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ANALYSIS OF TOTAL PHENOLICS AND ANTIOXIDANT ACTIVITY OF ALCOHOLIC AND AQUEOUS EXTRACT OF SOUTH INDIAN CURCUMA LONGA RHIZOMES

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ABSTRACT

The present study investigates the total phenolic content and antioxidant activity of *Curcuma longa* rhizomes alcoholic (50% methanolic, 96 % methanolic) and aqueous extracts by Folin-Ciocalteu assay and DPPH assay respectively. There was a statistically significant difference between groups as determined by one-way ANOVA. Bonferroni post hoc test revealed that total phenolic contents and antioxidant activity of *Curcuma longa* rhizomes was statistically significant; increasing from aqueous extract, followed by 50 % methanolic extract, and finally 96 % methanolic extract. The present study also confirms the significant and positive correlation existing between the antioxidant activity and phenolic content of *Curcuma longa* rhizomes.

KEYWORDS: **Phenolics, Antioxidant, *Curcuma longa*, Turmeric.**

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INTRODUCTION

Normal cellular processes liberates unstable free radical molecules, which also induced by external sources such as ionizing radiation, pesticides, and organic solvents., in the body.[1,2] such generated free radicals (reactive oxygen species) in the body finally results in cell damage.[3] although human beings, have natural antioxidant defenses that protect against oxidative damages.[4,5] still there exists inefficiency of natural antioxidant defenses which can be overcome with antioxidant rich diet that possessing inverse relationship with human disease occurance.[6] Free radicals also cause food deterioration due to lipid peroxidation, which leads to loss of nutritive value and functional properties of food.[7] lipid peroxidation occurs as a result of free radical chain reaction catalyst either by free radical, metal ions, oxygen or light.[8] Antioxidants are substances that counteract many oxidation reactions produced by free radicals, thus protecting and postponing tissues from damage. Functions of antioxidants are established through their actions such as scavenging reactive oxygen species, decreasing the localised oxygen concentration hence reducing molecular oxygen's oxidation potential and metabolizing lipid peroxides to non-radical substances etc.[9] Certain herbs considered as natural antioxidants, possessing compounds such as aromatic acids, flavonoids, tannins and anthraquinones, found to have effective role on reactive oxygen species (ROS) scavenging and prevention Of lipid peroxidation.[10] Past studies accomplished that certain medicinal herb considered as potential agent against oxidative damage.[11,12] Medicinal herb Turmeric (*Curcuma longa* L.), "belongs to family Zingiberaceae" is best known for its antioxidant properties. Turmeric is an important medicinal ingredient in the Indian system of medicine called Ayurveda and is commonly used as a spice and food preservative cultivated in tropical regions like, India, China etc, it posses several medicinal benefits such as antibacterial, antihelmintic, anticancer, antiparasitic, antiseptic,. antioxidative, anti-inflammatory, antirheumatic, antitumour, antiviral.[13] Turmeric is a vital source of bioactive compounds like antioxidants, polyphenols and flavonoids, which can be considered as the substitute of antibiotics used in food and food products. Present research was carried out to estimate and evaluate the relation between the total phenolic content (TPC) and antioxidant activity (AA) of aqueous and alcoholic extracts of rhizomes of *Curcuma longa*.

MATERIALS AND METHODS

Reagents and chemicals

Folin-Ciocalteu's (FC) phenol reagent was obtained from Merck (Germany). Sodium carbonate, Gallic acid, 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma (Germany), all other reagents and chemicals used were of analytical grade procured from local sources.

Sample collection

The plant samples rhizome part of Turmeric (*Curcuma longa* L.) is commonly cultivated in South India were undertaken for study was procured from the local medicinal plant traders and identified in the laboratory based on local names and pharmacognosy literature.

Sample preparation

Fresh samples were cleaned and washed with running tap water. Rhizome portions of turmeric were cut into small pieces and homogenized using a blender for 2 minutes separately. The homogenized sample was transferred into air-tight container and kept at –20°C before extraction. For powdered form, the homogenized sample was then kept in the freezer at –80°C overnight, freeze-dried for 3 days, grounded using a dry grinder and fine powders were obtained using a fine mesh sieve and stored at –20°C.

Sample extraction

Turmeric extracts were prepared by using three solvents methanol 50%, methanol 96% and water according to protocol as specified by (*Denisa Margina et al*[14]*., yan et.al.*[15]*)* with some modification. One gram of powdered samples transferred into a 3 different 200 ml volumetric flasks and 100 ml of 50%, 96% methanol and water were added to the samples separately. Mixture was shaken at 200 rpm for 120 minutes at 50°C.The mixture was then centrifuged at 3000 rpm for 15 minutes at room temperature and supernatant was saved. This supernatant was used for total phenolic content, and DPPH radical scavenging assays. The extracts were stored at –20°C.

Total Phenolic Contents

Total phenolic contents of all samples were determined using Folin-Ciocalteu assay as described by *Singlaton* and *Rossi.*[16] 1ml Samples were loaded in different test tube each and mixed thoroughly with 5ml Folin-Ciocalteu reagent (1/10 dilution). After 5 minutes, 4 ml of 7.5% sodium carbonate (Na2CO3) was added and allowed to react for 2 hrs at room temperature. The absorbance was measure at 765 nm using microplate reader spectrophotometers. Samples were measured in triplicates. Standard curve of gallic acid solution (10, 20, 40, 60, 80 and 100 ppm) was prepared using the similar procedure. The results were expressed as mg GAE/100 g extract sample.

Antioxidant activity

The antioxidant activities of all extracts were estimated by free radical scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. The evaluation was based on the method proposed by *Akowuah et al*.[17] 2 ml of 0.1 mM DPPH methanolic solution was added into 200 µl of sample extracts and 0.8 ml methanol. The mixture was thoroughly mixed and kept in the dark for 1 hr. The control was prepared by mixing 2 ml of DPPH and 1 ml methanol. The absorbance was measured at 517 nm using microplate reader spectrophotometers. Samples were measured in triplicates. Percentage of DPPH scavenging activity was calculated as follows:

$$\% \text{ inhibition of DPPH activity} = [(X - Y) / X] \times 100.$$

Where X = absorbance of the control and Y = absorbance of the sample.

Statistical analysis

Experimental data were analyzed using Excel (Microsoft Inc.) and SPSS version 17.0 software. Significant differences between samples were analyzed using analysis of variance (ANOVA) and Bonferroni multiple Comparison method (P< 0.05). Pearson's correlation was used to determine the correlation of data between TPC on AA. Data obtained were reported as mean ± standard deviation.

RESULTS AND DISCUSSION

Total phenolic content

Total phenolic contents of different extracts of *Curcuma longa* rhizomes were estimated using the diluted Folin-Ciocalteu reagent. Table 1, shows total phenolic content of 50 %, 96 % methanolic extract, and aqueous extract of *Curcuma longa* rhizomes. Results clearly showed that 96 % methanolic extract had the highest total phenolic content followed by 50 % methanolic extract and finally aqueous extract with mean value of 682.09 mg GAE/100 g extract, 530.96 mg GAE/100 g extract and 494.34 mg GAE/100 g extract, respectively. The finding in this study especially TPC of aqueous extract of *Curcuma longa* rhizomes was in agreement with study by *Tanzeela Nisar et al.*[18], which had mean value of 496.76 mg GAE/100 g extract. In present study there was a statistically significant difference between groups as determined by one-way ANOVA (F (2, 6) = 1434.912, p < 0 .001). Bonferroni post hoc test revealed that total phenolic contents of *Curcuma longa* rhizomes was statistically significant; increasing from aqueous extract (494.34 ± 3.95, p < 0 .001) , followed by 50 % methanolic extract (530.96 ± 3.58, p < 0 .001), and finally 96 % methanolic extract (682.09 ± 5.8, p < 0 .001).

Antioxidant activity

The antioxidant activity of different extracts of *Curcuma longa* rhizomes was estimated using the free radical scavenging effect on DPPH radical. Table 1 shows DPPH Inhibition (%) of 50 %, 96 % methanolic extract, aqueous extract of *Curcuma longa* rhizomes. Results were clearly showed that 96 % methanolic extract had the highest DPPH radical scavenging activity followed by 50 % methanolic extract and aqueous extract with mean value of 61.80, 37.35 and 29.09, respectively. The finding in this study especially AA of aqueous extract of *Curcuma longa* rhizomes was in agreement with study by *Tanzeela Nisar et al.*[18], which had mean value of 31.33. Another study by *Denisa Margina et al.*[14], with AA of aqueous and ethanolic (50%, 96%) *Curcuma longa* rhizome extracts approximately agrees with our study, with mean value 22.31, 37.25, 53.79 respectively. In present study there was a statistically significant difference between groups as determined by one-way ANOVA (F (2, 6) = 94.879, p < 0 .001). Bonferroni post hoc test revealed that antioxidant activity of different extracts of *Curcuma longa* rhizomes was statistically significant; increasing from aqueous extract (29.09 ± 1.08, p < 0 .001), followed by 50 % methanolic extract (37.35 ± 4.27, p < 0 .001), and finally 96 % methanolic extract (61.80 ± 2.83, p < 0 .001).

Table 1

Mean ± SD of total Phenolic content and DPPH Inhibition (%) of 50 %, 96 % methanolic extract, aqueous extract of Curcuma longa rhizomes

Parameters	50 % Methanolic extract (n = 3) (Mean ± S.D)	96 % Methanolic extract (n = 3) (Mean ± S.D)	Aqueous extract (n = 3) (Mean ± S.D)	p-Value *
Total Phenolic content (mg GAE/100g extracts)	**530.96 ± 3.58**	**682.09 ± 5.8**	**494.34 ± 3.95**	**0.001**
DPPH Inhibition (%)	**37.35 ± 4.27**	**61.80 ± 2.83**	**29.09 ± 1.08**	**0.001**

**** The mean difference is significant at the 0.05 level.***

The correlation between total phenolic content and antioxidant activity

A Pearson product-moment correlation coefficient was computed to assess the relationship between the total phenolic content and antioxidant activity of *Curcuma longa* rhizomes. Results of present study shows a positive correlation between the two variables, r = 0 .988, n = 9, p< 0.001 as similarly reported by *Cai et al.*[19], *Maizura et al.*[20] In the present study a significant and linear relationship exist between the antioxidant activity and phenolic content of *Curcuma longa* rhizomes. Increases in total phenolic content were correlated with increases in antioxidant activity of *Curcuma longa* rhizomes, thus indicating that phenolic compounds are the major responsible factor that contributes to antioxidant activity.

CONCLUSION

The results of present study concludes that total phenolic content and antioxidant activity of *Curcuma longa* rhizomes cultivated in south india, increases in order in three different extracts, i.e. Aqueous extract, followed by 50 % methanolic extract and 96 % methanolic extract respectively. Significant and positive correlation exists between the antioxidant activity and phenolic content of *Curcuma longa* rhizomes. Thus providing evidence that, the phenolic contents were the major responsible factors for antioxidant activity.

AUTHORS CONTRIBUTION

The study was done by Muthu Ramkumar (corresponding author), and the present study was supervised by Dr.S.Rajasankar in every aspects.

CONFLICT OF INTEREST

Conflict of interest declared none.

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