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Phytochemical profile and antimicrobial activity of turmeric grown in Andaman Islands

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Abstract

Turmeric is one of the important spice that has many medicinal properties. The co-2 variety of turmeric grown in Andaman and Nicobar Islands were subjected to phytochemical assay. The results showed that the rhizomes of turmeric had highest phenolic (31.62 mg/g GAE) and flavonoid content (18.02 mg/g GAE) in methanolic extract than aqueous extract. The antioxidant assay was done by DPPH activity of two extracts and were increased with increasing concentration (10-75 µg/ml), the DPPH activity was showed highest in methanolic extract (26.02 mg BHA/g). The antimicrobial scavenging activity was found to be 49.79% and 17.24% in methanolic and aqueous extract respectively. The methanol and aqueous extracts of the *C. longa* showed no antimicrobial activity against *R. solanacearum*, *C. gloeosporioides* and *Fusarium* spp., at all dilutions

Keywords: turmeric, Andaman Islands, antioxidant, antimicrobial activity

Introduction

Turmeric (*Curcuma longa* L., family: Zingiberaceae) is one of the most important and ancient spices of India and a traditional item of export. It is used as condiment, dye, drug and cosmetics in addition to its use in religious ceremonies. Rhizome is the economic part of turmeric and is well known for its medicinal properties along with its application in cosmetics and as natural dye in textile industry. Traditional Indian medicine uses turmeric powder for the treatment of biliary disorders, anorexia, coryza, cough, diabetic wounds, hepatic disorders, rheumatism and sinusitis. Various sesquiterpenes and curcuminoids have been isolated from the rhizome of *C. longa*, attributing a wide array of biological activities such as antioxidant anti-inflammatory wound healing, anticancer and antibacterial activity (Arulselvi *et al*, 2012)^[2]. As a powder, it has been in continuous use for its flavouring, as a spice in both vegetarian and non-vegetarian food preparations and it also has digestive properties (Govindarajan, 1980)^[12]. Turmeric is also a powerful anti-inflammatory and antioxidant (Song, 2001)^[23] and has been shown by scientists to protect the liver against a variety of toxic substances. The powdered rhizome is considered to be stimulating, carminative, purifying, anti-inflammatory, and anthelmintic (Eigner, 1999)^[9]. The medicinal property of turmeric is mainly due its bioactive compound curcumin. Spices play a major role in Andaman agriculture as they are well suited to grow in the plantation based cropping system of the Island. Turmeric is one of the major spices grown in both arecanut and coconut plantation as intercrops in organic system in Andaman Islands. Different varieties of turmeric are grown in the Island and Co-2 variety of turmeric released by Tamil Nadu Agricultural University (TNAU) is well adapted in the Island and front line demonstrations of this variety of turmeric is being undertaken in farmers' field in different parts of the Island. The phytochemical and anti-microbial study in any species of the plant will held in identification of effective compounds and their efficacy in scavenging free radicals. Though numbers of studies have been done in general on the phytochemical and antimicrobial activity of turmeric (*Curcuma longa* L.). The study on phytochemical profiling of turmeric in the Island is not studied so far. The influence of the region and the organic mode of cultivation may have an effect in the chemical constituents. Once the quality parameters on this particular variety of turmeric Co-2 is analyzed, suggestions for cultivation of this crop and the industrial application owing to its medicinal properties will help in increasing the area of cultivation of this particular crop in the Island due to its market demand.

Methods and Material

Plant material and sample preparation

The rhizomes of co 2 variety of *Curcuma longa* L. grown in glass house were harvested for further study. After washing the rhizomes were dried at 45 °C for 48 hours. The dried turmeric

slices were grinded into powder form by using high speed blender. One gram sample was mixed with 50 ml of 80% methanol and distilled water and kept for 1-2 day followed by centrifugation at 8000rpm for 10 min. This procedure was repeated till the powder was devoid of colour and filtered through Whatman No. 1 filter paper. The extract was concentrated by rotary evaporator and kept in a refrigerator for analysis.

Phytochemical assay

Phenols and flavonoids

About 1 g of powdered turmeric rhizomes were used for the analysis phytochemical compounds. The Folin-Ciocalteu colorimetric method was used to measure the total phenolic content (Bao *et al.*, 2005) [3]. 200 μ l of the extractions were oxidized with 1 ml of 0.5 N Folin-Ciocalteu reagent and then the reaction was neutralized with 1 ml of the saturated sodium carbonate (75 g/L). The absorbance of the resulting blue color was measured at 760 nm with a UV-2600 spectrophotometer (Simadzu, Japan) after incubation for 2 h at room temperature. Quantification was done on the basis of the standard curve of gallic acid. Results were expressed as milligram of gallic acid equivalent (mg GAE) per 100g of flour weight. The flavonoid content was determined by aluminium chloride colorimetric method which was modified from the procedure reported by Woisky and Salatino, 1998 [25]. Qualification was done using the Rutin as standard and the results was expressed as milligrams of rutin equivalent (mg RE) per 100 g of sample weight.

Antioxidant analysis

Total antioxidant activity was obtained by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) method (Rattanachitthawat *et al.*, 2010) [20] with some modification. Total antioxidant activity of the extracted turmeric rhizome was expressed as mg BHA/g sample equivalent, obtained from the calibration curve.

$$\% \text{ inhibition of DPPH radical} = (A_{\text{control}} - A_{\text{sample}}/A_{\text{control}}) \times 100$$

Where A_{control} is the absorbance of the control (without extract) and A_{sample} is the absorbance in the presence of the extract/standard.

The ABTS radical scavenging activity was determined by a colorimetric method (Re *et al.*, 1999) [21] with a little modification. Results were expressed in terms of Trolox equivalent antioxidant capacity (TEAC, μ M Trolox equivalents per 100 g dry weight).

$$\% \text{ inhibition of ABTS radical} = (A_{\text{control}} - A_{\text{sample}}/A_{\text{control}}) \times 100$$

The Ferric reducing-antioxidant power (FRAP) assay was done and is based on the reduction of the complex of ferric iron and 2, 3, 5-triphenyl-1, 3, 4-triaza-2-azoniacyclopenta-1,4- diene chloride (TPTZ) to the ferrous form at low pH (Benzie and Strain (1999) [4]. 0.9ml of prepared FRAP reagent is mixed with 0.1ml of diluted sample and the absorbance at 595 nm is recorded after a 15 min incubation at 37 °C and the results were expressed in mM of Fe^{2+} equivalents per grams of dry weight.

Nitric oxide scavenging activity was measured using the procedure described by Marcocci *et al.*, 1994 [17]. The percentage of inhibition was measured by the following formula:

$$\% \text{ inhibition of NO radical} = (A_{\text{control}} - A_{\text{sample}}/A_{\text{control}}) \times 100$$

The Metal chelating activity (MCA) of ferrous ion was measured using the method of Dinis *et al.*, 1994 [7]. The percentage of metal chelating activity was calculated by the following equation:

$$\% \text{ Metal chelating activity} = (A_c - A_s)/A_c \times 100$$

Where, A_c is the absorbance of the control and A_s is the absorbance of the extract/standard. Scavenging activity of hydrogen peroxide (H_2O_2) was estimated using the method of Ruch *et al.*, 1989 [22] with little modification. The amount of nitric oxide radical inhibition is calculated as

$$\% \text{ inhibition of } \text{H}_2\text{O}_2 \text{ radical} = (A_{\text{control}} - A_{\text{sample}}/A_{\text{control}}) \times 100$$

The IC_{50} (concentration for 50% inhibition) values of all the methods were calculated by using linear regression analysis. All the experiments were repeated thrice. Higher the IC_{50} value, lower is the scavenging activity.

The reducing power of turmeric extract was estimated in a method which involves the mechanism of single electron transfer system using ferric to ferrous reducing activity as determined spectrophotometrically from the formation of Perl's Prussian blue colour complex. This method is based on the principle of increase in the absorbance of the reaction mixtures described by Oyaizu (1986) [18]. The higher the absorbance of the reaction mixture the greater is the reducing power.

Antimicrobial activity

The phytopathogenic fungi, two Gram negative bacterial strains via, *Ralstonia solanacearum* and *Xanthomonas oryzae* and two fungal pathogens via, *Colletotrichum gloeosporioides* and *Fusarium* spp were collected from Plant Pathology laboratory, Division of Field Crops, Central Inland Agricultural Research Institute (CIARI), Port Blair. The plant pathogenic fungi were maintained on potato dextrose agar used for further experiments. Ten grams of the pulverized dried ginger rhizomes were extracted with 100 ml of methanol and 100 ml of sterilized distilled water by stirring for overnight on magnetic stirrer. The extract was filtered through whatman filter paper and reduced to dryness on a rotary evaporator at 40 °C. About 200 mg of both methanol and aqueous extracts was weighed, re-dissolved in 1 ml of the extraction solvent (methanol and water) and then tested for antimicrobial activities. From the stock (200mg ml^{-1}) preparation different dilutions (100mg/ml and 50mg/ml) were prepared for the antimicrobial activity.

In vitro antibacterial activity of turmeric rhizome extract against *R. solanacearum* and *X. oryzae* was determined by agar well diffusion assay. Each Petri dishes (size 9cm diameter) containing 15ml of Kings B medium (at 40 °C) was seeded with 100 μ l inoculums of target plant bacterial pathogens via, *R. solanacearum* and *X. oryzae* (1×10^8 CFU ml^{-1}). Media was allowed to solidify. Wells of 5 mm diameter were cut into solidified agar media with the help of sterilized cork borer. Aliquot 50 μ l of methanol and aqueous extracts of different dilutions were added in the respective well and the plates were incubated at 28 ± 2 °C for 24-48 h. The experiment was performed in triplicate under aseptic conditions.

In vitro antifungal activity of turmeric rhizome extract against *C. gloeosporioides* and *Fusarium* spp., was determined by paper disc method. Filter paper discs, 6 mm diameter, were sterilized by dry heat for 1 h at 160 °C oven temperature and impregnated with each of the test extracts by applying 10 µL of the extract solutions with different dilutions. In two conical flasks containing potato dextrose agar (PDA) media containing spore suspension of *C. gloeosporioides* and *Fusarium* spp., were poured into 14.5 cm in diameter petri plate and allowed to solidify. After the carrier solvents evaporated from the paper discs, they were placed on the surface of the medium; the plates were incubated for 4 days. The experiment was performed in triplicate under aseptic conditions.

Results and discussion

Phytochemical assay

Phenols and flavonoids

The rhizomes of turmeric had highest phenolic (31.62 mg/g GAE) and flavonoid content (18.02 mg/g GAE) in methanolic extract than aqueous extract (Table 1). Phenolic content in the study is four to six times higher as per report of (Wojdylo *et al.*, 2007 [26] (825.58 mg GAE/ 100g) and Kim *et al.*, 2011 [15] (582.8 mg GAE/100 g). Similarly, flavonoid content of methanolic extract is nearly two times more than aqueous extract (8.71 mg/g rutin). The reason may be due to tropical growing climate in the Islands where high phenolic compounds might have synthesized in the plant to defend against oxidative stress vis-à-vis reactive oxygen species (free radicals). This is in agreement with the report by Chen *et al.*, 2013 [6] who stated that when plants are stressed, an exchange occurs between carbon to biomass production or formation of defensive secondary compounds (Bryant, 1983) [5]. The higher values for methanolic extract may be due to the extraction power of solvent (80% methanol) to the phenolic compounds to the dilution media.

Antioxidant analysis

Results from our present study illustrated a significant decrease in the concentration of DPPH radical due to the scavenging ability of *Curcuma longa* and the reference compounds (Butylated hydroxyl toluene, BHT). There was a steady increase in the inhibition of the radicals with concomitant increase in concentration of the extract establishing dose dependence of the extract in scavenging DPPH radicals. DPPH activity of two extracts were increased with increasing concentration (10-75 µg/ml), being highest in methanolic extract (26.02 mg BHA/g) as shown in Table 1. The scavenging activity was found to be 49.79% and 17.24% in methanolic and aqueous extract respectively (Fig. 1). Phenolic compounds are high level antioxidants (Hall and Cuppett, 1997) [14] because they possess the ability to absorb and neutralize free radicals, quench active oxygen species and decompose superoxide and hydroxyl radicals (Duh *et al.*,

1999) [8]. IC₅₀ (concentration required for 50% reduction of scavenging activity) is shown in Table 2. The smaller IC₅₀ value, the higher DPPH scavenging activity. Here, the IC₅₀ of methanolic extract was found to be 168.73 µg/ml where for aqueous extract, the value increased to 1760 µg/ml, indicating the efficiency of alcoholic extract to quench DPPH free radicals

In ABTS assay, a concentration-dependent activity was observed (Fig 1). Higher concentrations of the extract were more effective in quenching free radicals in the system. At the concentration of 10 µg/ml, the ABTS activity was found to be 9.46 and 8.89 mg trolox/g in methanolic and aqueous extract indicating non-significant difference between them (Table 1). With the increase in concentration (10-75 µg/ml), the scavenging activity of turmeric to ABTS radical scavenging activity increased from 23.82 to 89.52 for methanolic extract and 12.82 to 59.44 for aqueous extract (Fig 2). The relative low scavenging potential for aqueous extract may be due to its low extract potential to remove the phytochemicals from its complex tissue to the solvent medium. Different radical scavenging capacity of the methanol extract against the tested radicals (DPPH and ABTS^{•+}) may be due to the different mechanisms involved in the radical-antioxidant reactions. These assays differ from each other in terms of substrates, probes, reaction conditions and quantification methods. Radical systems in antioxidant evaluations may influence the difference in the results obtained in an experiment (Yu *et al.*, 2002) [27]. Some compounds, though possessed ABTS^{•+} scavenging activity, did not exhibit DPPH scavenging activity (Wang *et al.*, 1998) [24]. Thus, comparison of assays is difficult and ranking of antioxidant activity is strongly dependent on the test system and on the substrate to be protected by the antioxidants (Frankel and Meyer, 2000) [11]. Due to low power of aqueous extract, its IC₅₀ value is 23 times higher than corresponding methanolic extract (Table 2). The reducing properties are generally associated with the presence of reductones (Pin-Der-Duh, 1998) [19], which have been shown to exert antioxidant action by breaking the free radical chain, by donating a hydrogen atom. Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. Our data on the reducing capacity of turmeric extract suggested that reductone-associated and hydroxide groups of compounds can act as electron donors and can react with free radicals to convert them to more stable products, and thereby terminate radical chain reactions. The reducing power of the extract increased and correlated well with the increasing concentration. A high absorbance value of the sample indicates its strong antioxidant activity. With the increase in concentration from 100 to 750 µg/ml (Fig. 2), the absorbance increased from 0.25 to 0.92 (methanolic extract) to 0.17 to 0.73 (aqueous extract) suggesting higher the electron donating ability with the increase in concentration.

Table 1: Phytochemicals of *Curcuma longa*

Extract	Phenol (mg GAE/g)	Flavonoids (mg rutin/g)	DPPH (mg BHT/g)	ABTS (mg trolox/g)	FRAP (mg Fe+2/g)	NO (mg Vit-C/g)	MCA (mg EDTA/g)	H ₂ O ₂ (mg Vit-C/g)	RP (mg BHT/g)
Methanolic	31.62	18.02	16.33	9.45	4.38	1.41	5.46	10.45	33.87
Aqueous	23.22	8.71	5.13	8.89	2.32	0.336	0.434	1.43	10.91

Table 2: IC₅₀ values (µg/ml) of *Curcuma longa* with different antioxidant methods

	DPPH	ABTS	FRAP	NO	H ₂ O ₂
Methanolic extract	168.43	20.54	14.12	58.17	24.74
Aqueous extract	1760	464.09	1355	584.5	41.59

Antimicrobial activity

The methanol and aqueous extracts of the *C. longa* showed no antimicrobial activity against *R. solanacearum*, *C. gloeosporioides* and *Fusarium* spp., at all dilutions. Against *X. oryzae*, the methanol extract of *C. longa* showed the

antibacterial activity (Table 3). The antimicrobial effect of the medicinal plants is well documented (Abirami *et al.*, 2014) [1]. The results of different studies provide evidence that some medicinal plants might indeed be potential sources of new antibacterial agents even against some antibiotic-resistant strains (Kone *et al.*, 2004) [16]. But, in present study, there was no antimicrobial activity in both methanol and aqueous extracts of *C. longa*, against the tested plant pathogens at the

specific dose. Our results are contradictory with some researchers who reported antimicrobial activity of plant extracts against pathogens (Habsah *et al.*, 2000; Fang *et al.*, 2003) [13, 10]. This variation may be because of the dose used in this study, the method of extraction of medicinal plants, the method of antimicrobial study, the genetic variation of plant, age of the plant or the environment.

Table 3: Antimicrobial activity of methanol and aqueous extracts of *C. longa*.

Target Pathogen	Methanol extract of <i>C. longa</i> (mm)			Aqueous extract of <i>C. longa</i> (mm)		
	200 mg/ml	100 mg/ml	50 mg/ml	200 mg/ml	100 mg/ml	50 mg/ml
<i>R. solanacearum</i>	-	-	-	-	-	-
<i>X. oryzae</i>	13	10	8	-	-	-
<i>C. gloeosporioides</i>	-	-	-	-	-	-
<i>Fusarium spp.</i>	-	-	-	-	-	-

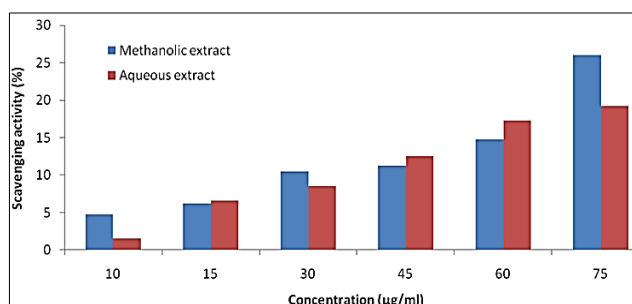


Fig 1: DPPH radical scavenging activities of turmeric extract

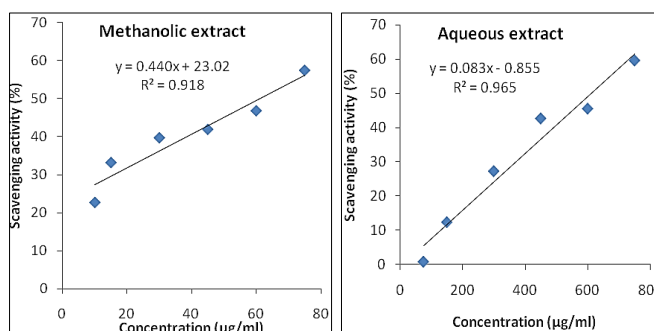


Fig 4: Nitric oxide radical scavenging activities of *Curcuma longa*

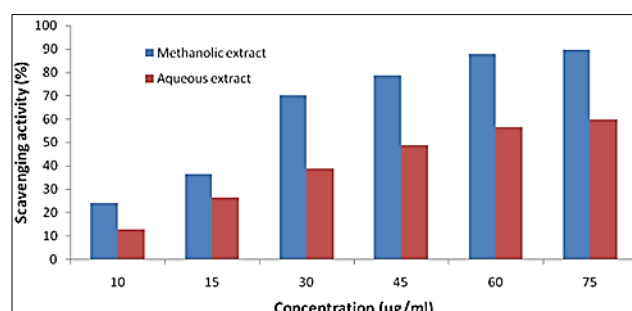


Fig 2: ABTS radical scavenging activities of turmeric extract

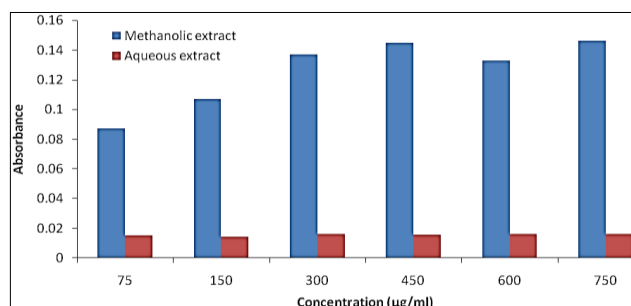


Fig 5: Metal chelating scavenging activities of *Curcuma longa* L. powder

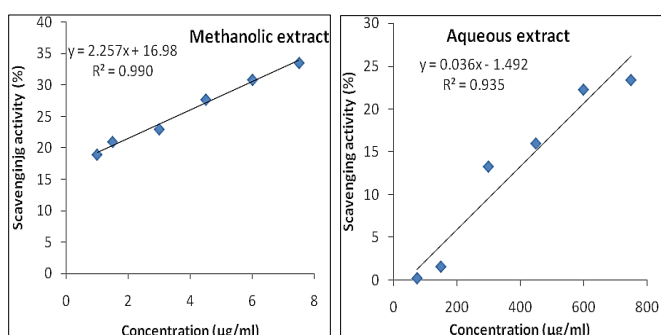


Fig 3: FRAP scavenging activities of *Curcuma longa*

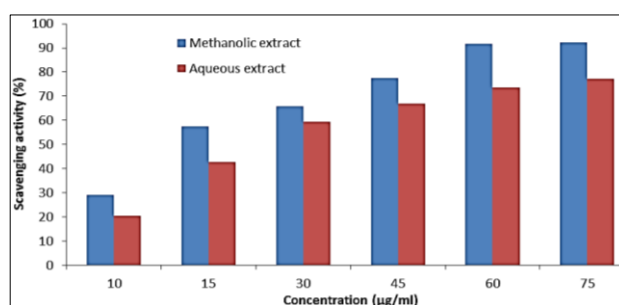


Fig 6: Hydrogen peroxide radical scavenging activities of *Curcuma longa*

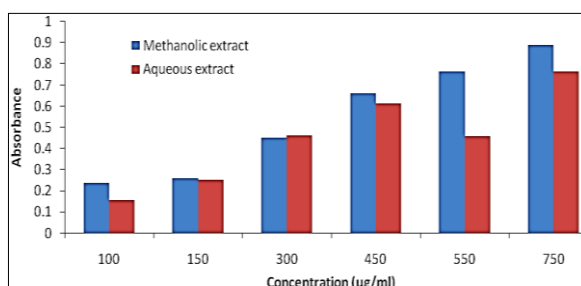


Fig 7: Reducing power of turmeric extract

The water and methanolic extracts of *C. longa* showed high content in phenolic and flavonoid compounds responsible for varieties for scavenging activity to maintain healthy cell. Highest scavenging activity in nitric oxide and hydrogen peroxide scavenging were observed. However, the powder is weak in scavenging DPPH and FRAP radicals. Again, the curcuma extract was effective only against *X. oryzae*. The reason may be either due to the dose of the extract or there may be some other solvents which may be able to extract high level of phenolic compounds that needs to be tested in future research.

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