Original Article

Investigation of Bioactivities and Physicochemical Properties of Four Different *Curcuma* Species Available in Sri Lanka

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Abstract

To enhance the value of the unexplored medicinal herbs in Sri Lanka, this study was focused on the physicochemical analysis and investigation of biological activities of four different *Curcuma* species abundantly dispersed in Sri Lanka namely, *Curcuma albiflora* Thwaites, *Curcuma aromatica* Salisb., *Curcuma longa* L. and *Curcuma zedoaria* Roxb. Many species in genus *Curcuma* were rich with phenolic compounds. The total phenolic content (TPC) of dried rhizomes of *Curcuma longa* was the highest of all (5.530 g of GA equivalents/1 g of dried rhizome). The average antioxidant properties in the DPPH test was reported in MeOH extracts of the rhizome and leaf samples of four species. Further the MeOH extracts exhibited the inhibitory activities toward -amylase enzyme with IC_{50} values ranging from 6.455 µg/ml to 307.475 µg/ml. The present study shows that Sri Lankan *Curcuma* species have a potency to be used as antioxidant and antidiabetic agents. This information particularly physicochemical constants such as moisture content, total ash, acid insoluble ash and water-soluble ash values will be helpful in standardization of quality, purity and authentication of these medicinal plants for preventing its raw materials adulteration.

Keywords: Antioxidant, Antidiabetic, Biological Activities, *Curcuma*, Physicochemical

Introduction

Use of medicinal plant products is gradually increasing in all over the world. Currently, thousands of plant metabolites are being successfully used in treating different diseases [1]. According to the estimates of World Health Organization, 80% of the world's population rely on plants as sources of drugs and 35% of drugs contain natural products in many countries. The genus *Curcuma* are annual and perennial rhizomatous herbs [2]. This herb is widely grown in Asia, Western Africa, Australia and South America like tropical regions of the world [3]. The exact number of *Curcuma* species available in the world is still debatable [4]. However out of 70 identified species, about 40 species are disclosed from India [5].

Curcuma species are used in different countries over the world as ingredients for food additives & preservatives, medicines, cosmetics as well as ornamentals [6]. *Curcuma* rhizome in some species are used to prepare yellow dyes [1]. Chest pain, spleen, enlarged liver, stomach ulcer, cough, hepatic disorders, skin diseases, diabetes, boils, blood purifier are some examples for the diseases controlled by medicines prepared from different parts of the *Curcuma* plants [7]. Different parts of these plant species have been considered as a basis of carbohydrates, starch, proteins, vitamins and minerals, fats [8]. Curcumin is identified as the most biologically active compound in *Curcuma* species [4]. *Curcuma* species are well known for many biological activities i.e; antiviral [9]; antihepatotoxic [10]; anti-inflammatory [11]; antirheumatic [12]; antifibrotic [8]; hypocholestraemic [13]; antimicrobial [10]; antidiabetic [14]; anticancerous [15]; antinociceptive [16] and gastroprotective properties [14].

Even *Curcuma* plants have different chemical compounds and medicinal uses, different regions have used various plant species under the same common name. For instance, in literature, *Curcuma albiflora* Thwaites, *Zingiber zerumbet* (L.) Roscoe ex Sm. and *Curcuma*

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zedoaria Roxb. are reported as 'Harankaha' in Sinhala although they are 3 different species [17].

Therefore, this study was aimed to investigate the physicochemical properties and different bioactivities of four *Curcuma* species abundant in Sri Lanka; *Curcuma albiflora*, *Curcuma aromatica* Salisb., *Curcuma longa* L. and *Curcuma zedoaria*. The present study will provide important information on differences and similarities of *Curcuma* plants grown in Sri Lanka by their physicochemical composition as well as in biological activities.

Material and Methods

General

Chemicals used for the experiments were purchased from Sigma Aldrich, unless otherwise stated. Samples were grinded by high speed universal disintegrator (Model HSD-80). Plant materials were extracted by an Ultrasonic Sonicator (BIOBASE model – UC-10A).

Sample Collection and Extraction

Fresh rhizomes and leaves of four *Curcuma* species were collected from dry and intermediate zones of Sri Lanka, *Curcuma albiflora* (Koslanda, Badulla district- IM2a), *Curcuma aromatica* (Miriswaththe, Badulla District-IM2a), *Curcuma longa* (Mihinthale, Anuradhapura district-DL1b) and *Curcuma zedoaria* (Miriswaththe, Badulla District- IM2a). Specimens of each species were authenticated by the National Herbarium, Peradeniya, Sri Lanka [Voucher numbers; *Curcuma albiflora*, PEK-CAL-26022020(UWU); *Curcuma aromatica* PEK-CAR-26022020(UWU); *Curcuma longa,* PEK-CL-26022020(UWU) and *Curcuma zedoaria*, PEK-CZ-26022020(UWU)].

Fresh rhizomes and leaves (250g) were cleaned and cut in to small pieces separately. Rhizomes were oven dried at 45 °C for 12 hours. Leaves were oven dried at 40 °C for 12 hours. Dried samples were grinded into a fine powder. Powders were stored in sealed containers for physicochemical analysis. 10g of fine powder was extracted with MeOH. MeOH was evaporated by HS-2005V type rotary evaporator with 40 °C of inner bulb temperature to obtain the plant extract. MeOH extracts of rhizomes and leaves were screened for total phenolic content, antioxidant activity and -amylase inhibition activities.

Analysis of Biological Activities

Total Phenolic Content (TPC)

Slightly modified Folin-Ciocalteu reagent method was used to analyse the TPC [18]. Series of Gallic acid (GA) was used to prepare a standard curve (5-500 µg/mL). A 100 µL of GA samples in the series were mixed with 100 µL of distilled water and 500 µL of 10 times diluted Folin-Ciocalteu reagent. After few minutes, 800 µL of Na_2CO_3 solution (7.5 %) was added. Then it was mixed well by a vortex meter and kept for 1 hr at room temperature. The absorbance was measured at the wavelength of 765 nm against the blank i.e distilled water. Test solution was prepared from extracts of dried rhizomes and leaves separately. The phenolic content was obtained from the standard curve as a mass of phenolics (g) GA equivalents (GAE) per 1g of rhizome/ leaves.

Antioxidant Activity

DPPH radical scavenging activity test was used for the experiment. The sample extracts and an ascorbic acid series was tested by 1,1-diphenyl-2-picrylhydrazyl (DPPH) test with slight modifications [19]. 0.75 mL of above solutions were mixed with 0.3 mL of DPPH solution. The mixture was kept in dark at 25 °C for 30 min. The absorbance was measured by micro plate reader at wavelength of 518 nm. The blank was prepared by mixing 0.3 mL MeOH and 0.75 mL of plant extract. The positive control was ascorbic acid. The reaction control was prepared by mixing 0.3 mL DPPH and 0.75 mL MeOH. Percentage of inhibition and IC_{50} values were calculated. The formula applied to calculate the inhibition percentage as follows.

$$\text{Inhibition Percentage} = \frac{Abs_{negative\ control} - Abs_{sample}}{A_{negative\ control}} \times 100$$

IC_{50} values were obtained by the curves plotted against the inhibition percentage and concentration.

-Amylase Inhibition Assay

96 mM of 3,5-dinitrosalicylic acid was used to prepare the dinitrosalicylic acid (DNSA) reagent. DNSA reagent was dissolved in 20 mL of deionized water. Sodium potassium tartrate (5.31 M) was dissolved in 8 mL of 2 M NaOH solution which is dissolved in 12 mL of deionized water [20]. Type 1-A, porcine pancreas, 20 mg protein/mL: 1184 U/mg of -Amylase was dissolved in phosphate buffer containing NaCl. The pH value of the phosphate buffer should be 6.9. Extracts were dissolved in deionized water and 1% dimethylsulfoxide (DMSO). 8 U/ mL -amylase was added to 100 µL of plant extract and kept for 30 min at 25 °C. Then, above mixture was added to a 100 µL of 0.5% starch solution. It was kept in water bath for 10 min at 37 °C. Finally, DNSA reagent (100 µL) was mixed. Then it was kept in a water bath for 15 min at 85 °C. After cooling the mixture was diluted with 900 µL of distilled water. In the same procedure negative controls were prepared by adding plant extracts with DMSO. DNSA reagent was added to the mixture before adding starch solution in preparation of the blanks and thereby the enzyme was denatured. Absorbances

were measured at the wavelength of 540 nm and dose response curves were used to analyse IC_{50} values. Acarbose standard was the positive control for the test.

Analysis of Physicochemical Properties

Determination of Weight Loss on Drying

The test was carried out for well mixed samples. 1g of rhizome and leaf powder of each species were transferred separately into petri dishes by distributing evenly. The depth was not exceeded 10 mm. Heated plates at 105 °C in hot air oven for 1 hr and then cooled in desiccators. Difference in weight was recorded as moisture content. As a percentage, moisture content was calculated separately.

Determination of Total Ash Value

The total ash was determined by incinerating 2 g of powdered samples in a porcelain crucible. The crucible was ignited and cooled before taking the weights. The samples were incinerated gradually by increasing the heat in a muffle furnace at 550 °C for 3 hrs. Ignition was done until a constant weight was observed. Then it was cooled in a desiccator. Then the percentages of the total ash values were calculated with reference to dried powdered samples.

Acid – Insoluble Ash

The ash in the crucible was washed by 25 ml of 2 N HCl. It was then boiled 5 min and filtered through a Whatman No: 42 ash less filter paper. The residue was washed twice with hot water. Then it was ignited to ash and cooled in desiccators. The remained ash sample was weighed and the acid insoluble ash of the sample was calculated with reference to the dried powder samples.

Water – Soluble Ash

The ash was washed in to a beaker using 25 ml water. It was boiled for 5 min and filtered through ash less filter paper (Whatmann No: 42). The residue was washed twice with hot water. Finally, it was ignited to ash and cooled. The weight of insoluble matter was subtracted from the weight of ash to obtain the water-soluble ash content. The percentage of water-soluble ash was calculated with reference to dried powder.

Statistical Analysis

Three replicates were used in all the experiments in this study. The findings were expressed as means along with Standard Deviation (SD). SD were calculated by Microsoft Excel 2019. Data were analysed by analysis of variance (ANOVA). Means were separated by Tukey pairwise comparison (P<0.05) with Minitab 17 statistical software.

Results and Discussion

The orange to reddish brown MeOH rhizome extracts and greenish MeOH leaf extracts from the *Curcuma* species showed positive results for antioxidant activity and -amylase activity. Further all samples were rich in phenolics. The total phenolic contents (TPC) of four *Curcuma* species are indicated in Table 1.

The TPC of dried rhizomes of *Curcuma longa* was found to be the highest among all and recorded as 5.530 g of GA equivalents/1 g dried rhizome. Rhizomes of *Curcuma aromatica* and *Curcuma longa* were recorded high phenolic contents compared to leaf while leaf samples of *Curcuma albiflora* and *Curcuma zedoaria* were shown to contain high phenolic contents compared to rhizomes. Previous studies indicated that total phenols in *Curcuma caesia* was 30 mg/g of the dry weight and that of *Curcuma longa* was 132 mg/g of the dry weight [21].

The phenolic contents in turmeric play a major role in its antioxidant activity and effectiveness of the products it uses. Turmeric has been reported to comprise many biological activities such as anti-inflammatory, hepatoprotective, antitumors, and antiviral activities [22]. The phenolic contents are closely related with their medicinal uses since phenolic compound can act as anti-oxidant by free radical scavengings/ antioxidant property [23]. The present findings provide basic information that make *Curcuma* species a suitable plant with economic importance having medicinal values, hence may be utilized as raw materials for pharmaceutical industries.

Table 1 Total Phenolic Content of Four *Curcuma* Species

Species	Total Phenolic Content (g of GA equivalents/ 1g dried powder)	
	Rhizome	Leaf
Curcuma albiflora	2.002 c ± 0.005	2.211b ± 0.006
Curcuma aromatica	2.404 b ± 0.006	2.063 d ± 0.002
Curcuma longa	5.530 a ± 0.012	2.292 a ± 0.008
Curcuma zedoaria	1.687 d ± 0.001	2.164 c ± 0.001

Means with different letters in the same column are significantly different at p<0.05

Therefore, the phenolic contents of Sri Lankan *Curcuma* Species are relatively high. Leaves of all four *Curcuma* species has almost similar TPC and hence can be used as a source of raw material.

The MeOH extracts of *Curcuma* species showed notable radical scavenging activity against the free radicals (Table 2). Enhanced antioxidant property was observed in the extract of *Curcuma longa* rhizome (IC_{50} 150.253 µg/ml) and well suited with the similar antioxidant activities of some *Curcuma* species reported by Xiang *et al*. (2018). Akter *et al*. (2019) reported that specially isolated compounds from *Curcuma longa* (Variety Ryudai gold) showed significant radical-scavenging activity (IC_{50} 26.4µg/mL) [24]. Further Tanvir *et al*. (2017) revealed that ethanolic extract of Khulna's mura (*Curcuma longa*) varieties from Bangladesh recorded IC_{50} value as 1.08 μg/mL [25]. However, the considerable antioxidant activity of the *Curcuma* species in present study could be a result of high anthocyanins and phenolic contents.

Interestingly, leaves of *Curcuma longa* and *Curcuma zedoaria* showed similar DPPH scavenging activity compared to rhizomes of *Curcuma longa,* the highest DPPH activity among rhizomes. Therefore, the big potential is available to utilize leaves not only the rhizomes of *Curcuma* as a source of antioxidants. *Curcuma albiflora* showed lowest DPPH activity in both rhizomes and leaves. The MeOH extracts of *Curcuma*

species exhibited high level of inhibition to -amylase enzyme under *in vitro* conditions (Table 3).

Inhibitory concentrations of crude extracts that required 50% inhibition against -amylase in *Curcuma aromatica, Curcuma longa* and *Curcuma zedoaria* rhizomes were highly significant compared to acarbose a clinical drug that act as the inhibitor of -amylase (28.273 µg/ml) determined under identical conditions. However, confirmation of antioxidant and antidiabetic activity of these species under *in vivo* conditions and in an animal model are essential to use as medicines.

According to the results obtained by Wahyu Widowati *et al*. (2018), curcumol, curcumin and bisdemethoxycurcumin isolated from *Curcuma longa* has shown significant inhibition activities against -amylase (13.25 ppm, 6.99 ppm and 1.79 ppm respectively) [26]. The results in the present study are in similar ranges with previous studies [26,27]. Studies conducted by Lekshmi *et al*., (2012) revealed that volatile oils extracted from dried rhizomes of *Curcuma* (IC_{50}=34.30 µM) showed higher -amylase inhibitory activity than acarbose (IC_{50}=296.3 µg/ml) [28]. Physicochemical parameters of dried powder as shown in the Table 4 were adhered to those in Indian Ayurvedic Pharmacopoeia. The physicochemical qualities were obtained as percentage by performing standard methods of analysis. The determination of physicochemical parameter is very important in identification of adulterants and improper handling of herbal materials.

Table 2 IC_{50} Values of DPPH Radical Scavenging Activity

Species	IC_{50} Values of DPPH radical scavenging activity (µg/ml)	
	Rhizome	Leaf
Curcuma albiflora	338.841 ± 0.240 b	371.996 ± 0.020 a
Curcuma aromatica	188.380 ± 0.342 c	212.080 ± 0.078 b
Curcuma longa	150.253 ± 0.273 d	162.633 ± 0.631 d
Curcuma zedoaria	389.051 ± 0.426 a	187.491 ± 0.547 c
Ascorbic acid (Standard)	0.565 ± 0.056 e	0.565 ± 0.056 e

Means with different letters in the same column are significantly different at $p < 0.05$

Table 3 IC_{50} Values of -amylase Inhibition Activity

Species	IC_{50} Values of -amylase Inhibition Activity (µg/ml)	
	Rhizome	Leaf
Curcuma albiflora	307.475 ± 0.051 a	37.399 ± 0.480 c
Curcuma aromatica	13.915 ± 0.023 c	51.295 ± 0.015 b
Curcuma longa	6.455 ± 0.117 e	30.275 ± 0.175 d
Curcuma zedoaria	9.492 ± 0.024 d	55.316 ± 0.092 a
Acarbose (Standard)	28.273 ± 0.615 b	28.273 ± 0.615 e

Means with different letters in the same column are significantly different at $p < 0.05$

Table 4 Physicochemical Parameters of *Curcuma* species

Physicochemical Constant	*Curcuma albiflora*		*Curcuma aromatica*		*Curcuma longa*		*Curcuma zedoaria*	
	R	L	R	L	R	L	R	L
Loss on Drying (W/W)%	18.00	4.20	20.00	3.80	3.10	19.40	4.20	19.80
Total Ash Value (W/W)%	5.20	2.40	4.60	7.00	11.60	6.80	5.00	7.50
Acid – Insoluble Ash (W/W)%	0.49	0.38	0.96	0.28	1.02	0.91	0.58	0.32
Water – Soluble Ash (W/W)%	13.91	7.21	15.21	9.56	4.65	2.27	20.10	12.40

R- Rhizome, L- Leaves

Ash values are important standards [29] to identification of drugs and purity analysis of crude drugs when in powder form [30]. Further, the total ash of a crude drug reflects the contaminations with inorganic materials [31]. Acid insoluble ash value is necessary to evaluate the quality of crude drugs. Acid insoluble ash represents the amount of silica present in the samples. In differentiating the contaminated minerals from natural ash of the herbal material, the comparison of total ash value and acid insoluble ash value of the sample could be used. Water soluble ash content could be used as a good indicator in detecting the quality of the drug or its incorrect drug preparations [32]. More water-soluble ash value appears in *Curcuma aromatica* rhizomes denotes that it is more soluble in water compared to the others. The average moisture content of *Curcuma* powder was found to be 12.53%. It is in the range of moisture content of quality starch of 10-13.5% to ensure better shelf life [32].

The results of physico-chemical analysis of present study indicated that it contains more moisture contents than the average value reported in previous studies. However, it is possible to have different results based on the method used to perform the moisture analysis.

Total ash value (12.30%), Acid insoluble ash value (1.05%) and water solube ash value (4.10%) of *Curcuma longa* reported from the previous studies [33] are very close to the values obtained from present study.

Conclusion

The genus *Curcuma* represents many species, some of them are fully explored but many species are not much studied. The present study showed that Sri Lankan *Curcuma* species have a great potential to be used as antioxidant and antidiabetic agents. In the present study rhizomes and leaf samples of four *Curcuma* species were thoroughly investigated for their physicochemical

characters to analyse their quality, safety and standardization to compare with different standards. The information from the present study will provide avenue to compare bioactivity of different *Curcuma* species with correct identification and authentication of these herbal materials to be utilized in herbal industry by preventing its adulteration.

Acknowledgments

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