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**Isha Singh**  
Medicinal & Aromatic Plants  
Section, Old IATTE Building,  
CCS Haryana Agricultural  
University, Hisar, Haryana,  
India

**VK Madan**  
Medicinal & Aromatic Plants  
Section, Old IATTE Building,  
CCS Haryana Agricultural  
University, Hisar, Haryana,  
India

## Effect of extraction techniques and solvents on antioxidant activity and sugars content of turmeric (*Curcuma longa* L.)

Isha Singh and VK Madans

### Abstract

The present study was planned to determine the effect of extraction techniques (mechanical shaking, refluxing, soxhlet extraction, and centrifugation) and solvents (acetone, ethanol and water) on antioxidant activity of turmeric rhizomes. Soxhlet extraction technique was found to be most efficient extraction technique for turmeric rhizomes as extracts obtained by soxhlet possess highest antioxidant activity. Amongst solvents, acetone extracts exhibited highest antioxidant activity. Extract yield and sugars content were estimated and their contents were also found to be highest in soxhlet extracts.

**Keywords:** *Curcuma longa*, extraction techniques, total phenols, flavonoids, curcumin

### Introduction

Turmeric (*Curcuma longa*) plant is a perennial herb belonging to the family Zingiberaceae and is grown extensively in south and south east tropical Asia. It is cultivated in warm, rainy regions like China, India, Indonesia, Jamaica and Peru. The rhizome of this plant is also referred to as 'root'. Turmeric is a spice of golden yellow colour and because of its colour, aroma and taste turmeric was named as 'Indian Saffron'. India produces about 80% of the world's supply of commercial turmeric. Turmeric contains protein (6.3%), fat (5.1%), minerals (3.5%), carbohydrates (69.4%), sugar (3%) and moisture (13.1%)<sup>[1]</sup>. Turmeric has a wide variety of phytochemicals including curcumin, zingiberene, curcumenol, curcumol, tetrahydrocurcumin, triethylcurcumin, turmerin, turmerone and turmeronols<sup>[2]</sup>. The most active component of turmeric is curcumin (diferuoyl methane) (2 - 5%) which is responsible for the yellow colour and comprises of curcumin I (94%), curcumin II (6%), and curcumin III (0.37%) which are found to be natural antioxidants<sup>[3]</sup>. Curcumin is an orange-yellow crystalline powder, practically insoluble in water and freely soluble in DMSO, acetone, ethanol, etc. Kaempferol, quercetin and rutin are the major flavonoids present in turmeric. The main phenolic constituents of turmeric are curcumin, ferulic acid and  $\rho$ -coumaric acid. Turmeric extract is an aleoresin consisting of volatile oil fraction and a heavy fraction of yellowish brown colour. Dried rhizomes contain 5 - 6% oil. The peculiar turmeric aroma is imparted by *ar*-turmerone. The essential oil obtained by steam distillation of rhizomes has  $\alpha$ -phellandrene (1%), sabinene (0.6%), cineol (1%), borneol (0.5%), zingiberene (25%) and sesquiterpine (53%)<sup>[4]</sup>. The rhizomes are used in folk medicines for treatment of biliary disorder, anorexia, cough, diabetic wounds, hepatic disorders, rheumatism and sinusitis<sup>[5]</sup>. Since turmeric has been used as a food additive to improve palatability, storage stability as well as traditional medicine throughout the world for thousands of years, a detailed analysis of its antioxidant activity is required. Thus, this study was carried out to determine the antioxidant activity of *Curcuma longa* L. and a comparison between the commonly used extraction techniques (mechanical shaking, refluxing, soxhlet extraction, and centrifugation) and solvents (acetone, ethanol and water) was also established. Extract yield and sugars contents were also determined.

### Experimental

#### Plant material

Dried turmeric rhizomes of variety BSR-2 were procured from Tamil Nadu Agricultural University, Coimbatore, India. Germplasm material of dried turmeric rhizomes was also procured to study its chemical profile and its comparison with the released variety of turmeric (BSR-2).

### Correspondence

**Isha Singh**  
Medicinal & Aromatic Plants  
Section, Old IATTE Building,  
CCS Haryana Agricultural  
University, Hisar, Haryana,  
India

### Preparation of extracts

Healthy turmeric rhizomes were selected, cut into small pieces and ground in warring blender to obtain a fine powder. Powdered samples were extracted by using following four extraction techniques and three solvents (acetone, ethanol, distilled water):

### Mechanical shaking

Two gram of powdered samples of turmeric rhizomes were extracted three times with 60, 40 and 30 mL of each solvent in conical flasks by shaking on a mechanical shaker for 2, 1 and 1 hrs respectively. Filtrates of each solvent from three extraction steps were pooled and their volumes were noted.

### Refluxing

Two gram of powdered samples of turmeric rhizomes were extracted by using a reflux condenser with approximately 75 mL of each solvent. The extraction was carried out at boiling temperature of the respective solvent for 5h. As solvent in round bottom flask boils, vapours rise into the condenser where they are converted back to liquid that drips into the round bottom flask. The extract was allowed to cool, filtered and residue was again extracted twice (each refluxing time 2h and 1h, respectively) with 75 mL of respective solvents. Filtrates of each solvent from three extraction steps were pooled and their volumes were noted.

### Soxhlet Extraction

Four gram of powdered samples of turmeric rhizomes were placed in a filter paper (Whatman No. 1) thimble in a classical soxhlet apparatus fitted with a 250 mL round bottom flask containing approximately 150 mL of each solvent. Extraction was performed at boiling temperature of respective solvent for 5h with completion of up to seven to eight cycles through siphon mechanism in case of extraction with acetone and ethanol. In case of extraction with water, time required for completion of one cycle was significantly more hence, with water, extraction was carried out for longer time with the completion of up to seven to eight cycles through siphon mechanism. After the completion of first extraction step, residue in thimble was again extracted twice (each extraction time 2h and 1h, respectively) with suitable amount of respective solvents. Filtrates of each solvent from three extraction steps were pooled and their volumes were noted.

### Centrifugation

Two gram of powdered samples of turmeric rhizomes were extracted three times with 60, 40 and 30 mL of each solvent in centrifuge tubes by centrifugation at 6000 rpm for 10 min. each Filtrates of each solvent from three extraction steps were pooled and their volumes were noted.

All the samples extracted by using above-mentioned techniques were performed in triplicate. Extracts were used for the estimation of total phenols, flavonoids and curcumin contents.

### Chemicals

Commercially available and highest purity chemicals were used for various experimental procedures.

### Estimation of extract yield

The extract yield was calculated by gravimetric method. Each extract was dried up completely in a beaker and extract yield was calculated by the difference in weight of beaker before

and after drying. Extract yield was expressed as gram per hundred gram (g/100g).

### Estimation of total sugars content

Total sugars were estimated by Phenol sulphuric method [6] using glucose as standard for which a calibration curve was obtained. Extracts were diluted to adjust the absorbance within calibration limits. To 1.0 mL of each extract, 2.0 mL of phenol solution (2%, w/v) was added followed by 5.0 mL concentrated sulphuric acid. Test tubes were allowed to cool for 30 minutes and absorbance was measured at 490 nm using UV-VIS-double beam spectrophotometer Model 2203 (Systronics Co.) against a blank. The amount of total sugars present was calculated from the calibration curve and results were expressed as milligrams per gram (mg/g).

### Estimation of reducing sugars content

Reducing sugars were estimated by the method of Nelson-Somogyi method [7-8] using glucose as standard for which a calibration curve was obtained. Extracts were diluted to adjust the absorbance within calibration limits. To 1.0 mL of each extract, 1.0 mL distilled water was added, followed by addition of 1.0 mL alkaline copper reagent, solution. Test tubes were shaken, covered with aluminum foil and heated in boiling water bath for 20 min. after cooling the test tubes at room temperature, 1.0 mL of arsenomolybdate reagent was added. The contents were mixed thoroughly and volume was made up to 10.0 mL with distilled water. The absorbance was measured at 520 nm using UV-VIS double beam Spectrophotometer Model 2203 (Systronics Co.) against a blank. The amount of reducing sugars was calculated from the calibration curve and results were expressed as milligrams per gram (mg/g).

### Estimation of non-reducing sugars content

The content of non-reducing sugars was calculated from the difference between the content of total sugars and that of reducing sugars.

Non-reducing sugars = Total sugars – Reducing sugars

### Estimation of antioxidant activity

Antioxidant activity was evaluated by  $\beta$ -carotene bleaching method of Hidalgo *et al.* [9] 1.0 mg of crystalline  $\beta$ -carotene was dissolved in 5.0 mL of  $\text{CHCl}_3$  and 0.1 mL of linoleic acid and 0.9 mL of tween 20 (200 mg) were added. The solvent was subsequently removed at 40°C in a vacuum evaporator and immediately the mixture was diluted with 250 mL of double distilled water. Aliquots (4 mL) of this emulsion were transferred into test tubes, to which were then added 0.2 mL of aliquots of test samples (1000  $\mu\text{g/mL}$  concentration level). A control containing 0.2 mL of respective solvent and 4.0 mL of emulsion was also used. The test tubes were covered with aluminium foil and placed in a water bath at 50°C. The absorbance at 470 nm was recorded with a UV-VIS double beam Spectrophotometer Model 2203 (Systronics Co.) at intervals of 30 min, until the colour of  $\beta$ -carotene disappeared from the control tubes. The above mixture without  $\beta$ -carotene served as blank. All determinations were carried out in triplicates. The antioxidant activity was calculated using the following equation:-

$$A_A(\%) = \frac{[(A_o)_{\text{control}} - (A_t)_{\text{control}}] - [(A_o)_{\text{sample}} - (A_t)_{\text{sample}}]}{[(A_o)_{\text{control}} - (A_t)_{\text{control}}]} \times 100$$

where,  $(A_0)_{\text{control}}$  and  $(A_0)_{\text{sample}}$  are the absorbance values measured at zero time of incubation for the control and sample, respectively and  $(A_t)_{\text{control}}$  and  $(A_t)_{\text{sample}}$  are the corresponding values at the end of the reaction time.

**Results and discussion**

**Extract yield**

In turmeric rhizomes (var. BSR-2), extract yield (g/100g) of extracts obtained by soxhlet technique was highest (23.82) followed by 13.83 in refluxing, 12.19 in centrifugation and 11.98 in mechanical shaking (Table 1). Similarly, in turmeric rhizomes (germplasm material) the corresponding values of extracts obtained by soxhlet technique, refluxing, centrifugation and mechanical shaking were 13.56, 7.82, 7.00 and 6.58, respectively. Amongst solvents, extract yield (g/100g) of water extracts of turmeric rhizomes (var. BSR-2) was highest (17.74) followed by ethanol (15.37) and acetone (13.26) extracts. Similarly, in turmeric rhizomes (germplasm material) the corresponding values were 10.70, 8.65 and 6.88 in water, ethanol and acetone extracts, respectively. Extract yield was higher in extracts obtained by soxhlet technique and refluxing in comparison to mechanical shaking and centrifugation, which may be due to more efficiency of hot solvent to extract more phytoconstituents in comparison to solvent at room temperature. Present findings are in agreement with previous investigations on seven medicinal plants showing that higher extract yields were obtained by refluxing (4.86-42.4 g/100g) technique in comparison to shaking (2.23-34.5 g/100g) which is due to the reason that hot solvent system under reflux state are more efficient for the recovery of antioxidant components thus offering higher extract yields [10]. The extract yield from *Quercus infectoria* galls. was highest in water extract (80.03%) followed by ethanol (45.77%) and acetone (43.57%) extracts showing that polar compounds in plants are easier to extract with more polar solvents [11]. Hence, the data of extract yield in present studies is in agreement with the studies of other research workers on different crops.

**Total sugars, reducing sugars, non-reducing sugars**

Contents of total sugars, reducing sugars and non-reducing sugars (mg/g) in various extracts of turmeric rhizomes varied widely. In turmeric rhizomes of var. BSR-2 (Tables 2, 3 and 4) the contents of total sugars, reducing sugars and non-reducing sugars were highest (37.96, 10.19 and 27.77 mg/g, respectively) in extracts obtained by soxhlet technique followed by refluxing (34.45, 8.83 and 25.62, respectively), mechanical shaking (29.51, 7.60 and 21.91, respectively) and centrifugation (28.99, 7.52 and 21.47, respectively). Amongst solvents, the contents of total sugars, reducing sugars and non-reducing sugars were highest (37.03, 9.66 and 27.37 mg/g, respectively) in water extracts followed by ethanol (32.48, 8.53 and 23.95, respectively) and acetone (28.68, 7.42 and 21.26, respectively) extracts. In turmeric rhizomes of

germplasm material, the contents of total sugars, reducing sugars and non-reducing sugars were 35.90, 9.78 and 26.12 mg/g, respectively in extracts obtained by soxhlet technique followed by refluxing (33.49, 8.68 and 24.81, respectively), mechanical shaking (28.53, 7.37 and 21.16, respectively) and centrifugation (28.13, 7.21 and 20.92, respectively). Amongst solvents, the contents of total sugars, reducing sugars and non-reducing sugars were highest (35.33, 9.35 and 25.98 mg/g, respectively) in water extracts followed by ethanol (31.54, 8.19 and 23.35, respectively) and acetone (27.67, 7.24 and 20.43, respectively) extracts (Tables 2, 3 and 4, respectively). Sugar molecules have many polar hydroxyl (-OH) groups and are highly polar in nature. Sugars form strong hydrogen bonds with one another and also with water molecules or ethanol molecules [12]. A molecule of sucrose (non-reducing sugar) has eight hydroxyl groups, three hydrophilic oxygen atoms (bound in a circle) and 14 hydrogen atoms. This enables the formation of hydrogen bonds with water molecules, hydration of sucrose molecules and therefore easy dissolution of sucrose in water. In non-aqueous solvents, sucrose solubility is significantly lower than water and hence, sucrose does not dissolve in non-polar solvents [13]. Hence, in present studies, the contents of total sugars, reducing sugars and non-reducing sugars in turmeric rhizomes were found to be higher in water extracts. It was reported that turmeric contained 3% sugars in it [1].

**Antioxidant activity**

In turmeric rhizomes of var. BSR-2 and germplasm material, antioxidant activity (%) of extracts obtained by soxhlet technique was highest (67.75 and 66.52, respectively) followed by refluxing (65.99 and 64.65, respectively), mechanical shaking (59.68 and 57.92, respectively) and centrifugation (58.44 and 56.46, respectively) at 1000 µg/mL concentration level (Table 5). Amongst solvents, antioxidant activity (%) of turmeric rhizomes (var. BSR-2 and germplasm material) extracts at 1000 µg/mL concentration level was highest in acetone extracts (76.14 and 75.04, respectively) followed by ethanol (74.35 and 72.65, respectively) and water (38.41 and 36.48, respectively) extracts (Table 5). These results are supported by a study showing that antioxidant activity of marine edible seaweeds *E. cottonii* and *Padina* species by using β-carotene bleaching method and reported that extracts prepared by soxhlet extraction technique showed higher antioxidant activity (34.72 and 28.67%) in comparison to extracts prepared by shaking (27.86 and 21.45%) [14]. Antioxidant activity of turmeric evaluated by β-carotene bleaching method was higher (92.45%) in ethanol extract in comparison to water (81.3%) extract [15]. Antioxidant activity by β-carotene bleaching method of asparagus and broccoli and reported that acetone extract had highest values of antioxidant activity coefficient (357 and 372) followed by methanol (336 and 372) and water (206 and 331) extracts [16].

**Table 1:** Extract yield (g/100g) of turmeric rhizomes extracts obtained by using different extraction techniques

Spices and part	Extraction↓Technique Solvent →	Extract Yield (g/100g)				
		Acetone	Ethanol	Water	Mean	Increase over T <sub>1</sub> (%)
Turmeric rhizomes (var. BSR-2)	Mechanical Shaking (T <sub>1</sub> )	10.00 ± 0.05	11.70 ± 0.05	14.25 ± 0.04	11.98	-
	Refluxing (T <sub>2</sub> )	11.72 ± 0.11	13.79 ± 0.07	15.99 ± 0.08	13.83	15.4
	Soxhlet (T <sub>3</sub> )	21.12 ± 0.05	24.08 ± 0.09	26.25 ± 0.04	23.82	96.3
	Centrifugation (T <sub>4</sub> )	10.20 ± 0.08	11.90 ± 0.09	14.46 ± 0.06	12.19	1.7
	Mean	13.26	15.37	17.74		
Turmeric rhizomes	Mechanical Shaking (T <sub>1</sub> )	5.12 ± 0.06	6.16 ± 0.05	8.46 ± 0.04	6.58	-

(germplasm material)	Refluxing (T <sub>2</sub> )	6.00 ± 0.09	7.68 ± 0.07	9.79 ± 0.06	7.82	18.8
	Soxhlet (T <sub>3</sub> )	10.72 ± 0.05	13.96 ± 0.04	16.00 ± 0.08	13.56	106.1
	Centrifugation (T <sub>4</sub> )	5.68 ± 0.06	6.78 ± 0.07	8.55 ± 0.05	7.00	6.4
	Mean	6.88	8.65	10.70		

**Table 2:** Total sugars (mg/g) in turmeric rhizomes extracts obtained by using different extraction techniques

Spices and part	Extraction↓Technique Solvent →	Total Sugars (mg/g)				
		Acetone	Ethanol	Water	Mean	Increase over T <sub>4</sub> (%)
Turmeric rhizomes (var. BSR-2)	Mechanical Shaking (T <sub>1</sub> )	25.33 ± 0.16	29.98 ± 0.13	33.23 ± 0.20	29.51	1.8
	Refluxing (T <sub>2</sub> )	30.57 ± 0.16	34.25 ± 0.17	38.54 ± 0.15	34.45	18.8
	Soxhlet (T <sub>3</sub> )	34.02 ± 0.31	36.24 ± 0.10	43.62 ± 0.41	37.96	30.9
	Centrifugation (T <sub>4</sub> )	24.81 ± 0.20	29.44 ± 0.35	32.73 ± 0.52	28.99	-
	Mean	28.68	32.48	37.03		
Turmeric rhizomes (germplasm material)	Mechanical Shaking (T <sub>1</sub> )	24.32 ± 0.14	29.06 ± 0.38	32.22 ± 0.20	28.53	1.4
	Refluxing (T <sub>2</sub> )	29.63 ± 0.22	33.42 ± 0.34	37.41 ± 0.26	33.49	19.1
	Soxhlet (T <sub>3</sub> )	32.67 ± 0.29	35.31 ± 0.22	39.73 ± 0.11	35.90	27.6
	Centrifugation (T <sub>4</sub> )	24.06 ± 0.41	28.37 ± 0.22	31.97 ± 0.27	28.13	-
	Mean	27.67	31.54	35.33		

**Table 3:** Reducing sugars (mg/g) in turmeric rhizomes extracts obtained by using different extraction techniques

Spices and part	Extraction↓Technique Solvent →	Reducing Sugars (mg/g)				
		Acetone	Ethanol	Water	Mean	Increase over T <sub>4</sub> (%)
Turmeric rhizomes (var. BSR-2)	Mechanical Shaking (T <sub>1</sub> )	6.52 ± 0.12	7.85 ± 0.04	8.43 ± 0.03	7.60	1.1
	Refluxing (T <sub>2</sub> )	7.82 ± 0.07	8.77 ± 0.13	9.90 ± 0.01	8.83	17.4
	Soxhlet (T <sub>3</sub> )	8.86 ± 0.05	9.90 ± 0.03	11.81 ± 0.01	10.19	35.5
	Centrifugation (T <sub>4</sub> )	6.48 ± 0.06	7.60 ± 0.07	8.49 ± 0.02	7.52	-
	Mean	7.42	8.53	9.66		
Turmeric rhizomes (germplasm material)	Mechanical Shaking (T <sub>1</sub> )	6.46 ± 0.05	7.38 ± 0.07	8.27 ± 0.04	7.37	2.2
	Refluxing (T <sub>2</sub> )	7.72 ± 0.12	8.56 ± 0.09	9.75 ± 0.02	8.68	20.4
	Soxhlet (T <sub>3</sub> )	8.47 ± 0.06	9.57 ± 0.10	11.30 ± 0.04	9.78	35.6
	Centrifugation (T <sub>4</sub> )	6.29 ± 0.07	7.24 ± 0.02	8.10 ± 0.03	7.21	-
	Mean	7.24	8.19	9.35		

**Table 4:** Non-reducing sugars (mg/g) in turmeric rhizomes extracts obtained by using different extraction techniques

Spices and part	Extraction↓Technique Solvent →	Non-reducing Sugars (mg/g)				
		Acetone	Ethanol	Water	Mean	Increase over T <sub>4</sub> (%)
Turmeric rhizomes (var. BSR-2)	Mechanical Shaking (T <sub>1</sub> )	18.81 ± 0.07	22.13 ± 0.12	24.80 ± 0.21	21.91	2.1
	Refluxing (T <sub>2</sub> )	22.75 ± 0.22	25.48 ± 0.10	28.63 ± 0.16	25.62	19.3
	Soxhlet (T <sub>3</sub> )	25.16 ± 0.26	26.34 ± 0.09	31.81 ± 0.41	27.77	29.3
	Centrifugation (T <sub>4</sub> )	18.33 ± 0.18	21.84 ± 0.28	24.24 ± 0.59	21.47	-
	Mean	21.26	23.95	27.37		
Turmeric rhizomes (germplasm material)	Mechanical Shaking (T <sub>1</sub> )	17.86 ± 0.09	21.68 ± 0.35	23.95 ± 0.16	21.16	1.2
	Refluxing (T <sub>2</sub> )	21.90 ± 0.11	24.86 ± 0.26	27.66 ± 0.27	24.81	18.6
	Soxhlet (T <sub>3</sub> )	24.20 ± 0.25	25.73 ± 0.31	28.43 ± 0.15	26.12	24.9
	Centrifugation (T <sub>4</sub> )	17.76 ± 0.34	21.13 ± 0.20	23.87 ± 0.24	20.92	-
	Mean	20.43	23.35	25.98		

**Table 5:** Antioxidant activity (%) of turmeric rhizomes extracts obtained by using different extraction techniques

Spices and part	Extraction↓Technique Solvent →	Antioxidant activity (%)				
		Acetone	Ethanol	Water	Mean	Increase over T <sub>4</sub> (%)
Turmeric rhizomes (var. BSR-2)	Mechanical Shaking (T <sub>1</sub> )	75.21 ± 0.06	73.33 ± 0.08	30.49 ± 0.11	59.68	2.1
	Refluxing (T <sub>2</sub> )	77.10 ± 0.10	75.28 ± 0.08	45.60 ± 0.12	65.99	12.9
	Soxhlet (T <sub>3</sub> )	78.17 ± 0.06	76.37 ± 0.13	48.71 ± 0.08	67.75	15.9
	Centrifugation (T <sub>4</sub> )	74.09 ± 0.15	72.42 ± 0.12	28.82 ± 0.09	58.44	-
	Mean	76.14	74.35	38.41		
Turmeric rhizomes (germplasm material)	Mechanical Shaking (T <sub>1</sub> )	74.09 ± 0.12	71.18 ± 0.15	28.50 ± 0.09	57.92	2.6
	Refluxing (T <sub>2</sub> )	76.36 ± 0.06	73.95 ± 0.09	43.63 ± 0.07	64.65	14.5
	Soxhlet (T <sub>3</sub> )	77.45 ± 0.13	75.32 ± 0.10	46.78 ± 0.08	66.52	17.6
	Centrifugation (T <sub>4</sub> )	72.24 ± 0.09	70.15 ± 0.18	26.99 ± 0.06	56.46	-
	Mean	75.04	72.65	36.48		

**Conclusion**

The results of present study showed that extract yield, sugars content and antioxidant activity of turmeric rhizomes varied with the type of extraction technique and solvent used.

Acetone extracts obtained from soxhlet extraction technique exhibited highest antioxidant activity.

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