



## QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT ACTIVITY OF *CURCUMA AMADA* ROXB: AN IMPORTANT MEDICINAL PLANT

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### Abstract

*Curcuma amada* Roxb. is a perennial, rhizomatous, aromatic herb belonging to the family Zingiberaceae. This plant is commonly known as "Amba ada", "Mango ginger" due to the flavour of rhizome resemble as raw mango. This plant is used in traditional systems of medicine (Ayurveda and Unani) from the ancient period for the treatment of various types of diseases. *C. amada* have also possessed several pharmaceutical properties such as antimicrobial, anti-inflammatory, analgesic, anticancer, anti hyperglyceridemic, antioxidant activity etc. The above said pharmaceutical activity may be shown due to the presences of various bioactive compounds including tannins, saponins, flavonoids, phenolics, alkaloids, curcumin, demethoxy curcumin, bis-demethoxy curcumin etc. Therefore, the present study was conducted to the preliminary screening of the phytochemicals, quantitative phytochemicals analysis, and antioxidant activity of rhizome and leaf powder sample of *C. amada*. In this study found that the contents of tannins, saponins, alkaloids, and flavonoids were higher in rhizome in comparison to leaf. Phenolics content was more in leaf sample than the rhizome. Antioxidant activity of rhizome was more than the leaf of *C. amada*. Acetone extract of the rhizome is higher than methanol extract of the rhizome.

**Keywords:** Antioxidant activity, *Curcuma amada* Roxb., Phytochemical analysis, Medicinal plant

### Introduction

*Curcuma amada* Roxb. is a perennial, rhizomatous, aromatic herb belonging to the family Zingiberaceae. This plant is commonly known as "Amahaldi", "Amba ada", "Mango ginger" due to the flavour of rhizome resembles as raw mango (Akter *et al.*, 2019; Tamta *et al.*, 2016). It is originated from the Indo-Malayan region and subsequently distributed in the tropics of Asia to Africa and Australia (Sasikumar, 2005). This plant is cultivated in a different state of India including Odisha (Tamta *et al.*, 2016) but commercial propagation was not reported till now. *C. amada* is used in traditional systems of medicine (Ayurveda and Unani) from ancient period as a coolant, appetizer, alexteric, antipyretic, aphrodisiac, diuretic, emollient, expectorant and laxative and to cure biliousness, itching, skin diseases, bronchitis, asthma, hiccup and inflammation due to injuries (Moon *et al.*, 2010; Policegoudra *et al.*, 2011). The rhizome of this plant along with common salt is used for the treatment of cold and cough and also used for the improvement of blood quality (Kapoor, 1990). Its rhizome has also been used for healing of wounds, cut and itching (Srivastava *et al.*, 2001). The leaf is used for contusions and sprains (Kirtikar and Basu, 1984; Rao *et al.*, 1989). While, the paste of the whole plant with peppers is used for the treatment of piles (Kirtikar and Basu, 1984; Kapoor, 1990). *C. amada* has possesses several pharmaceutical properties such as antimicrobial, anti-inflammatory, analgesic, anticancer, antihyperglyceridemic, antioxidant activity (Tamta *et al.*, 2016; Chowdhury *et al.*, 2015; Policegoudra *et al.*, 2011). The above said pharmaceutical activity may be

shown due to the presences of various bioactive compounds including curcumin, demethoxy curcumin, bis-demethoxy curcumin, phenol, terpenoids (Policegoudra *et al.*, 2011). The essential oil of its rhizome have possesses various medicinal properties and pharmaceutical activity (Gupta, 2001). Besides, this plant is used as a crucial ingredient for preparation of pickles, candies, sauces, curries, and salads (Shankaracharya, 1982; Gupta, 2001; Tamta *et al.*, 2016). In Odisha, peoples are eating *C. amada* rhizome adding in water rice along with curd for flavouring of rice.

Till now, there are a few numbers of reports available on phytochemical analysis and antioxidant activity study of *C. amada*. Therefore, the present study was conducted to evaluate qualitative and quantitative phytochemicals analysis and antioxidant activity of rhizome and leaf powder sample of *C. amada*.

### Materials and Methods

#### Collection and preparation of plant materials

*Curcuma amada* whole plants were collected from Medicinal Plant Knowledge Centre, Patrapada, Bhubaneswar, Odisha, India. Rhizome and leaf were separated from plant carefully and were washed under running tap water to remove the sand and soil adhered to the rhizome followed by distilled water. Then the rhizomes and leaf were separately cut into small pieces and dried under shade condition in room temperature to get constant weight. They were then coarsely powdered and stored in an airtight container at room temperature for phytochemicals analysis and antioxidant activity experiments.

### Preparation of plant extract and preliminary phytochemical analysis

Five grams of leaf and rhizome powder samples were soaked in 30 ml of different solvent systems like aqueous (distilled water), acetone, chloroform, ethanol, n-hexane, methanol, separately, covered with aluminium foil and were kept at room temperature for overnight. Then the extracts were filtered through filter paper (Whatman No. 1). The collected filtrates were used for the preliminary phytochemical analysis such as tannin, saponins, flavonoids, alkaloid, and phenolics compounds analysis were carried out by following standard procedures reported by Harborne (1973) and Behera *et al.* (2014).

### Quantitative phytochemical analysis

Leaf and rhizome powder sample was used for quantitative phytochemical analysis in terms of tannin, saponin, phenolic, and flavonoid as described by Behera *et al.* (2018a) and alkaloid content was estimated using the procedure described by Jain *et al.* (2016). The content of saponin, alkaloid, and flavonoid was expressed as mg/g in dry weight of leaf and rhizome sample. Quantitative analysis for phenolics and tannins was carried out based on gallic acid (GAE) and tannic acid (TAE), the standard curve was prepared and the data were presented in mg standard equivalent weight/g of the dry weight of rhizome and leaf sample of *C. amada*.

### Antioxidant activity by DPPH free radical scavenging assay

For the study of antioxidant activity of rhizome and leaf sample of *C. amada*, the extracts were prepared in methanol and acetone solvent system. Ten grams of rhizome and leaf powder was taken with 250 ml of above-said solvents separately in Soxhlet apparatus for 24 h. The extracts were filtered through filter paper (Whatman no. 1) and dried to get constant weight. Finally, the extracts were stored in the refrigerator at 4 °C for future study.

DPPH free radical scavenging activity of methanol and acetone extract of rhizome and leaf was estimated by the standard procedure described by Behera *et al.* (2018b) with minor modification. Different concentrations (10, 20, 40, 60, 80, and 100 µg/ml) of the sample were prepared by diluting the rhizome and leaf extract in methanol. Then 1 ml of different concentrations of both rhizome and leaf extract sample mixed with 1 ml of DPPH (0.15 mM in methanol) and 1 ml DPPH with 1 ml methanol was taken as control and kept in dark condition at room temperature for 30 min. Subsequently, the absorbance was recorded at 517 nm. The ascorbic acid was used as a standard, and the methanol was used as blank in this study.

## Results and Discussion

### Phytochemical analysis

Plants possess several metabolites including primary metabolites and secondary metabolites. Primary metabolites like protein, lipid, carbohydrates, and amino acids are present in different parts of the plant; these metabolites are used by the plant for their growth and development. While secondary metabolites such as tannin, saponin, flavonoids, phenolic, alkaloids, terpenoids, steroids etc are present in the plant parts for the protection of the plants from disease-causing germ/pathogen or in any adverse condition (Wink, 2015;

Behera *et al.*, 2018a). *C. amada* is an important medicinal plant protect from various types of diseases (Tamta *et al.*, 2016; Harit *et al.*, 2013; Policegoudra *et al.*, 2011) due to the presence of several secondary metabolites. In this study, to evaluate qualitative and quantitative phytochemicals are present in *C. amada* leaf and rhizome. Qualitative phytochemicals analysis i.e. alkaloids, flavonoids, phenolics, tannins, and saponins of both leaf and rhizome of *C. amada* was carried out using a different solvent system such as aqueous, acetone, chloroform, ethanol, n-hexane, and methanol. The types of phytochemicals were varied in plant parts along with the polarity of solvent systems. It was observed that alkaloids, flavonoids, phenolics, tannins, and saponins are present in aqueous, acetone, and ethanol extract of both leaf and rhizome sample of *C. amada* (Table 1). Flavonoids were present in chloroform extract of both leaf and rhizome of *C. amada*. Phenolics and tannins were present in n-hexane extract of rhizome sample of *C. amada*. The result of this study is agreed with the previous study reported by Hait and Deepak (2018). They were also reported that the phytochemicals like analysis of *C. amada* rhizome sample on different solvents like tannin, saponins, flavonoids, alkaloids and phenolics were present in water, acetone, methanol, ethanol, and chloroform solvent.

Preliminary phytochemicals analysis is not sufficient to evaluate the content of secondary metabolites present in leaf and rhizome of *C. amada*. Therefore, the quantitative phytochemical analysis was carried out in this study to evaluate the contents of phytochemicals including alkaloids, flavonoids, phenolics, tannins, and saponins in the leaf and rhizome sample of *C. amada*. This result was revealed that the secondary metabolites such as alkaloids, flavonoids, tannins, and saponins content were higher in rhizome sample in comparison to the leaf sample (Table 2). While phenolics content in leaf sample was higher (10.5 mg/g) than the rhizome sample (8.73 mg/g). Among all the evaluated phytochemicals, tannin was higher content (120 mg/g) in the rhizome of *C. amada*. Total flavonoids content of *C. amada* rhizome was estimated in the different solvent system by Yadav and Saravanan (2019). They found that the highest content of flavonoids present in methanol extract than ethyl acetate extract and aqueous extract. Tannins are known for its antifungal activity and flavonoids are played a key role in the development of the living system (Hait and Deepak, 2018). However, flavonoids and phenolics compound have possessed various biological activity including antioxidant, anti-inflammatory, anti-cancer, anti-diabetic, anti-pyptic activity (Hait and Deepak, 2018; Behera *et al.*, 2016; Behera *et al.*, 2014; Amin *et al.*, 2013; Das *et al.*, 2010). Thus, it may be believed that the medicinal and pharmaceutical activity of this plant is attributed to tannins, saponins, phenolics, flavonoids and alkaloids and other secondary metabolites present therein.

### Antioxidant activity of *C. amada*

Plants with antioxidant activity are useful in pharmaceutical industries for the preparation of new drugs (George and Britto, 2016). DPPH free radical scavenging activity is associated with antioxidant activity (Krishnaraj *et al.*, 2010). Different types of free radicals including hydroxyl radical can cause carcinogenesis, neurodegenerative, cardiovascular diseases, ageing and atherosclerosis, and associated with various diseases (Gupta *et al.*, 2015). These free radicals are reduced by antioxidant molecules (Gupta *et*

al., 2015; Saeidnia and Abdollahi, 2012). Therefore, in the present study, the antioxidant activity of both methanol and acetone extract of leaf and rhizome of *C. amada* was estimated by using DPPH scavenging activity method and ascorbic acid was used as standard. Both standards, as well as sample concentrations, were taken from 10 - 100 µg/ml. It was found that standard ascorbic acid has higher antioxidant activity in comparison to both methanol and acetone extract of leaf and rhizome sample of *C. amada* (Fig. 1). Among methanol and acetone extract, acetone extract was shown higher DPPH scavenging activity than methanol extract. *C. amada* rhizome part has possessed more antioxidant activity in acetone solvent system in comparison to methanol extract (Fig. 1). The rhizome of *C. amada* has shown more antioxidant activity than the leaf, which may be due to the presence of higher phytochemical content in rhizome in comparison to the leaf of *C. amada*. Yadav and Saravanan (2019) were also reported that the standard sample was showed more antioxidant activity in comparison to plant extract of *C. amada* rhizome in methanol solvent system, which is similar to this result. Antioxidant Activity of ethanol

and acetone extract of *C. amada* leaf was estimated by George and Britto (2016) using DPPH scavenging assay and observed that the antioxidant activity of ethanol extract of *C. amada* leaf was higher than acetone extract. Gupta et al. (2015) were found that methanol extract of rhizome shown maximum antioxidant activity than aqueous, ethyl acetate and dichloromethane extract of *C. amada* rhizome.

Nowadays, chemically synthesized drugs used for the treatment of various diseases, which can arise many snags. Bioactive compound rich plants are used for the preparation of herbal medicine in traditional (Ayurveda and Unani) systems of medicine for a long time. *C. amada* is an important spice which has several biological and medicinal properties. Therefore, the phytochemical analysis and antioxidant activity of leaf and rhizome of *C. amada* were estimated and found that rhizome contains more phytochemical contents and antioxidant potential than the leaf. This study was revealed that *C. amada* contains several secondary metabolites as well as antioxidant activity thereby justifying its ethnobotanical claims.

**Table 1:** Qualitative phytochemicals analysis of leaf and rhizome sample of *C. amada*

Solvents/ phytochemical	Alkaloids		Flavonoids		Phenolics		Tannins		Saponins	
	L	R	L	R	L	R	L	R	L	R
Aqueous	+	+	+	+	+	+	+	+	+	+
Acetone	+	+	+	+	+	+	+	+	+	+
Chloroform	-	-	+	+	-	+	-	+	-	-
Ethanol	+	+	+	+	+	+	+	+	+	+
n-Hexane	-	-	-	-	-	+	-	-	-	+
Methanol	+	+	+	+	+	+	+	+	+	+

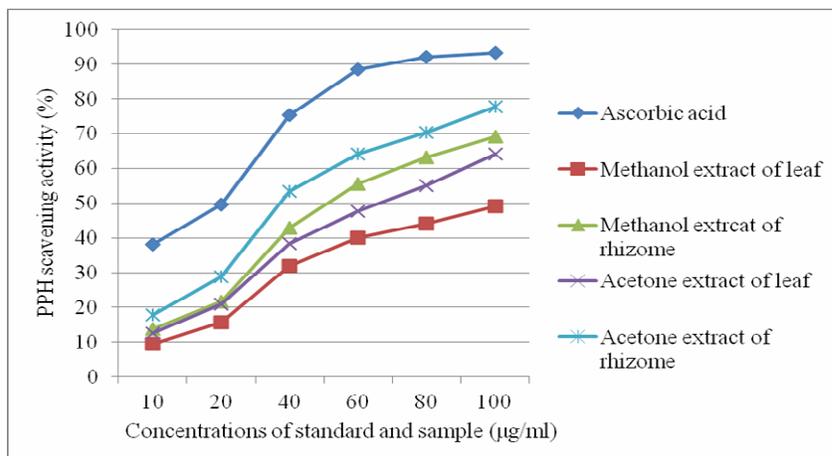
+ = Presence; - = Absence; L = Leaf; R = Rhizome

**Table 2:** Quantitative phytochemicals analysis of leaf and rhizome sample of *C. amada*

Phytochemicals	Leaf	Rhizome
Alkaloids (mg/g DW)	7.57 ± 0.23	12.11 ± 0.65
Flavonoids (mg/g DW)	8.02 ± 0.10	10.21 ± 0.54
Phenolics (mg GAE/g DW)	10.50 ± 0.37	8.73 ± 0.42
Tannins (mg TAE/g DW)	93.20 ± 0.65	120.82 ± 0.97
Saponins (mg/g DW)	22.68 ± 0.48	32.30 ± 0.70

Values in column presented in means ± standard deviation (SD).

DW: dry weight, GAE: gallic acid equivalent, TAE: tannic acid equivalent.



**Fig. 1 :** DPPH scavenging activity of methanol and acetone extract of leaf and rhizome of *C. amada*

**Conflicts of Interest:** The authors declare no conflicts of interest.

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