A Quantitative Estimation of Phytochemicals Anti-Diabetic and Anti-Oxidant Activities in crude extracts of *Zingiber officinale rhizome*

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ABSTRACT

In early days traditional plants used as medicine to cure diseases. *Zingiber officinale* commonly used as spice worldwide. The extracts of ginger used to cure throat infections, cold, cough. The plant samples were extracted with low to high polar solvents *viz.* ethyl acetate, methanol and water. Further crude extracts were screened for the presence of phytochemicals present in ethyl acetate, methanol, aqueous extracts such as flavonoids, alkaloids, steroids, glycosides etc. The quantitative estimation of phenols, flavonoids, alkaloids was performed with the help of double beam U.V spectrophotometer. The evaluation of antioxidant activity was determined by the DPPH method. To assess the antidiabetic activities of crude extracts, in vitroα-amylase inhibition method was performed and IC_{50} values were calculated. It is concluded that methanolic extract of *Zingiber officinale (rhizome)* is a potential source of antioxidant and the anti-diabetic properties.

Keywords: *Zingiber officinale,* Anti-diabetic activity, Antioxidant activity, DPPH, in vitro α-amylase inhibition.

INTRODUCTION

Zingiber officinale is used as Spice and condiment in the last 2,500 years. It was used as anti-inflammatory, anti-pyretic, antinausea agent and also used to treat tooth ache, arthritis, and to cure digestive problems, sorethroat[1] anti-tumorigenic, anti-inflammatory, anti-apoptotic, anti-diabetic, anti-cholesterol actions. Ginger is a strong anti-oxidant.Ginger (*Zingiber belongs to* Zingiberacae) is a medicinal plant that has been widely used in Chinese, unani,ayurveda herbal medicines all over the world [2]. *Zingiber officinale* herbaceous perinnial plant of the family Zingiberacae probably native to south-eastern Asia famous for its pungent aroma rhizome [3].

EXPERIMENTAL

Chemicals

The organic solvents used in the experiments were of analytical grade and purchased from Fisher scientific, Mumbai, India.

Plant Samples

The studied plant samples *Zingiber Officianale* cultivated in the month of may in Eluru of West Godavari district in coastal state Andhra Pradesh, India. The plant samples were authenticated at the botany department of St.Theresas college, Eluru. The plant samples were cleaned with water to remove any dust and debris. The cleaned fresh materials were shade air dry and chopped into a fine powder.

Plant Extractions

The dried sample powder of plant parts were weighed (Table-1) and packed into a soxhelet apparatus and extracted with the solvents n-hexane, ethyl acetate, methanol, and water. The extract was filtered by means of Whatman filter paper and then made it dry by means of a rotary evaporator. The final crude extracts were weighed and collected for further phytochemical assessments. The weight of the crude extract obtained in each solvent is noted in Table-1.

Table-1: TheWeights of the Crude Extracts Obtained in Each Solvent

S. No.	Compound	*Weight of the extract*	*% of the extract*
1	Sample	43.38	.....
2	Hexane	0.203	1.85
3	Ethyl acetate	0.473	2.19
4	Ethanol	2.418	10..26
5	Water	2.856	11.48

Preliminary Phytochemical Screening of the Plant Extractions

Phytochemical screening was conducted to the selected plant *Zingiber Officianale* crude extracts for the presence of alkaloids, phenols, saponins, flavonoids, carbohydrates, glycosides, steroids, triterpenoids, steroidal saponins by standard phytochemical procedures[4].

Preliminary phytochemical screening

Standard procedures were used for the phytochemical screening. The following tests were procedures followed for the phytochemical screening.

1. Test for steroids

Salkowski Test: Add few drops of plant extract to few mL of concentrated sulphuric acid, shake and on standing the lower layer turns red.

Liebermann Burchard's Test: To the plant extract, few drops of acetic anhydride is added and mixed well and 1 ml of concentrated sulphuric acid is added from the walls of the test tube, a reddish-brown ring is formed between the two layers.

2. Tests for triterpenoids

Salkowski Test: Add Few drops of concentrated sulphuric acid and plant extract, shake it and on standing, bottom turns golden yellow.

Liebermann Burchard's Test: Mix plant extract, few drops of acetic anhydride. 1 mL of concentrated sulphuric acid is added from the sides of test tube, a red ring is formed indicates triterpenes.

3. Test for Saponins

Foam Test: Few mL of plant extract is shaken with little quantity of water, the foam formed persists for 10 minutes. It shows the presence of saponins.

4. Test for Steroidal Saponin

The plant extract is hydrolyzed with sulphuric acid and extracted with chloroform. The chloroform layer is tested for steroids.

5. Tests for Triterpenoid Saponin

The plant extract is hydrolyzed with sulphuric acid and extracted with chloroform. The chloroform layer is tested for triterpenoids.

6. Tests for Alkaloids

Mayer's Test: The acid layer when mixed with Mayer's reagent (Potassium mercuric iodide solution) gives a creamy white precipitate.

Dragendorff's Test: The acid layer with few drops of Dragendorff's reagent (Potassium bismuth iodide) gives a reddish-brown precipitate.

Wagner's Test: The acid layer when mixed with few drops of Wagner's reagent (solution of iodide in potassium iodide) gives brown to red precipitate.

Hager's Test: The acid layer when mixed with few drops of Hager's reagent (Saturated solution of picric acid) gives a yellow coloured precipitate.

7. Test for Carbohydrates

Fehling's Test: To the plant extract add Fehling's A and B solutions and heated. It gives an orange-red precipitate confirms the presence of reducing sugar.

Molisch's Test: To the plant extract add Molisch's reagent and concentrated. sulphuric acid along the sides of the test tube, a reddish violet ring formed. It shows the presence of carbohydrate.

Benedict's Test: The plant extract added to Benedict's reagent and heated. The brown precipitate indicates the presence of sugar.

8. Test for Flavonoids

Ferric chloride Test: The alcoholic solution of plant extract added to freshly prepared ferric chloride solution blackish green colour is formed. It shows the presence of flavonoids.

Lead Acetate Test: The alcoholic solution of plant extract reacts with 10% lead acetate solution and given a yellow precipitate.

9. Test for Glycosides

Anthraquinone Test: The plant extract is shaken with either ammonia or caustic soda. The aqueous layer shows a pink colour.

Keller-killiani Test: This test is for cardiac glycosides. Mix plant extract and 0.4 mL of glacial acetic acids are mixed with ferrous chloride and 0.5 mL of concentrated sulphuric acid. The acetic acid layer shows a blue colour.

10. Test for Phenolic Compounds

Ferric chloride Test: Add the plant extract to ferric chloride solution then blue colour appears if hydrolyzable tannins are present and green colour appears if condensed tannins are present.

Test for chlorogenic acid: Mix the plant extract with aqueous ammonia and expose to air, the green colour is developed.

QUANTITATIVE ESTIMATION OF PHYTOCHEMICALS

Total Phenolic Content

To 0.4 mL of Folin-Ciocalteu's reagent FCR (diluted 1:10 v/v) add 1 mL of plant extract. After 5 min adds 4 mL of sodium carbonate solution. The final volume of the solution was made up to 10 mL with distilled water and kept for 90 min at room temperature. The absorbance of the sample was measured against the blank at 750 nm using a spectrophotometer. A calibration curve was constructed using catechol as standard and total phenol content of the extract was expressed in terms of catechol equivalent per gram. Total phenolic compounds present in two crude extracts of *Zingiber Officianale* was measured from the calibration graph ($r^2 = 0.998$).

Total Flavonoid Content

1 ml plant extract was added to 3 mL distilled water followed by adding 0.3mL 5 % $NaNO_2$. After 5 min at 25 °C, Aluminium chloride (0.3 mL, 10 %) was added. Subsequently, after 5 min, the reaction mixture was treated with 2 mL of 1 M sodium hydroxide. Lastly, the reaction mixture was made up to 10 mL with distilled water and the absorbance was measured at 510 nm. The entire flavonoid content was calculated from a calibration curve ($R^2 = 0.999$) and the results were stated as quercetin equivalent per gram.

Total Alkaloid content

Five mL pH 4.7 phosphate Buffer was added to 1 mL of plant extract and also add 5 mL BCG (bromo cresol green) solution, the mixture was shaken with 4 mL of chloroform. The extracts were taken in a 20 mL volumetric flask and then diluted the volume to 20 mL with chloroform. The absorbance of the complex in chloroform was measured at 470 nm. The total alkaloid content was calculated from a standardization curve ($R^2 = 0.998$) and the results were stated as atropine equal per gram.

Study of Biological Activities

Measurement of Antioxidant Activity (DPPH method)

The antioxidant activity of the *Zingiber Officianale* crude plant extracts was determined on the depending on their scavenging activity of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical. The plant extracts of 1 mL (1-500 µg/mL) of each solution of different concentrations were added to 3 mL of 0.004 % ethanolic DPPH free radical solution. The absorbance of the preparations was taken after 30 minutes at 517 nm by a UV spectrophotometer which was compared with the absorbance of standard ascorbic acid concentrations (1-500 µg/mL)[9]and absorbance of the DPPH for blank (without plant extract) also measured finally the % antioxidant activity was calculated by the following equation.

$$\%\ \text{Antioxidant Activity} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

Measurement of Anti-diabetic Activity

A mixture of1 mL of plant extract and 1 mL of α-amylase, were incubated in a test tube at 37 $^{\circ}$C for 10 min. After pre-incubation, 1 mL of 1% (v/v) starch solution was added to each tube and incubated for 15 min at 37 $^{\circ}$C. The reaction was terminated with 2 mL DNSA reagent, placed in boiling water bath for 5 min, cool to normal room temperature and add 5mL distilled water, and the absorbance was measured[10] at 546 nm using a UV-Visible spectrophotometer. The control reaction contains 100 % enzyme activity without any plant extract. The % inhibition of ȧ-amylase by each plant extract can be calculated using the following formula.

$$\%\ \text{Inhibition of}\ \alpha\ \text{amylase} = \frac{\text{Absorbance of control} - \text{Absorbance of extract}}{\text{Absorbance of control}} \times 100$$

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

Preliminary Phytochemical screening of the *Zingeber officianales* reveals the presence of steroids, alkaloids, flavonoids, phenolics, carbohydrates, and saponins,glycosides . The results are expressed as +ve for the presence and –ve for the absence of phytochemicals. The phytochemicals present in the Ethyl acetate, methanol, aqueous extracts of 2 plant samples were shown inTable-2.

Table-2: Phytochemical Screening of *Zingeber officianales(rhizome)*

S. No.	Screening Tests	*Zingeber officianales(rhizome)*		
		Ethyl Acetate Extract	Methanol Extract	Aqueous Extract
1.	Steroids			
(a)	Salkowski	−ve	+ve	+ve
(b)	Libermann	−ve	+ve	+ve
2.	Triterpenoids			
(a)	Salkowski	−v e	+v e	+v e
(b)	Liberman tests	−v e	+v e	+v e
3.	Saponins			
(a)	Foam	−v e	+v e	−v e
4.	Steroidal saponin			
(a)	Salkowski	−v e	+v e	−v e
(b)	Libermann	−v e	+v e	−v e
5.	Triterpenoid saponin	−v e	+v e	−v e
6.	Alkaloids			
(a)	Dragendorffs	−v e	+v e	+ve
(b)	Picric acid	−v e	+v e	+ve
7.	Carbohydrates			
(a)	Benedicts	+ve	+ve	+ve
(b)	Molisch	+ve	+ve	+ve
8.	Flavonoids			
(a)	Ferric chloride	−v e	+ve	+ve
9.	Glycosides	+ve	+v e	+v e
10.	Phenols			
(a)	$Fecl_3$	+ve	+ve	+ve
(b)	Chlorogenic	+ve	−ve	+ve

In the Phytochemical screening of *Zingeber officianales(rhizome)* an aqueous extract shows positive for steroids, carbohydrates, flavonoids, phenols. However, the Methanolic extract of *zingiber officianales* (stem) shows the higher intensity of flavonoids, steroids, saponins, triterpenoids, cardiac glycosides, carbohydrates.

Total Phenolic, Flavonoid, Alkaloid Contents

Phenolics are compounds with an OH group attached to an aromatic carbon. Phenolic compounds acts as antioxidants due to their ability to donate hydrogen, quench singlet oxygen and acts as metal chelators. The phenolic concentration could be the foundation for antioxidant activity. Consumption of phenolic-rich compounds plays a vital role in the dealing of various diseases like diabetes,cancer etc.

The quantity of phenolics present in different crude extracts of the *zingeber officianales* was determined by means of the Folin-Ciocalteus reagent by using UV spectrophotometer and

taking standard catechol and the total phenolics was expressed as mg equivalent of catechol per gram. The total amount of phenolics present in different extracts is noted down in Table-3.

Table-3: Results of Total Phenolics Present in Different Extracts

S. No.	Compound	*Zingeber officianales(rhizome)* absorbance	*Zingeber officianales(rhizome)* Amount mg/gm of the extract
1	Hexane	.....	....
2	Ethyl acetate	0.189	13.600
3	Methanol	0.221	22.222
4	Water	0.365	48.294

The aqueous extract of *Zinger officianales(rhizome)* shows a higher level of phenolics 48.294mg/gm while in the methanol extract shows 22.22mg/gm phenolics .

Flavonoids are a category of secondary plant metabolites with potential antioxidant potential and free radical scavenging activity[5]. The antioxidant activity influenced by the flavon nucleus, number, position, and types of hydroxyl substitutions of flavonoids[6]. The more the hydroxyl substitutions, the stronger the antioxidant activity. Flavonoids were showing their efficiency, insulin activity and release of glucose in insulin-sensitive tissues through various intracellular active signals. Flavonoids influence β-cell mass and maintain insulin sensitivity in peripheral tissues. The intake of flavonoids has protective effects against cancers,tumours[7].

The quantity of flavonoid content in different solvent extracts of the *Zinger officianales(rhizome)* , was estimated by the usage of aluminum chloride and standard quercetin. The total flavonoid amount was expressed as mg equivalent of quercetin per gram. The total flavonoids present in the various crude extracts are depicted in Table-4.

The methanolic extract of *Zinger officianales(rhizome)* shows a higher quantity of flavonoids 89.459 mg/gm.

Alkaloids have diverse and important physiological effects on humans and other animals. The medicinal properties of alkaloids are quite enormous for instance morphine and alkaloids is a powerful narcotic used for the relief of pains [8]. Alkaloids are nitrogenous substances, and plants accumulate them as a result of the availability of nitrogen. A heterocyclic ring containing one or more nitrogen atoms makes up an alkaloid. They can be found in the plant as N-oxides, salts, or Free States. In addition to carbon, hydrogen, and nitrogen, alkaloids also contain oxygen. Numerous pharmacological properties of alkaloids include antioxidant and anti-diabetic activity. The sum of alkaloids presents in different solvent extracts of the *Zinger officianales(rhizome)* was determined by using phosphate buffer and BCG (bromo cresol green) and atropine standard. The total alkaloid quantity was expressed as mg equivalent of atropine per gram. The total amounts of alkaloids present in different extracts are noted down in Table-5.

Table-4: Results of Total Flavonoids Content Present in Different Extracts

S. No.	Compound	*Zinger officianales(rhizome)* *absorbance*	*Zinger officianales(rhizome)* *Amount mg/gm*
1	Hexane	....	....
2	Ethyl acetate	....	....
3	Methanol	0.898	89.459
4	Water	0.564	24.465

The methanolic extracts of *Zinger officianales(rhizome)* show the highest quantity of alkaloids 47.103/gm respectively.

Table-5: Results of Total alkaloids Content Present in Different Extracts

S. No.	Compound	*Zingeber officianales(rhizome) absorbance*	*Zingeber officianales (rhizome) Amount/gm*
1	Hexane	....	....
2	Ethyl acetate	....	....
3	Methanol	0.589	47.103
4	Water	0.424	34.465

Antioxidant activity

S. No	Concentration in µg/mL	Ascorbic acid	*Zingeber officianales(rhizome)* *% of DPPH activity*		
			EtOAc	MeOH	Water
1	5	14.941	1.201	10.578	3.243
2	10	22.393	3.092	20.010	7.028
3	15	37.224	3.959	56.457	13.185
4	20	56.287	8.683	63.490	31.979
5	25	75.936	18.262	79.343	41.467
6	30	79.326	19.930	84.389	47.904
7	50	89.899	26.677	88.839	79.508
8	100	91.979	31.711	98.406	86.823

Antidiabetic activity

S. No	Concentration in µg/mL	Standard drug Acarbose	*Zingeber officianales(rhizome)* *% of inhibition*		
			EtOAc	MeOH	Water
1	5	13.418	0.0126	10.548	0.667
2	10	26.967	0.339	20.856	10.224
3	15	39.589	9.089	39.731	20.343
4	20	53.887	14.976	57.656	23.480
5	25	70.391	23.464	65.531	49.570
6	50	78.883	31.397	70.503	52.279
7	100	83.799	34.413	80.112	58.760
8	200	90.056	59.330	88.492	80.179

The present study validates the traditional use *of Zinger officianales(rhizome))* substitute for synthetic drugs with equal efficacies as natural antioxidants, antidiabetic agent because plants show equal DPPH IC_{50} with standard ascorbic acid and *α-amylase* inhibition activity equal to standard drug acarbose.

The aqueous extract showed maximum phenolics 48.294 µg/mL and methanolic extract showed maximum flavonoids 89.459 µg/mL, alkaloids 47.103 µg/mL. The antioxidant activity, antidiabetic activity is maximum in the methanolic extract due to the presence of flavonoids, alkaloids.

CONCLUSION

The hexane, ethyl acetate, methanol, and water extracts of plant sample of *Zingeber officianales(rhizome)* was analyzed for the presence of phytochemicals such as alkaloids, flavonoids, phenols etc and were further estimated quantitatively with the help of double beam U.V spectrophotometer. Moreover, the extracts of *Zingeber officianales(rhizome)* were also explored for antidiabetic and anti-oxidant activities using *in vitro α*-amylase inhibition method and DPPH method respectively. It was found that the methanol extract of *Zingeber officianales(rhizome))* was a potential source of anti-oxidant and the anti-diabetic properties compared to ethyl acetate and aqueous extracts.

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