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STUDIES ON NUTRITIONAL EVALUATION AND SECONDARY METABOLITES OF SOME SELECTED *CURCUMA* SPECIES COLLECTED FROM EASTERN GHATS OF UDAYAGIRI HILLS, GAJAPATHI DISTRICT, ODISHA

Kollana Y. Ramkumar*[1], Samuel David Raj[2], P. Suman[3], P. Rupa Vani[2], S. Hara Sreeramulu[3]

[1]Department of Biotechnology, Andhra University, Visakhapatnam.
[2]Department of Zoology Center for Research, Dr. VSK. Govt. Autonomous College, VSP.
[3]Department of Biological Sciences, IIIT Srikakulam, Etcherla, Srikakulam.

***Corresponding Author: Kollana Y. Ramkumar**
1Department of Biotechnology, Andhra University, Visakhapatnam.

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ABSTRACT
The current study was carried out to investigate the nutritional evaluation and secondary metabolites of fresh rhizome of some selected species of *Curcuma* viz., *C. amada, C. angustifolia, C. caesia, C. longa, C. roscoeana* and *C. zedoaria* which are collected from Eastern Ghats of Udayagiri Hills, Gajapathi District, Odisha, India. The dried and powdered rhizomes of each species were extracted with ethanol using Soxhlet extraction method. Phytochemical screening of ethanolic extracts of each species was performed qualitatively. Phytochemical screening of the ethanolic extract of six species showed the presence of Alkaloids, Flavonoids, Tannins, Terpenoids, Saponins, Phenolic compounds and Anthraquinones. Based on this study, it can be concluded that irrespective of the origin or variety, the species *Curcuma* is a good natural reservoir of energy and its nutritive components can be used as a food supplement.

KEYWORDS: Nutritional, Secondary metabolites, *Curcuma* species.

INTRODUCTION
The genus *Curcuma longa* L. (*Zingiberaceae*) represents a group of perennial rhizomatous herbs native to tropical and subtropical regions. Curcuma species are known for their antioxidant and blood purifier, clears skin color, removes wounds and shows antimicrobial properties against pathogens. *Curcuma* plants (rhizomes and leaves) have a camphoraceous aroma and contain many functional compounds such as phenolics, flavonoids and different antioxidant enzymes. *Curcuma* is extensively cultivated in tropical and subtropical regions of Asia, Australia, and South America (Ravindran et al. 2007). There are approximately 93–100 accepted *Curcuma* species, however the exact number of species is still controversial (Akarchariya et al., 2017). The genus is best known for being an essential source of coloring and flavoring agents in the Asian cuisines, traditional medicines, spices, dyes, perfumes, cosmetics, and ornamental plants (Leong-Skornikova and Newman, 2015). Several *Curcuma* species are used medicinally in Bangladesh, Malaysia, India, Nepal, and Thailand (Chuakul and Boonpleng, 2003) for treating pneumonia, bronchial complaints, leucorrhea, diarrhea, dysentery, infectious wounds or abscesses, and insect bites (Chuakul and Boonpleng, 2003; Basaka et al., 2010; Akarchariya et al., 2017). The rhizome is most commonly used part of the plant. The main active components of the rhizome are the nonvolatile curcuminoids and the volatile oil (Jayaprakasha et al., 2005; Lobo et al., 2009). Curcuminoids (curcumin, demethoxycurcumin, and bisdemethoxycurcumin) are nontoxic polyphenolic derivatives of curcumin that exert a wide range of biological activities (Itokawa et al., 2008).

The rhizomes of *Curcuma* species are used as carminative and stomachic, contusion and sprains (Sinha, 2001). *C. amada, C. angustifolia, C. caesia, C. longa, C. roscoeana* and *C. zedoaria* can be considered as lesser known species of *Curcuma*. They are used variously in the traditional system of medicines. *C. amada* is useful in bronchitis, asthma, sprains, skin diseases, and inflammation caused due to injuries. *C. angustifolia* is an aphrodisiac and useful in the treatment of leprosy, asthma, anemia, and leucoderma. Rhizomes of *C. caesia* are used for sprains and bruises and also employed in the preparation of cosmetics. The therapeutic uses of *Curcuma longa* is anticancer, antimicrobial, anti-inflammatory and free radical scavenging activity of the plant suggests a logical basis for its traditional use in foodstuff. The rhizome of *C. roscoeana* is used as an anti-inflammatory pain reliever, especially for shoulder

pain. It is believed to invigorate and improve the movement of blood and stimulate menstruation. *C. zedoaria* is said to be anti-mutagenic, anticarcinogenic, as well as anti-inflammatory (Jadhav, 2008, 2009).

Curcuma species have a high nutritional status like rich source of carbohydrates, proteins, alkaloids, flavonoids etc., vitamins or vitamin precursor found in curcumin which produces vitamin C, beta-carotene as well as polyphenol coupled with fatty acid and essential oil. The leaves are great source of vitamin and minerals (Chattopadhyan et al., 2004). The objectives of this work are to determine the proximate composition and secondary metabolites of *C. amada*, *C. angustifolia*, *C. caesia*, *C. leucorrhiza* and *C. zedoaria*. This study will give an imminent nutritional, phytochemical evaluation of ethanolic rhizome extracts of some selected *Curcuma* species collected from Eastern Ghats of Udayagiri Hills, Gajapathi District, Odisha, India.

MATERIAL AND METHODS

Six different species of the genus *Curcuma* i.e., *C. amada*, *C. angustifolia*, *C. caesia*, *C. longa*, *C. roscoeana* and *C. zedoaria* were collected from Eastern Ghats of Udayagiri Hills, Gajapathi District, Odisha, India. The rhizomes of each species were washed under tap water and rinsed with distilled water. The rhizomes were cut into pieces, dried under shade and finally dried in an oven at a temperature of 40°C for 2 days. The dried rhizomes of each species were pulverized by using grinder to obtain a powdered form.

Nutritional evaluation of *Curcuma* species

***Estimation of moisture*:** Moisture content was determined by using the standard method (AOAC, 2000). One gram of well-mixed sample was weighed accurately in clean, pre-heated dish of known weight by using sensitive balance. The uncovered sample and dish were kept in an oven provided with a fan at 105°C and let to stay 3 hr. The dish was covered and transferred to a desiccator and weighed after reaching room temperature.

***Determination of Protein content*:** The protein content of the sample was determined by micro Kjeldahl method (AOAC, 2000). It involves the conversion of organic nitrogen to ammonium sulphate by digestion of sample powder with concentrated sulphuric acid in a micro Kjeldahl flask. The digest was diluted, made alkaline with sodium hydroxide and distilled. The liberated ammonia was collected in a boric acid solution and total nitrogen was determined titrimetrically. The percentage of protein in the sample was calculated.

***Fat estimation*:** The dried samples left after moisture determinations were finely ground and the fat was extracted with chloroform and methanol mixture (AOAC, 2000). A dry and accurately weighed flask was fitted to the extractor, then solvent (Petroleum ether) was poured in to the flask, the extractor and condenser was fitted together. Water was allowed to flow through the condenser and heat was applied from an electrical heater. The extraction period is 6-8 hr. After that the solvent was distilled off, pour the oil in an empty petriplate, dried in oven for 30 min at 100°C, cooled and weighed.

***Ash content determination*:** Total ash was determined according to previous studies (AOAC, 2000). One gram of well mixed sample was weighed in a porcelain crucible of known weight. The crucible is ignited at 550-600°C in a muffle furnace until light gray ash was obtained. The content of the crucible was cooled in desiccator and weighed soon after it reached room temperature. Percentage of ash calculated from the increase in the weight of the crucible.

***Carbohydrate determination*:** The carbohydrate content was determined according to previous studies (Trevelyan and Harrison, 1952) by subtracting the total crude protein, oil, moisture and ash from the total dry matter.

Phytochemical Screening

Chemical tests were carried out qualitatively on each extract following standard procedures to identify the phytochemical constituents (Edoga et al., 2005; Tiwari et al., 2011).

Preparation of extract: 15 g of powder were extracted serially with 100 ml of ethanol in increasing order of their polarity and taken into the soxhlet apparatus which was run upto 12 hours. After that the extract was concentrated in rotary evaporator, dried in oven and stored at 40˚C in airtight bottles and was qualitatively tested for the presence of various phyto-compounds. About 50 mg of solvent free extract stirred with 3 ml of dilute hydrochloric acid and then filtered thoroughly. Then filtrate was tested carefully with various alkaloid reagents as follows.

Alkaloid determination: In a test tube containing 1 ml of extract, few drops of Dragendroff's reagent was added and the colour developed was noticed. Appearance of orange colour indicated the presence of alkaloids.

***AnalysisAnalysis of flavonoids*:** To the extract, a few drops of 10% sodium hydroxide solution were added. Formation of intense yellow colour which turns to colourless by addition of few drops of dilute acetic acid indicated the presence of flavonoids.

***Tannin determination*:** To the extract, a few drops of 10% lead acetate solution were added. Precipitate formation indicated the presence of tannin.

***Terpenoid Analysis*:** Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of conc. sulphuric acid, shaken well and allowed to stand. Appearance of red colour in the lower layer indicated the presence of steroids. Formation of reddish brown colour in the interface, after addition of

conc. sulphuric acid to the side carefully (without shaking) indicated the presence of terpenoids.

***Analysis of saponins*:** Crude extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously, then few drops of olive oil were added. The formation of stable foam was taken as an indication for the presence of saponins.

***Phenolic compound determination*:** To the test solution, few drops of 10% lead acetate solution was added. Formation of white precipitate indicated the presence of phenolic compounds.

***Anthraquinone test*:** The extracts (5 mL) were mixed with Benzene (10 mL), filtered and 10% ammonia solution (5 mL) was added to the filtrate. The mixture was shaken and the presence of pink, red or violet colour in the ammonia (lower phase) indicated the presence of anthraquinones.

RESULTS AND DISCUSSION

The current study is carried out to analyze the proximate composition and constituents of secondary metabolites of rhizome of some selected species of *Curcuma* which were shown in table 1 and table 2 respectively. Proximate analysis is usually the first step in the chemical evaluation of dried ingredients, where the material is subjected to a series of relatively simple chemical tests so as to determine the moisture, protein, fat and ash percentage (figure 1, table 1). In this study, more amount of moisture percentage was found in *C. roscoeana* (15.38 %) followed by *C. zedoaria* (13.90 %) and *C. angustifolia* (12.23 %), whereas protein content was found high in *C. longa* (19.81 %) followed by *C. angustifolia* (18.92 %) and *C. zedoaria* (17.32 %), while the percentage of fat was accumulated more in *C. angustifolia* (3.5) followed by *C. roscoeana* (3.2) and *C. zedoaria* (2.9 %), the ash percentage was found in between 1.2 % (*C. caesia*) and 3.6 % (*C. longa*) and finally the carbohydrate content was observed more in *C. caesia* (72.55 %) followed by *C. amada* (70.20 %) and *C. angustifolia* (63.02 %) respectively. The most important proximate component of fresh turmeric rhizome was its curcumin content with respect to processing and preparation of value added products. The results obtained are good in accordance with Mathai (1976). The essential nutritional composition of *Curcuma* species was similar in accordance with the report of the research work of Iniaghe et al., 2009 and El-Ghorab et al., 2010. It also adds bulk to the food and prevents the intake of excess starchy food and may therefore guard against metabolic conditions such as hypercholesterdemic and diabeties mellitus (Bamishaiye et. al., 2011).

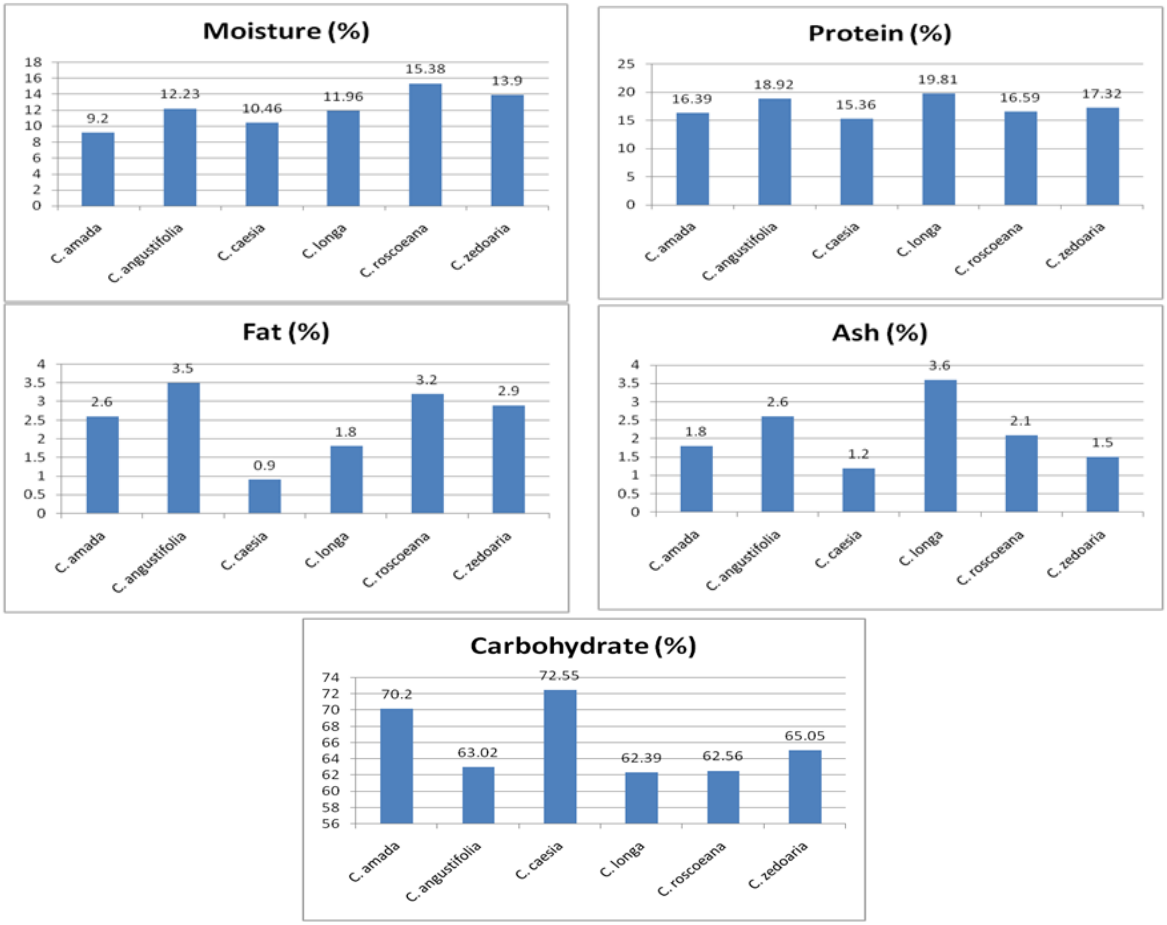

Figure 1: Moisture, Protein, Fat, Ash and Carbohydrate composition of rhizome of *Curcuma* species.

Table 1: Proximate composition of rhizome of *Curcuma* species.

Curcumin Species	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Carbohydrate (%)
C. amada	9.20	16.39	2.6	1.8	70.20
C. angustifolia	12.23	18.92	3.5	2.6	63.02
C. caesia	10.46	15.36	0.9	1.2	72.55
C. longa	11.96	19.81	1.8	3.6	62.39
C. roscoeana	15.38	16.59	3.2	2.1	62.56
C. zedoaria	13.90	17.32	2.9	1.5	65.05

The study carried out on the ethyl alcoholic extract of rhizomes discovered that the occurrence of medicinally active constituents of secondary metabolites such as Alkaloids, flavonoids, terpenoids, phenols, tannins and saponins which were found to be present in all the species. The secondary metabolite constituents studied in the six different species of *Curcuma* were represented in Table 2.

Table 2: Constituents of secondary metabolites in some selected species of *Curcuma*.

Curcumin Species	*C. amada*	*C. angustifolia*	*C. caesia*	*C. longa*	*C. roscoeana*	*C. zedoaria*
Alkaloids	-	+	+	+	+	-
Flavonoids	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+
Saponins	+	+	+	+	+	+
Phenolic compounds	+	+	+	+	+	+
Anthraquinone	+	+	+	+	+	+

Curcuma species have shown to have a wide spectrum of biological actions. These include anti-inflammatory, antioxidant, anticarcinogenic, antimutagenic, antifertility, antidiabetic, antibacterial, antifungal, hypotensive and hypocholesterolemic activities. Its anticancer effect is mainly mediated through induction of apoptosis (Bagchi, 2012). The contents of alkaloids in *Curcuma* species used in carminative, stomachic, contusion and sprain is also related to the high alkaloid in the rhizomes. Alkaloids found in some plants have been utilized for treatment in AIDS patients (Mc Mahon et al., 1995). The powdered form of the rhizome of *Curcuma anguistifolia* with honey is applied on the mucous membrane of oral cavity in stomatitis. The juice of *Curcuma anguistifolia* is rubbed on swelling and the paste is used to hasten the joining of fractures, cooling, demulcent and is also nutritious. It is also used for dysentery, dysuria and gonorrhea (Sudhir and Kumar, 2002).

Flavonoids are responsible for antioxidant as well as antimicrobial activity. The use of turmeric as an antiseptic in cleansing and disinfecting the skin and skin ulcers may be due to the high phenolic and flavonoids content since phenolic and flavonoids compounds have been reported to exert multiple biological effects including antioxidant, free radical scavenging, anti-inflammatory, anticarcinogenic and antiviral activities (Miller, 1996). Flavonoids are also known to be synthesized by plants in response to microbial activities (Dixon et al., 1993). Lipophilic flavonoids may also disrupt microbial membranes (Tsuchiya et al., 1996). The high content of tannin is also related with their medicinal applications since many human physiological activities such as stimulation of phagocytic cells, host-mediated tumour activities in a wide range of anti-infections have been assigned to tannin (Haslam, 1996). The growth of many fungi, yeasts and bacteria was inhibited by tannins (Chung et al., 1998). Further, tannins and terpenoids are attributed for analgesic and anti-inflammatory activities. Apart from these, tannins exhibit the property of astringency i.e., faster healing of wounds and inflamed mucous membrane (Okwu and Josiah, 2006).

Terpenoids exist in plants, as hydrocarbons (carotenes) or oxygenated derivatives which were accessory pigments in photosynthetic systems and give characteristic color to plant parts. They are a diverse class of phytochemicals including aromatic, non-aromatic, volatile and non-volatile constituents that play a key role in traditional herbal remedies and are being investigated for antibacterial, antineoplastic, and other pharmaceutical potentials (Tolstikova et al., 2006; Lage et al., 2010). Saponins, present in plants, have been suggested as possible anti-carcinogens. The proposed mechanisms of anti-carcinogenic properties of saponins include direct cyto-toxicity, immune modulatory effects. Likewise, alkaloids are a diverse group of secondary metabolites found to have antimicrobial activities by inhibiting DNA topoisomerase (Veerachari and Bpaiah, 2011). Phenolic compounds act as (a) metal chelators, (b) antimutagens and anticarcinogens and (c) antimicrobial agents (Proestos et al., 2005). In addition, Curcuminoids are a group of phenolic compounds composed mainly of curcumin, demethoxycurcumin and bisdemethoxycurcumin and have a wide range of medicinal and culinary uses (Chattopadhyay et al., 2004).

CONCLUSION

The present study revealed the proximate composition and secondary metabolites of some selected species of *Curcuma* from Eastern Ghats of Udayagiri Hills, Gajapathi District, Odisha, India. The results indicate that fresh *Curcuma* rhizomes exhibit good nutritional values that may be of immense utilisation for the development and value addition in food products. The presence of bioactive secondary metabolites in the rhizomes of *Curcuma* species were correlated with their medicinal applications since secondary metabolites are commercially employed as insecticides, fungicides, pharmaceuticals, while others are used as fragrances, flavourings, medicinal drugs and industrial materials.

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