Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 7, 2015

Original Article

VARIATION IN ANTIOXIDANT POTENTIAL OF *CURCUMA LONGA* L. COLLECTED FROM DIFFERENT ECOLOGICAL NICHES OF WESTERN HIMALAYAN REGION

NEELAM ARYA[a*], OM PRAKASH[a*], A. K. VERMA[b], VIVEKANAND[a], A. K. PANT[a]

[a]Department of Chemistry, [b]Department of Biochemistry, G. B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, 263145 India

Email: neeluchem@gmail.com

Received: 28 Jan 2015 Revised and Accepted: 08 May 2015

ABSTRACT

Objective: The present study aims to evaluate the nutritional content and antioxidant activities of twenty accessions of turmeric rhizome from various locations of Uttarakhand.

Methods: The antioxidant activity was evaluated by different method viz; DPPH radical scavenging activity, ferric reducing antioxidant power assay (FRAP), Hydroxyl radical scavenging activity, Nitric Oxide (NO) scavenging activity, Superoxide anion radical scavenging activity and nutritional value viz; total phenolic content, total flavonoid content, total ortho-dihydroxy phenols, total ascorbic acid content and total antioxidant capacity.

Results: Alcoholic extracts of different turmeric rhizomes showed wide variation in abilities to scavenge nitric oxide, hydroxyl, superoxide free radical, DPPH with IC_{50} values in the range of 39.03-76.14, 39.77-72.85, 43.77-77.13, 64.38-271.95 µg/ml and FRAP value 19.54-54.84 µmole. The total phenolic content, total flavonoid content, total ortho-dihydroxy phenols, total ascorbic acid content and total antioxidant capacity of different alcoholic extracts of rhizomes were found in the range of 11.19-51.49, 6.35-30.95, 0.93-8.12, 0.14-1.29 and 27.44-94.78 mg/g respectively.

Conclusion: The alcoholic extract of different accessions of *Curcuma longa* L. has shown excellent biochemical and antioxidant potential and therefore refers as an important natural source of antioxidant.

Keywords: Free radical scavenging, Total antioxidant capacity, Total phenolic, Total flavonoid, Total ascorbic acid.

INTRODUCTION

Plants contain a wide variety of free radicals scavenging molecules such as phenols, flavonids, vitamins, terpenoids that are rich in antioxidants, tend to be safer and possess antiviral, anti-inflammatory, anti-cancer, anti-tumour and hepato-protective properties [1,2]. Therefore, the evalution of antioxidant activity of various plant extracts is considered as an important step in the identification of their ability to scavenge the free radicals. Zingiberace family plants are well known for their antioxidant activity and diverse biological properties have been reported from rhizomes of these plants [3, 4].

The plant *Curcuma longa* L. commonly known as 'Turmeric' is a member of this family and is widely distributed throughout the tropics of Asia, Africa and Australia [5]. It is commonly used as an ingredient in various medicinal formulations [6]. It has also been considered as an analgesic in the treatment of menstrual disorder, rheumatism, and traumatic diseases due to a number of components, such as monoterpenoids, sesquiterpenoid, curcuminoids and turmerones respectively [7,8]. Curcumonoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin), the phenolic yellowish pigment of *Curcuma longa* L., have been suggested to have anti-microbial [9], anticarcinogenic [10], anti-inflammatory [11], hypocholesterolemic activities [12] and hepatoprotective [13]. There are several studies which state that these beneficial properties of *Curcuma longa* L. have been associated to the antioxidant activity [14-16]. The objective of present study was to estimate the biochemical and antioxidant activities of an alcoholic extract of turmeric rhizome collected from different altitudinal zones of hill and tarai regions of Uttarakhand, India. The study further analysed the variation in antioxidative activity in different climatic and geographical conditions along with the diversity on the basis of the correlation matrix by Principal Component Analysis. The antioxidant activity of alcoholic extract of *Curcuma longa* L. were evaluated by DPPH, FRAP, hydroxyl radical, nitric oxide radical, superoxide scavenging and also estimated the total phenolic, flavonoid, antioxidant capacity, ortho dihydroxy and ascorbic acid contents.

MATERIALS AND METHODS

Collection of plant material and extraction

Twenty accessions of rhizomes of *Curcuma longa* L. were collected from different altitudes viz. hill and tarai areas of Uttarakhand state (Garhwal and Kumaun region) in India in the month of October-November (table 1). Identification of plants was done by plant taxonomist, Department of Biological Sciences of G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand. The rhizomes were thoroughly washed under tap water and sliced. The air dried slices were powdered using blender. 1g of turmeric powder was mixed with 10 ml of methanol and kept for a weakness in an airtight test tube with intermediate shaking. After centrifugation the supernatant was collected and stored in deep freezer. Working solution of concentration 1 mg/ml was prepared from the stock solution for analysis.

Total phenolic content

The total phenolic content in extract of turmeric rhizomes was measured by Folin-Ciocalteu's reagent method [17]. Calibration curve was plotted by mixing 1 ml aliquots of 10, 20, 40, 60, 80, 100µg/ml of gallic acid solutions with 250 µl of Folin-Ciocalteu reagent and make up to 5 ml with distilled water.

After five min. 1 ml of saturated sodium carbonate and 1 ml of distilled water was added to it. The absorbance was measured after four hour incubation at 725 nm versus a prepared blank. 100 µl of extract was taken and make up to 1 ml with the same reagents as followed in construction of the calibration curve and the absorbance was measured. The content of total phenolic was expressed as mg gallic acid equivalent (GAE)/gm of fresh sample. All samples were analyzed in triplicate.

Total flavonoid content

The total flavonoid content was determined by the method of Choi [18]. 250 µl of extract was taken and mixed with 1.25 ml distilled waters and 75 µl of 5% sodium nitrite solution. After incubation of five min. add 150 µl of 10% aluminium chloride, 500 µl of 1M NaOH

and 275 µl of distilled water. Intensity of pink colour was measured at 510 nm. Standard curve was prepared from catechin stock solution at different concentrations and the amounts were calculated with the help of the calibration curve and expressed in mg catechin equivalent/gm of fresh sample. All samples were analyzed in triplicate.

Total ortho-dihydroxy phenols

Total ortho-dihydroxy phenols in an alcoholic extract of turmeric rhizomes was measured by Arnow's method [19]. 1 ml of extract from stock solution was mixed with 1 ml of 0.5N HCl and Arnow's reagent. After five min. 2 ml of 1N NaOH was added, mixed properly and absorbance was measured at 515 nm. The standard curve was prepared from Catechol (1 mg/ml) at different concentrations and the amount was calculated with the help of the calibration curve and expressed in mg catechol equivalent/gm of fresh sample. All samples were analyzed in triplicate.

Total antioxidant capacity

Total antioxidant capacity in extract of turmeric rhizomes was measured by Phosphomolybdanum method [20]. In this method, Mo (VI) is reduced to Mo (V) by the sample. In this assay, 100 µl of the extract is added in the eppendorf tube containing 1 ml of the reagent (0.6M sulphuric acid, 28 mM sodium phosphates and 4 mM ammonium molybdate) and heat at 95 ˚C for 90 minutes in the water. Its colour changes to green after cooling of the solutions and the absorbance was measured at 695 nm. The total antioxidant capacity was determined in terms of mg ascorbic acid equivalent/gm of fresh sample. All samples were analyzed in triplicate.

Total ascorbic acid content

Total ascorbic acid content was estimated according to the method of Roe and Keuther [21] with slight modification. In this method, 100 µl of extract was made up to 1 ml with distilled water. Different concentrations of ascorbic acid from 10-100µg/ml were used as the standard. Blank contain 1 ml of water. 0.5 ml of dinitro phenyl hydrazine (0.2%) was added to all the tubes, followed by 2 drops of 10% thiourea solution. The contents were mixed and incubated at 37 ˚C for three hours resulting in the formation of osazone crystals. The crystals were dissolved in cold 2.5 ml of 85% sulphuric acid. DNPH reagent and thiourea were also added after the addition of sulphuric acid. The tubes were cooled in ice and the absorbance was taken at 540 nm. The concentration of ascorbate in the samples was calculated and expressed in terms of mg ascorbate equivalent/gm of sample.

Ferric reducing antioxidant power assay (FRAP)

FRAP assay was performed according to the method described by Benzie and Strain [22]. FRAP reagent was prepared from acetate buffer of pH 3.6 (1.6 g sodium acetate and 8 ml acetic acid and makeup to 500 ml), 10 mM TPTZ solution in 40 mM HCl and 20 mM iron (III) chloride solution in proportion of 10:1:1 (v/v) respectively. First FRAP reagent was incubated at 37˚C in an oven for five min. then 200 µl of extract was added to 1.8 ml of FRAP reagent and mix well. The absorbance was measured at 593 nm after four min. and the samples were measured in three replicates. The standard curve of iron (II) sulphate (2000µM/ml) was prepared from different concentrations (50-500µg) by using the similar procedure and the result was expressed as µmol Fe (II)/g fresh sample.

2, 2-diphenyl-picryl hydrazyl (DPPH) radical Scavenging activity

The radical scavenging ability was determined as described by Braca [23] with slight modification. In this method, initially 0.002% of DPPH was prepared in alcohol and was kept in the dark place. Ascorbic acid (1 mg/gm) was used as standard and different concentrations of standard and plant extract was also prepared in alcohol. 1 ml of DPPH solution was mixed with 1 ml of sample solution and the standard solution separately. Incubate the samples for 30 min in dark at room temperature and recorded the absorbance at 217 nm. 1 ml of alcohol and DPPH solution was used as blank. The % inhibition was calculated using the following formula:

$$\text{Percent (\%) inhibition of DPPH activity} = [(A–B)/A] \times 100$$

Where A = absorbance of the control and B = absorbance of the sample.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of the extract was measured according to the method described by Halliwell [24]. 1 ml of the final reaction solution consisted of aliquots (500 µl) of various concentrations of the extract, 1 mM $FeCl_3$, 1 mM EDTA, 1 mM H_2O_2, 1 mM L-ascorbic acid, and 28 mM deoxyribose in phosphate buffer (pH 7.4). The reaction mixture was incubated for one hour at 37˚C, and further heated in boiling water bath for 20 min after adding 1 ml of 2.8% (w/v) trichloroacetic acid and 1 ml of 1% (w/w) thiobarbituric acid. The pink colour developed which was measured at 532 nm aganist a blank containing the phosphate buffer. Ascorbic acid was used as standard and % inhibition was calculated by the formula as mentioned earlier.

Table 1: List of collection site

Accessions No.	Collection site	District	Region	Altitude (feet)	Average Temp (˚C)	Average Humidity
1	Roorkee	Haridwar	Garhwal	879	24	35
2	Haridwar	Haridwar	Garhwal	1082	29	25
3	Herbertpur	Dehradun	Garhwal	1400	22	27
4	Kotdwar	Pauri Garhwal	Garhwal	1489	24	23
5	Dehradun	Dehradun	Garhwal	2155	24	40
6	Barswar	Pauri Garhwal	Garhwal	2647	19	28
7	Tehri	Tehri Garhwal	Garhwal	3061	20	26
8	Srinagar	Pauri Garhwal	Garhwal	3192	20	20
9	Joshimath	Chamoli	Garhwal	5157	16	23
10	Chamoli	Chamoli	Garhwal	5757	12	18
11	Khatima	Udham Singh Nagar	Kumaun	652	26	24
12	Pantnagar	Udham Singh Nagar	Kumaun	793	20	56
13	Ramnagar	Udham Singh Nagar	Kumaun	1131	14	68
14	Rudarpur	Udham Singh Nagar	Kumaun	1906	23	71
15	Dharchula	Pithoragarh	Kumaun	2982	11	36
16	Kapkot	Bageshwar	Kumaun	3418	8	43
17	Pithoragarh	Pithoragarh	Kumaun	4967	12	57
18	Almora	Almora	Kumaun	5416	10	61
19	Lohaghat	Champawat	Kumaun	5754	15	54
20	Munsyari	Pithoragarh	Kumaun	7200	2	53

Nitric Oxide (NO) scavenging activity

Nitric oxide scavenging activity of the extract was measured by the method with slight modification [25, 26]. In this method, the reaction mixture (3 ml) containing sodium nitropruside (10 mM) in phosphate buffer saline and the test extract (10-100µg/ml) was incubated at 25˚C for two hours. After incubation 1.5 ml of the reaction mixture was removed and 1.5 ml of the Griss reagent (1%

sulphanilamide in 2% orthophosphoric acid and 0.1% Napthylethyline diamine hydrochloride) was added. The absorbance of the chromophore formed was measured at 546 nm. Ascorbic acid was used as standard and % inhibition was calculated by the formula as mentioned in DPPH assay.

Superoxide anion radical scavenging activity

Superoxide anion radical scavenging activity was measured according to the method with slight modification [27]. In this method 1 ml of NitroBlue Tetrazolium (NBT) solution (156µM NBT in 100 mM phosphate buffer of pH 7.4), 1 ml of NADH solution (468µM in 100 mM phosphate buffer of pH 7.4) and 0.1 ml of sample solution of test extract (10-100µg) were mixed. The reaction started by adding 100 µl of Phenazine Metho Sulphate (PMS) solution (60µM PMS in 100 mM phosphate buffers of pH7.4). The reaction mixture was incubated at 25°C for five min, and the absorbance was measured at 560 nm against blank. Ascorbic acid was used as standard and the percentage of inhibition was calculated by the formula as mentioned in DPPH assay.

Statistical analysis

Means and standard errors of the means (SEM) of replicate scores were determined and subjected to Analysis Of Variance (ANOVA). Means was separated using the Duncan's New Multiple Rang Test. The relevant data were subjected to correlation and regression analysis. The significance level of correlation coefficient was checked on probability levels of $p \leq 0.05$ and $p \leq 0.01$.

The observations were recorded on10 biochemical and antioxidant activity to analyze the data by Principal Component Analysis through a software programme developed by IISR, New Delhi and non-hierarchical Euclidean cluster analysis was used for grouping all genotypes into clusters. All statistical analysis was carried out using Microsoft Excel Office (2003) and SPSS (version 16).

Table 2: Biochemical and IC 50 values of different antioxidant activities of 20 alcoholic extract of *Curcuma Longa* L.

Accession name	Phenolic (mg/gm)	Flavonoid (mg/gm)	Ortho dihydroxy phenol (mg/gm)	Total antioxi. Activity (mg/gm)	Ascorbic acid content (mg/gm)	FRAP (µmol Fe(II)/g)	DPPH (µg/ml)	OH-radical activity (µg/ml)	So-radical activity (µg/ml)	No-radical activity (µg/ml)
Haridwar	11.19±0.75^{a}	20.24±.69^{h,i}	0.93±0.08^{a}	54.56±1.18^{d}	0.73±0.01^{a}	20.42±0.31^{c}	271.95±1.56^{k}	54.45±0.46^{e}	63.58±0.24^{j}	44.99±0.27^{c}
Kotdwar	22.39±1.55^{b,c}	21.83±1.43^{i}	0.95±0.06^{a}	63.22±1.93^{f}	0.97±0.02^{c,d}	24.52±0.27^{a,b}	156.84±1.96^{h}	46.45±0.52^{c}	60.22±0.11^{h}	45.78±0.12^{d}
Herbatpur	40.30±1.55^{f,g}	12.70±1.05^{c,d}	1.38±0.05^{c}	35.33±1.20^{b}	0.56±0.01^{c,d,e}	51.54±0.38^{m}	146.79±2.03^{g}	58.40±0.49^{f}	65.30±0.33^{k}	63.89±0.07^{m}
Tahri	31.34±0.86^{d,e}	16.67±1.82^{d,e,f,g,h}	2.55±0.06^{f,g}	57.56±.40^{d,e}	0.36±0.01^{f}	49.43±0.27^{l}	99.31±2.89^{c,d}	66.37±0.68^{g}	71.45±0.45^{m}	62.05±0.19^{j}
Joshimath	38.81±0.43^{e,f}	13.89±1.73^{c,d,e,f}	1.13±0.06^{a,b}	48.11±2.48^{c}	0.49±0.02^{b,c,d}	25.75±0.30^{d}	136.58±1.97^{f}	44.20±0.67^{b}	75.33±0.23^{o}	74.11±0.38^{p}
Bharsar	32.09±1.14^{d,e}	16.27±.79^{c,d,e,f,g,h}	2.67±0.06^{g}	53.33±.58^{d}	1.29±0.01^{e}	20.51±0.27^{b}	172.03±2.91^{i}	66.31±0.50^{g}	72.65±0.14^{n}	67.57±0.19^{o}
Chamoli	48.51±1.97^{g,h}	27.38±1.19^{j}	8.12±0.06^{m}	79.33±2.03^{g}	0.84±0.03^{g}	28.17±0.27^{e}	131.59±4.04^{f}	54.56±0.03^{e}	51.64±0.53^{c,d}	39.79±0.12^{a}
Dharadun	20.90±0.75^{b,c}	7.54±1.73^{a,b}	2.17±0.06^{d}	44.33±1.76^{c}	0.28±0.01^{h,i}	43.14±0.45^{k}	172.98±2.61^{i}	70.13±0.66^{h}	58.29±0.55^{f}	76.14±0.23^{q}
Shrinagar	34.33±1.29^{f,g,h}	18.25±2.10^{f,g,h,i}	4.71±0.04^{k}	58.22±1.64^{d,e}	1.17±0.01^{a,b}	28.08±0.36^{e}	110.57±1.51^{e}	72.22±0.87^{h}	67.34±0.20^{l}	65.32±0.34^{n}
Roorkee	17.16±1.88^{a,b}	7.14±1.19^{a,b}	3.31±0.06^{j}	27.44±1.44^{a}	0.14±0.02^{i,j}	19.76±0.12^{a,b}	64.38±1.89^{j}	72.85±1.43^{i}	63.56±0.34^{i,j}	59.26±0.11^{k}
Dharchula	42.54±1.29^{f,g}	13.49±0.79^{c,d,e,f}	1.21±0.08^{b,c}	58.33±1.84^{d,e}	0.60±0.01^{j,k}	37.37±0.27^{j}	168.32±2.09^{j}	53.97±0.54^{e}	77.13±0.21^{p}	52.10±0.18^{j}
Munsiyari	25.37±1.14^{b,c,d}	13.10±2.06^{c,d,e}	2.26±0.09^{d,e}	94.78±1.97^{h}	1.11±0.01^{m}	52.03±0.30^{m}	103.20±3.30^{i}	44.20±0.76^{c}	62.64±0.31^{i}	49.27±1.03^{h}
Pithoragarh	51.49±0.75^{h}	19.05±0.69^{g,h,i}	4.05±0.09^{j}	60.89±1.09^{e,f}	0.57±0.02^{g,h}	41.90±0.23^{j}	173.85±1.90^{d,e}	47.29±0.60^{i}	46.73±0.19^{b}	48.82±0.09^{g,h}
Lohaghat	41.04±1.29^{f,g}	11.51±1.43^{b,c}	2.71±0.08^{g}	63.56±0.48^{f}	0.23±0.01^{l,m}	33.10±0.16^{h}	90.27±2.56^{i,j}	67.50±0.57^{g}	44.46±0.44^{a}	46.71±0.03^{e}
Almora	43.28±1.14^{f,g,h}	17.86±1.37^{e,f,g,h,i}	3.05±0.06^{h}	48.00±0.38^{c}	0.92±0.01^{f,g}	25.88±0.20^{d}	167.20±2.61^{b}	58.40±0.48^{f}	53.38±0.11^{e}	48.02±0.16^{f,g}
Pantnagar	27.61±0.75^{c,d}	20.24±2.06^{h,i}	5.57±0.07^{l}	64.78±0.59^{f}	0.21±0.02^{m,n}	30.77±0.23^{f}	65.93±5.29^{a}	42.55±0.68^{b}	47.49±0.34^{b}	41.85±0.17^{b}
Rudrapur	11.94±0.43^{a}	14.68±1.73^{c,d,e,f,g}	0.98±0.10^{a}	57.00±1.20^{d,e}	0.55±0.05^{k,l}	54.85±0.31^{n}	146.47±2.79^{g}	58.93±1.10^{f}	52.30±0.08^{d}	47.60±0.08^{e,f}
Kapkot	47.76±0.43^{g,h}	30.95±1.82^{j}	2.04±0.04^{e,f}	75.22±1.94^{g}	0.31±0.01^{b,c,d}	30.28±0.16^{f}	92.29±4.47^{b,c}	50.99±0.05^{b}	43.77±0.16^{a}	39.03±0.27^{a}
Ramnagar	21.39±1.14^{b,c}	21.03±1.05^{h,i}	2.55±0.09^{f,g}	55.67±0.77^{d}	0.45±0.03^{n}	31.91±0.36^{g}	167.37±.69^{i}	39.77±1.10^{a}	50.93±0.40^{c}	43.55±0.35^{c}
Khatima	12.69±1.14^{a}	6.35±1.05^{a}	4.12±0.06^{j}	65.67±2.71^{f}	0.16±0.03^{e}	19.54±0.30^{a}	140.59±.69^{f,g}	58.82±1.49^{f}	58.78±0.39^{g}	50.77±0.43^{i}

Superscriptlettersa,b,c,d,e,f,g,h,j,k,l,m,n,o,p,andqintherowindicatesignificantdifferenceatp < °.[05]. Valuesofexpressedasmean±standarderror(n=3)

Table 3: Correlation matrix (r) between biochemical, antioxidant activity of extract, altitude, temperature and humidity

Activity No.	Altitude 1	Temp 2	Humidity 3	Phenolics 4	Flavonoids 5	ODH phenols 6	TAA 7	Ascorbic acid 8	FRAP 9	DPPH 10	OH· activity 11	SO· Activity 12	NO· activity 13
1	1.00	0.811**	0.088	0.604**	0.200	0.139	0.501*	0.386	0.166	-0.004	-0.128	-0.078	-0.098
2		1.00	-0.272	0.629**	-0.418	-0.091	0.606**	-0.326	-0.171	0.041	0.404	0.158	0.378
3			1.00	-0.118	0.048	-0.145	0.097	-0.166	0.351	0.036	-0.291	-0.595**	-0.438
4				1.00	0.512*	0.237	0.162	0.201	-0.009	0.082	-0.132	-0.107	-0.135
5					1.00	0.323	0.463*	0.282	-0.103	-0.035	-0.455	-0.394	-0.567*
6						1.00	0.309	0.026	-0.327	-0.288	0.053	-0.364	-0.333
7							1.00	0.280	0.135	-0.159	-0.455	-0.309	-0.579*
8								1.00	-0.038	0.362	-0.036	0.327	0.082
9									1.00	0.073	-0.069	-0.020	0.052
10										1.00	-0.142	0.179	0.195
11											1.00	0.238	0.496*
12												1.00	0.703**
13													1.00

RESULTS AND DISCUSSION

Phenols, flavonoids and ortho dihydroxy phenols are known as nutritional secondary metabolites. In recent studies, it was observed that the local geoclimate, seasonal changes, external conditions such as light, temperature, humidity affect the composition of secondary metabolites. When plants are stressed, an exchange occurs between carbon to biomass production or formation of defensive secondary

compounds [28]**.** In present study nutritional profiling of all the 20 accessions of turmeric rhizomes were carried out by spectrophotometric analysis and results are shown in table 2.

The total phenolic content in extract of *Curcuma longa* varied from 11.19±0.75 to 51.49±0.76 mg gallic acid equivalent/g. The maximum phenolic content was found in the sample collected from Pithoragarh and minimum was found in the sample from Haridwar. The total flavonoid content varied from 6.35±1.05 to 30.95±1.82 mg chatechin equivalent/g, with maximum in the sample collected from Kapkot (Bageshwar) and minimum in the sample collected from Khatima. The amount of total Orthodihydroxy phenol (ODP) was determined with the catechol reagent. The experimental results showed the variation in the quantity of ODP among the alcoholic extracts of different *Curcuma long,* and varied from 0.93±0.08 to 8.12±0.06 mg catechol equivalent/g. The maximum ODP content was found in the sample collected from Chamoli and minimum in the sample collected from Haridwar.

Table 4: eigen vector, eigen root and associated variation for principal component in turmeric rhizome based on biochemical and antioxidant activity

S. No.	Characters	Eigen vector									
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	Total phenolic content	0.227	0.441	0.290	0.416	0.046	-0.023	-0.101	-0.331	-0.390	-0.472
2	Total flavonoid content	-0.012	0.243	-0.322	0.122	0.563	-0.055	0.567	-0.283	0.318	0.003
3	Ortho dihydroxy phenol	0.495	0.139	0.450	0.004	0.415	-0.232	-0.196	0.353	0.253	0.285
4	Total Antioxydant activity	0.324	-0.067	-0.206	0.245	0.083	0.811	-0.255	-0.105	0.114	0.186
5	Total Ascorbic acid content	-0.612	-0.273	0.222	0.546	0.320	0.016	-0.234	0.120	0.163	-0.070
6	FRAP	0.065	-0.023	-0.118	0.071	-0.221	-0.370	-0.446	-0.601	0.460	0.133
7	DPPH scavenging activity	0.121	-0.441	0.575	0.088	-0.166	0.104	0.478	-0.376	0.011	0.209
8	OH^-scavenging activity	-0.438	0.487	0.372	-0.453	0.095	0.310	-0.123	-0.238	0.053	0.218
9	SO^-scavenging activity	0.047	-0.403	-0.100	-0.313	0.551	-0.102	-0.253	-0.317	-0.496	0.053
10	NO^-scavenging activity	0.105	0.232	0.158	-0.372	0.104	0.174	-0.050	0.001	0.428	-0.738
11	Eigen root	3.136	1.728	1.375	1.206	0.822	0.624	0.399	0.292	0.233	0.185
12	Percent variation	31.36	17.28	13.75	12.06	8.22	6.24	3.99	2.92	2.33	1.85

Table 5: Non hierarchical cluster of 20 turmeric genotypes

Cluster number	Number of genotypes	Entry name
I	2	Dharadun, Roorkee
II	3	Pithoragarh, Pantnagar, Kapkot
III	4	Herbatpur, Tahri, Joshimath, Dharchula
IV	5	Haridwar, Kotdwar, Munsiyari, Rudrapur, Ramnagar
V	2	Lohaghat, Khatima
VI	1	Chamoli
VII	3	Bharsar, Shrinagar, Almora

Table 6: Average distance of intra and inter-cluster centroids

	I	II	III	IV	V	VI	VII
I	(1.666)						
II	5.232	(1.844)					
III	3.102	4.033	(1.563)				
IV	4.422	3.197	3.049	(2.379)			
V	2.971	3.245	3.474	3.265	(1.666)		
VI	6.729	3.037	5.713	4.852	4.855	(0.000)	
VII	3.775	4.081	2.754	3.258	3.611	4.361	(1.650)

The intra cluster distances are shown in parenthesis.

Antioxidants are the compounds responsible for the reduction of oxidative degradation by scavenging free radicals, per-oxide radicals, metal chelators and metal reducing agents. In present study, the Total Antioxidant Capacity (TAC) of all the twenty accessions of turmeric rhizomes were estimated by phosphomolybednum method and result revealed that the values of TAC varied from 27.44±1.44 to 94.78±1.97 mg ascorbic acid equivalent/g. The maximum value was found in the sample collected from Munsiyari whereas sample collected from Roorkee showed the minimum amount of total antioxidant capacity. The antioxidant power was also determined by FRAP assay (Ferric Reducing Antioxidant Power Assay). The FRAP values of all the extracts were calculated. The results varied from 19.54±0.30 to 54.84±0.31 µmole$FeSO_4$equvalient/g. Among all the extracts, the sample collected from Rudrapur (Udhamsingh nagar) showed the highest FRAP value while the sample collected from Khatima showed the lowest FRAP value. Ascorbic acid content of *Curcuma longa* L. rhizomes in different collections varied from 0.14±0.02 to 1.29±0.01 mg/g. Maximum ascorbic acid content was found in the sample collected from Bharsar whereas sample collected from Roorkee showed minimum amount of ascorbic acid content. A significant variation was observed in Ascorbic acid content in *Curcuma longa* L. rhizome.

DPPH has been widely used to evaluate the free radical scavenging potential of various antioxidant substances. Results from present study illustrated a significant decrease in the concentration of DPPH radical due to the scavenging ability of extract of different samples of *Curcuma longa* and the reference compounds (EDTA and Ascorbic acid). Percent DPPH radical scavenging was expressed as IC_{50} values of the extracts i.e., the inhibitory concentration which causes 50% inhibition of DPPH radicals. The IC_{50} values of EDTA and Ascorbic acid were observed to be 28.95±2.25 and 31.03±2.07 while in different samples varied from 64.38±1.89 to 271.95±1.56µg/ml (table 2). It was observed that DPPH free radical scavenging activity of extracts and standard increased with increasing of concentration and lower IC_{50} value indicated a higher DPPH free radical scavenging activity. The highest free radical scavenging activity was found in the sample collected form Roorkee and lowest free radical scavenging activity was found in the sample collected from Haridwar.

Hydroxy radical scavenging by the reference compound (Ascorbic acid and Qurecitin) and extract of different turmeric rhizome samples were quantified by measuring the inhibition of the degradation of 2-deoxyribose by hydroxyl radical and expressed as IC_{50} values of the extract and reference compound. The IC_{50} values in Ascorbic acid and Qurecitin were found to be 34.48±1.26 and 27.63±0.72µg/ml and in different turmeric samples varied from 39.77±1.10 to 72.85±1.43µg/ml (table 2). The observations showed that OH free radical scavenging activity of extracts and standard was increased with increasing of concentration. The highest free radical scavenging activity was found in the sample collected form Ramnagar and lowest was found in the sample collected from Roorkee.

In superoxide radical scavenging activity the extracts of different *Curcuma longa* rhizome and reference compound were determined by the PMS-NADH superoxide generating system and the results were expressed as IC_{50} values of the extracts and reference compound. The IC_{50} value of Ascorbic acid and Qurecitin was found to be 25.78±0.85 and 30.15±0.37µg/ml and in different samples ranged from 43.77±0.16µg/ml in Kapkot to 77.13±0.21µg/ml in Dharchula (table 2). Result showed that superoxide radical scavenging activity increases with increasing the concentration of extract and standard and the lower IC_{50} value indicated a higher radical scavenging activity.

All extracts of *Curcuma longa* and reference compound had a significant ($P<0.05$) dose-related effect on the scavenging of Nitric oxide radical. The IC50 value in ascorbic acid and Qurecitin was found to be 17.83±0.25 and 15.66±0.61µg/ml and in different samples ranged 39.03±0.27µg/ml in Kapkot to 76.14±0.23µg/ml in Dehradun. Result showed that nitric oxide radical scavenging activity increases with increase of concentration of extract and standard and lower IC_{50} value indicated higher NO radical scavenging activity.

In this study, we also observed the effect of altitude, temperature and humidity on the different antioxidant activities of all twenty accessions of turmeric rhizome. All the statistically significant correlation coefficients were given in table 3. Result showed that total phenolic contents and total antioxidant activity were positively correlated with altitude and temperature at the level of 0.01. Superoxide free radical scavenging activity was negatively correlated with humidity at the level of 0.01. Total phenolic content was positively correlated with total flavonoids contents and total flavonoids content was positively correlated with total antioxidant content at the level of 0.05 and negatively correlated with nitricoxide free redical scavenging activity. Total antioxidant assay showed negative correlation with nitricoxide free redical scavenging activity at the level of 0.05. Hydroxyl and superoxide free radical scavenging activity were positively correlated with nitric oxide free radical scavenging activity at the level of 0.05 and 0.01.

Several reports revealed that stimulation of polyphenol synthesis and accumulation generally varies in responses to biotic and abiotic stresses [29]**.** Lower temperature favours anthocyanin accumulation but reduces cell growth. If we take an example of strawberry cell culture, maximum anthocyanin content was obtained at 15° C and it was about 13-fold higher than obtained at 30° C [30]**.** In another study, deficiencies in nitrogen, phosphate, potassium, sulphur, magnesium and iron level were also reported to increase phenolic concentration from roots [31]**.**

Non-hierarchical euclidean cluster analysis

Non-hierarchical cluster analysis based on Principal Component Analysis [32] was found to be more useful measure for estimating the variability in germplasm under study. For the Principal component analysis each genotype was identified on the basis of correlation matrix as a single point in a standardized multidimensional space [33]**.** The principal component analysis of 20 turmeric accessions based on correlation matrix of antioxidant activity and bioactive compounds yielded the 10 eigen roots (eigen values) and eigen vectors. These values and associated percentage of variation explained by eigen root have been presented in table 4. First 8 components explained 95.82 percent variation. The maximum variation of 31.36 percent was explained first by latent vector followed by 17.28 percent second vector and so on. In the present study 20 accessions of turmeric rhizomes were grouped into seven non-overlapping clusters (table 5) determined by 'F' Test. The Intra Cluster Distance (D) ranged from 2.379 to 0.00 (table 6). The maximum Intra cluster distance was noted in cluster IV (2.379). This cluster accommodated 5 accessions and was considered to be the biggest one. The second biggest cluster was III having 4 accessions respectively. The maximum Inter cluster Distance (6.0729) was found between cluster I and VI, whereas minimum distance (2.754) was found between cluster III and VII. The grouping of accessions in clusters reflects the relative divergence of clusters and allows a convenient selection group of accessions with their overall phenotypic similarity for hybridization programme facilitating better exploitation of germplasm.

The accessions present in cluster VI recorded maximum value of total phenolic content (48.51 mg/gm), total flavonoid content (27.38 mg/gm), total ortho-dihydroxy phenols (8.12 mg/gm) and total antioxidant capacity (79.33 mg/gm). The accessions in VII cluster recorded maximum total ascorbic acid content (1.13 mg/gm). Similarly cluster III recorded maximum value of ferric reducing antioxidant power assay (FRAP) (41.02 µmol Fe (II)/g) and Superoxide anion radical scavenging activity (72.30 µg/ml). The accessions in cluster IV recorded maximum value of DPPH activity (169.17µg/ml). The accessions in cluster I recorded maximum value of Hydroxyl radical scavenging activity (71.49µg/ml) and Nitric Oxide (NO) scavenging activity (67.70µg/ml).

CONCLUSION

The alcoholic extract of *Curcuma longa* L. has shown excellent biochemical and antioxidant potential and therefore refers as an important natural source of antioxidant. In present study twenty turmeric accessions were analysed for total antioxidant activity, total phenolic content, total flavonoid content, total ortho-dihydroxy phenol, total ascorbic acid content and their different radical scavenging activity. We correlate the antioxidant potential with the geographical and climatic conditions such as altitude, temperature and humidity and revealed that the occurrence of bioactive compounds can be affected by these conditions and hence may affect the antioxidant properties of turmeric.

ACKNOWLEDGEMENT

The authors are grateful to Dr. D. S. Rawat, Assistant Professor and Plant Taxonomist, Department of Biological sciences of G. B. Pant University of Agriculture and Technology, Pantnagar for the identification of plant specimens and the first author is also thankful to the University Grants Commission (UGC) for providing financial assistance.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Cai YZ, Sun M, Corke H. Antioxidant activity of betaline from plants of the *Amaranthacea*. J Agric Food Chem 2003;51:2288-94.
2. Lim YY, Murtijaya J. Antioxidant properties of Phyllanthus amarus as affected by different drying methods. lebensmittle wissenschaft. Technol 2007;40:1664-9.
3. Keawpradub N, Zaeoung S. Cytotoxic and free radical scavenging activities of Zingiberaceous rhizomes. Songklanakarin J Sci Technol 2005;27:799-812.
4. Stoilova I, Krastanov A, Stoyanova A, Denev P, Gargova S. Antioxidant activity of ginger extract. Food Chem 2007;102:764-70.
5. Ravindran PN. Turmeric the golden spice of life. Turmeric the genus Curcuma. Taylor Francies 2006;14(1):1-14.
6. Aggarwal BB, Sundaram C, Malani N, Ichikawa H. Curcumin; the Indian solid golid. Adv Exp Med BIol 2007;595:1-75.
7. Tang W, Eisenbrand G. Chinese drugs of plant origin. Springer Verlag, Berlin, Heidelberg. Germany 1992;613-9.
8. Lee H-S, Shin WK, Song C, Cho KY, Ahn YJ. Insecticidal activities of ar-turmerone identified *Curcuma longa* L. rhizome against *Nilaparvata lugens* (Homoptera: Delphacidae) and *Plutella*

xyostella (Lepiodoptera: Yponomeutidae). J Asia-Pac Entomol 2001;4(2):181-5.

9. Wang Y, Lu Z, Wu H, Lv F. Study on the antibiotic activity of microcapsule curcumin aganist foodborn pathogens. Int J Food Microbiol 2009;136:71-4.
10. Kuttan R, Bhanumathly P, Nirmala K, George MC. Potential anticancer activity of turmeric (*Curcuma longa*). Cancer Lett 1985;29(2):197-202.
11. Wang X, Jiang Y, Wang YW, Huang MT, Ho CT, Hauang Q. Enhancing anti-inflammation activity of curcumin through O/W nanoemulsions. Food Chem 2008;108:419-24.
12. Ammon HPI, Wahl MA. Pharmacology of *Curcuma longa* L. Plant Med 1991;57:1-7.
13. Kiso Y, Suzuki H, Watanabe N, Oshima Y, Hikino O. Antihepatotoxic principles of *Curcuma longa* rhizomes. Plant Med 1983;49:185-7.
14. Devasagayam TPA, Sainis KB. Immune system and antioxidants especially those derive from Indian medicinal plants. Indian J Exp Biol 2002;40:639-55.
15. Jayaprakasha GK, Rao LJ, Sakariah KK. Antioxidant activities of curcumin, demethoxycurcumin and bisdemethoxycurcumin. Food Chem 2006;98:720-4.
16. Maheshwari RK, Shing AK, Gaddipati J, Srimal RC. Multiple biological activities of curcumin; A short review. Life Sci 2006;78:2081-7.
17. Singlenton UL, Rossi JA. Colorimetry of total phenolics with phosphomolibdic phosphotungstic acid reagent. Am J Enol Vitic 1965;16:144-58.
18. Choi Y, Jeong H, Lee J. Antioxidant activity of methanolic extracts from some grains consumed in Korea. Food Chem 2007;130:130-8.
19. Arnow LE. Colorimetric determination of the components of 3,4-dihydroxyphenylalanin-tyrosine mixtures. J Biochem 1937;118:531-7.
20. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem 1999;269:337-41.
21. Reo JH, Kuether CA. Determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenylhydrazine derivate of ascorbic acid. J Biochem 1943;143:399-406.
22. Benzie IFF, Strain JJ. The ferric reducing ability of plasma as a measure of antioxidant power; the FRAP assay. Anal Biochem 1996;239:70-6.
23. Braca A, Sortino C, Politi M. Antioxidant activity of flavonoids from Licania licaniaeflora. J Ethnopharmacol 2002;79:379-81.
24. Halliwell B, Gutteridge JMC, Aruoma OI. The deoxyribose method; a simple "test-tube" assay for determination of rate constants for reaction of hydroxyl radicals. Anal Biochem 1987;165:216-9.
25. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JK, Tannenbaum SR. Analysis of nitrate, nitrite and 15N in biological fluids. Anal Biochem 1982;126:131–6.
26. Marcocci L, Maguire JJ, Droy-Lefaix MT, Packer L. The nitric oxide scavenging property of Ginkgo biloba extract Egb 761. Biochem Biophys Res Commun 1994;201:748-55.
27. Nishimiki M, Rao NA, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulphate and molecular oxygen. Biochem Biophys Res Commun 1972;46:849-53.
28. Bryant JP, Chapin FSI, Klein DR. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. Oikos 1983;40:357-68.
29. Dixon RA, Paiva NL. Stress-induced phenyepropanoid metabolism. Plant Cell 1995;7(7):1085-97.
30. Zhang W, Seki M, Furusaki S. Effect of temperature and its shift on growth and anthocyanin production in suspension cultures of strawberry cells. Plant Sci 1997;127:207-14.
31. Chalker-Scott L, Fnchigami LH. The role of phenolic compounds in plant stress responses; low temperature stress physiology in crops (paul HL. Ed. CRC press: Inc, Boca Raton, Florida Entomologist; 1989. p. 40.
32. Hotelling H. Analysis of a complex of statistical variables into principal components. J Educational Psychology 1933;24:417-41.
33. Lohani M, Singh D, Singh JP. Genetic diversity assessment through principal component analysis in potato. Veg Sci 2012;39(2):207-9.