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Estimation of carbohydrate, protein and secondary metabolites of selected *Curcuma* species from the Northern coastal region of Odisha, India

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Abstract

The presence of phytochemicals is not uncommon in the species belonging to the genus *Curcuma* owing to its medicinal properties. It is used since ancient times in a variety of Indian medications. Being edible, it also serves to supply the body with protein and carbohydrates besides other nutrients present in it. The study was undertaken to determine the composition of carbohydrate, protein and phytochemicals of some selected species of *Curcuma* viz., *C. longa*, *C. caesia*, *C. aromatica*, *C. amada* and *C. angustifolia* collected from the Northern coastal region of Odisha, India. The present study reveals that among these species highest amount of protein was present in *Curcuma longa* (48.79%) whereas highest amount of carbohydrate was present in *Curcuma angustifolia* (20.36%). Alkaloid, Glycoside and phenolics are the common secondary metabolites present in all these five *Curcuma* species.

Keywords: *Curcuma*, carbohydrates, proteins, phytochemicals

Introduction

Since ancient times, traditional systems of medication have been used across the globe and the medicinal plants have played a pivotal role in the treatment of various ailments. Consequently, in recent practices, plant secondary metabolites have been substantially used as a source of therapeutics^[1]. As compared to synthetic drugs, antimicrobial agents that owe their origin to plants are not associated with side effects are also inexpensive, easily accessible and affordable^[2]. The genus *Curcuma* (Family: Zingiberaceae) was coined by Linnaeus in 1753 in 'Species Plantarum'. The word likely had its origin from the Arabic word 'Kurkum' meaning yellow colour^[3]. The genus entails a group of perennials, rhizomatous herbs that are indigenous to the tropical and subtropical regions of the world^[4]. Roughly 93-100 accepted *Curcuma* species are found across the world^[5]. Among these, about 40 species are reported from India^[6]. However, the exact number of species is still debatable^[7]. The plants belonging to the genus possess a camphoraceous aroma^[8]. The rhizome having non-volatile curcuminoids and the volatile oil as the active constituents, is the most used plant part^[9]. For its medicinal and nutritive properties, the genus has a global economic value^[10].

Curcuma has been used since time immemorial in different mythology and traditional medicines^[10]. The rhizomes are used as carminative and stomachic and treat sprains^[5]. Certain obscure *Curcuma* species are *C. aromatica*, *C. angustifolia*, *C. caesia*, *C. amada* and *C. longa*. These species carry enormous importance in the field of medicine. *C. aromatica* is used in native medicine for skin diseases, sprain, bruise, in snake poison and to enhance complexion, in treating dysentery and gastric ailments. The rhizome paste with milk is consumed for the treatment of indigestion, rheumatism and dysentery. Leaves of the plant have proven beneficial in healing wounds and fractured bones. In consonance with Ayurveda, several diseases related to skin, cardiovascular and respiratory system can be cured by this drug^[11]. *C. angustifolia* is used in treating diarrhoea, dysentery, and colitis; reduces the Pitta Dosh and helps to check bleeding, also used for healing stomach ulcers. Application of the rhizome powder with honey to the mucous membrane of oral cavity have been salutary in healing stomatitis. It is also helpful in typhoid, fever, ulceration of the bowels and bladder^[12]. The rhizomes of *C. caesia* are useful for sprains, bruises and are also employed in the preparation of cosmetics^[5]. *C. amada* has been emphasized as an appetizer, antipyretic, diuretic, emollient, expectorant, laxative, aphrodisiac and alexiteric.

It cures biliousness, itching, skin diseases, bronchitis, asthma, hiccup, and inflammation due to injuries^[3]. The rhizomes of *C. longa* possess a prospective medicinal property.

Turmeric plant has been regarded as an excellent natural antiseptic, disinfectant, anti-inflammatory and analgesic by traditional Ayurvedics. The plant has also been used frequently to aid digestion, improve intestinal flora and to treat skin irritations^[13].

A range of biologically active secondary metabolites present in plants are cumulatively referred to as phytochemicals. The flavour and colour of edible plant and the beverages derived from them owe to the phytochemical constituents of the plant. They exercise a range of potentially important biological effects on human cells^[14]. *Curcuma* species are rich in alkaloids, flavonoids, tannins, terpenoids, saponins, phenolic compounds and anthraquinones^[15, 16].

Curcuma species are nutritionally rich and contains a good amount of carbohydrates, proteins, alkaloids, flavonoids, etc.^[5]. Carbohydrates are the most widely distributed organic compounds in Earth and are one of the most important ingredients in foods of raw materials^[17, 18]. They make up much of our food and are vital to national economies^[17]. The dietary carbohydrates comprise of a diverse group of substances including a range of physical, chemical, and physiological properties. Principally carbohydrates are substrates for energy metabolism^[19]. They may occur inherently or be added to food products to provide nutrients and are mostly added to improve the texture and overall quality of food product^[18]. They may also be immunomodulatory and affect Calcium absorption. These properties influence the overall health by contributing to the control of the body weight, diabetes, ageing, cardiovascular disease, bone mineral density, etc.^[19]. Proteins are the most abundant and ubiquitous biomolecules. A great variety of protein can be isolated from a single cell^[20]. They are present in large amounts than any other biomolecule and make up over 50 per cent of the dry weight of cells. They are designated as the unique macromolecules for being the foundation of every reaction occurring in the biological system^[21]. They virtually facilitate every process that take place in a cell and exhibits an array of functions^[20]. Dietary protein is of ample importance as is evident by the fact that the solid matter of the active tissues of the body is largely composed of protein^[22].

India is endowed with a variety of *Curcuma* species, which are of tremendous importance. The nutritive aspect of many Indian species is yet to be exploited^[23]. The conservation of plant biodiversity stresses the need to explore both current and long-term issues. It highlights the need to domesticate plants^[24]. Domestication carries enormous importance which is evident from the fact that Charls Darwin (1859) devoted the first chapter of his book "The Origin of Species by Means of Natural Selection" to variation under domestication^[25]. The term "Domestication" has been used for a long time but is still struggling to have a satisfactory definition^[26]. The increased adaptation of plants and animals for cultivation or rearing and utilization by humans is the outcome of a selection process that was carried out consciously by humans or if it was a consequence of cultivation of plants and rearing of animals by humans^[25]. The domestication practices carried out by humans is considered as the most important developments in the history of humans^[26]. The transition from hunter-gatherer societies to settled agriculturalists occurred independently and lead to the domestication of crops and livestock^[27]. Domestication results from the interaction of plant or animal, human and environmental factors^[25]. It is estimated that about 2500 plant species have undergone domestication worldwide,

with more than 160 families contributing one or more crop species^[27].

The objective of this work is to estimate the carbohydrate, protein and phytochemical constituents of the selected *Curcuma* species and to raise consciousness about conservation and domestication of the species containing a decent amount of the above-mentioned macronutrients and phytochemicals, so that most of the people could be benefitted.

Materials and Methods

1. Collection of plant specimen

Healthy rhizomes of *Curcuma amada*, *Curcuma aromatica*, *Curcuma angustifolia*, *Curcuma caesia* and *Curcuma longa* were collected from the natural wild habitats of Northern Coastal Region of Odisha. Field surveys in this region were conducted during the flowering season of the plant i.e., during the month of July to October to know the natural habitat and distribution pattern of these plants. The local Vaidyas or Kavirajs (traditional medicinal practitioners) and other knowledgeable persons of the tribal villages were interviewed during the field visits about the uses of this plant and the plant species were identified with the help of regional flora books^[28, 29].

2. Determination of Protein Content

(i) Plant Extract Preparation

500 mg of dry rhizome powder was mixed by adding 5 ml of PBS and was centrifuged at 5000 rpm for 10 minutes. The supernatant was used for extraction.

(ii) Estimation of Protein Content

The protein content of the sample was estimated by Lowry's Method^[30]. Reagent A was prepared by adding 50 ml of 2% Sodium Carbonate to 50 ml of 0.1N Sodium hydroxide solution. 10 ml of 1.56% Copper sulphate solution was mixed with 10 ml of 2.37% Sodium Potassium tartrate solution to prepare Reagent B. 2 ml of Reagent B and 100 ml of Reagent A was mixed to prepare Reagent C. 2 ml of 2N Folin-Ciocalteu Reagent was mixed with equal volume of water just prior to use. 0.1 ml of the sample was taken and the volume was made up to 1 ml. 5 ml of Reagent C was added and the sample was allowed to stand for 10 minutes at room temperature. Then 0.5 ml of Folin-Ciocalteu Reagent was added and it was incubated at room temperature in dark for 30 minutes. Then the absorbance was taken at 660 nm. The amount of protein content in the sample was calculated with the help of the calibration curve of BSA.

3. Determination of Carbohydrate Content

(i) Plant Extract Preparation

500 mg of dry rhizome powder was hydrolysed with 2.5 ml of 2.5 N HCl by keeping it in a boiling water bath for 90 minutes. Then the sample was cooled to room temperature and the volume was made up to 50 ml. The sample was then centrifuged at 5000 rpm for 10 minutes and the supernatant was used for analysis.

(ii) Estimation of Carbohydrate Content

Total carbohydrate content was determined by anthrone method^[31]. The anthrone reagent was prepared by adding 200 mg of anthrone with 100 ml of ice-cold 95% H₂SO₄. 1 ml of plant extract was taken and 4 ml of anthrone reagent was added to it and heated for 8 minutes in a boiling water bath and the green to dark green colour development was

observed. The absorbance was taken at 630 nm. The carbohydrate content in the sample was calculated with the help of standard curve of glucose.

4. Phytochemical Assay

(i) Plant Extract Preparation

Near about 30 gm of air-dried plant material were taken in a conical flask containing 200 ml of ethanol and was plugged with cotton wool. Then it was kept on orbital shaker for 48 hours with the speed of 150 rpm at room temperature. After that extracts were filtered with Whatman No. 1 filter paper and the supernatant was collected. Then it was stored at 4 °C in air tight containers. The tests for different phytochemicals were carried out for all the three different types of extracts.

(ii) Screening test for Quality Analysis

(a) Test for alkaloid

2 ml of 1% HCl and 6 drops of both Mayer's Reagent and Dragendroff's Reagent were added to it. An organic precipitate was formed which indicated the presence of alkaloid in the sample.

(b) Test for flavonoid

About 10 drops of aqueous extract of plant material was taken in a test tube and 5 ml of dilute ammonium solution was added to it. Then little amount of concentrated H₂SO₄ was added slowly. A yellow colour was observed which confirmed the presence of flavonoids and it disappeared on standing.

(c) Test for Glycosides

5 ml of plant extract was taken in a test tube and was treated with 2 ml of glacial acetic acid with one drop of ferric chlorine solution. Then 1 ml of concentrated H₂SO₄ was added over it gently. A brown ring of the interface was formed which indicated the presence of a deoxy sugar of the glycosides. A violet ring might appear below the brown ring where as in the acetic acid layer, a greenish ring might form just gradually through thin layer.

(d) Test for Phenols

2 ml of plant extract was taken in a test tube and 3 ml of ethanol was added to it. Then a pinch of FeCl₃ was added to it. A greenish yellow colour was formed which indicated the presence of phenols.

(e) Test for Tannins

5 ml of plant extract was taken in a test tube and few drops of 1% of lead acetate were added to it. A yellow precipitate was formed which indicated the presence of tannins.

(f) Test for Saponins

The plant extract with 20 ml of distilled water was agitated in a graduated cylinder for about 15 minutes. The formation of 1 cm layer of foam indicated the presence of saponins.

Result and Discussion

The study was carried out to estimate the composition of secondary metabolites and the protein and carbohydrate content of rhizomes of some selected *Curcuma* species.

1. Determination of Protein Content

During the present investigation, highest protein content was found in *Curcuma longa* (48.79%), followed by *C. caesia* (18.53%) and *C. amada* (13.19%). *C. aromatica* (10.29%) and *C. angustifolia* (9.18%) showed relatively lesser protein content. (Table-1)

Table 1: Relative protein content in different *Curcuma* species

<i>Curcuma</i> species	Protein %
<i>C. amada</i>	13.19
<i>C. angustifolia</i>	9.18
<i>C. aromatica</i>	10.29
<i>C. caesia</i>	18.53
<i>C. longa</i>	48.79

The above results are similar to the studies conducted by some other investigators on the nutritional evaluation of various species of *Curcuma* such as among *C. amada*, *C. angustifolia*, *C. caesia*, *C. longa*, *C. roscoeana* and *C. zedoaria* [5, 32]

Turmeric (*C. longa*) serves as a good source of protein. The powdered form of the rhizome is used as a spice, food preservative and colouring agent [33]. Turmerin found in the rhizomes of turmeric is a water-soluble protein with potent antioxidant properties [34]. In India, turmeric is used as a household remedy for reducing pain, swelling, wound injury and various types of inflammation. Several skin infections can be cured by the application of turmeric paste on the site of infection [35]. The *C. caesia* is very helpful to diabetic patients [36].

2. Determination of Carbohydrate Content

In this study, the carbohydrate content of the selected *Curcuma* species was found to be lying in the range of 19-21%. The carbohydrate content was highest in *C. angustifolia* (20.36%) followed by *C. caesia* (20.29%) and *C. aromatica* (19.93%). Comparatively lesser carbohydrate content was estimated in *C. longa* (19.91%) and *C. amada* (19.51%). (Table-2)

Table 2: Relative Carbohydrate content in different *Curcuma* species

<i>Curcuma</i> species	Carbohydrate %
<i>C. amada</i>	19.51
<i>C. angustifolia</i>	20.36
<i>C. aromatica</i>	19.93
<i>C. caesia</i>	20.29
<i>C. longa</i>	19.91

The above results are similar to the studies conducted by some other investigators on the nutritional evaluation of carbohydrate content of various species of *Curcuma* [5, 8].

Mango ginger (*C. amada*) is mainly used in the manufacture of pickles and culinary preparations [37]. Inclusion of *C. amada* in diet offers a vital option for the treatment of high-fat and high-sugar diet (HFHS)- induced obesity and memory loss [38]. Juice of *C. angustifolia* is rubbed on swelling to provide relief and its paste beside being nutritious is used to hasten the joining of fractures and provides a cooling effect [39].

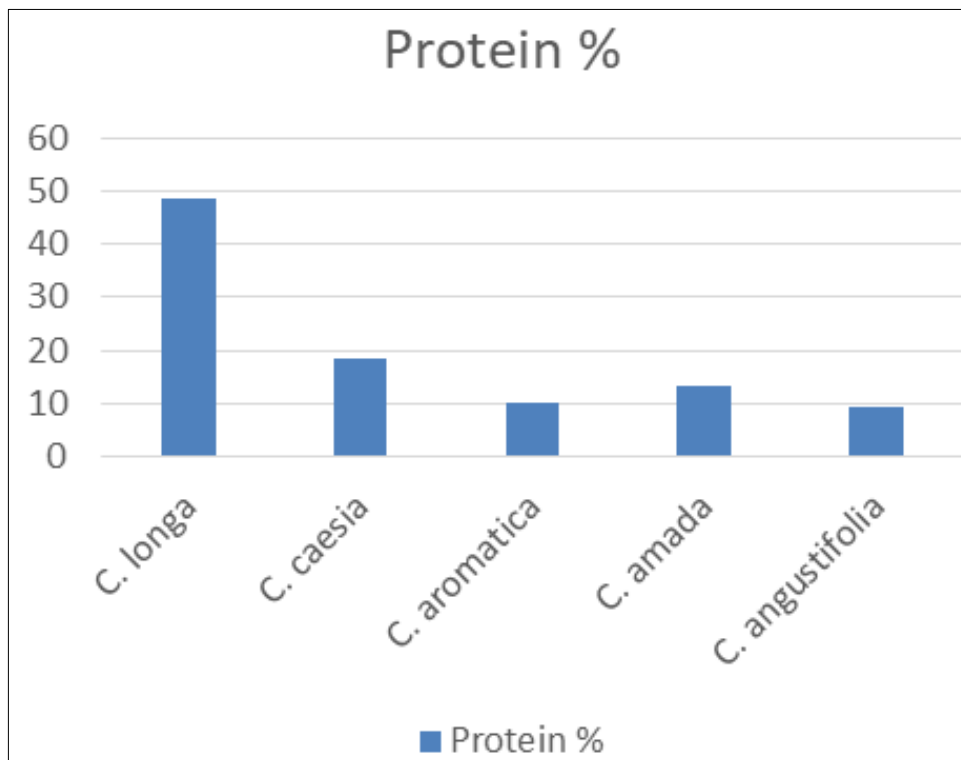


Fig 3: Relative Protein Content

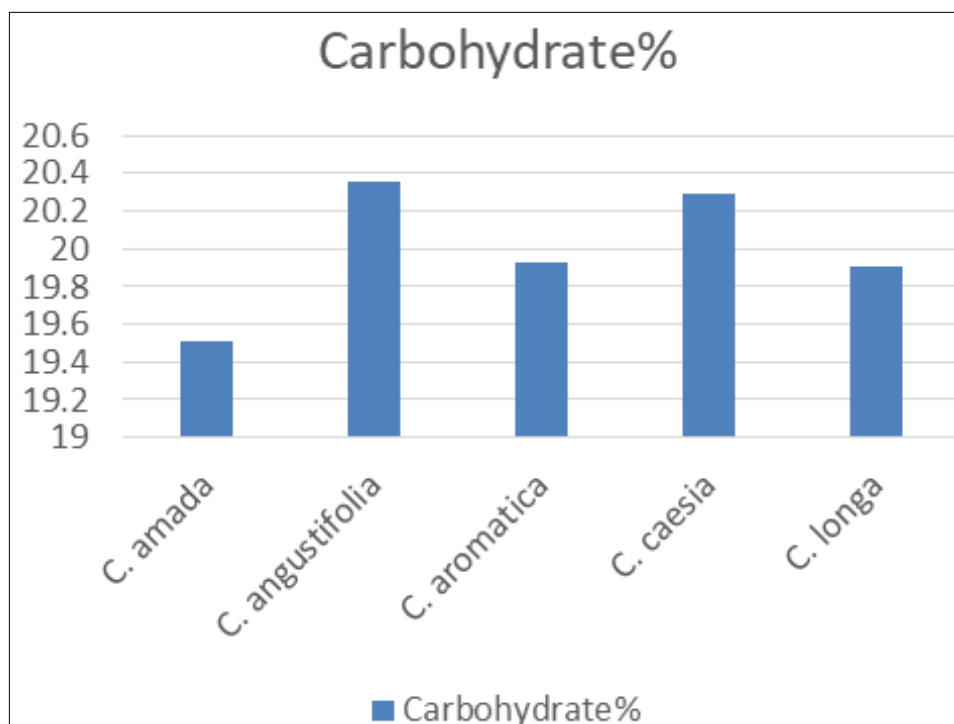


Fig 4: Relative Carbohydrate Content

3. Phytochemical assay

Results obtained for qualitative screening of phytochemicals in rhizome of *C. amada*, *C. longa*, *C. angustifolia*, *C. aromatica* and *C. caesia* have been presented in Table 3. Total six phytochemicals were screened in which three were found to be present in all the rhizome extracts. These were glycoside, tannins and alkaloids. Remarkably flavonoids, phenols, saponins, tannin and alkaloids were present in the rhizome of these plants. This suggests that the rhizomes have extensive potentials of phytochemicals.

During the present investigation alkaloids, glycosides, phenolics and tanin were found in the dried rhizome powder of *C. longa* and *C. angustifolia* while alkaloids, flavonoids, glycosides, phenolics and tannins were present in dried rhizome powder of *C. amada*. Alkaloids, flavonoids, glycosides, phenolics and saponin were found in the rhizome powder of *C. aromatic*. There was the presence of alkaloids, flavonoids, glycosides, phenolics, tannins and saponins in rhizome of *C. caesia*. (Table-3)

Table 3: Qualitative analysis of different bioactive compounds from the rhizome of the *Curcuma* species

Sl. No	Scientific Name	Plant part used for analysis	Alkaloid	Flavonoid	Glycoside	Phenolics	Tannin	Saponin
1	<i>Curcuma amada</i>	Dried Rhizome	+	+	+	+	+	-
2	<i>Curcuma angustifolia</i>	Dried rhizome	+	-	+	+	+	-
3	<i>Curcuma aromatica</i>	Dried Rhizome	+	+	+	+	-	+
4	<i>Curcuma caesia</i>	Dried Rhizome	+	+	+	+	+	+
5	<i>Curcuma longa</i>	Dried Rhizome	+	-	+	+	+	-

Phytochemical analysis of different species of *Curcuma* conducted by other investigators also reveals the presence of different types of phytochemicals which are responsible for their nutritional and medicinal importance. Ten phytochemicals, viz., carbohydrates, proteins, starch, amino acids, steroids, glycosides, flavonoid, alkaloid, tannins and saponins were isolated in the methanolic extracts of rhizomes of *C. longa* [40]. Ten phytochemicals were analysed from methanolic extracts of *C. longa* [41]. Six phytochemicals, viz., alkaloids, flavonoids, tannins, saponins, cardiac glycosides and phenolic were identified from the aqueous extracts of turmeric [42]. According to the percentage of yield, ethanolic extract gives more percentage than methanolic extracts [43].

Owing to the presence of bio actives like alkaloids, flavonoids, tannins, saponins, glycosides and phenolic substances in the rhizomes of *C. longa*, *C. caesia*, *C. aromatica*, *C. amada* and *C. angustifolia*, these species are pharmacologically very active. They possess several properties like antimicrobial, antidiabetic, anti-inflammatory properties, etc.

Conclusion

The study analysed the composition of carbohydrates, proteins, and secondary metabolites in some selected *Curcuma* species from the Northern Coastal region of Odisha, India. All most all five *Curcuma* species contain different amount of carbohydrates and proteins. All most all species contain medicinally important secondary metabolites for which they possess anti-microbial, anti-inflammatory, anti-cancer, anti diabetic, anti-oxidant and anti-allergic properties. The results implies that the *Curcuma* species are not only medicinally important but also nutritionally very rich. As such some of these species should be used as our common diet. Public awareness should be raised for the conservation and domestication of its potential wild species for the daily house hold uses.

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