Tamta et al., *Cogent Chemistry* (2016), 2: 1168067
http://dx.doi.org/10.1080/23312009.2016.1168067

cogent chemistry

MEDICINAL CHEMISTRY | RESEARCH ARTICLE

Chemical composition and *in vitro* antioxidant potential of essential oil and rhizome extracts of *Curcuma amada* Roxb

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Abstract: The chemical composition of hydro-distilled essential oil from rhizomes of *Curcuma amada* was analysed. Nineteen compounds representing 77.31% of the total essential oil were identified by GC and GC-MS. Rhizomes were extracted by various solvents with different polarities using various antioxidant assay systems. The petroleum ether extract showed potent DPPH radical scavenging activities (IC_{50} = 18.98 ± 0.05) and reducing power (A_{700} = 0.861 ± 0.001). Ethyl acetate extract exhibited remarkable nitric oxide radical scavenging activity (IC_{50} = 5.97 ± 0.09) higher than that of ascorbic acid (IC_{50} = 6.05 ± 0.02). The essential oil showed promising superoxide radical scavenging activities (IC_{50} = 15.30 ± 0.03 µg/ml) as compared with ascorbic acid (IC_{50} = 15.28 ± 0.01). The results indicate that the oil and organic extracts from rhizomes of *C. amada* could serve as an important bioresource of antioxidants for food and pharmaceutical industries.

Subjects: Bioscience; Food Science & Technology; Health and Social Care

Keywords: *Curcuma amada*; β-myrcene; essential oil; GC-MS; antioxidant activity

Received: 13 January 2016
Accepted: 11 March 2016
First Published: 13 April 2016

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Reviewing editor:
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Anita Tamta a student of G.B.P.U.A&T, Pantnagar pursued her MSc in Biochemistry. Her thrust area for master's thesis was natural products and she worked on the phytochemical analysis and antioxidant activity of *Curcuma amada* of family Zingiberaceae under the co supervision of Dr. Om Prakash (Professor, Chemistry, Corresponding author) who has research experience in the area for more than 15 years and had externally funded national projects, funded by University Grant Commission, New Delhi. His thrust area of research is Natural products. His work in the field can be evidenced by more than 55 papers published in National and international journals of repute. His current research interest focuses mainly on the analysis of bioactive natural compounds and their biological activity determination. In the present study, the essential oil composition of *C. amada* and antioxidant potential of its oil and extracts were studied. So that traditional uses of this herb can be authenticated by scientific outcomes and judicious application of the herb.

PUBLIC INTEREST STATEMENT

The plants of family Zingiberaceae are big repository of secondary metabolites. A number of plants from this family are used in traditional system of medicine for cosmetics, anti-inflammatory, antiulcer, antioxidant and antimicrobial properties .*Curcuma amada* Roxb. is an important aromatic plant in the countries of Indian subcontinent having morphological resemblance with ginger and flavour of raw mango (*Mangifera indica*). The synthetic antioxidant possesses toxic effects including carcinogenic effect. There is intensive search programme throughout the world to look for new natural antioxidants. In the present study, we are reporting *in vitro* antioxidant potential with essential oil composition of *C. amada*. The extracts and essential oil revealed good antioxidant potential. The essential oil possesses β-myrcene as major compound. Besides its medicinal properties, the herb possessing antioxidant potential thus it may be good source as neutraceutical and β-myrcene. It will also be helpful to upgrade the scientific knowledge of traditional people.

1. Introduction

Antioxidants present in vegetables, beverages and fruits were providing health-promoting ingredients in human diet and also responsible for the prevention and treatment of radical-mediated disorderness (Middleton, Kandaswami, & Theoharides, 2000). The usefulness of artificial antioxidants such as butylated hydroxytoulene (BHT) and butylated hydroxyanisole (BHA) are under scrutiny due their suspected role in carcinogenesis (Pokorný, 1991). Thus, there is an urgent need of natural additives as potential antioxidants having an important role in preventing a variety of stress-related diseases (Noguchi & Niki, 1999).

Curcuma amada Roxb. (Zingiberaceae) is a perennial, rhizomatous, aromatic herb commonly known as Amada or "Amahaldi" or "mango ginger" due to the raw mango-like aroma of the rhizome. It is found wild as well as in cultivation in various parts of world. In India, it is cultivated in Gujarat, West Bengal, Uttar Pradesh, Karnataka, Tamil Nadu, Konkan and in the hills of Western coast of India but it is not cultivated anywhere commercially (Ghani, 1998). Mango ginger is used medicinally as a coolant, astringent and to promote digestion. In addition, to this, it is used as a basic ingredient in pickles, preserves, candies, sauces, curries and salads (Verghese, 1990; Shankaracharya, 1982). Its rhizome has carminative properties as well as being useful as a stomachic (Husain et al., 1992). Its rhizome has traditionally been used for healing of wounds, cuts and itching (Srivastava, Srivastava, & Shah, 2001). It possesses antifungal, anti-inflammatory, analgesic, anticancer and antihyperglyceridemic properties (Chowdhury et al., 2015; Ghosh, Gupta, & Chandra, 1980; Mujumdar, Naik, Dandge, & Puntambekar, 2000; Gupta, 2003; Kumari Bai & Shannukanada, 2015). The rhizome extracts of Curcuma were observed to be potent antimutagenic properties based on its antioxidative activity (Pushparani Devi, Mazunder, & Priyadarshini Devi, 2015). Its rhizomes are used for the manufacture of oleoresin, essential oil, etc. (Gupta, 2001).

Therefore, the aim of the present study was to evaluate the chemical composition of the essential oil from the rhizome of *C. amada* by GC/GC–MS and to analyse the antioxidative properties of the essential oil and various organic extracts.

2. Material and methods

2.1. Source of plant material

The rhizomes of *Curcuma amada* were collected from Khatim, Uttarakhand in the month of October 2012 and verified by Dr. D.S. Rawat (Plant taxonomist) G. B. Pant University of Agriculture and Technology, Pantnagar.

2.2. Isolation of essential oils

Fresh crushed rhizomes of *C. amada* subjected to Clevenger's type apparatus for 8 h separately extraction of distillate by dichloromethane followed by drying over anhydrous Na_2SO_4 and removal of solvent yielded 0. 052% of essential oil. Five hundred gram of grinded material was subjected for successive solvent extractions using Soxhlet apparatus. The yields of extracts in different solvents from rhizome have been recorded in Table 1.

2.3. GC analysis of essential oils

GC analysis of the essential oil was performed in Nucon-GC 5765 system.

2.4. GC-MS analysis of essential oils

The GC-MS data were obtained on GC MS-QP 2010 plus with following conditions.

The compounds were identified by matching their mass spectra and GC retention indices with those in NIST-MS Wiley Library, comparing with literature reports and published data (Adams, 1995).

Table 1. Yield of extracts from rhizome of *Curcuma amada*

Extract	Yeild(g)
Petroleum eather	3.00
Cyclohexane	0.81
Ethyleac etate	2.17
Chloroform	2.12
Acetone	1.20
Methanol	2.50

2.5. Determination of antioxidant activity

2.5.1. 2,2′-Diphenyl picryl hydrazyl free radical scavenging activity

This scavenging effect on the DPPH radical was determined according to the methods developed earlier (Cuendet, Hostettmann, Potterat, & Dyatmiko, 1997; Singh, Marimuthu, Murali, & Bawa, 2005; Yen & Duh, 1993). Various amounts of essential oil (5, 10, 15, 20 and 25 µl) and extracts (5, 10, 15 µg and 20 and 25 µg) were mixed with 5 ml of 0.004% methanolic solution of DPPH. Each mixture was placed for 30 min in the dark and the absorbance of the samples was read at 517 nm using UV-spectrophotometer. Fresh DPPH solution was prepared daily, stored in an amberlight bottle in dark at 4°C between the measurements. The control and standard were subjected to the same procedure except for the control, where there was no addition of the sample and for the standards 5, 10, 15, 20 and 25 µg of the sample were replaced with 5, 10, 15, 20 and 25 µg of BHT, catechin and gallic acid. A lower absorbance indicates higher radical scavenging power. DPPH radical scavenging activity was calculated by following equation.

$$\text{DPPH Radical scavenging activity } (\%) = [1 - \frac{A_t}{A_o} \times 100]$$

where A_t is the absorbance of the sample and A_o is the absorbance of the control at 517 nm.

2.5.2. Nitric oxide radical scavenging activity

Nitric oxide (NO) was generated from sodium nitroprusside (SNP) and was measured by the Griess reagent. It is based on the principal that SNP in aqueous solution at physiological pH spontaneously generates NO, which interacts with oxygen to produce nitrite ions that can be estimated by the use of Griess Reagent. Scavengers of NO compete with oxygen leading to reduced production of NO. Two millilitres of SNP (10 mM) in phosphate buffer saline (PBS) pH 7.4 was mixed with different concentrations of extract (5–20 µg/ml) dissolved in acetone and incubated at 25°C for two and half hours. The samples from the above were reacted with 1 ml of Griss reagent (1% sulphanilamide, 0.1% naphthylethylenediamine dichloride and 2 ml orthophosphoric acid). Pink colour will arise. Absorbance was reset at 546 nm. Ascorbic acid was taken as standard (Naskar et al., 2010).

Nitric oxide scavenging activity was calculated by following equation.

$$\text{Nitric Oxide scavenged } (\%) = [1 - \frac{A_t}{A_o} \times 100]$$

where A_t is the absorbance of the sample and A_o is the absorbance of the control at 546 nm.

2.5.3. Super oxide radical scavenging activity

1 ml of Nitroblue terazolium (156 Mm), 1 ml Nicotinamide adenine dinucleotide (reduced) (468 Mm) and 0.1 mL of Phenanzine methosulphate solution (PMS) in 0.1 M of phosphate buffer solution (pH 7.4) were added to 0.1 ml extract of different concentrations (5, 10, 15 and 20 µg) and essential oil

of 5, 10, 15 and 20 µl then incubated at 25°C for 5 min and absorbance was read at 560 nm against blank containing all reagent except PMS. Ascorbic acid was taken as standard (Wei et al., 2010). Super oxide radical scavenging activity was calculated by following equation.

$$\text{Superoxide radical scavenged } (\%) = [1 - \frac{A_t}{A_o} \times 100]$$

where A_t is the absorbance of the sample and A_o is the absorbance of the control at 560 nm.

2.5.4. Hydroxyl radical scavenging activity

The hydroxyl radical scavenging capacity of different polarity extracts was evaluated by the method described by Ramalingam, Sudini, Boddupalli, and Anisetti (2012), Olabinri et al. (2010). 60 µl $FeSO_4.7H_20$ (1 mM), 90 µl aqueous 1,10 Phenanthrolein monohydrate (1 mM), 2.4 (0.2 M) phosphate buffer (pH 7.8) and 150 µl of H_2O_2(.17 mM) in 1.5 ml of different concentrations of essential oils (5, 10, 15 and 20 µl) and extracts (5, 10, 15 and 20 µg). Absorbance was taken at 560 nm. Control of 5, 10, 15 and 20 µl concentration with no extract was taken and different concentrations of ascorbic acid (5, 10, 15 and 20 µg) were used as standard. Hydroxyl radical scavenging activity was calculated by following equation.

$$\text{Hydroxyl radical scavenged } (\%) = [1 - \frac{A_t}{A_o} \times 100]$$

where A_t is the absorbance of the sample and A_o is the absorbance of the control at 560 nm.

2.5.5. Reducing power activity

The reducing power of the essential oil and extracts was determined by the method reported earlier (Yen & Duh, 1993; Singh et al., 2005). Different amounts of essential oils (5, 10, 15 µl and 20, 25 µl) and extracts (5, 10, 15 µg and 20, 25 µg) were mixed with 2.5 ml of the phosphate buffer (200 mM, pH 6.6) and 2.5 ml of 1% potassium ferricynide, $K_3[FeCN_6]$. The mixtures were incubated at 50°C. After incubation, 2.5 ml of 10% trichloroacetic acid was added to the mixtures, followed by centrifugation at 650 rpm for 10 min. The upper layer (5 ml) was mixed with 5 ml of distilled water and 1 ml of 0.1% ferric chloride and absorbance of the resultant solution was measured at 700 nm using UV-spectrophotometer. The control and standard were subjected to the same procedure except for the control, where there was no addition of the sample and for standard 5, 10, 15 µg and 20, 25 µg of the sample were replaced with 5, 10, 15 and 20 µg, 25 µg of BHT, catechin and gallic acid. Absorbance at 700 nm is plotted against the different amounts of essential oils. An increase in the absorbance indicates increase in reducing power.

2.5.6. Effect on the chelating activity of Fe^{2+}

This method is based on the principle of the Fe^{2+-} chelating ability of the antioxidant by measuring the ferrous iron-ferrozine complex formed at 562 nm (Hsu, Chen, Weng, & Tseng, 2003). To different concentrations of oil (5–25 µl) and extract (5–25 µg) were added 0.1 ml of 2 mM $FeCl_2.4H_2O$, 0.2 ml of 5 mM ferrozine and methanol to make up the volume to 5 ml. The solutions were mixed and allowed to react for 10 min. The absorbance at 562 nm was measured; a lower absorbance indicated a higher ferrous iron chelating capacity. The chelating activity on Fe^{+2} of the oil was compared with that of EDTA (0.01 mM) and Citric acid (0.025 M). Chelating activity was calculated by following equation.

$$\text{Chelating activity } (\%) = [1 - \frac{A_t}{A_o} \times 100]$$

where A_t is the absorbance of the sample and A_o is the absorbance of the control at 562 nm.

3. Results and discussion

3.1. GC and GC-MS analysis of essential oils

The hydro-distillation of rhizome of *C. amada* essential oil showed the presence of 65 compounds, of which 19 were identified on the basis of GC and GC-MS analysis. The total identified compounds contribute to 77.31% of the oil. The major constituents identified in the oil are β-myrcene (40%), β-pinene (11.78%), ar-curcumene(10%), camphor (3.21%), E-decahydronepthane (1.77%), α-pinene (1.48%) perillene (1.81%), α-terpinol (1%), safrole (1.03%), α-zingiberene (1.2%), β-elemene (1.0%), β-ocimene (1.01%) and minor constituents are camphene (0.24%), 1,8-cineole (0,06%), Limonene/β-phellandrene (0.27%), Curzerenone (0.36%) caryophyllene oxide (0.22), borneol (0.12%). The composition of essential oil of *C. amada is* presented in Table 2.

The cis- and trans hydro-ocimene, ocimene and myrcene were found to be the major compounds present in the volatile oils of *C. amada*, which indicates that the aroma of mango ginger is a mixture of characteristic compounds found in both raw mango and turmeric reported by Rao, Rajanikanth, and Seshadri (1989). The rhizome essential oil of *Curcuma amada* from Lucknow revealed, ar-curcumene (28.1%), β-curcumene (11.2%), camphor (11.2%) and curzerenone (7.1%), 1,8-cineole (6.0%) as major components (Srivastava et al., 2001). The essential oil of *Curcuma amada* Roxb. contains α-pinene, α- and β-curcumene, camphor, cuminyl alcohol, myristic acid and turmerone. Car-3-ene and cis-ocimene contribute the characteristic mango odour of the rhizome. Its rhizomes yield 1% essential oil containing d-α-pinene 18%, ocimene 47.2%, linalool 11.2%, linalyl acetate 9.1% and safrole 9.3% (Chopra, Nayar, & Chopra, 1980).

3.2. Determination of antioxidant activity

3.2.1. Scavenging activity of DPPH radical

DPPH radical is a very fast method to evaluate the antioxidant activity. It is a stable free radical, which has been widely accepted as a tool for estimating free radical scavenging activities of antioxidants (Sanchez-Moreno, 2002). In the radical form, this molecule had an absorbance at 517 nm which disappeared after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule. The use of DPPH assay provides an easy and a rapid way to evaluate antioxidants by spectrophotometer (Devi, Mazumder, & Devi, 2015). DPPH forms purple colour when dissolved in solution and altered to yellowish colour with interaction of rhizome extract of Curcuma (Pushparani Devi et al., 2015). DPPH radical scavenging activity was studied for essential oil and different extracts of *C. amada* at selected dose levels (Table 3). Lower IC_{50} value indicates higher antioxidant activity. Of all samples studied, the petroleum ether extract had the strongest free radical scavenging activity with an IC_{50} value of 18.98 ± 0.05 in comparison to standard BHT (5.09 ± 0.02). With an increase in the concentration of extracts, an increase in the scavenging activity was observed for all the extracts.

3.2.2. Nitric oxide radical scavenging activity

Nitric oxide (NO) was generated from sodium nitropruisside (SNP) and was measured by the Griess reagent. It is based on the principal that SNP in aqueous solution at physiological pH spontaneously generates NO, which interacts with oxygen to produce nitric ions that can be estimated by the use of Griess reagent. Scavengers of NO compete with oxygen leading to reduction and production of NO (Naskar et al., 2010). The NO radical scavenging activity of extracts in a dose-dependent manner at 546 nm has been presented with respect to the standard antioxidant ascorbic acid (Table 4).The highest nitric oxide radical scavenging activity observed in ethyl acetate extract (5.97 ± 0.09 µg/ml). The order of increasing scavenging activity: Essential oil < Cyclohexane < Petroleum ether < Chlorof orm < Acetone < Ethyle acetate. The present findings imply that organic extracts of *C. amada* are nitric oxide scavengers and their nitric oxide scavenging activity attributed to their antioxidant activity.

Table 2. Composition (%) of *Curcuma amada* Roxb. rhizome essential oil

S.No.	Constituent	Our findings	Choudhury, Rabha, Kanjilal, and Ghosh (1996)	Srivastava et al. (2001)	Singh, Singh, Lampasona, and Catalan (2003)	Dutt and Tayal (1941)
1	Propanone	–	–	–	0.19	–
2	Tricyclene	–	–	–	t	–
3	α-pinene	1.48	0.9	0.4	0.70	18.0
4	Camphene	0.24	–	1.7	0.18	–
5	Sabinene	–	–	–	t	–
6	β-pinene	11.78	4.9	0.6	4.64	–
7	β-myrcene	40.2	88.6	0.2	80.54	–
8	p-cymene	–	–	–	–	–
9	Limonene/ β-phellandrene	0.27	0.1	0.4	0.13	–
10	1,8-cineole	0.06	0.1	6.0	0.06	–
11	(Z)-β-ocimene	–	2.4	–	0.22	47.2 E&Z
12	(E)-β-ocimene	1.01	–	–	1.88	–
13	(E)-decahydro naphthalene	1.77	–	–	–	–
14	2-nonanone	–	–	0.1	–	–
15	Linalool	–	–	0.4	–	11.2
16	Perillene	1.81	0.4	–	1.47	–
17	(E)-thujone	–	–	–	–	–
18	(E)-sabinol	–	–	–	–	–
19	Camphor	3.21	–	11.2	t	–
20	Isoboneol	–	–	4.5	–	–
21	Borneol	0.12	–	1.3	–	–
22	terpinen-4-ol	–	–	0.2	0.09	–
23	α-trepineol	1.00	–	0.8	–	–
24	linalyl acetate	–	–	–	–	9.1
25	bornyl acetate	–	–	–	–	–
26	Safrole	1.03	–	–	–	9.3
27	δ-elemene	–	–	0.2	–	–
28	α-copaene	–	–	–	0.13	–
29	β-elemene	1.00	–	2.8	–	–
30	β-(E)-caryophyllene	0.75	–	0.2	0.53	–
31	β-gurjunene	–	–	–	0.07	–
32	α-(E)-bergamotene	–	–	0.2	–	–
33	α-humulene	–	–	–	0.05	–
34	ar-curcumene	10.00	–	28.1	–	–
35	β-selinene	–	–	0.6	–	–
36	α-selinene	–	–	0.5	–	–
37	α-zingiberenne	1.2	–	1.4	–	–
38	α-muurolene	–	–	–	0.07	–
39	β-curcumene	–	–	11.2	–	–

(*Continued*)

Table 2. (*Continued*)

S.No.	Constituent	Our findings	Choudhury, Rabha, Kanjilal, and Ghosh (1996)	Srivastava et al. (2001)	Singh, Singh, Lampasona, and Catalan (2003)	Dutt and Tayal (1941)
40	Cubebol	–	–	–	–	–
41	Spathulenol	–	–	0.3	–	–
42	caryophyllene oxide	0.22	–	0.5	0.34	–
43	Curzerenone	0.36	–	7.1	0.14	–
44	α-muurolol	–	–	–	0.04	–
45	β-bisabolol	–	–	0.5	–	–
46	Germacrone	–	–	0.3	–	–
47	Zerumbone	–	–	0.2	–	–
48	Others	22.69	2.6	18.1	8.53	5.2
Total %		77.31	74	81.9	91.47	94.8

Note: *t* = trace (>0.1%).

Table 3. Free radical scavenging activity (DPPH) of the extracts and essential oil *Curcuma amada*

Sample	IC50 (µg/ml)
Petroleum ether extract	18.98 ± 0.05
Cyclohexane extract	21.19 ± 0.08
Ethyleacetate extract	26.14 ± 1.95
Chloroform extract	23.74 ± 0.26
Acetone extract	22.01 ± 1.26
Methanol extract	25.06 ± 0.07
Essential oil	25.06 ± 0.07
BHT	5.09 ± 0.02
Catechin	7.53 ± 0.14
Gallic acid	8.46 ± 0.11

Note: Values are given as mean ± SD of triplicate experiments.

Table 4. Nitric oxide radical scavenging activity of the extracts and essential oil of *C. amada*

Sample	IC50 (µg/ml)
Petroleum ether extract	11.14 ± 0.32
Cyclohexane extract	15.74 ± 0.13
Ethyleacetate extract	5.97 ± 0.09
Chloroform extract	7.23 ± 0.23
Acetone extract	7.18 ± 0.05
Methanol extract	6.41 ± 0.04
Essential oil	18.40 ± 0.01
Ascorbic acid	6.05 ± 0.02

Note: Values are given as mean ± SD of triplicate experiments.

Table 5. Superoxide radical scavenging activity of the extracts and essential oil of *C. amada*

Sample	IC50 (µg/ml)
Petroleum ether extract	19.63 ± 0.05
Cyclohexane extract	17.65 ± 0.11
Ethyleacetate extract	20.49 ± 0.05
Chloroform extract	18.68 ± 0.05
Acetone extract	18.60 ± 0.06
Methanol extract	16.97 ± 0.03
Essential oil	15.30 ± 0.03
Ascorbic acid	15.28 ± 0.01

Note: Values are given as mean ± SD of triplicate experiments.

Table 6. Hydroxyl ion radical activity of the extracts and essential oil *C. amada*

Sample	IC50 (µg/ml)
Petroleum ether extract	12.84 ± 0.04
Cyclohexane extract	17.52 ± 0.21
Ethylacetate extract	19.27 ± 2.42
Chloroform extract	16.70 ± 0.30
Acetone extract	11.47 ± 0.14
Methanol extract	9.30 ± 2.00
Essential oil	12.84 ± 0.04
Ascorbic acid	7.92 ± 0.37

Note: Values are given as mean ± SD of triplicate experiments.

3.2.3. Superoxide radical scavenging activity

Superoxides are produced from molecular oxygen due to oxidative enzymes of body as well as via non enzymatic reactions such as auto-oxidation by catecholamines. The presence of flavanoids in the rhizome of *C. amada* is responsible for the antioxidant activity and the extracts possess many free hydroxyl substitution, which might have great antisuperoxide properties (Siddhuraju, Mohan, & Becker, 2002). In the PMS/NADH-NBT system, superoxide anion derived from dissolved oxygen by PMS/NADH coupling reaction reduces NBT. The decrease in the absorbance at 560 nm with antioxidants thus indicates the consumption of superoxide anion in the reaction mixture. All the extracts exhibited strong superoxide radical scavenging activities. The highest superoxide radical scavenging activities were observed in essential oil (IC_{50} = 15.30 ± 0.03 µg/ml) compared to that of standard (Table 5). The order of increasing superoxide scavenging activity: Ethyl acetate < Petroleum ether < Chloroform < Acetone < Cyclohexane < Methanol < essential oil.

3.2.4. Hydroxyl ion radical scavenging activity

Hydroxyl radicals subtract hydrogen atoms from lipids, which after reaction with oxygen gives peroxyl radicals initiating lipid peroxidation. They are also causing chemical modifications in sugars, purines and pyrimidines, resulting in DNA mutations, as occur with the formation of 8-hydroxydeoxyguanosine (8-OHdG) from 2-deoxyguanosine (Herraiz & Galisteo, 2015). Hydroxyl radical is an extremely reactive oxidizing radical that will react with most biomolecules at diffusion controlled rates. The highest scavenging activity observed in methanol extract (9.30 ± 2.00 µg/ml) as compared to standard (Table 6). The order of increasing hydroxyl ion scavenging activity: Ethyleacetate < Cycl ohexane < Chloroform < Petroleum ether/Essential oil < Acetone < Methanol.

Table 7. Chelating activity of the extracts and essential oil of *C. amada*

Sample	IC50 (µg/ml)
Petroleum ether extract	20.72 ± 0.10
Cyclohexane extract	8.30 ± 0.13
Ethyl acetate extract	4.52 ± 0.18
Chloroform extract	3.21 ± 0.56
Acetone extract	9.71 ± 0.16
Methanol extract	57.17 ± 2.00
Essential oil	9.01 ± 0.47
EDTA	11.56 ± 0.08
Citric acid	4.97 ± 0.16

Note: Values are given as mean ± SD of triplicate experiments.

Table 8. Reducing power of the extracts and essential oil *C. amada*

Sample	Reducing power (%)	
	Dose level (5 µl/µg)/ml	Dose level (25 µl/µg)/ml
Petroleum ether extract	0.347 ± 0.001	0.831 ± 0.001
Cyclohexane extract	0.136 ± 0.002	0.310 ± 0.001
Ethyleacetate extract	0.165 ± 0.003	0.509 ± 0.002
Chloroform extract	0.280 ± 0.002	0.610 ± 0.003
Acetone extract	0.135 ± 0.001	0.262 ± 0.003
Methanol extract	0.151 ± 0.002	0.300 ± 0.001
Essential oil	0.184 ± 0.001	0.315 ± 0.004
Catechin	0.455 ± 0.006	0.623 ± 0.004
Gallic acid	0.575 ± 0.003	0.715 ± 0.003

Note: Values are given as mean ± SD of triplicate experiments.

3.2.5. Effect on the chelating activity of Fe^{2+}

Ferrozine, a chelating reagent, was used to indicate the presence of chelator in the reaction type. Ferrozine forms a complex with free Fe^{3+} but not with Fe^{2+}. In presence of chelating agents, the complex formation between ferrous and ferrozine is disturbed, resulting in decrease of the colour of the complex. Measurement of colour reduction therefore allows estimating the metal chelating activity of the coexisting chelator (Yamaguchi, Ariga, Yoshimura, & Nakazawa, 2000).

The chelating activity of Fe^{2+} of the essential oil and the organic extracts is shown in Table 7. The highest chelating activity was observed in Chloroform extract (3.21 ± 0.56) followed by Ethyl acetate (4.52 ± 0.18). Moderate activity was observed Cyclohexane (8.30 ± 0.13), Acetone (9.71 ± 0.16), Essential oil (9.01 ± 0.47) and Petroleum ether extract (20.72 ± 0.10) as compared to known standards, while methanol extract showed little chelating activity (57.17 ± 2.00) as compared to positive controls, Citric acid (4.97 ± 0.16), EDTA (11.56 ± 0.08). There are several examples of the photochemical activity of Fe(III) complexes such as irradiation of Fe(III) complexes with various carboxylic organic acids by visible light results in electron transfer from the ligand to the Fe(III) ion and subsequently the formation of a redox-active Fe(II) ion and a set of free radicals (Timoshnikov, Kobzeva, Polyakov, & Kontoghiorghes, 2015).

3.2.6. Reducing power activity

The extracts exhibited reducing power activity with different potentials. The maximum reducing power was observed in Petroleum ether (A_{700}=0.861 ± 0.001), followed by Chloroform (A_{700} = 0.610 ± 0.003), Ethyl acetate (A_{700} = 0.509 ± 0.002), Cyclohexane (A_{700} = 0.310 ± 0.001),

Methanol (A_{700} = 0.300 ± 0.001) and Acetone (A_{700} = 0.262 ± 0.003) at 25 µg in comparison to the standards BHT (A_{700} = 0.550 ± 0.008 – 0.735 ± 0.009), Catechin (A_{700}= 0.455 ± 0.006 – 0.623 ± 0.004) and Gallic acid (A_{700} = 0.575 ± 0.003 – 0.715 ± 0.003) (Table 8). Essential oil exhibited moderate reducing power in a dose-dependent manner with maximum power (0.315 ± 0.004) for 25 µl and minimum (0.184 ± 0.001) for 5 µl dose at 700 nm with respect to standard antioxidants BHT, Gallic acid and Catechin. It has been indicated that the antioxidant potential of certain compounds is related to their reducing power (Siddhuraju et al., 2002) and serve as an important indicator of prospective antioxidant activity in a plant extracts. Determination of the ferric reducing antioxidant power is a simple direct test of antioxidant capacity. In this study, assay of reducing activity was based on the reduction of ferric to the ferrous form in the presence of reductants (antioxidants) in the tested samples. The Fe^{2+} was then monitored by measuring the formation of Perl's Prussian blue at 700 nm. The reducing power of a compound may serve as a significant indicator of its potential antioxidant activity.

4. Conclusion

The study revealed the potential antioxidant and radical scavenging activity of organic extracts and essential oil of *Curcuma amada* rhizomes indicates its protective role against oxidative damage and as an important natural antioxidant. The curcumis of curcuminoide family found in spice turmeric is a potent antioxidant scavenging ROS and induced antioxidant response (Elsayed, 2016). The residual oil and extract due to its antioxidant activity could be utilized in pharmaceutical sector and food industries. Further research in this direction will be utilized for strengthening its real potential in various sectors.

Funding

This work was supported by University Grants Commission [F. No. 41-234/2012(SR)].

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Citation information

Cite this article as: Chemical composition and *in vitro* antioxidant potential of essential oil and rhizome extracts of *Curcuma amada* Roxb, Anita Tamta, Om Prakash, H. Punetha & A.K. Pant, *Cogent Chemistry* (2016), 2: 1168067.

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