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

Anita Tamta¹, Om Prakash²*, H. Punetha¹ and A.K. Pant²

**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 (IC₅₀ = 18.98 ± 0.05) and reducing power (A₇₀₀ = 0.861 ± 0.001). Ethyl acetate extract exhibited remarkable nitric oxide radical scavenging activity (IC₅₀ = 5.97 ± 0.09) higher than that of ascorbic acid (IC₅₀ = 6.05 ± 0.02). The essential oil showed promising superoxide radical scavenging activities (IC₅₀ = 15.30 ± 0.03 μg/ml) as compared with ascorbic acid (IC₅₀ = 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.

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

**ABOUT THE AUTHORS**

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 nutraceutical 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 ingredi-
ents in human diet and also responsible for the prevention and treatment of radical-mediated dis-
orderliness (Middleton, Kandaswami, & Theoharides, 2000). The usefulness of artificial antioxidants
such as butylated hydroxytoluene (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 rhi-
zome. 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 stom-
achic (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 antihyperglycemic 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 ex-
traction of distillate by dichloromethane followed by drying over anhydrous Na₂SO₄ 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).
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).

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

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 Phenazine 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.

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), Olabini et al. (2010). 60 μl FeSO₄.7H₂O (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 H₂O₂(.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.

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 ferricyanide, K₃[Fe(CN)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 Fe²⁺

This method is based on the principle of the Fe²⁺-chelating ability of the antioxidant by measuring the ferrous iron-ferrrozine 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 FeCl₂. 4H₂O, 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 Fe²⁺ 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.

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-decahydronephane (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 IC₅₀ value indicates higher antioxidant activity. Of all samples studied, the petroleum ether extract had the strongest free radical scavenging activity with an IC₅₀ 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 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 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 < Chloroform < 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.
| 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    | Isoborneol                | –            | –                                            | 4.5                      | –                                         | –                    |
| 21    | Borneol                   | 0.12         | –                                            | 1.3                      | –                                         | –                    |
| 22    | terpinen-4-ol             | –            | –                                            | 0.2                      | 0.09                                      | –                    |
| 23    | α-trepenol                | 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)-bergomotene         | –            | –                                            | 0.2                      | –                                         | –                    |
| 33    | α-humulene                | –            | –                                            | –                        | 0.05                                      | –                    |
| 34    | α-curcumumene             | 10.00        | –                                            | 28.1                     | –                                         | –                    |
| 35    | β-selinene                | –            | –                                            | 0.6                      | –                                         | –                    |
| 36    | α-selinene                | –            | –                                            | 0.5                      | –                                         | –                    |
| 37    | α-zingiberene             | 1.2          | –                                            | 1.4                      | –                                         | –                    |
| 38    | α-muuralene               | –            | –                                            | –                        | 0.07                                      | –                    |
| 39    | β-curcumumene             | –            | –                                            | 11.2                     | –                                         | –                    |

(Continued)
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.
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 (IC₅₀ = 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 < Petroleum ether/Essential oil < Acetone < Cyclohexane < Methanol < essential oil.

| Sample           | IC₅₀ (μg/ml) |
|------------------|-------------|
| Petroleum ether extract | 19.63 ± 0.05 |
| Cyclohexane extract         | 17.65 ± 0.11 |
| Ethylacetate 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.

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: Ethylacetate < Cyclohexane < Chloroform < Petroleum ether/Essential oil < Acetone < Methanol.

| 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.
3.2.5. 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), Methanol (57.17 ± 2.00) 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<sub>700</sub> = 0.300 ± 0.001) and Acetone (A<sub>700</sub> = 0.262 ± 0.003) at 25 μg in comparison to the standards BHT (A<sub>700</sub> = 0.550 ± 0.008 – 0.735 ± 0.009), Catechin (A<sub>700</sub> = 0.455 ± 0.006 – 0.623 ± 0.004) and Gallic acid (A<sub>700</sub> = 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<sup>2+</sup> 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 curcumin 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.

References
Adams, R. P. (1995). Identification of essential oil components by gas chromatography/mass spectroscopy. Carol Stream, IL: Allured Publishing.

Chopra, R. N., Nayar, S. L., & Chopra, I. C. (1980). Glossary of Indian Medicinal Plants CSIR. New Delhi: Council of Scientific & Industrial Research.

Choudhury, S. N., Rabha, L. C., Kanjilal, P. B., & Ghosh, A. C. (1996). Essential oil of Curcuma amada Roxb. from Northeastern India. Journal of Essential Oil Research, 8, 79–80. http://dx.doi.org/10.1080/10412905.1996.9700560

Chowdhury, F. H., Mohammad, Al-Amin, Kazi, M. R., Sarker, A., Alam, M. M., Chowdhury, M. H., ... Sultana, G. N. (2015).

Dictionary of Indian medicinal plants (546 p.). Lucknow: CIMAP.
Kumari Bai, C., & Shannukananda, P. (2015). Analgesic activity of aqueous extract of Curcuma amada (mango-ginger) in male albino winster rats. Journal of Evidence Based Medicine & Healthcare, 2, 6662 p.

Middleton, E. J., Kandaswami, C., & Theoharides, T. C. (2000). The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease, and cancer. Pharmacological Reviews, 52, 673–751.

Mujumdar, A. M., Naik, D. G., Dandge, C. N., & Puntambekar, H. M. (2000). Anti-inflammatory activity of Curcuma amada Roxb. in albino rats. Indian Journal of Pharmacology, 32, 375–377.

Naskar, S., Islam, A., Mazumdar, U. K., Saha, P., Halder, P. K., & Gupta, M. (2010). In vitro and in vivo antioxidant potential of hydro-methanolic extract of Phoenix Dactylifera. Journal of Scientific Research, 5, 102–105.

Pokorny, J. (1991). Natural antioxidants for food use. Trends in Food Science & Technology, 2, 223–227.

Pushparani Devi, H., Mazunder, P. B., & Priyadarshini Devi, L. (2015). Antioxidant and antimutagenic activity of Curcuma caesia Roxb, rhizome extracts. Toxicology Reports, 2, 423–428.

Ramalingam, R., Sudini, S., Boddupalli, I. M., & Anisetti, R. N. (2012). Antioxidant, free radical scavenging and in vitro cytotoxic studies of ethanolic extracts of Leucas indica var. lavandulifolia and Leucas indica var. nagalpuramiana. Asian Pacific Journal of Tropical Biomedicine, 2, S1637–S1642.

Rao, A. S., Rajanikanth, B., & Seshadri, R. (1989). Volatile aroma components of Curcuma amada Roxb. Journal of Agricultural and Food Chemistry, 37, 740–743.

Sanchez-Moreno, C. (2002). Review: Methods used to evaluate the free radical scavenging activity in foods and biological systems. Food Science and Technology International, 8, 121–137.

Shankaracharya, N. B. (1982). Mango ginger. Indian Cocoa. Areca nut Spices Journal, 5, 78–80.

Singh, G., Singh, D. P., Lamposano, M. P., & Catalian, C. (2003). Curcuma amada Roxb – Chemical composition of rhizome oil. Indian Perfumer, 47, 143–146.

Singh, G., Marimuthu, P., Murali, H. S., & Bawa, A. S. (2005). Antioxidative and antibacterial potentials of essential oils and extracts isolated from various spice materials. Journal of Food Safety, 25, 130–145.

Srivastava, A. K., Srivastava, S. K., & Shah, N. C. (2001). Constituents of the rhizome essential oil of Curcuma amada Roxb. from India. Journal of Essential Oil Research, 13, 63–64.

Timoshnikov, V. A., Kobzeva, T. V., Polyakov, N. E., & Kostogriphos, G. J. (2015). Inhibition of Fe(II) and Fe(III)-induced hydroxyl radical production by the iron-chelating drug deferiprone. Free Radical Biology and Medicine, 78, 118–122.

Yen, G. C., & Duh, P-D. (1993). Antioxidative properties of methanolic extracts from peanut hulls. Journal of the American Oil Chemists’ Society, 70, 383–386.