Antioxidant and Phytochemical Studies of the Rhizome Extracts of Curcuma amada Roxb and Zingiber officinale Rosc

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Abstract Various reactive species which are either radicals or non radicals formed during normal metabolic processes. Oxidative stress has been identified as a major causative factor in the development and progression of several life threatening diseases, including neurodegenerative and cardiovascular disease, Antioxidants play a vital role in preventing or delaying oxidation and scavenging free radicals. Plants have been used as exogenous antioxidants from several years. In this contest, the rhizome extracts of two important medicinal plants Curcuma amada Roxb and Zingiber officinale Rosc were investigated for antioxidant properties and phytochemical constituents. Antioxidant capacity of different solvent extracts of these plants was estimated by scavenging diphenyl picryl hydrazyl (DPPH), nitric oxide (NO) and hydrogen peroxide. The petroleum ether and alcohol extracts of Curcuma amada showed stronger antioxidant activity with IC50 values of 26-30µg/ml and 25-29µg/ml, respectively in all the methods while methanol extract showed moderate activity, while petroleum ether and ethanol extracts Zingiber officinale reported good scavenging activity in all the three methods with IC50 ranging from 25-30µg/ml except hydroxyl radical scavenging method. Petroleum ether extracts of both the test plants exhibited good inhibition of free radicals generated by DPPH, hydrogen peroxide and nitric oxide when compared to standard ascorbic acid. The phenolic substances are commonly present in all the studied extracts. Among the two medicinal plants tested, Zingiber officinale has better scavenging activity in all the performed methods followed by Curcuma amada. In the present study, potent antioxidant activity of Zingiber officinale and Curcuma amada extracts leads to scientific validation of these plants. A natural substance which is a part of daily diet and nutritional supplement with antioxidant property constitutes a new source of herbal drug.

Keywords Antioxidants, Free Radicals, Extracts, Solvents, Phytochemicals

1. Introduction

Oxygen in ground state is less reactive, but active oxygen species in other forms such as single oxygen, super oxide radical anion, hydrogen peroxide, etc., are greatly toxic and produce oxidative stress in plants [1]. They are continuously produced by a body via enzymatic and non-enzymatic reactions like respiratory chain reaction, phagocytosis and prostaglandin synthesis.
Oxidative stress due to free radical has been identified a major source for developing to exert a toxic effect in turn to cause various diseases such as neurodegenerative and cardiovascular problems [2]. The free radicals are deactivated by antioxidants. Antioxidants play a vital role in preventing or delaying oxidation, even at relatively small concentration and thus have various physiological roles in plant body [3].

Antioxidants are materials at relatively low concentrations significantly inhibit the role of oxidation at targets. Due to continuous generation of partially reduced form of oxygen by constitutive metabolic pathways, a number of protective antioxidant enzymes such as, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHPX), glutathione reductase (GSHRX), glutathione-s-transferase (GST) and non-enzymatic antioxidants are required to deal with toxic species [4]. The use of synthetic antioxidants such as Butylated hydroxyanisole (BHA) and butylated hydroxyltoluene (BHT) has to be thought twice due to its carcinogenicity [5]. Hence, it is better to turn on to natural compounds especially derived from plants and dietary sources that provide a large number of antioxidants [6]. Plants are good source of exogenous antioxidants. About two-thirds of the plant species in the world have medicinal properties, and many of these have excellent antioxidant capacity [7]. Hence antioxidant potential of plants has received a great deal of attention. Moreover, many plants are considered good source of nutrition and hence they are recommended for their therapeutic values [8].

Dietary materials which are antioxidants play an important role in one’s overall health. The human body needs more antioxidants than it has the ability to produce, thus a regular supply of antioxidants is necessary for the body in order to protect it against cellular damage caused by oxidative stress. Antioxidants are abundantly found in vegetables, fruits, leaves, oilseeds, cereal crops etc. [9]. The literature reports the antibacterial and antioxidant activity of various rhizome extracts. In this contest, the rhizome extracts of two important medicinal plants Curcuma amada Roxb and Zingiber officinale Rosc were investigated for antioxidant properties and phytochemical constituents.

Curcuma amada Roxb plant of the family Zingibaraceae is an important spice. It is commonly called Mango ginger. It is also known as amra haridra, amahaldi, taldiha, banahaldi in different regions. Mango ginger is a perennial herb which is morphologically similar to the ginger but with a mango taste. Rhizomes of mango ginger are mainly used for making pickles because of its mango taste. The major constituents found in its rhizomes are curcuminoids, phenolic compounds and essential oils [10].

Curcuma amada Roxb is a perennial, rhizomatous, aromatic herb plant growing around 80 cm tall and rhizomes are of mild yellow colour. The plants are adapted to growing in areas of seasonal drought in monsoonal forests, in which they prefer a humus-rich, moist but well-drained soil in a shaded position. It is found wild as well as cultivated in various parts of world. In India, the plant is cultivated in tropical areas of Gujarat, West Bengal, Uttar Pradesh, Karnataka, Tamilnadu, Konkan, and in the hills of western coast of India. Mature Mango ginger plants can be harvested after 6 months of germination. Its rhizome used as a mild ginger – flavoured spice in pickle [11]. It has numerous biological activities such as antioxidant, antibacterial, anti-inflammatory, antifungal and analgesic. In Ayurveda and Unani medicine, rhizome of mango ginger is used for treating coughs and other chest complains such as bronchitis and a wide range of digestive problems such as stomach pain, loss of appetite, indigestion, for healing of wounds, cuts itching in skin diseases, asthma and inflammation. Also, it is used as carminative, expectorant, appetizer and laxative [12].

Zingiber officinale Rosc is an important spice of the family Zingibaraceae. It is commonly called ginger. It is cultivated in tropical and subtropical countries of the world, while in India in the states like Kerala, Arunachal Pradesh, Orissa, Meghalaya, West Bengal, Tamil Nadu, Andhra Pradesh and Karnataka which is a rain fed crop propagated by seed tubers and should be harvested from the fifth month after sowing. Ginger is a plant whose rhizome is widely used as a spice for folk medicines and considered a universal medicine by India’s ayurvedic herbalists. Its sweet taste has made it a popular herb and used in breads, candies, and tonics. Ginger is known to work as a digestive aid by increasing the production of digestive fluids and saliva, which helps to relieve indigestion, gas pains, diarrhoea and stomach cramping. It is to be strong antioxidant and good antimicrobial agent for sores, wounds and protects the liver and stomach, also useful for bowel disorders, circulatory problems, arthritis, fever, headache, motion sickness, hot flashes, muscle pains, nausea and vomiting [12].

2. Materials and Methods

2.1. Collection and Extraction of Plant Material

Rhizome of two medicinal plants Curcuma amada and Zingiber officinale were selected for the determination of antioxidant activity. The Curcuma amada and Zingiber officinale rhizome (Figure 1) were collected that were grown in and around Mysuru. The rhizome were washed, cut into small pieces and dried for few days in shade at room temperature. Air dried rhizome were powdered using a warring blender.
Solvent extraction was carried out using soxhlet apparatus. Powdered material (100 mg) was placed in a porous thimble in the upper chamber and in the lower boiling flask; the extracting solvent (200 ml) was added. The flask was heated by temperature controlled heating mantle. The round bottom flask was filled with different solvents in the following order petroleum ether, methanol and ethanol based on polarity from low to high. The temperature was set up based on the boiling point of the solvents. The solvent was heated to reflux and extracted. The sample filled in the thimble was extracted till colorless extract was obtained on the top of the extractor. The collected extract was re-concentrated separately for testing by the reduced pressure. After complete evaporation of the solvent, each of the solvent extracts was weighed and preserved in brown air tight bottle.

2.2. Determination of Antioxidant Activity

Antioxidant capacity of different extracts of was estimated by scavenging diphenyl picryl hydrazyl (DPPH), nitric oxide (NO) and hydrogen peroxide.

2.3. DPPH Radical Scavenging Assay

Free radical- scavenging action of the different extracts was measured in terms of hydrogen donating or radical-scavenging capacity using stable radical DPPH as described by Blois method [13]. The stock solutions were prepared by dissolving 0.001 g of the extracts in one ml of DMSO. Different concentrations i.e., 20, 40, 60, 80 and 100 μg of stock solution were made up to 2 ml with methanol. Methanolic solution of the DPPH (0.1 mmol) was prepared and a volume of 1 ml of the same was added separately to each of above for testing solutions. The mixture of solution and extract was shaken strongly, incubated for 30 min and then the absorbance was recorded at 517 nm. Each experiment was performed in triplicates and values are reported as the mean ± Standard Deviation (SD). Ascorbic acid (AA) was used as a standard parallel to the test compound and DMSO served as a negative control. The ability of the extracts to scavenge the DPPH radical was calculated using the following equation:

\[ \text{DPPH scavenging effect (\%)} = \left( \frac{A_c - A_b}{A_c} \right) \times 100 \]

where \( A_c \) and \( A_b \) are the absorbance values of the control and test sample.

2.4. Hydroxyl Radical Scavenging Assay

Capability of the compounds to effectively scavenge hydrogen peroxide was determined by the method of Ruch et al. [14] and compared with butylated hydroxyanisole (BHA) which is used as a standard. The Fenton system generated hydroxyl radical (OH·) in aqueous media. The stock solutions were prepared by dissolving 0.001 g of the extracts in one ml of DMSO. The assay mixture (5ml) contained following reagents: safranin (11.4μmol), EDTA–Fe(II) (40μmol), \( \text{H}_2\text{O}_2 \) (1.76μmol), the extract solution (4, 8, 12, 16 and 20μl) and a phosphate buffer (0.067 mol, pH 7.4). The assay mixtures were incubated at 37°C for 30 min in water. The scavenging effect of the extracts on hydroxyl radicals generated by Fenton’s system is quantified spectrophotometrically at 532 nm. Butylated
hydroxyanisole was used as a standard. Each experiment was done in triplicates and values are reported as the mean ± standard deviation (SD). The suppression ratio for OH⁻ was calculated using the following formula:

\[
\text{Suppression ratio} (\%) = \left( \frac{A_0 - A_i}{A_0} \right) \times 100
\]

where \(A_0\) = Absorbance of the control; \(A_i\) = Absorbance of the test compound/extract.

2.5. Nitric Oxide Scavenging Assay

Nitric oxide radical scavenging capacity was determined according to the method of Green et al. [15]. Griess reagent was used to quantify the nitrite, which is produced by the nitroprusside at physiological pH. Sodium nitroprusside 10 mM (1.5 ml) in phosphate buffer (pH 7.4) was mixed with various concentrations (20, 40, 60, 80 and 100 µg) of 1 ml extract and the mixture was incubated at 25°C for 150 min and sodium nitroprusside spontaneously generates nitric oxide during this duration. After incubation, 1.5 ml Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% naphthylethylenediamine dihydrochloride) was added and incubated at room temperature for 30 min. The final volume of the test solution was made-up to 4 ml with the phosphate buffer (pH 7.4). The reduced nitrite ions are quantified by the detection of active secondary radical scavenging by different extracts of Curcuma amada (Table 2).

\[
\text{Nitric oxide scavenging effect} (\%) = \left( \frac{A_c - A_i}{A_c} \right) \times 100
\]

\(A_c\) = absorbance in presence of sample of extract

2.6. Phytochemical Analysis

Phytochemical analysis of extracts was carried out for the detection of active secondary metabolites or different constituents such as tannins, alkaloids, flavonoids, terpenoids, steroids, carbohydrates, proteins and saponins. The extracts were reconstituted in methanol and 1 ml of each extract was subjected to standard phytochemical analysis according to the procedure described by Harborne [16].

3. Results

3.1. Yield of the Solvent Extract

Among the solvent extracts, yield was very good and higher in methanol extract (105.4 g/kg and 102.6 g/kg) than ethanol and petroleum ether extracts in both the screened plants (Table 1).

### Table 1. Yield of the solvent extracts

| Plants          | Solvent Extract | Yield per kg |
|-----------------|-----------------|-------------|
| Curcuma amada   | Petroleum ether | 19.8 g      |
|                 | Methanol        | 105.4 g     |
|                 | Ethanol         | 13.4 g      |
|                 | Petroleum ether | 16.3 g      |
| Zingiber officinale | Methanol      | 102.6 g     |
|                 | Ethanol         | 12.3 g      |

3.2. Antioxidant Activity of Curcuma amada

Free radical scavenging activity of the extracts was evaluated by hydrogen donating ability using stable radical DPPH. The ability of the extracts to neutralize hydroxyl radical was expressed as 50% inhibitory concentration (IC₅₀) in µg/ml. The alcohol extract of Curcuma amada was most active with IC₅₀ value at 25µg/ml. Petroleum ether and methanol extracts showed good activity with IC₅₀ values at 30µg/ml and 29µg/ml, respectively. So, all the extracts showed higher radical scavenging activity compared to standard ascorbic acid (33µg/ml). In hydroxyl radical scavenging assay, hydroxyl radical generated through Fenton system in aqueous media was inhibited by the scavenging activity of the extract which is expressed as IC₅₀ values and compared with the standard ascorbic acid. The antioxidant activity of Curcuma amada was more potent in petroleum ether extract with IC₅₀ values 27µg/ml followed by alcohol extract at 31µg/ml and methanol extract at 35µg/ml. So all the extracts showed good antioxidant activity compared to standard ascorbic activity (32µg/ml) except methanol extract. The formation of nitrite by the reaction of sodium nitroprusside with oxygen is inhibited by scavenging activity of the extract which is expressed as IC₅₀ values and compared with standard ascorbic acid. The petroleum ether and alcohol extracts showed stronger antioxidant activity with IC₅₀ values at 26µg/ml and 29µg/ml respectively and methanol extract showed moderate activity with IC₅₀ value at 31µg/ml. Hence, lower the IC₅₀ value higher will be the scavenging activity (Table 2).

### Table 2. IC₅₀ values of DPPH, Hydrogen peroxide and Nitric oxide radical scavenging by different extracts of Curcuma amada

| Solvent extracts | DPPH IC₅₀ (µg/ml) | H₂O₂ IC₅₀ (µg/ml) | Nitric oxide IC₅₀ (µg/ml) |
|------------------|------------------|------------------|--------------------------|
| Petroleum ether  | 30±0.65          | 27±0.56          | 26±0.97                  |
| Methanol         | 29±0.58          | 35±0.62          | 31±0.53                  |
| Ethanol          | 25±0.35          | 31±0.47          | 29±0.83                  |
| Ascorbic acid    | 33±0.53          | 32±0.98          | 34±0.61                  |

The antioxidant effects of Curcuma amada by DPPH, hydrogen peroxide and nitric oxide radical scavenging methods increases with increase in concentration and the data are depicted in Figures 2, 3 and 4, respectively.
3.3. Antioxidant Activity of Zingiber officinale

Results of DPPH radical scavenging assay of *Z. officinale* showed that all the solvent extracts have higher scavenging efficiency in the order petroleum ether (24µg/ml) > methanol (26µg/ml) > alcohol (30µg/ml) indicating higher activity compared to standard ascorbic acid (33µg/ml). Results of hydroxyl scavenging assay of *Z. officinale* showed that petroleum ether was most active with the IC₅₀ value of 25µg/ml while alcohol extract showed moderate scavenging activity at 30µg/ml, but methanol extract showed least activity at 39µg/ml which is less than that of standard ascorbic acid (32µg/ml). So, all the plant extracts except methanol have better...
scavenging activity compared to ascorbic acid. The nitric oxide scavenging efficiency was strongest only in petroleum ether extract with IC$_{50}$ value 23µg/ml, both alcohol and methanol extracts showed good scavenging activity at 25µg/ml and 26µg/ml, respectively as compared to standard ascorbic acid (34µg/ml) (Table 3).

The antioxidant activity of *Zingiber officinale* by DPPH, hydrogen peroxide and nitric oxide radical scavenging methods increases with increase in concentration and the data are depicted in Figures 5, 6 and 7, respectively.

**Table 3.** IC$_{50}$ values of DPPH, Hydrogen peroxide and Nitric oxide radical scavenging by different extracts of *Zingiber officinale*

| Solvent extracts | DPPH (µg/ml) | H$_2$O$_2$ (µg/ml) | Nitric oxide (µg/ml) |
|------------------|--------------|--------------------|---------------------|
| Petroleum ether  | 24±0.94      | 25±0.99            | 23±0.65             |
| Methanol         | 26±0.85      | 39±0.71            | 26±0.68             |
| Ethanol          | 30±0.73      | 30±0.49            | 25±0.53             |
| Ascorbic acid    | 33±0.53      | 32±0.98            | 34±0.61             |

**Figure 5.** DPPH free radical scavenging activity of different extracts of *Z. officinale* and standard ascorbic acid at different concentrations (20–100µg/ml)

**Figure 6.** Hydroxyl radical scavenging activity of different extracts of *Z. officinale* and standard ascorbic acid at different concentrations (20–100µg/ml)
3.4. Phytochemical Analysis of Solvent Extracts of *Curcuma amada* and *Zingiber officinale*

The petroleum ether extract of *Curcuma amada* showed presence of flavonoids, tannins and proteins. Ethanol extract showed presence of terpenoids, tannins, carbohydrates, saponins and methanol extract showed presence of terpenoids, tannins, carbohydrates and flavonoids (*Table 4*).

**Table 4.** Phytochemical composition of solvent extracts of *Curcuma amada*

| Phytochemical compounds | Petroleum ether | Methanol | Ethanol |
|-------------------------|----------------|---------|--------|
| Flavonoids              | +              | +       | _      |
| Terpenoids              | _              | +       | +      |
| Tannins                 | +              | +       | _      |
| Steroids                | _              | _       | _      |
| Carbohydrates           | _              | +       | +      |
| Proteins                | +              | _       | _      |
| Saponins                | _              | _       | _      |

Petroleum ether extract of *Zingiber officinale* showed presence of flavonoids, terpenoids, tannins, proteins and saponins, ethanol showed presence of flavonoids, terpenoids, tannins, proteins and saponins and methanol extracts showed the presence of flavonoids, terpenoids, tannins, carbohydrates, saponins and proteins (*Table 5*).

**Table 5.** Phytochemical composition of solvent extracts of *Zingiber officinale*

| Phytochemical compounds | PE | Methanol | Ethanol |
|-------------------------|----|---------|--------|
| Flavonoids              | +  | +       | +      |
| Terpenoids              | _  | +       | +      |
| Tannins                 | +  | +       | +      |
| Steroids                | _  | _       | _      |
| Carbohydrates           | _  | +       | +      |
| Proteins                | +  | _       | +      |
| Saponins                | +  | +       | +      |

4. Discussion

Free radicals produced in body as fragments that are containing a number of unpaired electrons in its outermost molecular or atomic orbitals [17]. These free radicals damage the body cells and disrupt the body metabolism and cause health problems such as cardiac diseases, cancer etc. Hence, these free radicals can be inhibited by the use of antioxidants. This antioxidant neutralizes free radicals and protects the body from cell damage. These antioxidants are present in a number of green leafy vegetables, citrus fruits, etc. The antioxidant activity can be recorded by free radical scavenging capacity, inhibition of lipid peroxidation, metal ion chelating ability and reducing ability.

DPPH, hydroxyl radical and nitric oxide scavenging methods are selected for the determination of antioxidant activity in the current study. The scavenging ability of the samples is the measure of antioxidant activity. In DPPH method, a stable radical is used as a substrate to evaluate the antioxidant activity of *Curcuma amada* and *Zingiber officinale* extracts. The hydrogen donating ability is responsible for the effect of antioxidants by DPPH radical scavenging. Hence, the DPPH scavenging activities of the extracts were taken as parameter to check their antioxidant potential. The petroleum ether, methanol and ethyl alcohol extracts of *Curcuma amada* have good scavenging activity with IC$_{50}$ values ranging from 25-30µg/ml which is lesser than the standard ascorbic acid (33µg/ml). All the extracts of *Zingiber officinale* have good scavenging activity at concentrations lower than that of standard.

Hydroxyl radicals are the most reactive reduced form of dioxygen that are known to initiate cell damage [18]. The
petroleum ether (27µg/ml) and ethyl alcohol (31µg/ml) extracts have showed good scavenging activity compared to standard ascorbic acid (32µg/ml). Petroleum ether extract of *Z. officinale* have exhibited very good scavenging activity.

Nitric oxide radical scavenging activity was determined by the ability of the extract to inhibit the formation of nitric oxide ions that can be produced by the interaction of nitric oxide generated by sodium nitroprusside in aqueous solution at physiologically pH [19]. Petroleum ether, methanol and ethyl alcohol extracts of *Curcuma amada* have exhibited good scavenging activity with IC$_{50}$ values ranging from 26-31µg/ml lesser than that of ascorbic acid (33µg/ml). Also, all solvent extracts of *Z. officinale* showed good scavenging activity with least I$_{50}$ values ranging from 23-26µg/ml.

Petroleum ether extracts of both the test plants exhibited good inhibition of free radicals generated by DPPH, hydrogen peroxide and nitric oxide when compared to standard ascorbic acid. Methanol and ethanol extracts of all the plants exhibited moderate and good scavenging activity.

The study of antioxidant activity of *Curcuma amada* was done by earlier workers with various solvent extracts mainly methanol and ethanol extracts [20, 21, 22]. In the present study antioxidant activity of extracts of petroleum ether also proved to have good scavenging activity which has not been reported in literature. This indicates that even petroleum ether along with other solvents like methanol, ethanol, acetone, ethyl acetate and chloroform also have potent radical scavenging capacity [9, 23].

Antioxidant activity of *Zingiber officinale* showed that petroleum ether and ethanol extracts have good scavenging activity in all these three methods with IC$_{50}$ ranging from 25-30µg/ml except hydroxyl radical scavenging method. The literature reports antioxidant activity done by DPPH, ABTS, AFCA and FRAP methods [24, 25] but in the present work in all three DPPH, hydroxyl radical and nitric oxide scavenging methods showed good antioxidant activity which confirms the free radical scavenging ability of *Zingiber officinale*. In literature there are some reports of antioxidant activity using solvent extracts of hexane, acetone, methanol, ethyl acetate and ethanol [26, 27, 28]. In the present study the solvents like petroleum ether, methanol and ethanol were used and all of them have showed good results. The literature study reported methanol and ethanol extracts as potent radical scavengers [26, 24, 29] but the present study reports petroleum ether extract also showed good scavenging capacity, which confirms it also a potent extract for scavenging. The rhizome of *Z. officinale* have good scavenging capacity and it can be used for potential applications as antioxidants in food systems and various solvent extracts played an important role in the modulation of oxidative stress [30].

The phytochemical analysis of all the solvent extracts of *Curcuma amada* and *Zingiber officinale* rhizomes flavonoids were commonly present in all the studied extracts. Terpenoid present in all the solvent extracts except petroleum ether extract of C. amada and Z. officinale. Tannins were present in all the extracts of C. amada and Z. officinale. Saponins were present only in alcohol extract of C. amada and petroleum ether, methanol and ethanol extracts of Z. officinale. So it was observed that maximum phytochemicals were present in Z. officinale.

The phenolic substances are commonly present in all the studied extracts [31]. The scavenging activities of phenolic substances may be due to active hydrogen donating ability of hydroxyl substituent, since phenolic compounds present in the extract are good electron donors and may contribute to the conversion of hydrogen peroxide to water. The correlation between phenol content and antioxidant activity can be influenced by extraction procedure assay methods, solvents and variation in the nature of compounds responsible for antioxidant activity [32]. Hence, among the two medicinal plants tested, *Zingiber officinale* has better scavenging activity in all the performed methods followed by *Curcuma amada*.

There are several methods followed for the determination of antioxidant activity. The chemical complexity of extracts which is a mixture of group of compounds with different functional groups, polarity and chemical behavior may lead to scattered results depending on the method employed [33]. Therefore, an approach with multiple assays for evaluating the antioxidant potential is appropriate.

### 5. Conclusions

The antioxidants efficacies of the plant materials have remarkable properties as therapeutic agents in combating various diseases caused by oxidative stress. The chemical constituents present in the herbal medicine or plants are a part of the physiological functions and hence are thought to have better compatibility with human body. Hence, the use of natural antioxidants mainly from medicinal plants rich in phenolic compound has to be encouraged and validated with scientific experimental investigations. In conclusion, in the present study potent antioxidant activity of *Zingiber officinale* and *Curcuma amada* extracts leads to scientific validation of these plants. A natural substance which is a part of daily diet and nutritional supplement with antioxidant property constitutes a new source of herbal drug.

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