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Research Article

ANTIOXIDANT POTENTIAL AND SIMULTANEOUS ESTIMATION OF QUERCETIN, RUTIN, AND GALLIC ACID IN *CURCUMA* SPECIES

SOWMYA PRIYA M[1], SANGILIMUTHU A[1]*, KARPAGAVALLI M[2], RAMKUMAR M[1], JOSPHIN NIRMALA A[1]

[1]Department of Biotechnology, Karpagam Academy of Higher Education, Coimbatore - 641 021, Tamil Nadu, India. [2]Department of Pharmaceutical Chemistry, Karpagam College of Pharmacy, Coimbatore - 641032. Tamil Nadu, India. Email: smuthu.al@gmail.com

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ABSTRACT

Objective: This study was designed to provide simple and cost-effective methods to quantify the biologically active phytoconstituents such as rutin, quercetin, and gallic acid from *Curcuma* species and evaluation of the antioxidant potential of different parts with different solvent extracts of *Curcuma* species.

Methods: Ultraviolet-visible spectrophotometer was used for the analysis of quercetin, rutin, gallic acid and total flavonoid content of *Curcuma* species extracts. Antioxidant potential of *Curcuma* species extracts were evaluated using 2-2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity.

Results: Ethyl acetate extract of *Curcuma aromatica* rhizome, aerial part contain higher quantity of quercetin and rutin compared to the other extracts, and also *Curcuma species* such as *Curcuma longa* and *Curcuma amada* contains high antioxidant capacity. The total flavonoid content was high in ethyl acetate extract of *Curcuma aromatica* as 88.35±0.25 µg/g dry weight of quercetin equivalents.

Conclusion: Different extracts of *Curcuma* species possess good free radical scavenging activity and the IC_{50} of *Curcuma amada* aerial part, *Curcuma longa* aerial part, and *Curcuma aromatica* rhizome was 61.65±1.75, 62.95±1.85, and 89.40±0.15 (µg/ml), respectively. The *Curcuma* species contains high total flavonoid content and antioxidant potential.

Keywords: *Curcuma aromatica, Curcuma longa, Curcuma amada,* Rutin, Quercetin.

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INTRODUCTION

Curcuma is a major genus in the family of Zingiberaceae and it contains nearly 100 species [1]. It is a rhizomatous herb distributed throughout the world; most of the species are present in China. *Curcuma* species being used as traditional medicine for various diseases in developing countries among the traditional healers and also used as traditional Chinese medicine for various illness among the Chinese communities. The *Curcuma* species has been traditionally used in India for several human illnesses. In *Curcuma* species, *Curcuma longa* rhizome powder has been used for cough, wound healing from the ancient time. Among the scientific communities, various parts of *C. longa* have been proved to have an antioxidant [2], antibacterial [3], anticancer [4], hepatoprotective effects [5], and anti-inflammatory [6] potentials due to the presence of major bioactive principles such as α-turmerone and β-turmerone in essential oil extracted from *C. longa* rhizome [2], and it contains curcumin, demethoxycurcumin, and bisdemethoxycurcumin identified with high-performance liquid chromatography coupled with electrochemical detection methods [7]. Different organic solvents such as petroleum ether, chloroform, benzene, methanol, and aqueous fractions of *C. longa* demonstrated against *Staphylococcus aureus* found that petroleum ether and methanol fractions possess higher antimicrobial activity [8]. The essential oil extracted from *C. longa* leaves and it acts as potential candidate for the oxidative mediated damage by *in vitro* 2-2-diphenyl-1-picrylhydrazyl (DPPH) and ABTS free radical scavenging methods [9]. Another major species from the genus of *Curcuma* is *Curcuma aromatica* and *Curcuma amada* which have various pharmaceutical applications. Essential oil from *C. aromatica* is very active against various free radicals [10] and contains camphor, vinyldimethylcarbinol, bomeol, and cubenol. Potent larvicidal compounds as 9-oxoneoprocurcumenol and neoprocurcumenol from petroleum ether extract of *C. aromatica* against mosquito larvae [11] and also, ethyl acetate extract of *C. aromatica* rhizome contains curcumin, demethoxycurcumin, and β-sitosterol-3-O-b-D-glucopyranoside [12]. The methanol extract of *C. amada* leaves and rhizome has shown good anticancer potentials against MCF-7 cell lines [13]. The chloroform extract of *C. amada* rhizome contains amadannulen and crude extract possesses antioxidant activity on DPPH, superoxide, lipid peroxidation, and antibacterial activity against *Micrococcus luteus, Bacillus cereus,* and *Bacillus subtilis* [14,15].

The rhizomes of *C. longa, C. aromatica,* and *C. amada* are used as traditional medicine and food. However, it is difficult to distinguish their pharmacological activities as their chemical characteristics are obviously different. Therefore, quantitative analysis of chemical characteristics such as quercetin, gallic acid, and rutin is very important for ensuring the efficacy of the herbs. Mostly, after harvesting the rhizomatous part of the *Curcuma* species, the aerial part burned in their vegetation field as waste to avoid that, there should be attempts to quantify the pharmaceutical important biomolecules in useful manner using simple and cost-effective methods such as ultraviolet (UV)-visible spectrophotometer and Fourier transform infrared spectroscopy.

Quercetin is a flavonol found in many plants and it is used as an ingredient in various food preparations. Quercetin is synthesized in plants by phenylpropanoid biosynthetic pathway with key enzymes such as phenylalanine ammonia-lyase, cinnamate-4-hydroxylase, and 4-coumaroyl-CoA ligase. It is used for treating high cholesterol, heart disease, diabetes, cataracts, hay fever, peptic ulcer, schizophrenia, inflammation, asthma, gout, viral infections, chronic fatigue syndrome, preventing cancer, and for treating chronic infections of the prostate [16]. Rutin is the glycoside between the flavonol quercetin

and the disaccharide rutinose, and also it is one of the phenolic compounds found in various medicinal plants. Rutin was first isolated from *Ruta graveolens* which is the source of the name. It inhibits the platelet aggregation [17] and decreases capillary permeability, improving blood circulation, anti-inflammatory activity, and it inhibit aldose reductase [18,19]. Gallic acid is a type of phenolic acid and it is formed from 3-dehydroshikimate with shikimate dehydrogenase to produce 3,5-didehydroshikimate of tautomerizes to form the gallic acid [20], and it is found in a number of plants containing high antioxidant activity.

Antioxidants have been widely used as additive to provide protection against oxidative degradation of foods [21]. Although many synthetic chemicals such as flavonoids, phenolic compounds are found to be strong radical scavengers and also, these compounds have serious side effects [22]. In this context, attempts to quantify the quercetin, rutin, and gallic acid in different solvent extract of *C. longa, C. aromatica,* and *C. amada* aerial parts and rhizomatous parts and revealed the free radical scavenging abilities of the extracts as natural sources are important.

The objective of our work was to assess and compare the phytochemical components and antioxidant properties of different solvent extracts of *Curcuma* species aerial parts and rhizomatous parts of *C. longa* L. (turmeric), *C. amada* Roxb (mango-ginger), and *C. aromatica* Salisb (wild turmeric).

METHODS

Collection of the plant samples

The *C. longa*, *C. amada,* and *C. aromatica* species were collected at the Tamil Nadu Agriculture University, Coimbatore, from November 2014 to January 2015, and the species were identified and authenticated by the Botanical Survey of India, Coimbatore - 641 003, Tamil Nadu, India, and the voucher specimen was deposited at the same institute for future reference.

Chemicals

Petroleum ether, chloroform, ethyl acetate, methanol, ethanol, gallic acid (Himedia), quercetin (Himedia), rutin (Himedia), aluminum chloride, potassium acetate, and DPPH were used, and all the chemicals including the solvents were of analytical grade.

Extract preparation

The collected plant materials (aerial part and rhizome) were washed thoroughly in tap water, chopped, air dried for 1-2 weeks at 35-40°C, and pulverized in an electric grinder. The 50 g dry plant materials were taken for sequential extraction with increasing polarity of the solvents (petroleum ether, chloroform, ethyl acetate, and methanol) finally concentrated to get powdered for further quantified and antioxidant studies were carried out [23].

UV-visible spectrophotometer analysis of quercetin, rutin, and gallic acid

The major phytochemicals were screened with respective standard compounds such quercetin, rutin, and gallic acid using UV-visible spectrophotometer of *Curcuma* extracts. Preprepared defatted ethyl acetate, chloroform, and methanol extracts of *C. longa, C. amada,* and *C. aromatica* aerial parts and rhizomes were taken for this analysis and dissolved with respective solvents and the maximum absorbance bands compared with that of standard compounds.

Estimation of total flavonoid content

The content of total flavonoid in the ethyl acetate, chloroform, and methanolic extracts of *C. longa, C. aromatica,* and *C. amada* aerial part and rhizome was assessed [24]. 1 milliliter of each extract was mixed with 0.5 ml of 2% aluminum chloride ethanol solution. After 1 hr incubation at room temperature, the absorbance was measured at 415 nm. A yellow color indicated the presence of flavonoid. The total flavonoid content was calculated as quercetin equivalent (mg GE/g).

Antioxidant activity

DPPH radical scavenging activity

The ability of ethyl acetate, chloroform, and methanolic extracts of C. longa, C. aromatica, C. amada aerial part and rhizome extracts to scavenge DPPH radicals was assessed according to a method described earlier with slight modifications [25]. Briefly, aliquots of the extract 100-500 µg/ml were mixed with 3.0 mL DPPH (0.5 mmol/L in methanol), the resulting absorbance was recorded at 517 nm after 30 minutes incubation at 37°C. The percentage of scavenging activity was derived using the following formula, Percentage of inhibition (%)

$$=[(A_{control}-A_{sample})/A_{control}] \times 100$$

Where $A_{control}$–absorbance of DPPH

A_{sample} - absorbance of reaction mixture (DPPH with Sample).

RESULT AND DISCUSSION

Estimation of total flavonoid content

After evaporation of solvents, the final concentrated extracts were diluted with respective solvents (mg/ml) and taken for total flavonoid analysis with quercetin equivalence. Aliquoted the quercetin (20, 40, 60, 80, and 100 µg/ml) and plotted regression calibration curve found which was Y=0.7245x-0.7641 R^2=0.9857 (Fig. 1). Different extract of *C. longa, C. amada,* and *C. aromatic* aerial parts and rhizome revealed their total flavonoid contents and high amount of flavonoid content was present in ethyl acetate extract of *C. aromatica* rhizome when compared to other extracts. Rhizome extracts contain more flavonoid content than aerial parts extracts (Table 1).

The total flavonoid content were presents in the selected *Curcuma* species in the following order: *C. aromatic* rhizome > *C. aromatic* aerial part > *C. amada* rhizome > *C. amada* aerial part > *C. longa* rhizome > *C. longa* aerial part [26]. Compared to all the extracts *C. aromatica* rhizome contains more flavonoid contents. Flavonoids are one of the most diverse and widespread groups of natural compound contains antioxidants, anticancer, antidiabetic, antiaging, and these compounds prevent cardiovascular disease.

UV-visible spectrophotometer analysis of quercetin, rutin and gallic acid

The quercetin, rutin, and gallic acid were dissolved in methanol and screened their maximum absorbance peak (λ max) values of the substances using UV-visible spectral studies and found to be 375 nm, 257 nm, and 310 nm, respectively. Same manner preprepared ethyl acetate, chloroform, and methanol extracts of *C. longa, C. amada,* and *C. aromatica* aerial parts and rhizomes were dissolved in respective solvents and performed full-length scan against blank between 200 and 800 nm and observed the maximum absorbance peak of the extracts. The absorbance values (λ max) were compared to the standard quercetin, rutin, and gallic acid.

Fig. 2 shows the absorbance bandwidth of the ethyl acetate extract of *Curcuma* species aerial part and rhizomatous parts found to be *C. aromatica* rhizome ethyl acetate extract (CA2REA), *C. aromatica*

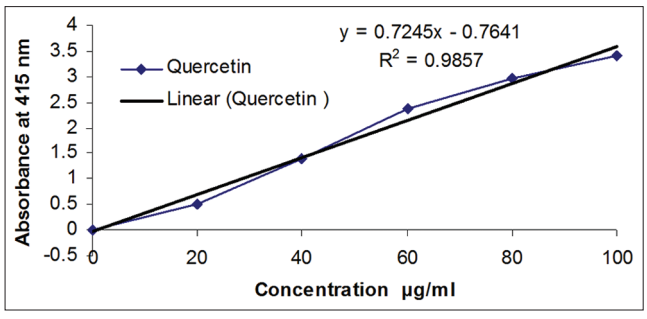

Fig. 1: Total flavonoid content with quercetin equivalent

aerial part ethyl acetate extract (CA2AEA), and *C. amada* aerial part ethyl acetate extract (CA1AEA) contains strong peak values were nearby the quercetin and rutin λ max, it shows these two substances present in the crude extracts. *C. longa* rhizome ethyl acetate extract (CLREA), *C. longa* aerial part ethyl acetate extract (CLAEA) contains weakest peak values were nearby the quercetin and rutin λ max, it shows these two substances present in the extracts with lower concentration.

Fig. 3 shows the chloroform extract of *Curcuma* species was screened with UV-visible spectrophotometer and the absorbance peak values compared to the quercetin, rutin, and gallic acid UV-visible spectra. The result shows *C. longa* aerial part chloroform extract (CLACh), *C. aromatic* aerial part chloroform extract (CA2Ach), and *C. amada* aerial part chloroform extract (CA1Ach) absorbance band exactly match with rutin UV-visible spectra.

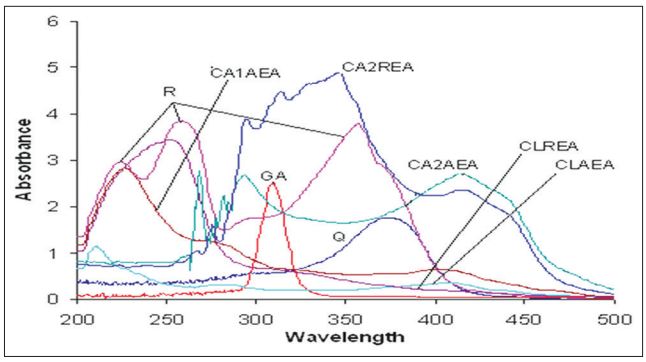

Fig. 2: Ultraviolet-visible spectra of ethyl acetate extracts of *Curcuma* sp.

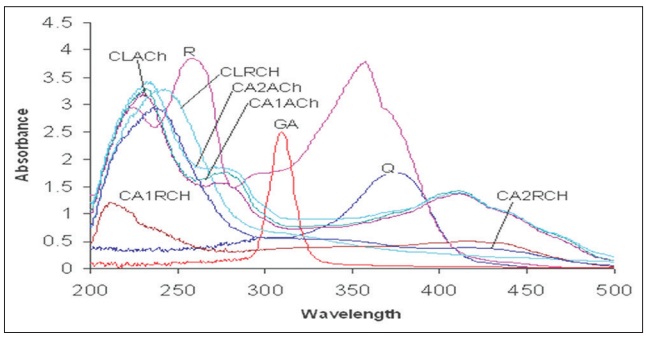

Fig. 3: Ultraviolet-visible spectra of chloroform extracts of *Curcuma* sp.

Fig. 4 shows, *C. aromatica* rhizome methanol extract (CA2RM), *C. aromatica* aerial part methanol extract (CA2AM) UV-visible spectra possess more similar absorbance band to rutin and quercetin, it shows extract contains rutin and quercetin and rest of the UV-visible spectra of extracts show the lowest absorbance values in the respective standard band area.

Antioxidant activity

DPPH radical scavenging activity

The photometric evaluation of the antioxidant capacity of defatted ethyl acetate, chloroform, methanolic extracts of *C. longa, C. aromatica,* and *C. amada* aerial part and rhizome showed good antioxidant capacity (Fig. 5). Significant decreases were observed in preprepared DPPH radical due to the scavenging ability of the extracts [27]. The ability of DPPH radical scavenging is higher in *C. aromatica* rhizome ethyl acetate extract compared to other extracts.

The DPPH free radical scavenging activity of *Curcuma* species possess ability to scavenge DPPH free radicals as equal to the standard antioxidant L-ascorbic acid [30]. It produced hydrazine by converting

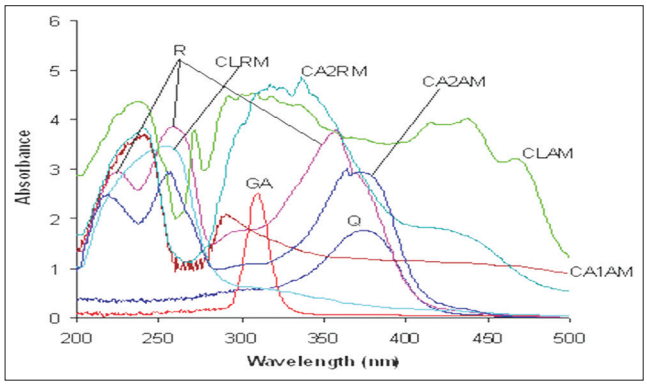

Fig. 4: Ultraviolet-visible spectra of methanol extracts of *Curcuma* sp.

Table 1: Total flavonoid contents of Curcuma species with quercetin equivalence

S.No.	Plant materials	Solvents used for extraction		
		Ethyl acetate µg/mg dry weight	Chloroform µg/mg dry weight	Methanol µg/mg dry weight
1	*Curcuma longa* rhizome	24.15±1.25	6.15±0.12	46.15±1.25
2	*Curcuma amada* rhizome	69.75±1.95	10.15±0.95	69.25±2.15
3	***Curcuma aromatica rhizome***	**88. 35±0.25**	**17.22±1.05**	**75.25±0.95**
4	*Curcuma longa* aerial part	25.55±0.75	1.25±0.05	4.15±0.15
5	*Curcuma amada* aerial part	31.30±1.65	7.45±0.15	38.25±2.95
6	*Curcuma aromatica* aerial part	84.00±1.85	12.35±0.65	14.05±1.65

Table 2: IC_{50} values of *Curcuma* species extracts with DPPH free radical scavenging activity

S.No.	Plant materials	IC_{50} values		
		Ethyl acetate extract (µg/ml)	Chloroform extract (µg/ml)	Methanol extract (µg/ml)
1	*Curcuma longa* rhizome	300.15±1.85	391.15±1.15	415.55±1.45
2	*Curcuma amada* rhizome	91.15±1.15	231.25±1.25	275.13±2.10
3	*Curcuma aromatica* rhizome	**89.40±0.15**	>500	92.35±1.75
4	*Curcuma longa* aerial part	**62.95±1.85**	381.18±1.55	>500
5	*Curcuma amada* aerial part	**61.65±1.75**	250.45±2.15	410.25±1.25
6	*Curcuma aromatica* aerial part	>500	>500	>500
7	Standard L-ascorbic acid	61.15±1.25		

DPPH: 2-2-diphenyl-1-picrylhydrazyl

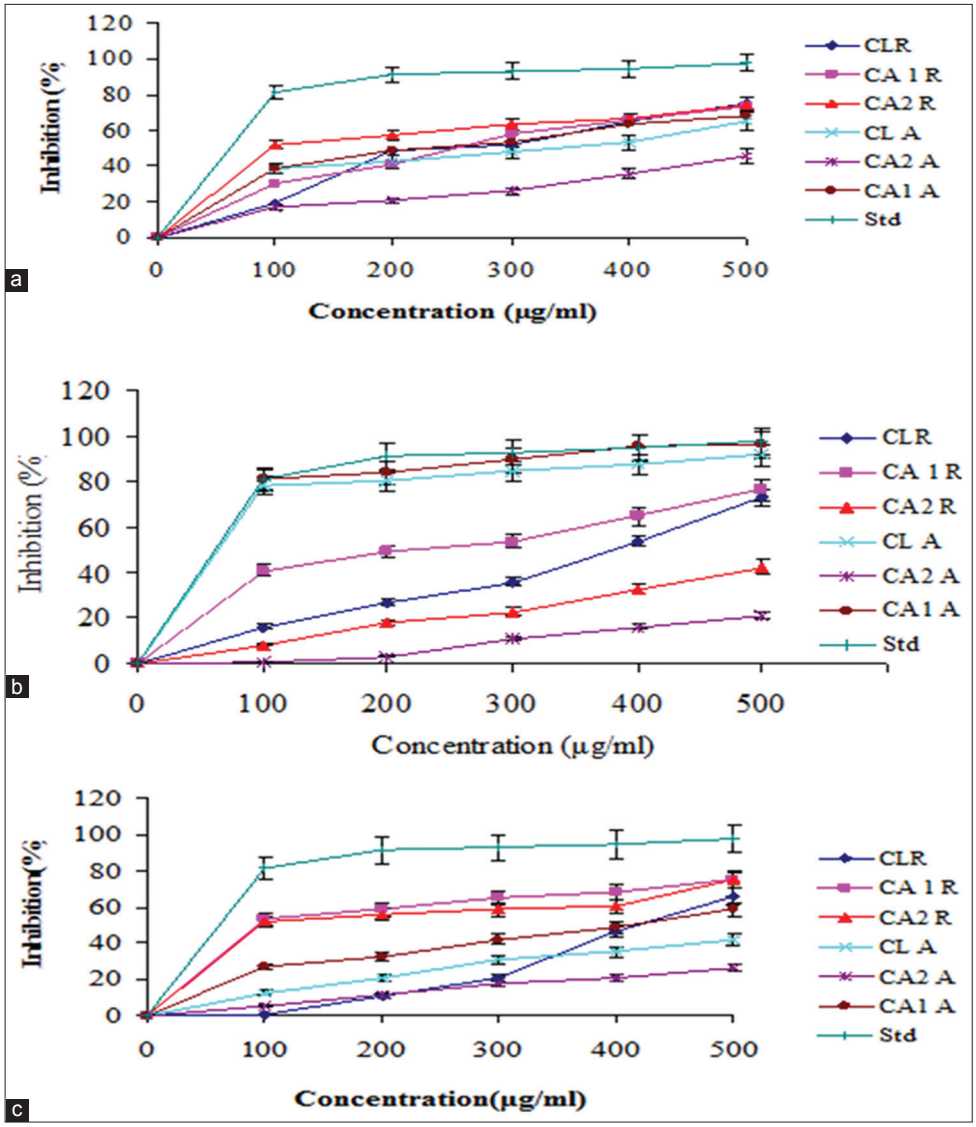

Fig. 5: 2-2-diphenyl-1-picrylhydrazyl free radical scavenging activity of *Curcuma* species (a) ethyl acetate extract, (b) chloroform extract, and (c) methanol extract. (*Curcuma longa* rhizome [CLR], *Curcuma amada* rhizome [CL1R], *Curcuma. aromatica* rhizome [CA2R], *C. longa* aerial part [CLA], *C. amada* aerial part [CA1A], and C. aromatica aerial part [CA1A])

the unpaired electrons to paired electron due to the hydrogen donating ability of the extract [28]. The IC_{50} values were shown in Table 2 and *C. longa* and *C. amada* extracts showed the IC_{50} values very nearer to the standard antioxidant L-ascorbic acid [29].

CONCLUSION

In conclusion, the total flavonoid contents and antioxidant potentials of de-fatted ethyl acetate, chloroform, and methanol extracts of Curcuma longa, Curcuma aromatica, Curcuma amada aerial parts and rhizome were revealed. Among the *Curcuma* species, *C. aromatica* rhizome – ethyl acetate extract was found to be an effective antioxidant with high total flavonoid contents. Ethyl acetate extract of *C. aromatica* rhizome and aerial part contains higher quantity of quercetin and rutin compared to the other extracts. Among the different solvent extracts of each parts, ethyl acetate extracts showed maximum total flavonoid content and high antioxidant capacity than the other extracts such as ethyl acetate > methanol > chloroform.

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