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Study of secondary metabolite constituents and curcumin contents of six different species of genus *Curcuma*

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Abstract

Curcuma is a rhizomatous perennial plant which belongs to family Zingiberaceae having various ethnomedicinal significance. In this study, six different species of genus *Curcuma* i.e., *C. amada*, *C. caesia*, *C. angustifolia*, *C. leucorrhiza*, *C. longa* and *C. zedoaria* were screened for the presence of major secondary metabolites and curcumin contents were also analyzed. 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 phenols, flavonoids, alkaloids, terpenoids, tannins and saponins. Further, the curcumin contents of the ethanolic extracts of *C. longa*, *C. zedoaria*, *C. angustifolia*, *C. leucorrhiza*, *C. amada* and *C. caesia* were 125mg/100g, 88mg/100g, 71mg/100g, 15mg/100g, 11mg/100g and 8mg/100g of extract powder respectively.

Keywords: *Curcuma*, secondary metabolites, curcumin contents.

1. Introduction

The genus *Curcuma*, locally called 'haldi', a rhizomatous flowering plant belonging to family Zingiberaceae, comprising about 50 species in tropical regions and about 28 species in India, have been used in traditional systems of medicine (Ayurveda, Siddha, and Unani) for a long time. Among them, the most studied is *C. longa* which is known to possess tremendous therapeutic potency [1]. *C. amada*, *C. angustifolia*, *C. caesia*, *C. leucorrhiza* and *C. zedoaria* can be included lesser known species of *Curcuma* (Photo plate 1 to 6). They are also used variously in the traditional system of medicines. *C. amada* is useful in bronchitis, asthma, sprains, skin diseases, and inflammation caused due to injuries. Rhizomes of *C. caesia* are used for sprains and bruises and also employed in the preparation of cosmetics. *C. angustifolia* is an aphrodisiac and useful in the treatment of leprosy, asthma, anemia, and leucoderma. *C. zedoaria* is said to be antimutagenic, anticarcinogenic, as well as anti-inflammatory [2-3]. Rhizome infusion of *C. leucorrhiza* is given orally in stomach pain and indigestion.

Secondary metabolites, a group of bioactive substances, having diverse classes of compounds like alkaloids, terpenoids, phenols, flavonoids, tannins, saponins, etc., are produced through secondary metabolism in different plants. The medicinal value of plants lies in these chemical substances that have definite physiological action on the human body [4]. Phytochemical analysis of ethnomedicinal plants for secondary metabolites is an important area of fundamental research because of its relevance for the discovery of therapeutic agents and providing clues for new sources of bioactive compounds. Further, Curcumin, chemically curcuminoids, the main yellow bioactive component of *Curcuma longa* and its allied species, has been shown to have a wide spectrum of biologically actions including anticancer effect [5]. The present study was undertaken for screening of secondary metabolites such as alkaloids, terpenoids, phenols, flavonoids, tannins, saponins and to verify the variation of total curcumin contents in rhizomes of six *Curcuma* species (*C. amada*, *C. angustifolia*, *C. caesia*, *C. leucorrhiza*, *C. longa* and *C. zedoaria*) found in different agro-climatic regions of Assam and India, in order to explore their therapeutic potential.

2. Materials and Methods

Collection and Processing of Plant Material

Six different species of genus *Curcuma* i.e., *C. amada*, *C. angustifolia*, *C. caesia*, *C. leucorrhiza*, *C. longa* and *C. zedoaria* were collected from different agro-climatic region of

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Assam, North East India. The plants were processed and analyzed. The rhizomes of each plant were washed in tap water and then rinsed in 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 plant were pulverized by using grinder to obtain a powdered form.

Preparation of Extracts of Plant Material

Plant extracts of each plant were prepared using ethanol as extracting solvent. 100g of the dried and powdered plant material (rhizome) was extracted with 400ml of ethanol at 65 °C for 2 days using Soxhlet extraction method. After filtering and evaporating to dryness, the crude ethanolic extract was obtained.

Phytochemical Screening

Chemical tests were carried out qualitatively on each extract following standard procedures to identify the phytochemical constituents [6-7].

1. Test for alkaloids

Dragendroff's test: 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.

Mayer's test: To 1 ml of the extract, 2 ml of Mayer's reagent was added, a dull white precipitate indicated the presence of alkaloids.

Wagner's test: To 1 ml of the extract, 2 ml of Wagner's reagent was added. Appearance of a reddish brown precipitate indicated the presence of alkaloids.

Hager's test: Extracts were dissolved individually in dilute hydrochloric acid and filtered. Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids was confirmed by the formation of yellow coloured precipitate.

2. Test for flavonoids

Alkaline reagent test: To the test solution, a few drops of 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.

Lead acetate test: To the test solution, a few drops of lead acetate solution were added. Formation of yellow precipitate indicated the presence of flavonoids.

3. Test for phenolic compounds

Lead acetate test: To the test solution, a few drops of 10% lead acetate solution were added. Formation of white precipitate indicated the presence of phenolic compounds.

Ferric chloride test: To the test solution, a few drops of ferric chloride solution were added. A dark green colour indicated the presence of phenolic compounds.

4. Test for tannins

Lead acetate test: To the test solution, a few drops of 10% lead acetate solution were added. Precipitate formation indicated the presence of tannin.

5. Test for terpenoids

Salkowski's test: 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 of interface after

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

6. Test for saponins

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

Determination of curcumin content

0.1 g dry powder of each was dissolved in 50 ml absolute ethyl alcohol separately. The content was refluxed over heating mantle for one hour. The solution was cold and decanted into a volumetric flask. The extract volume was made up to 50 ml by adding ethyl alcohol freshly. Absorbance was measured directly at 425 nm in a spectrophotometer. The optical density was recorded. Then the extract was diluted with absolute ethyl alcohol two times and ten times respectively. Absorbance was measured at 425 nm in the same spectrophotometer for both the solutions.

Curcumin content was determined by using the following formula:

Curcumin contents mg/100gm=

$$\frac{0.0025 \times A_{425} \times \text{Volume made up} \times \text{Dilution factor} \times 100}{0.42 \times \text{Weight of the sample} \times 1000}$$

(Since 0.42 absorbance at 425 nm = 0.0025 gm)

3. Results and Discussion

The present study carried out on the ethyl alcoholic extract of rhizomes revealed the presence of medicinally active secondary metabolites. The studied secondary metabolite constituents in the six different species of *Curcuma* is summarized Table 1 and total curcumin contents is presented in Table 2 and figure 1. Alkaloids, flavonoids, terpenoids, phenols, tannins and saponins were found to be present in all the species (Table 1). Various experiments have been demonstrated that phenolic compounds such as flavonoids, phenolic acids, tannins, etc. are potential antioxidant and antioxidant activity of these compounds is due to their ability to scavenge free radicals. Accumulation of free radicals can cause pathological conditions such as asthma, arthritis, inflammation, neuro-degeneration, heart disease, aging effect, etc. [8]. Additionally, phenolic compounds act as (i) metal chelators, (ii) antimutagens and anticarcinogens, (iii) antimicrobial agents [9]. The growth of many fungi, yeasts and bacteria was inhibited by tannins [10]. Further, tannins and terpenoids are attributed for analgesic and anti-inflammatory activities. Apart from these, tannins contribute property of astringency i.e., faster the healing of wounds and inflamed mucous membrane [11]. 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 [12]. Total curcumins content study of the five species found to range from 8mg/100g to 125mg/100g (Table 2 and Figure 1). *C. longa* had the highest curcumins content (125mg/100g) followed by *C. zedoaria* (88mg/100g), *C. angustifolia* (71mg/100g), *C. leucorrhiza* (15mg/100g) *C. amada* (11mg/100g) and *C. caesia* (8mg/100g). Curcumin has been shown to have a wide spectrum of biological actions.

These include in its anti-inflammatory, antioxidant, anticarcinogenic, antimutagenic, antifertility, antidiabetic, antibacterial, antifungal, hypotensive and hypocholesterolemic activities. Its anticancer effect is mainly mediated through induction of apoptosis [5]. *C. longa*, the most studied species of *Curcuma*, is known to possess tremendous therapeutic potency [1]. The medicinal properties of *C. longa* can be attributed due to the presence of curcumin and secondary metabolites. In case of *C. amada*, *C. angustifolia*, *C. caesia*, *C. leucorrhiza* and *C. zedoaria*, also, showed the presence of most of the secondary metabolites, however, total curcumins content analysis revealed remarkable variation.

4. Conclusion

In conclusion, since time immemorial turmeric (*C. longa*) has been used as medicine for the treatment of various diseases, colorant and food additive. Presence of all the secondary metabolites under study having higher amount of curcumins is found to be impressive. In case of *C. amada*, *C. angustifolia*, *C. caesia*, *C. leucorrhiza* and *C. zedoaria*, however, some members showed lower level of curcumin contents (8mg/100g, 11mg/100g and 15mg/100g in *C. caesia*, *C. amada* and *C. leucorrhiza* respectively in comparison to 125mg/100g in *C. longa*); moreover, presence of most of the studied secondary metabolites is found to be encouraging and indicates the therapeutic potential of the species.



Plate 1. *Curcuma amada* Roxb.



Plate 2. *Curcuma angustifolia* Roxb.



Plate 3. *Curcuma caesia* Roxb.



Plate 4. *Curcuma leucorrhiza* Roxb.



Plate 5. *Curcuma longa* L.



Plate 6. *Curcuma zedoaria* Rosc.

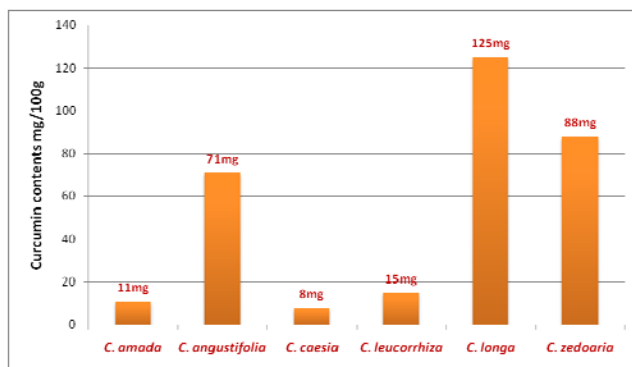
Table 1: Secondary metabolite constituents in the six different species of *Curcuma*

Secondary metabolites	Tests	<i>C. amada</i>	<i>C. angustifolia</i>	<i>C. caesia</i>	<i>C. leucorrhiza</i>	<i>C. longa</i>	<i>C. zedoaria</i>
Alkaloids:	Dragendorff's test	-	+	+	-	+	-
	Mayer's test	+	+	+	+	+	+
	Wagner's test	+	-	+	+	+	+
	Hager's test	+	-	+	-	+	-
Flavonoids:	Alkaline test	+	+	+	+	+	+
	Shinoda test	+	+	+	+	+	+
Terpenoids:	Lieberman test	+	+	+	+	+	+
	Salkowski test	+	+	+	+	+	+
Phenols:	Lead acetate test	+	+	+	+	+	+
	Ferric chloride test	+	+	+	+	+	+
Tannins:	Ferric chloride test	+	+	+	+	+	+
	Alkaline test	+	+	+	+	+	+
Saponins	Frothing test	+	+	+	+	+	+

Note: '+' indication of present; '-' indication of absent.

Table 2: Content of total curcumin in the six different species of *Curcuma*

Name of plant species	Curcumin contents mg/100g
<i>C. amada</i>	11
<i>C. angustifolia</i>	71
<i>C. caesia</i>	8
<i>C. leucorrhiza</i>	15
<i>C. longa</i>	125
<i>C. zedoaria</i>	88

**Fig 1:** Comparative analysis of curcumin contents in the six different species of *Curcuma*.

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