



PHYSICOCHEMICAL AND PHYTOCHEMICAL INVESTIGATION OF THREE DIFFERENT SPECIES OF *CURCUMA RHIZOME*

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ABSTRACT

The Zingiberaceae, the largest family in the Zingiberales, comprises nearly 50 genera and 1000 species and is pantropical concentrated mainly in the old world, chiefly in Indomalaysia. Members of the family yield species, dyes, perfumes, medicines and a number of ornamental species are cultivated for their ornamental flowers. *Curcuma* is one of the important 'Rasayana' drugs mentioned in Ayurveda.

In the present study three successive extractions of plant rhizomes viz. *C. amada*, *C. malabarica* and *C. zedoaria* were undertaken by using various solvent like alcohol, hydro-alcohol and water in their increasing polarity and the extracts thus obtained were subjected for their phytochemical analysis, followed by Thin Layer Chromatographic examination by optimizing the solvent system.

Keywords: Zingiberaceae, Rasayana, Curcumin, Cinnamaldehyde, Camphor.

INTRODUCTION

Plants have been a source of medicine for thousands of years, and phytochemicals continue to play an essential role in Indian culture and health care¹. The origin of the effective drugs is given in our traditional texts with their formulations, in this view studies pertaining for testing of folklore medicinal plants for pharmacological studies has been taken up².

Most of the epidemiological studies established a link between phytochemicals and the range of biological activities that impart health benefits in human beings which dates back to Charaka Samhita. Scientific research supports the biological activity of many of the phytochemicals in isolation and combination. They were copiously used in Ayurveda and other traditional medicine. The groups of spices are considered to be the storehouse of active phytochemicals. Several groups of polyphenols (anthocyanins, proanthocyanidins, flavanones, isoflavones, resveratrol and ellagic acid), non-nutrient chemical and dietary constituents are currently used in the pharmaceutical industry. The various spices belonging to the genus *Curcuma* are well known for their multiple uses as medicines, cosmetics, dyes, flavourings and nutraceuticals³.

In developing countries, the practice of medicine still relies heavily on plant and herbal extracts for the treatment of human ailments and as a dietary supplements for health care. Dietary agents consist of a wide variety of biologically active compounds that are ubiquitous in plants, and many of them have been used as traditional medicines^{4,5,6}. Traditionally, curcuma drugs called "Ukon" and "Gajutsu" in Japanese have been used in oketsu syndromes (caused by the obstruction of blood circulation) in Chinese medicine⁷.

Out of *Curcuma* family *Curcuma longa* is the most important species. There are extensive in-vitro and in-vivo investigations on turmeric extracts (ethanol, acetone, water and ethyl acetate extracts) or "pure" active "curcumin" powder over last half century. So the present study is focused on *C. amada*, *C. malabarica* and *C. zedoaria*. *C. amada* and *C. zedoaria* are distributed throughout India in the wild and cultivated forms; where as *C. malabarica* occur in south India⁸.

Curcuma amada Roxb., Zingiberaceae, (Mango-ginger) is a perennial herb cultivated as an annual with rhizomes having characteristic odour of raw mangoes. The rhizome finds extensive use in the indigenous systems of medicine⁹. *C. amada* is found the wild in parts of West Bengal, and is cultivated in Gujarat, Uttar Pradesh, Kerala, Karnataka, Tamil Nadu and the north-eastern states⁸.

Curcuma zedoaria a medicinal tuber plant belonging to this family, is a close relative of *Curcuma longa*. Various parts of *Curcuma zedoaria* are used in Ayurveda and other folk and tribal system of medicines¹⁰. *Curcuma zedoaria* is a constituent of a wide variety of ayurvedic preparations like Dasamularishtam, Valiya Rasnadi Kashayam, and so forth. The rhizome is used for curing stomach diseases, toothache, blood stagnation, leucoderma, tuberculosis, enlargement of spleen, and for promoting menstruation in traditional medicine in Asia¹⁰. *Curcuma zedoaria* (Christm) Roscoe has many components such as essential oils, oil-resin, terpenic compounds and other constituents, with a wide spectrum of biological properties. Presence of extract and the essential oil has cineol, camphene, alpha-pinene, camphor and other compounds this plant has been used in folk medicine, against digestive and gall bladder disorders, cough, hepatic disorders and halitosis, besides presenting anti-inflammatory and antimicrobial activities¹¹. Extracts of *Curcuma zedoaria* and *Curcuma malabarica* tubers show antimicrobial activity¹².

In the present study, physicochemical evaluation of three different species i.e. *C. amada*, *C. malabarica* and *C. zedoaria* has been later up. The extraction was carried out in three different solvent system i. e. alcoholic, hydro-alcoholic and water system and their comparison by TLC profiling.

MATERIAL AND METHODS

The rhizomes of three different species from *C. amada*, *C. malabarica* and *C. zedoaria* was collected from the different geographical region and used for the study.

Reagent and chemicals

All the reagent and chemicals used for the study were of analytical grade and purchased from authorized dealer of the company.

Physico-chemical Evaluation

Physico-chemical parameters such as the total ash, acid insoluble ash, acid soluble ash, water insoluble ash, water soluble ash were determined as per standard methods. Considering the diversity of chemical nature and properties of contents of drugs, three different solvents water, alcohol and hydroalcohol were used for the determination of extractive values as per standard methods¹³⁻¹⁵. All determinations were carried in three experiments and the results were presented as mean $\pm$ standard error of mean (SEM).

Extraction process

The preliminary phytochemical screening of the plant involves extraction of the plant material and identification of the plant active constituents.

Preparation of extracts

Method of extraction

Continuous hot percolation process by using Soxhlet apparatus

Materials

1. Soxhlet apparatus
2. Alcohol
3. Distilled water
4. Shade dried coarse powder of rhizome of different sp. of *Curcuma*.

METHODS

Preparation of extract

The shade dried powdered material (60 mesh, 100g) of each species was extracted with alcohol, hydroalcohol (30:70) and water. The extracts obtained was concentrated under vacuum (-760mmHg) and stored in a desiccator. The weight of the each extract is given in tablet.

Development Method

One dimensional ascending method by using standard protocol was followed. Different extracts were applied 1cm above from the base of the TLC plates. Development was done using petroleum ether: ethyl acetate (7:3) solvent systems.

Visualization

Developments of chromatograms were carried out by spraying anisaldehyde-H₂SO₄ reagent followed by gentle heating at 105°C till the spots were developed^{16,17}.

Documentation

After visualization as mentioned above different spots were detected. The Rf values of those spots were recorded carefully and the chromatograms were documented by photography and UV light.

Phytochemical Screening

The TLC profile from the various extract demonstrates the presence of cinnamaldehyde and camphor. The details of Rf values are given in table-3.

The detail about phytochemical constituents present in various extracts is summarized in table-4.

Phytochemical Evaluation

1. Test for Steroid

To 2ml of extract add 2ml chloroform and 2 ml conc. H₂SO₄. Shake well, chloroform layer appear red and acid layer show greenish yellow fluorescence¹⁸.

2. Test for Glycoside

To the solution of the extract add glacial acetic acid, few drops 5% ferric chloride and concentrated sulphuric acid are added, and observed for a reddish brown coloration at the junction of two layers and the bluish green color in the upper layer¹⁹.

3. Test for Flavanoid

To 4 ml of extract add 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added, red color was observed for flavonoids and orange color for flavones²⁰.

4. Test for Alkaloid

To 0.5g of each extract adds 5ml of 1% aqueous hydrochloric acid and kept in water bath; 1ml of the Filtrate is to be treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow colored precipitate indicates the presence of alkaloids.

5. Test for Tannin

To 0.5 ml of extract solution, 1 ml of water and 1-2 drops of ferric chloride solution was added. Blue color was observed for gallic tannins and green black for catecholic tannins¹⁹.

6. Test for Saponin

To 1 ml extract add 2 ml distilled water and shake it. Persistent foam was observed.

Table 1: Physicochemical Properties of *Curcuma* sp.

Quantitative standards	<i>C. amada</i>	<i>C. malbarica</i>	<i>C. zedoaria</i>
Total Ash Value (in %w/w)	5.28 $\pm$ 0.29	6.09 $\pm$ 0.22	7.50 $\pm$ 0.16
Acid Insoluble Ash (in %w/w)	0.39 $\pm$ 0.18	0.39 $\pm$ 0.18	0.55 $\pm$ 0.15
Aqueous soluble Ash (in %w/w)	10.67 $\pm$ 0.32	15.89 $\pm$ 0.22	19.61 $\pm$ 0.73
Moisture content (in %w/w)	7.96 $\pm$ 0.35	11.02 $\pm$ 0.18	5.35 $\pm$ 0.94

Table 2: Percentage yield in successive solvent extraction

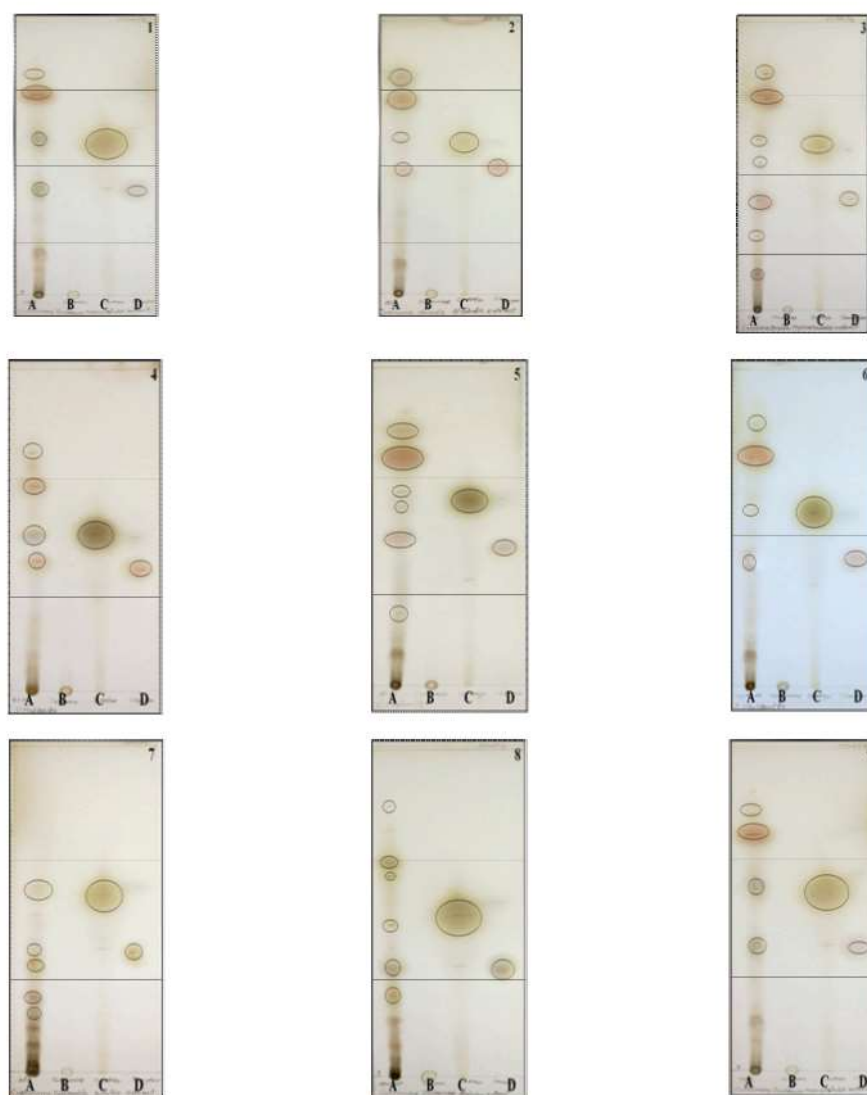
Extracts	<i>C. amada</i>	<i>C. malbarica</i>	<i>C. zedoaria</i>
Water soluble extractive	30.48%	10.97%	5.96%
Alcohol soluble extractive	6.54%	9.49%	7.17%
Hydro-alcohol soluble extractive	6.51%	10.59%	14.11%

Table 3: TLC analysis of extracts of plant with their Rf values

Curcuma Sp.	Plate No.	Extract	Curcumine	Cinnamaldehyde	Camphor
C. Amada	Plate a	0.53, 0.64, 0.79	-	0.37	0.53
	Plate b	0.51, 0.59, 0.73, 0.82	-	0.35	0.56
	Plate c	0.56, 0.70, 0.79	-	0.38	0.55
C. malbarica	Plate d	0.39, 0.64, 0.74	-	0.33	0.52
	Plate e	0.09, 0.56, 0.72	-	0.32	0.54
	Plate f	0.55, 0.61, 0.71, 0.80	-	0.32	0.57
C. zedoaria	Plate g	0.22, 0.32, 0.38	-	0.37	0.56
	Plate h	0.61, 0.74, 0.80	-	0.39	0.59
	Plate i	0.26, 0.52, 0.67	-	0.36	0.55

Table 4: Phytochemical constituents present in various extracts

Phytochemical constituents	Curcuma amada	Curcuma malbarica	Curcuma zedoaria
Steroids/Triterpenoids	+	+	+
Glycoside	+	+	-
Flavonoids	+	+	+
Alkaloids	-	-	-
Tannins	-	-	-
Saponins	-	-	-

Figure 3: TLC finger printing profile of *Curcuma* sp.

(Spot A- extract, B-Curumine standard, C-Cinnamaldehyde standard, D-Camphor standard)

1. TLC profile of water soluble extractive of *C. amada*, 2. TLC profile of alcohol soluble extractive of *C. amada*, 3. TLC profile of hydro-alcohol soluble extractive of *C. amada*, 4. TLC profile water soluble extractive of *C. malabarica*, 5. TLC profile of alcohol soluble extractive of *C. malabarica*, 6. TLC profile of hydro-alcohol soluble extractive of *C. malabarica*, 7. TLC profile water soluble extractive of *C. zedoaria*, 8. TLC profile of alcohol soluble extractive of *C. zedoaria*, 9. TLC profile of hydro-alcohol soluble extractive of *C. zedoaria*

RESULTS

Physico-chemical Evaluations

The physicochemical studies of rhizome of *Curcuma* Sp. are summarized in table-1.

The percentage yield in successive solvent extraction is summarized in table-2.

DISCUSSION

The physical evaluation furnished different ash values, extractive values in three different species of *Curcuma*. Total ash, acid insoluble ash and water soluble ash values were also determined.

Three different solvents viz. Water, alcohol, hydro-alcohol soluble was selected for extractive values study. This showed water soluble extractive value was found to be more than alcohol soluble extractive value however it was less than hydro-alcohol soluble extractive value.

The TLC analysis was performed by usual technique using the optimized solvent systems specific for the various classes of compound. Detecting reagent was used to visualize the chromatograms. Thus a variety of standardization parameters viz. physico-chemical, phytochemical and chromatographical parameters were studied and a data was generated to ensure the quality of plant material for future reference.

CONCLUSION

The methanolic extracts of the studied plants showed the presence of bioactive compounds in all the three species, amongst the three *Curcuma amada* and *Curcuma malabarica* have maximum bioactive compounds. Thus these two are pharmacologically and medicinally more important than *Curcuma zedoaria* which has been discussed.

The TLC profile of the three different extracts also put the information that cinnamaldehyde and camphor is present in all the three species of *Curcuma* but curcumin is totally absent or in such a less quantity to detect by TLC finger printing.

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