



Preliminary Phytochemical Analysis and Comparative Study of Antioxidant Potential in Extract of Rhizome of *Curcuma Species* and *Zingiber species*

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Abstract : *Curcuma* and *Zingiber* belong to different genera of the same family *Zingiberaceae*. Phytochemical analysis on *Curcuma* and *Zingiber* was carried out using standard methods. The result revealed the presence of alkaloids, tannins, phenolic compounds, saponins, steroids and flavonoids in the rhizome extract of both the plant species. The result of the present study revealed that the total polyphenol content and total tannin content of *Curcuma* were higher than those that of *Zingiber* while total flavonoid content of *Zingiber*

was higher than *Curcuma*. Both the rhizome extracts exhibited high antioxidant potential. DPPH radical scavenging activity was done in the methanolic extracts of rhizome and the IC_{50} value of *Curcuma* was lower than *Zingiber* which may lead to the conclusion that *Curcuma* possessed higher antioxidant activity as compared to *Zingiber*. The present data suggest that methanolic extracts of *Curcuma* and *Zingiber* can be used as a good source of antioxidants.

Keywords: *Zingiber*, *Curcuma*, phytochemical, antioxidants

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Introduction :

Curcuma has been suggested to have anti-microbial, anti-carcinogenic, anti-inflammatory, hypocholesterolemic activities and hepatoprotective. There are several studies which state that these beneficial properties of *Curcuma* have been associated to the antioxidant activity (Arya et al, 2015). Ginger extracts demonstrated effective antioxidant properties and their consumption may reduce or delay the progression of diseases that oxidative stress causes due to lack of antioxidant supplementation (Tohma et al, 2017). Plants with medicinal properties have been found to contain various antioxidants that are present as secondary metabolites which are synthesized as defence mechanism. (Mahboubi, 2019) Antioxidant capacity is related with compounds capable of protecting a biological system against potential harmful effects of processes or reactions involving reactive

oxygen and nitrogen species (ROS and RON). A direct correlation between the amount of polyphenols detected in the selected vegetal products and the antioxidant capacity can be observed. Polyphenols have been intensively investigated for their antioxidant properties. It seems that the mechanism involves the modulation of oxidative stress (Scalbert et al 2005).

The objective of the work was the phytochemical analysis of the rhizome extract along with the evaluation of the in-vitro antioxidant activity of crude methanol extract of *Curcuma* spp. and *Zingiber* spp. and its different fractions through different free radical scavenging assay and also analysis of the data collected comparing the antioxidant potencies of *Curcuma* spp. and *Zingiber* spp.

Materials and Methods:

The present research work was conducted in the Department of Botany, Patna Women's College, Patna, during the period August-October, 2019. To get the desired results, methods are needed for purification, detection and identification of different constituents present in the plants. The results of the preliminary phytochemical screening provide an experimental basis for the use of medicinal plants in traditional therapy. The phytochemical constituents are responsible for the beneficial pharmacological actions of these plants. Antioxidant assay of plant extract reveals its antioxidant potency which can lead to the formation of natural antioxidants.

Plant Materials

Fresh rhizomes of turmeric (*Curcumin* spp.) and ginger (*Zingiber* spp.) was purchased from Rajapur Pul sabji market of Patna during August 2019 and will be grown in pots, and plant extract was prepared out of the plant rhizome. The plant extract was tested for antioxidant potential.

The plant extracts were subjected to tested for-

Preliminary phytochemical analysis:

Test for alkaloids: A quantity (3ml) of concentrated extracts were taken into a test tube and 1 ml HCL was added, the mixture was heated gently for 20 minutes and then cooled and filtered, the filtrate was kept in the brown bottle for the test to be done.

Wagner's test:

- 1 ml of extract was mixed with Wagner's reagent, and the brown reddish precipitate is formed. This indicates the presence of alkaloids.

Dragendroff's test -:

- 2 drops of dragendroff's reagent and 1 ml of the extract were mixed. The formation of creamy ppt indicates the presence of alkaloids.

Mayer's test -:

- 1 ml of the extract was mixed with Mayer's reagent. The presence of yellow coloured ppt confirmed alkaloids.

Test for tannin

- 4ml extract was taken and treated with 4 ml FeCl₃. The formation of green colour indicates the presence of condensed tannin.

Test for saponin-:

5 ml extract and 20 ml of distilled water were mixed and then agitated in graduated cylinder for 15 minutes. The formation of foam indicates Saponin.

Test for flavonoid:

Alkaline reagent test:- The extract was treated with 10% NaOH solution. The formation of intense yellow colour indicates the presence of Flavonoid. It turned colourless when 2 drops of diluted acid were mixed.

Mg turning test:- The extract was treated with Mg turning and conc. HCl was added to this solution, and then 5ml of 95 % ethanol was added. The formation of crimson red colour indicates the presence of flavonoid.

Test for phenol:

Ferric chloride test:- The extract was treated with 4 drops of Alcoholic FeCl₃ solution. The formation of bluish black colour takes place. This indicates the presence of Phenol.

Test for steroids:

1ml extract was mixed with 10 ml of chloroform & equal volume of concentrated H₂SO₄ acid was added from the side of test tube. The upper layer turns red and H₂SO₄ layer showed yellow with

green fluorescence. This indicates the presence of steroid.

Quantitative estimation of phytochemicals

Estimation of total polyphenol content : The total polyphenol content (TPC) of the plant extracts was estimated spectrometrically according to the Folin-Ciocalteu method (Singleton et al., 1999).

The Folin-Ciocalteu's reagent was diluted 10 folds. 0.4 mL of the extract (0.25 mg/mL) was mixed with 1.6 mL of 7.5% sodium carbonate solution. Then, 2 mL of 10-fold diluted Folin-Ciocalteu reagent was added and the final reaction mixture was incubated for an hour placing it in the dark. The intensity of the blue-coloured complex was measured at 765 nm using spectrophotometer and using standard solution of gallic acid at different concentrations.

The content of total phenolic content was expressed as mg gallic acid equivalent (GAE/g) of a fresh sample.

The sample was analysed in triplicate.

Estimation of total tannin content : The total tannin content (TTC) in the plant extracts was estimated using the Folin-Ciocalteu method with tannic acid as a standard. (Afroz et al., 2014)

Briefly, 0.1 mL of the solution containing 1 mg of the extract was treated with 7.5 mL of distilled water, and 0.5 mL of Folin Ciocalteu's reagent was added in it. 1 mL of 35% sodium carbonate and 0.9 mL of distilled water were added to the above mixture and the solution was mixed and then incubated for 30 minutes.

The intensity of the developed blue-coloured complex was measured at 725 nm. The results were expressed as mg of tannic acid equivalent (TE) per 100 g of plant sample.

Estimation of total flavonoid content : The total flavonoid content (TFC) was estimated using an aluminum chloride colorimetric assay. (Chang et al., 2002)

First, 1 mL of the extract (1 mg/mL) was treated with 0.3 mL of 5% sodium nitrite and then it was added to the reaction mixture. After 5 minutes, 0.3 mL of 10% aluminum chloride was added. Subsequently, after 6 minutes, another 2 mL of 1 M sodium hydroxide (NaOH) was added, followed by the immediate addition of 2.4 mL

of distilled water to produce a total volume of 10ml volume. The colour intensity of the flavonoid-aluminum complex was measured at 510 nm. The total flavonoid content was determined as Quercetin equivalent and was expressed as g of QE/100 mg of plant sample.

Evaluation of antioxidant activity by radical scavenging assay

- DPPH radical scavenging assay
- For 1mM DPPH Solution Preparation: 4 mg of DPPH was treated with 100ml of 99% methanol. The DPPH solution was kept in volumetric flask and then it was covered well with aluminium foil and kept in cool and dark condition (Ghasemzadeh et. al., 2012).
- For extract preparation: 1g of powdered extract was dissolved in 100 ml of 99% methanol. Then it was kept in shaking water bath for 2-3 hrs at room temperature. The extracts were taken out and centrifuged at 6000 rpm for 15 minutes. The supernatant was filtered and kept in viols.
- Preparation of different concentrated extract : The extract was diluted with methanol to form different concentrated solutions (50, 100, 150, 200, 250µg/ml).
- Reaction mixture preparation : 1mg of various concentration of extracts was treated with 1.0 ml of 1mmol DPPH sol.
- Preparation of blank solution : 1 ml of methanol and 1ml of DPPH solution were used as blank.

The samples were then . incubated for 30 minutes in dark at room temperature and the absorbance was recorded at 517nm.

Result and Discussion :

Phytochemical analysis : Preliminary phytochemical analysis was done by the plant extracts of curcuma and Zingiber in their methanolicethanolic and aqueous extract. The data shown in Table 1 and table 2 shows phytochemical analysis of aqueous, methanolic and ethanol extracts of curcuma spp and zingiber spp, respectively.

Table 1. Showing phytochemical analysis of plant extracts of *Curcuma* spp

Phytochemical test	Aqueous	Ethanollic	Methanolic
Test for alkaloids	+	+	+
Test for flavonoid	++	++	++
Test for phenol	+	++	++
Test for saponin	+	+	+
Test for tannin	+	+	+
Test for steroids	+	+	++

Table 2. Showing phytochemical analysis of plant extracts of *Zingiber* spp

Phytochemical test	Aqueous	Ethanollic	Methanolic
Test for alkaloids	+	+	+
Test for flavonoid	++	++	++
Test for phenol	+	-	+
Test for saponin	+	+	+
Test for tannin	+	+	+
Test for steroids	+	+	+

The result of the preliminary phytochemical screening reveals the presence of various phytochemical constituents, i.e., phenol, tannin, alkaloid, saponin, flavonoid, and steroids. The result of the present work was similar to the result of (Sawant et al., 2013).

The result of preliminary phytochemical screening of ethanolic, methanolic and aqueous extract of Turmeric showed the presence of alkaloids, tannins, phenolic compounds, saponins and flavonoids.

And in case of Zinger, it was seen that the ethanolic, methanolic and aqueous extracts contained alkaloids, flavonoids, phenols, saponins, tannins. However, the presence of phenols was not observed in the ethanolic extract.

Quantitative Estimation of Phytochemicals

- Estimation of total polyphenol content

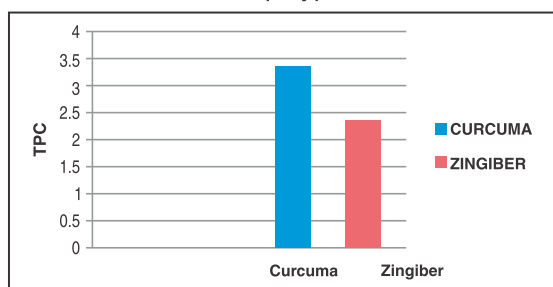


Fig. 1. Showing variation of Total Polyphenol Content

The total phenolic content in extract of *Curcuma* was 3.39±0.21 mg gallic acid equivalent/100g and that of *Zingiber* was 2.40±0.12 mg equivalent/100g. The results shows that *Curcuma* exhibits more phenolic content

- Estimation of total flavanoid content

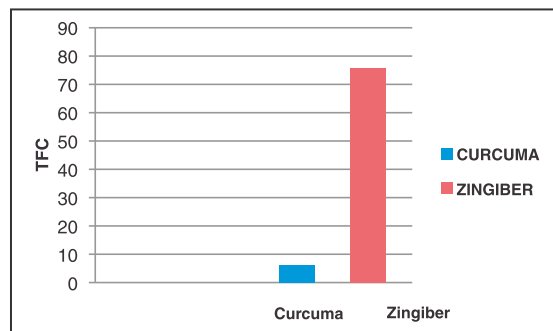


Fig. 2. Showing variation of Total Flavanoid Content

The total flavanoid content was found to be 6.70±0.85mg catechin equivalent/100mg for Curcuma and 76.65±0.98mg catechin equivalent/100mg for Zingiber.

- Estimation of total tannin content

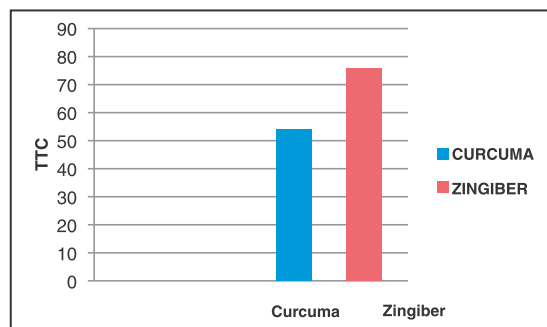


Fig. 3. Showing variation in Total tannin content

Total tannin content was found to be 55.35±0.88 mg tannic acid equivalent/100g for Curcuma and 76.64±0.75 mg tannic acid equivalent/100 g for Zingiber.

Table 3. Showing results for Quantitative estimation of Extract

PHTOCONTITUENTS	TOTAL PHENOLIC CONTENT (mg GAE/100g)	TOTAL FLAVANOID CONTENT (mg CE/100g)	TOTAL TANNIN CONTENT (mgTAE/g)
<i>Curcuma</i> spp.	3.39±0.21	6.70±0.85	55.35±0.88
<i>Zingiber</i> spp.	2.40±0.12	76.65±0.98	76.64±0.75

The data present in the table III, revealed that the total polyphenol content and total tannin content of *Curcuma* spp. was higher than that of *Zingiber* spp. while total flavonoid content of *Zingiber* spp. was higher than *Curcuma* spp. The result was similar to the result of Arya et al, 2015 in which TPC, TFC and TTC of different *Curcuma* varieties were determined.

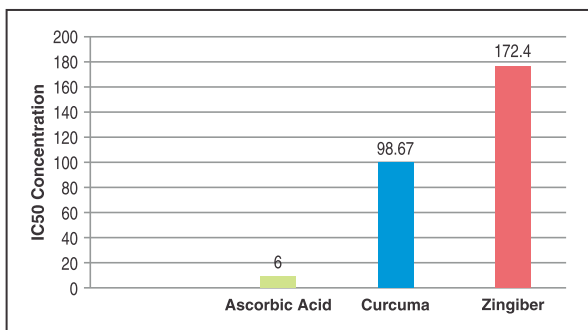


Fig. 4. Showing IC₅₀ concentration of different sample

Table 4. IC₅₀ value of different samples

Material	IC ₅₀
Ascorbic acid	6µg/ml
Zingiber	172.4µg/ml
Curcuma	98.67µg/ml

Table IV determines the IC₅₀ values of different samples, the standard ascorbic acid had an IC₅₀ value of 6µg/ml, for *Curcuma* it was about 98.67µg/ml whereas that for *Zingiber* it was about

172.4 µg/ml. By this study it may be considered that *Curcuma* spp. has better antioxidant activity as compared to *Zingiber* spp. The results for *Curcuma* was very similar with the result of Khosropannah M et al. 2016 who provided an analysis of antioxidant assay in Curcumin.

The results for *Zingiber* was very similar to the results obtained by Mahboubi, 2019 reviewed the composition and bioactivity of *Zingiber officinale* Rosc.

Both the rhizome extracts exhibited high antioxidant potential. DPPH radical scavenging activity was done in the methanolic extracts of rhizome and the IC₅₀ value of *Curcuma* spp. was lower than *Zingiber* spp (Tilak et al., 2014).

Conclusion :

Today, antioxidant properties of the plants can replace the synthetic antioxidants. The result of the present study showed that both of the plant extracts exhibited potent antioxidant potency.

Methanolic extract exhibited high free radical scavenging activity due to the presence of phenolic compound. Turmeric showed maximum radical scavenging activity and higher amount of phenolic compound whereas ginger possessed higher quantity of TFC.

In the present study, the data suggest that methanolic extract of curcuma and zinger can be used as good source of natural antioxidants.

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