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Research Article
Comparison of Curcuminoids Content, *in vitro* Anti-oxidant and Anti-diabetic Activity of *Curcuma longa* Collected from Four Different Countries

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Abstract

Background and Objectives: Turmeric, also known as *Curcuma longa* is being used as a traditional medicine throughout Asian countries from ancient time. The study aimed to quantify the concentration of curcumin in turmeric samples grown in four different geographical conditions such as Oman, Yemen, India (Asia) and Tanzania (Africa) and to investigate the effect of varying concentrations of curcumin on the *in vitro* anti-oxidant and anti-diabetic activity. **Materials and Methods:** Four types of turmeric samples were collected from Oman, India, Yemen and Tanzania. The curcumin content was determined in methanol and acetone extracts by UV-Vis spectrophotometer. Curcuminoids were also isolated, extracted and quantified from each sample. The *in vitro* anti-oxidant activity of turmeric samples were investigated by DPPH and Thiobarbituric acid assay methods while anti-diabetic activity was assessed by measuring the α-amylase inhibitory activity. **Results:** The results showed that the concentrations of curcumin, the biologically active constituent of turmeric, vary greatly among the four varieties. The methanolic extract of Omani variety contained the maximum curcumin content while the highest percentage yield of curcuminoids was isolated from the methanolic extract of Yemeni turmeric. It was found that methanol is the better solvent than acetone for the extraction of curcumin as well as curcuminoids. All turmeric extracts exhibited dose dependent anti-oxidant and anti-diabetic activity. Tanzanian sample showed the highest percentage inhibition of DPPH free radical and lipid peroxides (56.07 and 48.15%, respectively) while Yemeni sample possessed the highest anti-diabetic activity (38.06%). **Conclusion:** It may be concluded that curcumin content in turmeric depends upon the geographical location and climatic conditions where it is grown. The biological activity of turmeric depends upon its total curcuminoids content including curcumin.

Key words: Anti-diabetic, anti-oxidant, curcumin, turmeric, thiobarbituric acid

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Medicinal plants have a rich history of treating and combating diseases since ancient time. Medicinal plants have provided mankind with numerous biologically active molecules such as morphine, digoxin, atropine, reserpine, quinine, paclitaxel etc. and therefore, medicinal plants are considered as potential and reliable source of identifying new drug molecules. The rhizomes of *Curcuma longa* L. (family: Zingiberaceae) commonly known as turmeric in English is a widely used spice throughout the world especially in Asian continent. Turmeric is known as Kurkum in Arabic and Haldi in Hindi[1]. It is highly regarded as a universal panacea in complementary and alternative medicine (CAM) because of its broad spectrum of biological, medicinal and therapeutic activities in a variety of human diseases including cancer[2].

Turmeric contains approximately 60-70% carbohydrates, 6-8% proteins, 6-13% water, 5-10% fat, 3-7% essential oils, 3-7% dietary minerals, 2-7% dietary fibers and 1-6% mixture of curcuminoids. Chemically, turmeric includes diarylheptanoids, a class of curcuminoids (i.e., diferuloyl methane), such as curcumin, demethoxycurcumin and bis demethoxycurcumin[3]. The essential components of turmeric are polyphenolic curcuminoids namely curcumin, demethoxycurcumin and bisdemethoxycurcumin (Fig. 1) which are chiefly responsible for its yellow color[4]. It is one of the best natural coloring agents used in herbal, food and cosmetic industry.

A large number of published research articles have reported the activity of compounds like curcumin and sodium curcuminate isolated from *C. longa* L. as being potent anti-inflammatory agents. Demethoxycurcumin and bisdemethoxycurcumin have also been shown to exhibit anti-oxidant activity[5]. Research also supports the opinion of a wide-ranging health benefit of curcumin-related compounds in the treatment of multiple diseases that consists in its capacity to reduce the oxidative stress[6]. Both anti-oxidant and prooxidant activity have also been reported for curcumin and its analogues[7,8].

A broad interest in the activities of curcumin prompted us to investigate and compare the anti-oxidant activity using several methods in crude organic extracts of turmeric.

Recent studies have shown turmeric and its active principal constituent curcumin to be beneficial in cancer, asthma, diabetes, inflammation, hepatitis, liver disorder, skin diseases, bacterial infections, amenorrhea etc[9]. The US- FDA found curcumin to be safe in clinical studies and has approved an acceptable daily intake level of 0.1-3 mg kg^{-1} b.wt. But the content of natural pigment and bio-active phytoconstituents of turmeric varies greatly depending on geographical location[10] and thus a difference in biological activity is expected. Hence, this study was designed to (i) Determine the concentration of curcumin in turmeric samples grown in four different geographical conditions such as Oman, Yemen, India (Asia) and Tanzania (Africa) and (ii) To investigate the effect of varying concentrations of curcumin on the *in vitro* anti-oxidant and anti-diabetic activity. The findings of this study would be useful to pharmaceutical, food, color and cosmetic industries for selecting the source of turmeric for isolation of curcumin. The study will also provide a justification for its use in various chronic ailments such as diabetes, arthritis, cancer etc in traditional system of medicine.

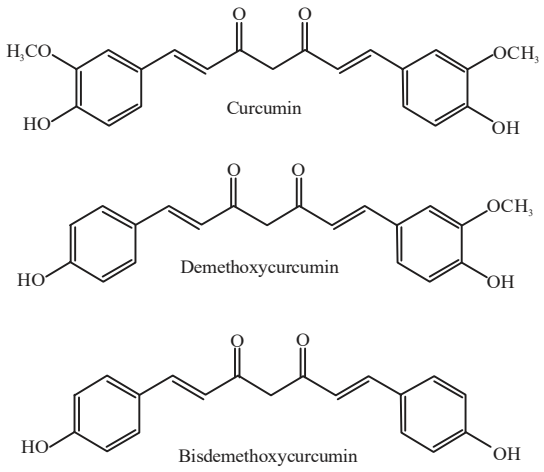

Fig. 1: Chemical structure of curcuminoids present in turmeric

MATERIALS AND METHODS

Chemicals: All solvents and chemicals used in this study were of analytical grade and were purchased from the local supplier.

Collection and processing of plant material: The dried turmeric rhizomes were purchased from the local markets of four different countries viz. Oman, India, Yemen and Tanzania. The samples were identified by the faculty member of Department of Pharmacy, Oman Medical College, Oman. The rhizomes were powdered using domestic grinder and then sieved (mesh 20) to obtain powder of uniform particle size. The samples were kept in air tight container and protected from light until further use.

Extraction and quantification of curcumin: An accurately weighed sample (2 g) from each turmeric powder was dissolved in 100 mL of acetone and methanol separately in a

round bottom flask. The mixture was refluxed for 1 and 2 h on a heating mantle at an optimum temperature of 50-60°C. The extract was filtered and the clear filtrate was collected. The volume was made up to 100 mL with the respective solvent. An aliquot of 0.5 mL was taken and further diluted with acetone/methanol to 50 mL in a volumetric flask. The percentage of curcumin in the diluted methanol and acetone extract was quantified by measuring the absorbance at λ_{max} 425 nm by using double beam UV spectrophotometer[11].

$$\text{Total curcumin (\%)} = \frac{\text{Absorbance}}{\text{1680x concentration of sample}} \times 100$$

Isolation of total curcuminoid: The original acetone or methanol extract of turmeric was concentrated under vacuum until it was reduced to half of the volume and then it was refrigerated for 24 h for crystallization. The curcuminoids were precipitated from the reddish-oily residue by adding hexane. The solid sample so obtained was filtered with vacuum suction to obtain dried crystalline powder which was orange-yellow in color. The solid was weighed using a digital balance to calculate the percentage yield.

In vitro anti-oxidant activity of methanolic extract of turmeric

DPPH free radical scavenging assay: The free radical scavenging activity of the methanolic extracts of four turmeric samples was determined using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay method[12]. The assay mixture contained 2 mL of 1.0 mM DPPH radical solution prepared in methanol and 1 mL of turmeric extract solution of different concentrations (0.1-0.8 mg mL^{-1}). The solution was rapidly mixed and incubated in dark at room temperature for 30 min. The absorbance of each solution was measured at 517 nm using double beam UV-VIS spectrophotometer. The DPPH radical solution with 1 mL methanol was taken as blank. The percentage radical scavenging was calculated by the following formula:

$$\text{Free radical scavenging activity (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Lipid peroxidation inhibition assay: The anti-oxidant activity of turmeric was investigated by lipid per-oxidation inhibition assay using thiobarbituric acid (TBA). In this modified thiobarbituric acid-reactive species (TBARS) assay[13]. The lipid peroxides formed using egg homogenate was measured. Briefly, 0.5 mL of egg homogenate (10% v/v) was mixed with 0.1 mL of the turmeric extract of different concentration (25-100 μg mL^{-1}). The volume was then made up to 1.0 mL with distilled water. Thereafter, 0.05 mL of ferrous sulphate (0.07 M) was added to induce lipid per-oxidation and the mixture was incubated at 37°C for 30 min. Then, 1.5 mL of 20% acetic acid followed by 1.5 mL of 0.8% w/v TBA in 1.1% w/v sodium dodecyl sulphate (DDS) was added. The resulting mixture was vortex mixed and heated at 95°C for 1 h. After cooling, 5 mL of butanol was added and the mixture was centrifuged at 3000 rpm for 10 min. The absorbance of the organic upper layer was measured at 532 nm and the percentage inhibition was calculated with the formula:

$$\text{Inhibition (\%)} = 1 - \frac{T}{C} \times 100$$

where, C is the absorbance value of the control treated in the same way but without sample.

Determination of α-amylase inhibitory activity: The anti-diabetic activity of turmeric was evaluated by determining the percentage inhibition of α-amylase using a modified starch iodine method[14]. Briefly, 1 mL of turmeric extract of concentration (10, 40 and 80 mg mL^{-1}) was mixed with 1 mL of substrate-potato starch (1% w/v), 1% w/v α-amylase and 2 mL of acetate buffer (0.1M, pH 7.2) and the resulting mixture was incubated for 60 min at room temperature. After the incubation, 0.1 mL of 1% w/v iodine solution prepared using potassium iodide was added to the mixture. Absorbance of the mixture was recorded at 565 nm. Acarbose was used as a positive control:

$$\text{Inhibition of } \alpha\text{-Amylase (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

RESULTS

Quantification of curcumin: In this study, curcumin content in turmeric rhizomes samples grown in four different countries was quantified in two organic solvents of varying polarity. The acetone and methanolic turmeric extracts showed absorption maxima (λ_{max}) at 425 nm. The results illustrated in Fig. 2 shows a variation in curcumin content (%) in each sample. The curcumin content in both organic extracts of turmeric was found in the following order Omani>Indian>Yemeni> Tanzanian. The curcumin content of methanolic extract of

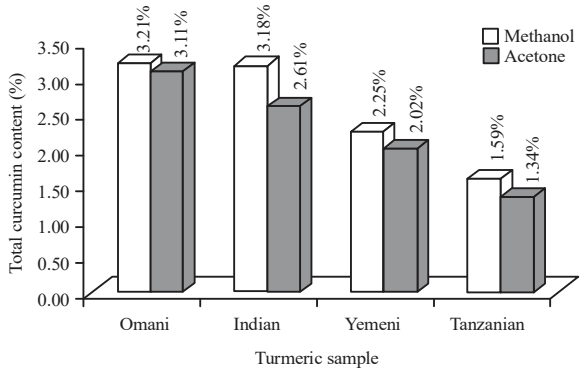

Fig. 2: Percentage of total curcumin in dried powder of turmeric rhizomes collected from four different countries

Indian and Omani turmeric variety was 3.21 and 3.18% which is slightly higher than the curcumin content of plant cultivated in most tropical countries. The percentage content of curcumin in methanol extract was found to be 1.59-3.21% while acetone extract showed lower content (1.34-3.11%). The curcumin content in methanol solvent is slightly higher than the acetone extract indicating it to be a solvent of choice for the extraction of curcumin. The variation in curcumin content in four turmeric samples could be attributed to the difference is climatic factors such as temperature, type of soil, rainfall, hour of day light, phenological stage and harvesting time. Thus, the results indicate that the Omani and Indian variety of *Curcuma longa* have similar curcumin content which is higher than the Tanzanian and Yemeni varieties.

Isolation of curcuminoids: The mixture of total polyphenolic curcuminoids present in turmeric i.e., curcumin, demethoxycurcumin and bisdemethoxycurcumin (Fig. 1) was isolated, purified and their percentage yield was calculated with reference to the dried rhizome powder in both the organic extracts. The percentage yield of curcuminoids content isolated from all four turmeric samples varied in amount and is presented in Table 1. Thin layer chromatography (TLC) chromatogram of all samples developed using chloroform: methanol (21:4) solvent system showed the presence of three spots. The R_f values of all the detected spots were comparable with the values reported in the literature.

Anti-oxidant activity of turmeric by using DPPH: The results of *in vitro* free radical scavenging of turmeric extracts investigated by DPPH assay method are presented in Table 2. The highest anti-oxidant activity was noted at the concentration of 0.8 mg mL^{-1} of turmeric extract. The

Table 1: Percentage yield of total curcuminoids extracted with methanol and acetone solvents

Turmeric sample	Weight of total curcuminoids (g)		Yield (%)	
	Methanol	Acetone	Methanol	Acetone
Omani	0.104	0.096	5.20	4.80
Indian	0.107	0.133	5.35	6.65
Yemeni	0.162	0.098	8.10	4.90
Tanzanian	0.143	0.085	7.15	4.25

Table 2: Anti-oxidant activity of turmeric by DPPH method

Concentration (mg mL^{-1})	Inhibition (%) of DPPH radical (Mean±SD)			
	Omani	Indian	Yemeni	Tanzanian
0.1	42.33±10.1	47.17±7.10	35.37±10.1	52.37±3.9
0.4	47.67±4.1	47.20±4.02	45.17±6.0	54.40±4.8
0.8	49.20±1.1	48.80±8.20	53.57±4.9	56.07±2.2

Table 3: Anti-oxidant activity of different samples of turmeric using Thiobarbituric acid

Concentration (µg mL^{-1})	Inhibition (%) of Lipid peroxides (Mean±SD)			
	Omani	Indian	Yemeni	Tanzanian
25	7.83±3.88	18.37±10.8	14.21±0.28	9.83±4.71
50	26.64±3.56	36.30±2.33	21.54±8.48	36.24±13.26
100	44.50±1.81	40.10±13.71	27.52±1.31	48.15±1.45

percentage inhibition of DPPH radical by Omani, Indian, Yemeni and Tanzanian turmeric at concentrations (0.1, 0.4 and 0.8 mg mL^{-1}) was found to be 42.33, 47.67 and 49.20, 47.17, 47.20 and 48.8, 35.37, 45.17 and 53.57 and 52.37, 54.40 and 56.07, respectively.

Anti-oxidant activity of different samples of turmeric using thiobarbituric acid: The damage caused by oxidative stress can effectively be measured by using TBARS. In this experiment, Thiobarbituric acid (TBA) was used to assess the antioxidant activity of different turmeric samples at three different concentrations viz. 25, 50 and 100 µg mL^{-1} (Table 3).

Evaluation of anti-diabetic activity using α-amylase inhibitory activity: The α-amylase inhibitory activity of turmeric extract was examined by starch iodine method at three different concentrations. The results of α-amylase inhibitory activity is presented in Table 4.

DISCUSSION

The color, quality, therapeutic value and cost of turmeric basically depends on its curcumin content. Hence, the curcumin level of turmeric is of significant economic and therapeutic importance[15]. The R_f values of all the detected spots were comparable with the values reported in the literature[16].

Table 4: Determination of anti-diabetic activity by α- amylase inhibition

Concentration ($\mu g\ mL^{-1}$)	Inhibition (%) of α-amylase enzyme (Mean±SD)				
	Omani	Indian	Yemeni	Tanzanian	Acarbose
10	15.43±5.2	10.33±4.5	29.33±4.6	4.43±2.71	25.50±2.5
40	24.17±8.4	11.10±1.8	31.43±5.8	23.71±3.2	53.20±5.8
80	29.10±6.8	37.84±5.6	38.06±9.5	29.67±1.2	65.40±3.8

The turmeric rhizome should contain not less than 5.0% w/w of the total curcuminoids as per the standards of ASEAN Herbal medicine while WHO recommendation is not less[17,18] than 4.0% w/w. The data presented in Table 1 indicates that the curcuminoids content in all turmeric samples is higher than the recommended levels by WHO and ASEAN herbal medicine. It was interesting to note that the Yemeni variety showed the maximum curcuminoids content though its curcumin content was lower than the Omani and Indian varieties. The variation in curcuminoids content is perhaps due to difference in climatic condition of each place[19]. The yield of curcuminoids in methanol extract was slightly better than the acetone extract except for the Indian variety.

The percentage inhibition of DPPH radical was observed to be directly proportional to the concentration of samples[20]. The results clearly indicated that the best activity was shown by Tanzanian variety at all concentrations. Our results are in agreement with the previous studies[21,22]. The antioxidant activity at 0.8 mg mL^{-1} was observed in the following order, Tanzanian>Yemeni>Indian>Omani. Though, the Tanzanian and Yemeni turmeric had the lower curcumin content yet they exhibited the better anti-oxidant activity in comparison to Indian and Omani variety which were rich in curcumin content. Thus, it could be inferred that the anti-oxidant activity of turmeric does not depend solely on curcumin content but it is related to its polyphenolic curcuminoids content.

The damage caused by oxidative stress can effectively be measured by using TBARS[23]. According to the results presented in Table 3, the percentage inhibition of lipid peroxides increased with increasing the concentration of the respective curcumin sample. Among the four turmeric samples, the maximum inhibition was shown by Tanzanian sample at the concentration of 100 $\mu g\ mL^{-1}$ followed by Omani, Indian and Yemeni varieties. The Yemeni variety exhibited the lowest anti-oxidant activity in TBARS assay. However, no correlation could be drawn between the curcumin content and inhibition (%) of lipid peroxides.

One of the therapeutic approaches to lower the elevated blood glucose level is to inhibit the breakdown of polysaccharides by inhibiting the carbohydrate hydrolysis caused by α-amylase enzyme[24]. The result of α-amylase inhibitory activity shows that the turmeric samples possess moderate anti-diabetic activity in comparison to standard drug acarbose. The highest percentage inhibition was shown by Yemeni variety (38.06%) while Omani variety exhibited lowest activity (29.1%) at the 80 $\mu g\ mL^{-1}$ in comparison to the acarbose which showed 65.4% inhibition at the same concentration.

CONCLUSION

The results of this comparative study performed on four turmeric varieties collected from four different countries revealed that curcumin content in turmeric varies greatly depending upon the geographical location and climatic conditions where they are grown. The methanolic extract of Omani variety contained the highest curcumin content while Yemeni variety was rich in curcuminoids. The findings of this study could be used by drug, food, color and cosmetic industries for selecting the source of turmeric for isolation of curcumin. The results of this study also confirmed that turmeric extract exhibit α-amylase inhibitory activity and possess useful anti-oxidant activity, thus it can be used along with hypoglycemic therapy for an effective management of blood glucose level in diabetic patients and in other chronic diseases.

SIGNIFICANCE STATEMENT

This study has demonstrated that the rhizome of *Curcuma longa* has the ability to improve the diabetic conditions by inhibiting α-amylase and via suppression of oxidative stress. This study will help the researcher to uncover the critical areas of diabetes that previous research studies were not able to explore.

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