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Comparison of phytochemical properties of Indian and Sri Lankan turmeric rhizomes (*Curcuma longa*)

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Abstract
Turmeric, *"Curcuma longa"* is a prominent spice widely consumed throughout the world. Phytochemical properties of Indian and Sri Lankan Turmeric rhizomes were qualitatively as well as quantitatively analyzed. Two Indian and three Sri Lankan samples were collected and subjected to analyze antioxidant capacity using DPPH radical scavenging assay and total phenolic content according to Folin –Ciacaltea method. Qualitative analysis of phytochemicals with respect to Saponin, Tannin, Alkaloids, Flavonoids and Sterols depicted positive results for both Indian and Sri Lankan samples whereas quantification of antioxidant potential and phenol content showed variations among both types of turmeric. This was attributed to structural factors of each compound. The total Phenolic content and DPPH free radical scavenging capacity among the turmeric types were ranging from 627.4 to 422.68mg GAE/100g and from 7.7 to 3.48µg/ml respectively; which shows that both types are rich in phytochemicals.

Keywords: antioxidants, phenols, phytochemicals, turmeric

1. Introduction
Turmeric is a prominent spice which belongs to the Zingeberacaea family found particularly in Asian countries including India and Sri Lanka too. It is obtained from the rhizome of the plant *Curcuma longa* [1]. Turmeric has a tumour inhibition property and it can generate a protective effect on DNA [2]. In Sri Lankan traditional Ayurvedic medicine turmeric is also widely being used. This particular spice has an anti-inflammatory effect which can cure gastritis, toothaches, chest pains and menstrual difficulties [3]. Sri Lankans use turmeric powder as a sanitizing agent by dissolving turmeric power in water and it is sprayed or spilled, especially in the morning in front of their shops and boutiques. They believe that the disease-causing microorganisms are destroyed and it can bring prosperity to their business. In addition to that there is a practice among Sri Lankans and Indians to bath patients who are suffering from communicable diseases using turmeric powder dissolved water. There are some evidences to prove that the consumption of turmeric has the capability of treating skin diseases [4]. Curcumin is the major active compound found in turmeric and it is used to treat for various diseases due to the active nature of curcumin. Other than that Curcumin has anti-inflammatory, antimicrobial, antibacterial and antioxidant properties too. Curcumin has the ability of scavenging the free radicals formed in the body, it suppresses the production of B- cell activation factor and decreases the activation of signal transduction pathway [5]. The use of curcumin alone is less effective than using as whole turmeric. It has been proven that usage of curcumin alone in small quantities is not harmful. However, using of this poly phenol in human trials has not been approved yet [3]. The antioxidant capacity of curcumin is attributed to its unique conjugated structure, which exists in an equilibrium between the diketo and keto-enol forms that are strongly favoured by intramolecular H-bonding [6]. Since demethoxycurcumin and bisdemethoxycurcumin have similar structures like curcumin, Curcumin shows typical radical-trapping ability as a chain-breaking antioxidant. Turmeric powder is widely used in food industry as a spice, food colouring agent as well as a preservative [7]. There are about 110 species under the genus *curcuma*. Out of them only 20 species have been undergone through a phytochemical study. *Curcuma longa* is the species which is widely being studied and about 235 compounds have been identified. They are categorized under phenolic compounds and terpenoids. Therefore, this study is typically focused in determining phytochemicals and their properties of Turmeric.

2 Materials and Methodology

2.1 Plant material

Indian turmeric samples were purchased from Colombo and a local-market while local turmeric samples were collected from 3 places such as local market, National Spice Garden and Local home garden. The five different types of turmeric samples were ground using a stainless steel laboratory scale grinder which was used only for the grinding purpose of turmeric. Turmeric rhizomes were ground for 2 minutes until passing through 1mm aperture size. Then the samples were labelled and were preserved in dry stoppered containers (I.S Specification No I.S 1797 – 1985 Methods of Test for Spices and Condiments / A.O.A.C 17th edn 2000, Official Method 920.164 Preparation of Test sample). Grinded samples were utilized for phytochemical analysis.

2.2 Determination of the phytochemical content of Turmeric rhizome

Alkaloid

About 50 mg of Solvent free extract was stirred with 3 ml of dilute hydrochloric acid and then filtered thoroughly. The filtrate was tested carefully with various alkaloid reagents as follows:

Terpenoid and steroid

Four milligrams of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet colour was observed for terpenoid and green bluish colour for steroids.

Flavonoid

Four millilitres of extract was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red colour was observed for flavonoids and orange colour for flavones.

Tannins

To 0.5 ml of extract solution 1 ml of water and 1-2 drops of ferric chloride solution were added. Blue colour was observed for gallic tannins and green black for catecholic tannins.

Saponins

About 0.2 g of the extract was shaken with 5 ml of distilled water and then heated to boil. Frothing (appearance of creamy miss of small bubbles) shows the presence of saponins

2.3 Determination of total phenolic content

Total phenolic content of Turmeric extracts was determined using the Folin–Ciocalteu (F-C) reagent, according to the method described by Cheng *et al* (2007) [8]. An aliquot of 0.125 ml of diluted extract was added to 0.5 ml of deionized water and 0.125 ml of the (F-C) reagent. After shaking, the mixture was incubated for 3min at room temperature. Then, 1.52 ml of 7% Na_2CO_3 solution was added. The volume obtained was adjusted to 3 ml using distilled water, mixed vigorously, and held for 90 min at ambient temperature. The absorbance of the solution was then measured at 760 nm against a blank. The total phenolic content was expressed as mg of Gallic acid equivalents (GAE) per gram of dry weight through the calibration curve of Gallic acid. The sample was analysed in three replications. Gallic acid was used as the standard phenol. A dilution series was prepared using the Gallic acid stock solution.

2.4 Determination of the antioxidant content; DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity

For the DPPH assay, the procedure followed the method of Mansouri *et al* (2005) [9] with some modifications. The stock solution was prepared by dissolving 3 mg of DPPH in 100 ml methanol to obtain an absorbance of 0.900±0.02 units at 517 nm using UV-Vis Spectrophotometer. 3ml of methanol was used as the blank sample. Control sample was prepared by mixing 2ml DPPH solution in 2ml methanol. For each turmeric sample a serial dilution was prepared and 1.5 ml of each diluted sample was mixed with 1.5 ml DPPH solution and incubated at room for 30 minutes in a dark place. The absorbance was read at 517 nm. The percentage inhibition of absorbance was calculated according to the following equation.

$$\% \text{ inhibition} = [A\text{control} - A\text{sample} / A\text{control}] \times 100\%$$

Where,

$A_{control}$ = Absorbance value of the DPPH solution of the control sample

A_{sample} = Absorbance value of the DPPH solution in the presence of extracted black cumin

The calculated percentage inhibition of absorbance at 517nm was plotted as a function of concentration of extracted turmeric sample and the sample concentration which gives the 50% inhibition activity was estimated as the IC_{50} value from regression analysis using the software MINITAB R17. Gallic acid was used as the standard antioxidant. Six different concentrations of Gallic acid; 1, 2, 3, 4, 5, 6 mg/L were prepared and from each Gallic acid solution 2 ml was mixed with 2 ml DPPH solution and the absorbance was measured at 517 nm after 10 minutes of incubation at room temperature in a dark place. The percentage inhibition was calculated for each dilution of Gallic acid solution and was plotted as a function of concentration of standard antioxidant. The concentration which gives the 50% inhibition activity was estimated as the IC_{50} value from regression analysis using the software MINITAB 17 for standard reference.

3. Result and Discussion

3.1 Qualitative analysis of phytochemicals in turmeric rhizomes of 5 different types

The phytochemical presence in five types of Turmeric rhizomes were qualitatively analysed in terms of saponin, tannin, alkaloids, flavonoids and results are given in table 1.

Table 1: Qualitative analysis of phytochemical in turmeric rhizome

Type	Saponin	Tannin	Alkaloids	Flavonoid	Sterol
Indian 1	+	+	+	+	+
Indian 2	+	+	+	+	+
Local 1(Matale)	+	+	+	+	+
Local 2 (Research centre)	+	+	+	+	+
Local 3(Home garden Matale)	+	+	+	+	+

*Matale: Matale is a district of central province of Sri Lanka

According to the results given in table 1, all types of turmeric contained phytochemicals. But their quantity can be varied base on their genetics, location of cultivation, climatic conditions etc. Presence of these phytochemicals indicates that turmeric rhizomes contained some medicinal properties. Saponins are active antifungal agents which have soapy characteristics. Tannins are also known as antimicrobial

agents and they are water soluble polyphenols that can precipitate proteins [10]. Therefore, tannins are able to prevent the development of microorganisms by precipitating microbial protein and making nutritional proteins unavailable for them. Apart from that tannins can inhibit the growth of many fungi, yeasts, bacteria, and moulds.

Tannins have various physiological effects like anti - irritant, antisecretolytic, antiphlogistic, antimicrobial and antiparasitic effects. Phytotherapeutically tannin-containing plants such as turmeric plants are used to treat nonspecific diarrhoea, inflammations of mouth and throat and slightly injured skins [10, 11]. Due to the presence of flavonoids, turmeric can scavenge hydroxyl radicals, superoxide anions and lipid peroxy radicals [11]. Ikpeama *et al*, [12] also states that turmeric contains saponin, tannin, flavonoids, alkaloids and sterols. Therefore, the results of the phytochemical determination tests in this research matches with their findings.

3.2 Determination of total phenolic content in rhizomes of 5 different types of turmeric

The total phenolic content of five types of Turmeric was determined and it was expressed as mg per GAE (Gallic Acid Equivalent) for 100g of dry powder. Since turmeric shows many health benefits and antimicrobial functions, quantification of phenolic content is important. The Total Phenolic Content of turmeric was determined using Folin – Ciacaltea and the results are illustrated in the table 2

Table 2: Total Phenolic content of turmeric rhizomes

Turmeric type	Poly phenol content (mg GAE/100g)
Indian 1 (Colombo)	545.66^{a}±10.81
Indian 2 (Matale)	545.65^{a}±13.39
Local 1(Matale)	486.52^{b}±13.78
Local 2 (Research centre)	627.46^{c}±11.57
Local 3(Home garden Matale)	422.68^{d}±11.79

*****Data presented as mean values for triplicates with duplicate measurements in each replicate ± S.D (n=6). a,b,c,d letters in same column are significantly different at ($p < 0.05$) level.

According to the table 2, while local sample (Research centre) has the highest total phenolic content of 627.46±11.57, the lowest content was reported in Local 3 sample (Home garden Matale) which was 422.68±11.79 mg GAE/100g.The Indian turmeric types contain almost equal amounts of TPC. The local 1 (Matale) contains 486.52±13.78mg GAE/100gTotal Phenolic Content. Even though the Total Phenolic Contents of Indian 1 and Indian 2 are almost equal, Indian 2 has the highest standard deviation than the Indian 1. Since the Local 2 is a genetically developed variety, it may be incorporated with relevant genes to produce more phenolic compounds. Further, the Indian types are also developed for export purpose; they also contained high phenolic content. The mechanism of the chemical reaction happened in the analysis is reduction of phosphotungstic to phosphotungstic blue and that causes the increment of absorbance due to increase in the number of aromatic phenolic groups [13, 14]. According to published findings, Total Phenolic Content of Turmeric is about 582.8mg GAE/100g. Under this circumstance, local 2 has the highest amount of TPC comparatively published value; however, all other 4 types had lower values. In a recent study, Liu *et al* [15] has observed TPC in Turmeric rhizomes was 712.4mg GAE/100g. In this research study they have used Chinese turmeric and according to that Chinese turmeric have more TPC than Sri Lankan and India Turmeric. The data obtained from the study was statistically analysed and results revealed that there was a significant difference between the means of TPC in the 5 turmeric types at 95% confidence level ($P < 0.05$).

3.3 Determination of antioxidant content of 5 different types of Turmeric rhizomes

The antioxidant contents of 5 types of turmeric rhizomes were determined using DPPH assay and the DPPH free radical scavenging capacity is used as the principle. The analysis was done using the inhibition percentage and the relevant IC_{50}value. The results pertaining to the IC_{50} values are given in the table 3

Table 3: IC_{50} values of the different turmeric types

Turmeric type	IC $_{50}$ Value (µg/ml)
Indian 1 (petta)	4.58^{a}±0.04
Indian 2 (Matale)	4.58^{a}±0.02
Local 1 (Matale)	5.86^{b}±0.11
Local 2 (Research centre)	3.44^{c}±0.09
Local 3 (Home garden Matale)	7.70^{d}±0.14

*****Data presented as mean values for triplicates with duplicate measurements in each replicate ± S.D (n=6). a,b,c,d letters in same column are significantly different at ($p < 0.05$) level.

IC $_{50}$ value is used to evaluate the antioxidant capacity and DPPH is a stable radical which is widely used to evaluate the free radical scavenging activity in many plant extracts [16]. The stable DPPH shows a maximum absorbance at 515nm and it can be reduced by an antioxidant as follows.

$$DPPH^{\cdot} + AH \rightarrow DPPH\text{-}H + A^{\cdot}$$

The IC_{50} values were obtained by constructing a calibration curve which is prepared by calculating inhibition percentage values using the following equation as a function of concentration VS inhibition percentage.

$$\% \text{ Inhibition} = [A_{control} - A_{sample}/A_{control}] \times 100$$

Antioxidant activity was determined by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay. The principle of DPPH assay is reducing the stable free radical DPPH to yellow coloured diphenyl- picrylhydrazyl by the antioxidant present in turmeric. The method is based on the reduction of DPPH in methanolic solutions in the presence of an antioxidant as a result of in forming of non-radical form of DPPH-H in the reaction solution. DPPH analysis is also one of the tests used to prove the ability of components of the turmeric extracts to act as donors of hydrogen atom [17]. This shows that when the IC $_{50}$ value is lower it has a high antioxidant potential as it needed less amount of the material to scavenge the DPPH radical.

According to the data given in table 3, Local 1, Matale contains the highest IC $_{50}$ value (7.70±0.14µg/ml) and the Local 2, which contains the lowest value (3.44±0.09 µg/ml) which depict that highest antioxidant capacity is in Local 2 and lowest in Local 1. The Indian types (1 and 2) contain almost equal amounts of IC_{50} with equal antioxidant potentials. According to various researches the turmeric varieties in West Bengal, India contains IC_{50} value of 5.99µg/ml [18] and Indonesian types contains a high IC_{50} value of 8.33µg/ml [19] which shows that observed results are in compliance with these published findings. The values for IC_{50} except the Local 3 are lower than the IC_{50} value of West Bengal, Indian turmeric which are better in antioxidant activity and all the 5 turmeric types contain low IC_{50} than the Indonesian turmeric. The mean values for IC_{50} were analysed

using one-way parametric ANOVA and according to the results at 95% confidence level, there is a significant difference between the mean IC_{50} values in the 5 turmeric types ($p<0.05$).

4. Conclusion

Turmeric is a rich source of antioxidants and phenols which has many phytochemicals like Tannin, Sterols, Flavonoids and alkaloids. This spice can be utilized as a medicinal plant in many fields due to its natural medicinal properties.

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