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Estimation of Total Phenolics and Free Radical Scavenging of Turmeric (*Curcuma longa*)

[1]Tanzeela Nisar, [2]Muneeb Iqbal, [1]Ahmad Raza, [1]Madiha Safdar, [1]Fatima Iftikhar and [1]Marwa Waheed

[1]National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan
[2]Department of Physiology, University of Veterinary and Animal Sciences, Lahore, Pakistan

Abstract: Turmeric "*Curcuma longa"* member of Zingiberaceae family is well known for its curative potential against diseases. Disease preventive role of turmeric and other plant foods is due to the bioactive components such as phenolic acids, tannins and flavonoids. For the current study, antioxidant activity of turmeric extracts was investigated against DPPH free radical scavenging activity. Proximate analysis and total phenolic contents of ethanol, methanol and water extracts of turmeric were also carried out. The total phenolic content (TPC) means related to three solvents at their different concentrations i.e. Ethanol (60%), Ethanol (80%), methanol (60%), methanol (80%) and aqueous extract were 678.76 mg GAE/ 100 g, 745.76 mg GAE/ 100 g, 523.87 mg GAE/ 100 g, 682.43 mg GAE/ 100 g and 496.76 mg GAE/ 100 g respectively. Free radical scavenging activities of three solvents of turmeric at their different concentrations i.e. Ethanol (60%), Ethanol (80%), methanol (60%), methanol (80%) and aqueous extract were 46.16%, 52.19%, 35.41%, 49.83% and 31.33% respectively. Results indicated that ethanolic extract showed better phenolic acids and DPPH scavenge activity than methanol and water extracts.Proximate composition showed that turmeric contained moisture (13.02%), crude protein (6.47%), crude fat (5.33%), crude fiber (4.80%), ash (3.49%) and NFE (69.89%).

Key words: Turmeric · Ethanol · Antioxidant · DPPH scavenge activity

INTRODUCTION

Plants based foods have been reported for their antioxidant properties since old days. They are potential source in combating against different diseases [1, 2]. Spice is a natural substance or mixture of substances that are taken from the flowers, seeds, roots, trunks or leaves of numerous plants and provide specific colour, smell, odor and taste to the food. Above 400,000 species of flowering plants are known for having antimicrobial activities.Spices have an extensive history of their use in developing and advanced countries owing to their tremendous antimicrobial and antifungal activities [3-5].

Turmeric (*Curcuma longa* L.)"belongs to family Zingiberaceae" is best known for its antioxidant and antimicrobial properties against foodborne pathogens. It is widely consumed as a flavoring, preservative and coloring agent in South Asia, India and China. It is well notorious for its unique medicinal properties. It is cultivated in tropical regions like Pakistan, China, Peru and India [6, 7].*Curcuma longa* is considered as native to the India. It is grown commercially in many countries of south Asia, China and India. It is well known for culinary use as a key constituent of curry powder. It is an approved food additive In the United States. Turmeric is normally called haldi or haridra in India. It is also called as "Manjal" and its powder as ManjalThool in Tamil language. It is also known as "Indian saffron", as it was broadly used as a substitute to the more costly saffron spice [8,7]. Turmeric has healthy influence on digestive system and it also enhances the mucin secretion in the digestive tract. In classical literature several actions of turmeric have been specified like antibacterial, antithelmintic, anticancer, antiparasitic, antiseptic, antioxidative, anti-inflammatory, antirheumatic, antitumour, antiphlegmatic, antiviral, astringent, aromatic, blood purifier, clear skin colour, remove wound maggots, hepatoprotective, stop liver obstruction, heals wound, stimulant and sedative [9]. Nowadays, *Curcuma longa* is used widely in the food industries, as a coloring agent as well as an additive to impart flavor in curries. Turmeric is a prompt source of bioactive compounds like antioxidants, polyphenols and flavonoids, which may be the substitute of antibiotics used in food and food products. Turmerone,

Corresponding Author: Tanzeela Nisar, National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakitsan.

zingiberene, ar-turmerone and curlone are included in volatile constituents of turmeric while the nonvolatile constituents are curcuminoids [10]. The prophylactic behavior of these spices and other medicinal plant is due to the presence of bioactive compounds such as phenolic acids [2].

The bioactive element of *Curcuma longa* is curcumin. The characteristic color of turmeric is because of curcuminoids, first time identified in 1842 by Vogal. Curcuminoids are the phenolic compounds that are predominantly present in turmeric. Curcuminoids mainly comprise of curcumindemethoxycurcumin and bisdemethoxycurcumin. Among Curcuminoids, Curcumin is the main element which is responsible for the biological functions of turmeric. Curcumin is an orange-yellow crystalline substance that is insoluble in water. It is thought to be the powersful bioactive portion of turmeric [11]. Present research was carried out to evaluate the antioxidant activity of turmeric using DPPH assay.

MATERIAL AND METHODS

Procurement of Raw Material: Freshly harvested local cultivar of turmeric rhizomes were procured from Horticulture section of Ayub Agriculture Research Institute (AARI), Faisalabad. The poultry meat was procured from local market. All the reagents were made available in Food Microbiology and Biotechnology Lab. in National Institute of Food Science and Technology, University of Agriculture, Faisalabad.

Sample Preparation: Turmeric rhizomes were cleaned under running tap water in order to remove adherent sand and clay particles. Afterwards turmeric rhizomes were steamed for 10 min. Then rhizomes were cut into slices. These cut slices were oven dried at 40 °C for 48 hours. After drying turmeric slices were grinded into powder form by using high speed blander. The resultant powder was analyzed for its chemical attributes.

Proximate Analysis: Analysis for moisture, ash content, crude protein, crude fat, crude fiber and nitrogen free extract (NFE) of powder turmeric was carried out according to their respective methods AOAC [12]. All the tests were carried out in triplicates.

Preparation of Extracts: Turmeric extracts were prepared by using three solvents; methanol and ethanol and water at two different concentrations 60% and 80% according to protocol as specified by (Mukhtar and Ghori, [13] with some modifications. All the extracts were placed in the orbital shaker at 300 rpm, for 1 hour at a constant temperature of 25 °C. Further, solvent extracts (aqueous methanol and aqueous ethanol) except the water extracts were filtered through Whatman filter paper and then concentrated through rotary evaporator (Eyela, Japan). The filtered extracts were stored at 4 °C and used for further studies.

Table 1: Treatments for Turmeric powder extraction

Treatment	Solvent	Percentage
T_1	Ethanol	60%
T_2	Ethanol	80%
T_3	Methanol	60%
T_4	Methanol	80%
T_5	water	100%

Total Phenolic Contents: Total phenolic contents (TPC) in turmeric powder were estimated using Folin-Ciocalteu method as described by Wojdylo *et al.* [14] with little modifications. The mechanism is based on the reduction of phosphotungstic acid to phosphotungstic blue and as result absorbance increases due to rise in the number of aromatic phenolic groups. For the purpose, 50 μL of each prepared extract was separately added to test tubes, each containing 250 μL of Folin-Ciocalteu's reagent and 750 μL of 20% sodium carbonate solution and final volume was made up to 5ml with distilled water. After two hours, absorbance was measured at 765 nm using UV/visible light Spectrophotometer (CECIL CE7200) against control having all reaction reagents except sample extract. Total polyphenols were estimated and values were expressed as gallic acid equivalent (GAE; mg gallic acid/100g) using the following formula:

$$C = c \times V / m$$

where
C = Total phenolic contents (mg/g plant extract, in GAE)
c = Concentration of gallic acid (mg/mL)
V = Volume of extract (mL)
m = Weight of turmeric extract (g)

Free Radical Scavenging Ability (DPPH assay): The turmeric extracts were analyzed for DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity using the method as described by Maizura*et al.* [15]with some modifications. Sample solutions were prepared by dissolving 0.025mL of sample extract in 10mL of ethanol.

A fresh solution of DPPH mixed in ethanol (6×10^{-5}M) was prepared before measurements, 3mL of this solution was mixed with 77iL extract in 1cm path length microcuvettes. The resultant samples were placed in dark at room temperature for about 15 minutes. Decrease in absorbance was measured afterwards, at 515nm on UV/visible light spectrophotometer. Absorbance for blank sample having the same amount of ethanol and DPPH solution as that for sample extract was also estimated at 515nm on UV/visible light spectrophotometer. Free radical scavenging activity was measured as:

Reduction of absorbance (%) = [(AB - AA) / AB] × 100

AB = Absorbance of blank sample at t = 0 minute
AA = Absorbance of tested extract solution at t = 15 minute

RESULTS AND DISCUSSIONS

Proximate Analysis: The proximate analysis of raw material is an important tool to evaluate the quality of raw material. For this purpose, *Curcuma longa* was investigated for different parameters to find out its composition and over all nutritional value (Table 2). Proximate composition showed that turmeric contained moisture (13.2±0.35%), crude protein (6.47±0.17%), crude fat (2.7±0.14%), crude fiber (4.80±0.13%), ash (3.49±0.09%) and NFE (69.05±1.81%).

The date obtained was comparable with the earlier results. According to Lim *et al*. [5] the *Curcuma longa* (powder) contained moisture, crude protein, carbohydrates, crude fat, crude ash and crude fiber as 14.8±0.12%, 6.9±0.03%, 69.4±0.36%, 1.5±0.3% 2.5±0.01% respectively. The moisture, protein and NFE results are in agreement with our above mentioned findings while fat, fiber and ash are highly inconsistent with our results.

According to Chainani[10] turmeric contains fat (5.1%), protein (6.3%), carbohydrates (72.4%), minerals (3.5%) and moisture (12.1%). The protein, moisture and ash contents results are in agreement with our above mentioned findings while fat results are highly inconsistent with our results.

Khan *et al*. [16] studied turmeric powder to explore its chemical composition and resulted that it possessed dry matter (95.3%), ash contents (6.95%), crude protein (7.5%), ether extract (2.7%), neutral detergent fiber (38.2%), acid detergent fiber (19.8%) and acid detergent lignin (6.8%).

Table 2: Composition of turmeric

Constituents	Quantity (%)
Moisture	13.2±0.35
Crude protein	6.47±0.17
Crude fat	2.7±0.14
Crude fiber	4.80±0.13
Ash	3.49±0.09
NFE	69.05±1.81

Values are expressed as means ± standard deviation

According to them ash contents 6.94% are highly inconsistent in our results. The variations between my findings and above mentioned findings of various researchers are may be due to difference in varieties and cultivation practices.

Total Phenolic Contents: The health promoting effects associated with the phenols has necessitated their quantification in the various food and food products. Globally consumers are now gaining awareness day by day to the diet related health problems and shifting their diet pattern on natural plant based food and food products that are rich in polyphenolic compounds. Total phenolic compounds directly indicate the antioxidant activity.Total phenolic content of the ethanol, methanol and aqueous extracts of turmeric were measured by Folin's reagent. Results are presented as mg of gallic acid equivalent (GAE) per 100 gram of extracts (Table 4). Total phenolic content (TPC) means related to three solvents at their different concentrations i.e. Ethanol (60%), Ethanol (80%), methanol (60%), methanol (80%) and aqueous extract were 678.76 mg GAE/ 100 g, 745.76 mg GAE/ 100 g, 53.87 mg GAE/ 100 g, 682.43 mg GAE/ 100 g and 496.76 mg GAE/ 100 g respectively. Results showed that the highest value of total phenolic content (TPC) was recorded in ethanolic extract (80%) while the lowest for aqueous extract.

Results related to research work of Kaur and Kapoor[17] have sorted the present research outcomes. They evaluated the bioactive phenolic compounds and antioxidant potential of Asian spices and vegetables including *Curcuma longa* for its application in different functional foods. They analyzed that the total phenolic content in turmeric varied from 1.72 to 7.46 g GAE/ 100g.

It was found that total phenolic contents in dry powder of turmeric were 825.58 mg GAE/ 100g and our results are highly consistent with their work [14]. Different extraction methods and solvents have a significant effect on extraction yield as well as their antioxidant potential. Kim *et al.* [18] studied the total phenolic content in turmeric extract and they found that total phenolic content in turmeric extract was 582.8 mg GAE/100 g extract.

Table 3: Mean squares for total phenolic contents (mg GAE/100g) of turmeric extracts

SOV	Df	SS	MS	F
Treatments	4	142328	35582.1	55.5**
Error	10	6412	641.2	
Total	14	148740		

* = Significant

**= Highly significant

NS=Non-significant

Table 4: Means for total phenolic contents (mg GAE/100g) of turmeric extracts

Treatment	TPC (mg GAE/100g)
T_1	678.76±16.96^c
T_2	745.76±14.91^a
T_3	523.87±10.47^d
T_4	682.43±13.64^b
T_5	496.76±12.41^f

Mean carrying same letter are statistically non-significant

T_1= 60% ethanol extract of turmeric

T_2= 80% ethanol extract of turmeric

T_3 = 60% Methanol extract of turmeric

T_4 = 80% Methanol extract of turmeric

T_5 = Aqueous extract of turmeric

It was revealed that ethanolic extracts of turmeric exhibited higher antioxidant potential than its aqueous extract under similar conditions. Antioxidant activity of methanol extracts of turmeric was studied by Shan *et al.*[19] and estimated its phenolic content which was 6.3 mg GAE/ g dry weight of sample. In a recent study Liu *et al.* [20] studied the polyphenol contents and antioxidant capacity of ethanol extracts of turmeric which showed a total phenolic content of 712.4 mg GAE/ 100g dry weight of sample.

Free Radical Scavenging Activity (DPPH assay): Mean squares regarding DPPH scavenging activity of extracts are presented in Table 5Antioxidant activity was determined by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay.The principle of DPPH assay; in DPPH assay the antioxidant plant extracts were able to reduce the stable free radical DPPH to yellow colored dipheny-picrylhydrazyl. The method is based on the reduction of DPPH in alcoholic solutions in the presence of an antioxidant, due to the formation of non-radical form of DPPH-H in the reaction solution.DPPH analysis is also one of the tests used to prove the ability of components of the turmeric extracts to act as donors of hydrogen atom [21].

Mean squares for DPPH illustrated highly significant effect of solvent, their concentration and interaction on free radical scavenging activity of turmeric powder extract (Table 5). Means for free radical scavenging activities of three solvents at their different concentrations i.e. Ethanol (60%), Ethanol (80%), methanol (60%), methanol (80%) and aqueous extract were 46.16%, 52.19%, 35.41%, 49.83% and 31.33% respectively. It was shown in Table 6 that indicated free radical scavenging activities was maximum for ethanolic extract (80%) followed by methanol (80%) and the lowest for aqueous extract.Turmeric powder was extracted with 50% ethanol. According to them, turmeric has antioxidant activity (50% v/v) measured through DPPH assay. Moreover, Kaur and Kapoor [17] assessed the free radical scavenging activity of turmeric powder by extracting it with water and ethanol. It was concluded that ethanol gave more extraction yield than water and measured the antioxidant potential by DPPH assay. According to them, ethanolic and water extracts of turmeric contained 62.45% and 41.3% antioxidant activity respectively.

Moreover, Antioxidant activities weredetermined by Tilak*et al.* [22] of some edible plants including turmeric. Plant material extracted by using water and methanol as a solvent. According to them, antioxidant potential of methanolic extract was higher than the water extract. According to them, methanolic and water extracts of turmeric contained 52% and 38% antioxidant activity respectively.

Table 5: Mean squares for DPPH assay and antioxidant activity of turmeric extracts

SOV	df	SS	MS	F
Treatments	4	142328	35582.1	55.5**
Error	10	6412	641.2	
Total	14	148740		

** = Highly significant

* = Significant

NS = Non-significant

Table 6: Means for DPPH assay (%) of turmeric extracts

Treatment	DPPH (%)
T_1	46.16±1.24^c
T_2	52.19±1.51^a
T_3	35.41±1.17^d
T_4	49.83±1.42^b
T_5	31.33±0.95^e

Mean carrying same letter are statistically non-significant

T_1 = 60% ethanol extract of turmeric

T_2 = 80% ethanol extract of turmeric

T_3 = 60% Methanol extract of turmeric

T_4 = 80% Methanol extract of turmeric

T_5 = Aqueous extract of turmeric

CONCLUSIONS

Proximate composition showed that turmeric contained moisture (13.2±0.35%), crude protein (6.47±0.17%), crude fat (2.7±0.14%), crude fiber (4.80±0.13%), ash (3.49±0.09%) and NFE (69.05±1.81%). The total phenolic content (TPC) means related to three solvents at their different concentrations i.e. Ethanol (60%), Ethanol (80%), methanol (60%), methanol (80%) and aqueous extract were 678.76 mg GAE/ 100 g, 745.76 mg GAE/ 100 g, 523.87 mg GAE/ 100 g, 682.43 mg GAE/ 100 g and 496.76 mg GAE/ 100 g respectively. Results showed that the highest value of total phenolic content (TPC) was recorded in ethanolic extract (80%) while the lowest for aqueous extract. Research indicated that free radical scavenging activities was maximum for ethanolic extract (80%) followed by methanol (80%) and the lowest for aqueous extract.

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