containing acid-insoluble matter was transferred to the original crucible, dried on a hot plate, and ignited to a constant weight. The residue was allowed to cool in a desiccator and weighed.^[4,5,8] The content of the acid-insoluble ash (in mg/g) of air-dried material was calculated as follows.

% Acid insoluble ash = $\frac{\text{weight ash}}{\text{weight of sample}} \times 100$

Determination of water-soluble ash

Water (25 mL) was added to the crucible containing the total ash, covered with a watch glass, and boiled gently for 5 min. The watch glass was rinsed with 5 ml of hot water and added to the crucible. The water-insoluble

matter was collected on an ashless filter paper and washed with hot water. The filter paper containing the water-insoluble matter was transferred to the original crucible, dried on a hot plate, and ignited to a constant weight.^[4,5] The water-soluble ash content was calculated using the following equation

% Water soluble ash = $\frac{\text{total ash content} - \text{water insoluble residue in total}}{\text{ash weight of sample}} \times 100$

Foreign matter analysis

Foreign matter presence may be due to faulty collection of crude drugs or due to deliberate mixing. It was separated from the drug so that the results obtained taste are important parts of the morphology of a particular drug.^[4,5]

Preliminary phytochemical screening of Ginger rhizome extracts

The qualitative screening of ginger (*Zingiber officinale*), contained information on the screened from ginger. Alkaloid and flavonoid were present in ginger ethanol extract. Phenol and phytate were moderate in ginger ethanol extract while flavonoid, saponin, and alkaloid were also found in ginger extract. Steroid, anthraquinone, tannin, and saponin were extracted in in ginger ethanol extract while phenol and tannin were also present in ginger Ethanol extract.^[4,5,6]

Total Phenolic Content

Total phenolic content was estimated by the Folin–Ciocalteu colorimetric method, based on the procedure of Azlim Almey, 2010, using gallic acid as a standard phenolic compound. A linear calibration curve of gallic acid with an R² value of 0.9917 was obtained. Figure 3 shows the mean TPC of the Ginger extracts measured using the GAE equation of $Y = 0.001x + 0.002$ (R² = 0.9065), whereby Y = absorbance at 765nm and X = concentration of total phenolic compounds in mg per ml of the extract.^[4,5,6,7]

Total Tannin Content

Total tannin content was estimated by the Folin– Denis colorimetric method, based on the procedure of Azlim Almey, 2010, using Tannic acid as a standard. A linear calibration curve of gallic acid with an R² value of 0.9065 was obtained (not shown). Figure 4 shows the mean TTC of the Ginger extracts measured using the TAE equation of $Y = 0.005x + 0.1079$ (R² = 0.9065), whereby Y = absorbance at 276nm and X = concentration of total phenolic compounds in mg per ml of the extract.^[4,5,6]

In-vitro antioxidant activity of *Zingiber officinale*

In-vitro antioxidant activity of *Zingiber officinale*, will be performed using two different methods.

Hydrogen Peroxide (H₂O₂) Scavenging Activity

In this test, varying concentrations of the test substance (50 to 800 µg/ml) were assayed. Test solution: H₂O₂ solution (40 mmol/l in phosphate buffer): phosphate

buffer (pH 7.4) at 1:0.6:3.4 ml was added to the test tube. The absorbance of the reacting solution versus blank including extract solution plus phosphate buffer (1:4, ml) was checked spectrophotometrically at 230nm. The control consisted of phosphate buffer: H₂O₂ solution (3.4:0.6, ml). the equation was used for % H₂O₂ inhibition.^[5,6]

Reducing Power Ability

Prepare the different concentrations of samples (1.0 ml). Mix it with 2.5 ml of phosphate buffer (50 mM, pH 7.0) and 2.5 ml of 1% potassium ferricyanide. Incubate at 50°C for 20 min. Then add 2.5 ml of trichloroacetic acid

(10%) to the mixture, and centrifuge at 3000 rpm for 10 min. Finally, mix 1.25 ml from the supernatant with 1.25 ml of distilled water and 0.25 ml FeCl₃ solution (0.1%, w/v). Measure the absorbance at 700 nm. Carry out the assays in triplicate. Increased absorbance values indicate a higher reducing power.^[5,6]

RESULTS

The ginger extracts harvested in two different seasons were successfully evaluated using various parameters for their physicochemical characteristics, quantitative estimation of their secondary metabolites and their antioxidant activity.

Table 1: Physicochemical characteristics.

Physicochemical Character	GNOV	GAPR
Extractive Value	2.4	2.03
Total ash (%w/w)	8.33	9.03
Acid insoluble ash ash (%w/w)	1.6	1.25
Water insoluble ash ash (%w/w)	5.23	6.52
Foreign organic matter (%w/w)	1.53	1.66
Loss on drying ash (%w/w)	13.52	11.09
Moisture Content	12.12	5.43

Table 2: Phytochemical screening of Zingiber officinalis.

Sr. No.	Chemical test	GNOV	GAPR
1.	Alkaloid	+	+
2.	Carbohydrate		
	Molish test	+	+
	Fehlings test	+	+
3.	Glycosides	-	-
4.	Tannin	+	+
5.	Phenolic	+	+
6.	Flavonoid	+	+
7.	Steroid	-	-
8.	Saponins	+	+

Total Phenolic Content

The total phenolic content of both ginger samples was calculated using the FC reagent method, and the numerical content was compared with the std. curve of

the Gallic Acid and is represented as the GAE (Total Gallic Acid Equivalent). The Ethanolic extracts, of GNOV, showed a of (13.23 ±0.13 mg/g) and the GAPR showed a TPC of (9.38 ±0.13 mg/g).

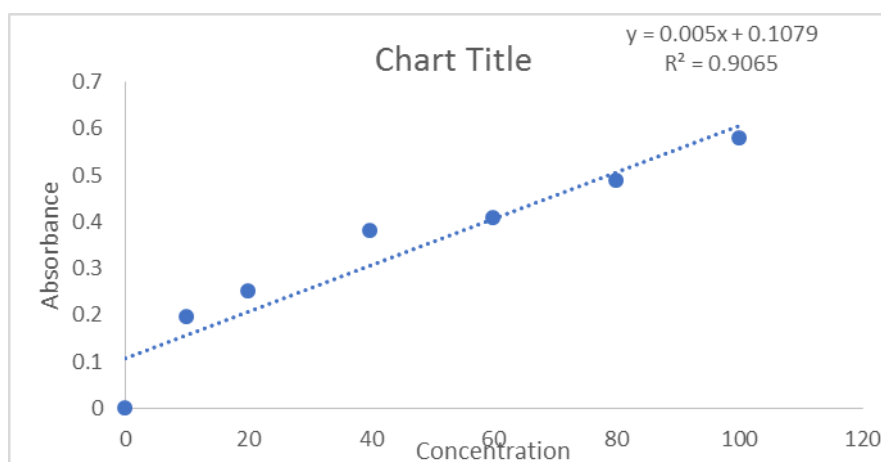


Figure 1: Standard curve of GAE.

Total Tannin Content

Total tannin content was estimated by the Folin– Denis colorimetric method and the numerical content was compared with the std. curve of the Tannic Acid and is

represented as the TAE (Total Tannic Acid Equivalent). The Ethanolic extracts, of GNOV, showed a of $(16.85 \pm 0.08 \text{ mg/g})$ and the GAPR showed a TPC of $(14.32.38 \pm 0.08 \text{ mg/g})$.

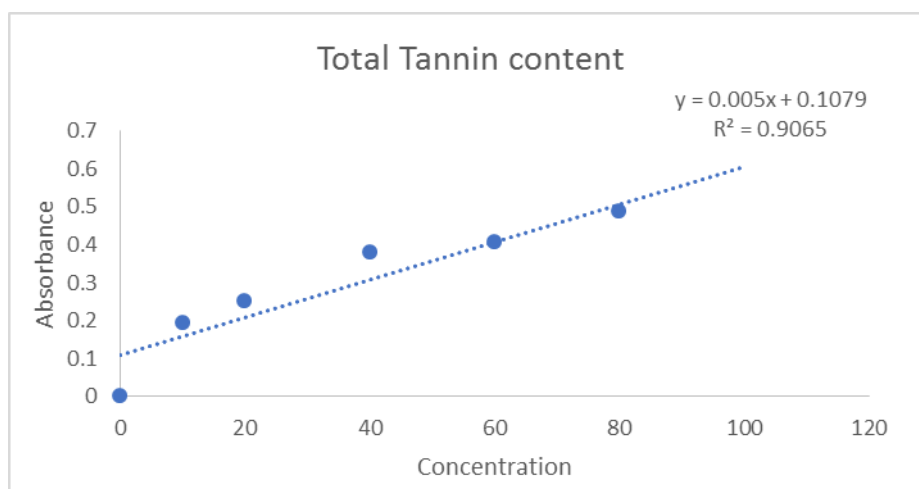


Figure 4: Standard curve of Tannic Acid.

In-vitro antioxidant activity of *Zingiber officinale*

In the present study, both ginger extracts in various concentrations were tested for their free radical scavenging activity in different in vitro methods. It was concluded that free radicals were scavenged by the test extracts.

Hydrogen Peroxide Scavenging Assay

Hydrogen peroxide (H_2O_2) scavenging activity of natural antioxidants present in extracts has been determined widely by measuring decimers of 11:0, in an incubation system containing H_2O , and the scavenger using the classical UV- method at 230 nm.

Table 3: Antioxidant Activity (Hydrogen Peroxide) of Ascorbic acid.

Sr. No.	Concentration	Absorbance	% Inhibition	IC 50 Value
1	0	0	0	444.20 $\mu\text{g/ml}$
2	50	0.589	6.50794	
3	100	0.486	22.8571	
4	200	0.399	36.6667	
5	400	0.356	43.4921	
6	600	0.198	68.5714	
7	800	0.128	79.6825	

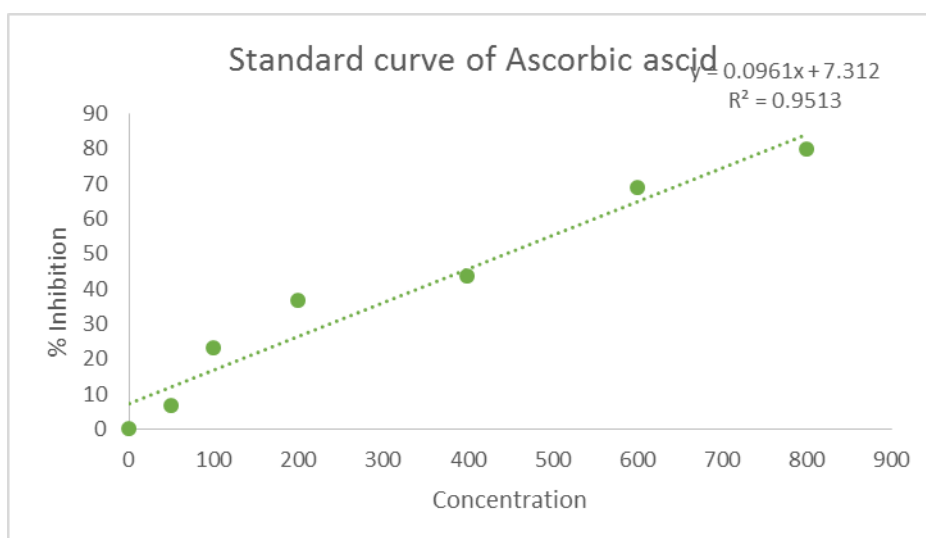
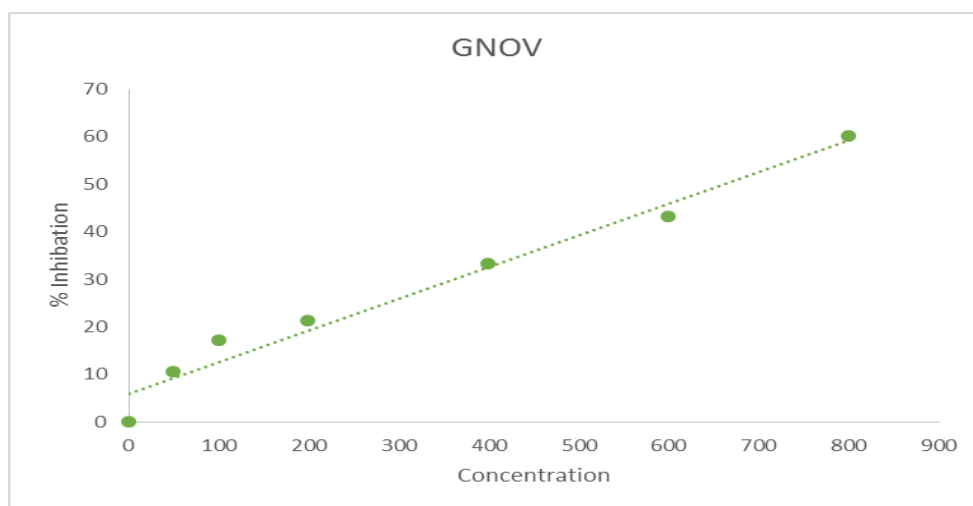


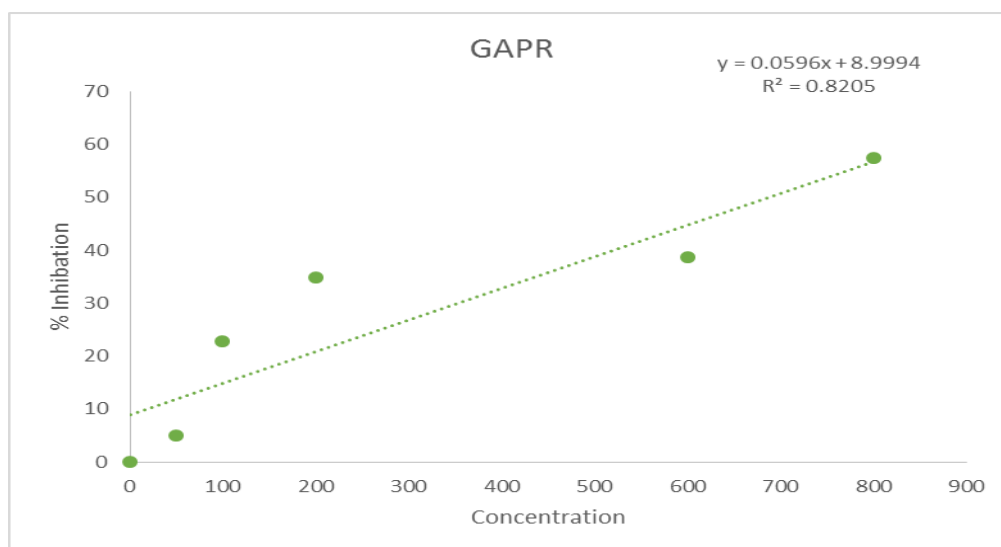
Figure 5: Graph of Hydrogen peroxide radical scavenging assay of Ascorbic acid.

Table 4: Antioxidant Activity (Hydrogen Peroxide) of GNOV.

Sr. No.	Concentration	Absorbance	% Inhibition	IC 50 Value
1	0	0	0	660.55 µg/ml
2	50	0.564	10.4762	
3	100	0.522	17.1429	
4	200	0.496	21.2698	
5	400	0.421	33.1746	
6	600	0.358	43.1746	
7	800	0.252	60	

**Figure 6: Graph of Hydrogen peroxide radical scavenging assay of GNOV.****Table 5: Antioxidant Activity (Hydrogen Peroxide) of GAPR.**

Sr. No.	Concentration	Absorbance	% Inhibition	IC 50 Value
1	0	0	0	687.93 µg/ml
2	50	0.599	4.92063	
3	100	0.487	22.6984	
4	200	0.411	34.7619	
5	400	0.387	38.5714	
6	600	0.269	57.3016	
7	800	0.183	70.9524	

**Figure 7: Graph of Hydrogen peroxide radical scavenging assay of GAPR.**

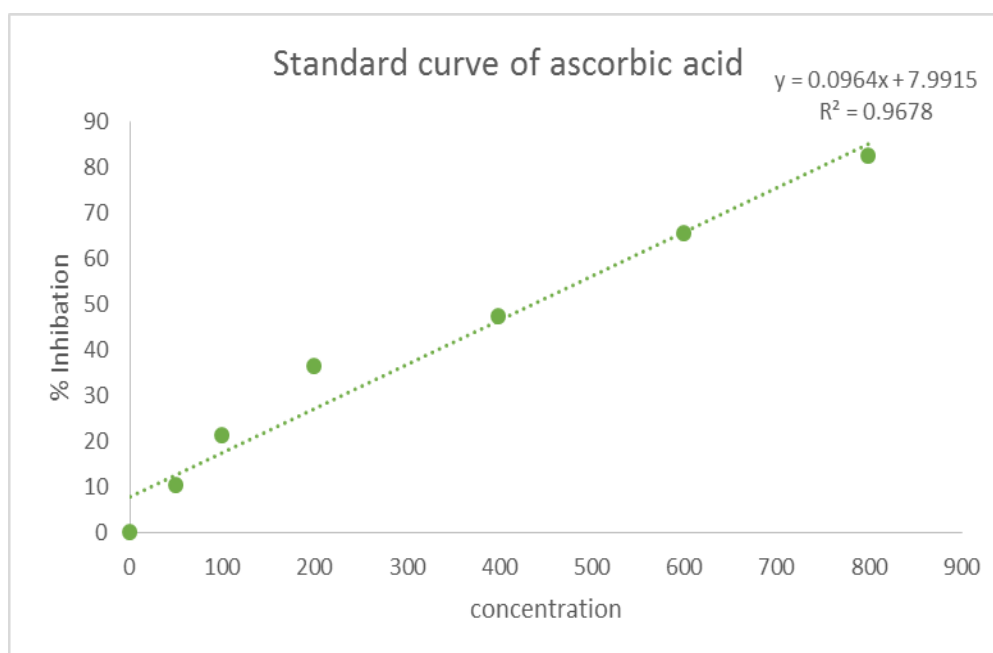
Reducing Power Radical Scavenging Assay

The reducing properties are generally associated with the presence of reductones, which have been shown to exert

antioxidant activity. The reducing capacity of a compound serves as an indicator of its antioxidant activity.

Table 6: Antioxidant Activity (Reducing Power) of Ascorbic acid.

Sr. No.	Concentration	Absorbance	% Inhibition	IC 50 Value
1	0	0	0	435.77 µg/ml
2	50	0.556	10.467	
3	100	0.489	21.256	
4	200	0.396	36.2319	
5	400	0.327	47.343	
6	600	0.214	65.5395	
7	800	0.109	82.4477	

**Figure 8: Graph of Reducing power radical scavenging assay of Ascorbic acid.****Table 7: Antioxidant Activity (Reducing Power) of GNOV.**

Sr. No.	Concentration	Absorbance	% Inhibition	IC 50 Value
1	0	0	0	526.25 µg/ml
2	50	0.589	5.15298	
3	100	0.524	15.62	
4	200	0.463	25.4428	
5	400	0.422	32.0451	
6	600	0.354	42.9952	
7	800	0.302	51.3688	

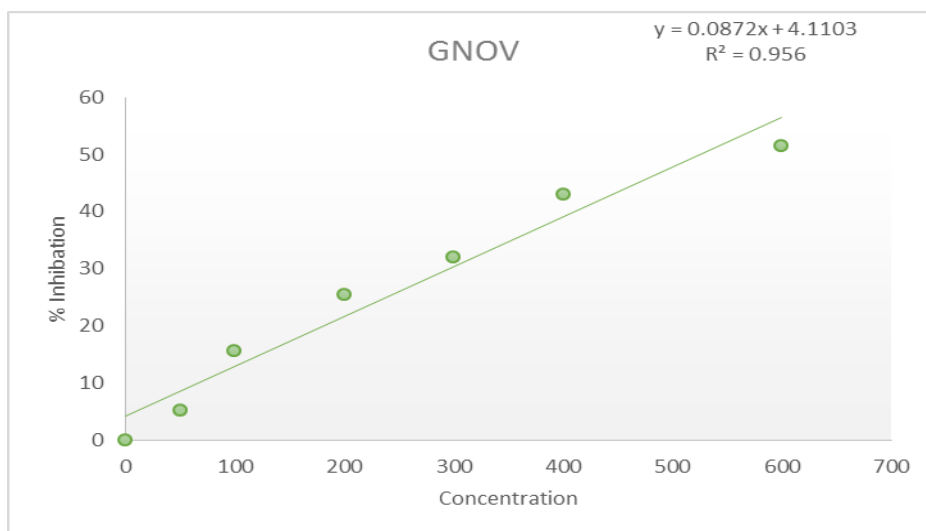


Figure 9: Graph of Reducing power radical scavenging assay of GNOV.

Table 8: Antioxidant Activity (Reducing Power) of GAPR.

Sr. No.	Concentration	Absorbance	% Inhibition	IC 50 Value
1	0	0	0	539.34 µg/ml
2	50	0.479	22.8663	
3	100	0.421	32.2061	
4	200	0.389	37.3591	
5	400	0.354	42.9952	
6	600	0.286	53.9452	
7	800	0.238	61.6747	

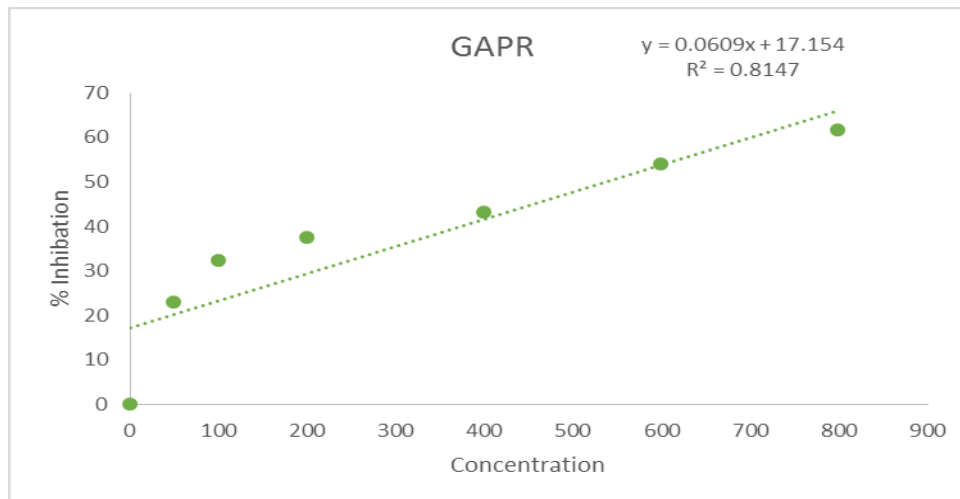


Figure 10: Graph of Reducing power radical scavenging assay of GAPR.

DISCUSSION AND CONCLUSION

The comparative pharmacognostical study of *Zingiber officinale* roscoe harvested in different seasons was performed successfully. Both samples were studied using various parameters and showed presence of the secondary metabolites such as carbohydrates, tannins, phenols, flavonoids, steroids, and saponins. The quantitative estimation of both samples was performed where the GNOV sample showed the presence of higher GAE and TAE as compared to the GAPR. The antioxidant study of both samples was also performed

using 2 different methods where the GNOV showed better antioxidant activity as compared to the GAPR. The above study shows the systematic comparison between the *Zingiber officinale* Roscoe samples harvested and collected at different times of year. The study can also be used to determine the better yield of plant cultivated in different season. The study can also influence the standardization parameters for the quality control of *Zingiber officinale* on the industry level evaluations and help ease the process.

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