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PHYTOCHEMICAL ANALYSIS OF *CURCUMA CAESIA* ROXB. AND *CURCUMA AROMATICA SALISB.* OF UPPER BRAHMAPUTRA VALLEY, ASSAM, INDIA

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ABSTRACT

The present study deals with phytochemical and TLC analysis of two ethnomedicinally important Zingiberaceae medicinal plants Curcuma caesia Roxb. and Curcuma aromatica Salisb. from Upper Brahmaputra Valley (UBV) Zone of Assam, India. Preliminary phytochemical screening of rhizome in different solvents were conducted and were found to exhibit positive test for different phytoconstituents like tannin, phenols, flavonoids, alkaloidas , terpanoids, saponins, amino acids etc. The total phenol and flavonoid contents in Curcuma caesia is 63.42 mg/g, gallic acid equivalent and 32.87 mg/g, quercetin equivalent respectively. Similarly, in Curcuma aromatica it is 59.8 mg/g, gallic acid equivalent and 32.47 mg/g, quercetin equivalent respectively. TLC analysis of Methanol extract confirmed the presence of alkaloids, flavonoids, tannins and phenols in the rhizome of both the plants.

KEY WORDS

Curcuma caesia; C. aromatica; phytochemicals; UBV zone; TLC.

INTRODUCTION

Man's dependence on plant life for food as well as for health has been as old as human existence. In spite of considerable progress in the synthetic drugs, plant and plant products are still considered to be the major sources of medicaments and have extensive use in ethno-medicine and traditional system of medicine (Nath and Borah, 2011). Assam (24°2' - 27°6' N latitudes and 88°8' - 96° E longitudes) is blessed with rich heritage of plant kingdom. There are so many plants that are traditionally claimed to be useful against many diseases and are being used till the date by various ethnic communities inhabiting in Assam. Plants, since times immemorial, have been used virtually in all the cultures as a source of medicine (Singh and Dubey, 2012). In the developing world, especially in rural areas, herbal remedies continue to be a primary source of medicine. India has a long tradition of the application of plant remedies. The northeasternrn part of India is very rich in medicinal plants diversity.

Zingiberaceae, is a Monocotyledonous family of yielding spices, condiments, dyes, perfumes and medicines besides many ornamental species cultivated for their showy flowers (Borah and Sharma, 2012). Zingiberaceae is well known for its immense medicinal values and is widely distributed throughout the tropics, particularly in Southeast Asia. India is one of the richest and diverse regions for Zingiberaceae, having 22 genera and about 170 species. The NE region of India is a zone of greatest concentration of Zingiberaceae members where 19 genera and about 88 species are reported (Prakash and Mehrotra, 1995). Most of the Zingiberaceae members are found here at wild states and many of them are lack behind of scientific investigation. Literature survey has pointed out the importance of the plants as herbal medicine but reports on ethnobotanical study of Zingiberaceae members in an effective way are scanty.

The most important genera coming under Zingiberaceae are *Curcuma, Kaempferia, Hedychium, Amomum, Zingiber, Alpinia, Elettaria* and *Costu* (Joy et al., 1998).

Plants of genus *Curcuma* belongs to Zingiberaceae family is known for their high therapeutic potentials. The genus *Curcuma* in Assam is represented by *Curcuma longa* Linn. Curcuma *aromatica* Salisb., *Curcuma amada* Roxb., *Curcuma angustifolia* Roxb., *Curcuma caesia* Roxb. etc. Some work has been carried out on *Curcuma longa* and *Curcuma amada* from this region, but *Curcuma caesia and Curcuma aromatica* are two widely used medicinal plants found in wild state is very less attended.

Ehnobotanical study reveals that both the plants are used by different ethnic communities of study site to cure various ailments including dysentery, stomach ache, indigestion, constipation, worm infection, gastric, toothache, skin diseases, itching, sprains etc. Therefore, the present study deals with phytochemical analysis of two important Zingiberaceae medicinal plants *Curcuma caesia* Roxb. and *Curcuma aromatica Salisb*. from Upper Brahmaputra Valley Zone, Assam, India in order to validate the ethnobotanical claims.

MATERIALS AND METHODS

About the study site:

Assam is situated in North-East India. Assam can be broadly divided into the Six Agro-climatic regions. The Upper Brahmaputra Valley (South) zone comprises the Districts of Tinsukia, Dibrugarh, Sibsagar, Jorhat, Golaghat and newly formed district Charaideo and Majuli covering an area of 16,013 sq.km accounting for 20.40 percent of total area of Assam and is extended between 26.45° and 27.15° N latitudes and 94.25° and 95.25° E longitudes. It has elevation of 86.6 Mtrs. The average annual rainfall is 108.44 cm and temperature vary between 15°-35°C (Acharyy and Sharma, 2004). Soil is alluvial and suitable for cultivation. Semi evergreen-deciduous forest and grassland are the dominating vegetation type of the study site. The Climate is characterised by very wet summer months followed by sunny winters.

Analysis of odour, colour and moisture content of the rhizome of *Curcuma caesia and Curcuma aromatica:*

Vertical sections of fresh rhizomes were made to analysis the odour and colour of the rhizome. Moisture content were also determined using the ISTA methods ,1996 (Sarangthem, K. and Haokip, M.J., 2010) as follows-

Moisture content (%) = Original Weight-Over dry Weight/ Original Weight × 100

Phytochemical analysis

Collection of plant materials

The fresh rhizome of *Curcuma caesia* Roxb. and *Curcuma aromatica* Salisb were collected from three districts of Upper Brahmaputa Valley of Assam, India viz. Sivasagar, Jorhat and Golaghat in the month of October – November 2017. The rhizome was then washed carefully to remove all the dirt and cut into small pieces and were shade dried separately. The well dried rhizome pieces were then grounded to fine powder using a mixer grinder and preserved separately in airtight containers with proper labelling for future use.

Preparation of plant extract

20 gm of powder of both the species was macerated overnight separately with 150 ml of ethanol, methanol and ethyl actate. Then, the macerated drug was kept for extraction in Soxhlet apparatus at 50º C for 5 hours. Then, the extract was collected and concentrated by evaporating and the extracts were kept in refrigerator at 4^0C until use.

Qualitative phytochemical screening

Preliminary phytochemical screening of different extracts of rhizome of *C. aromatica* and *C. caesia* were done by using standard procedures (Harbone, 1998; Edeoga *et al*, 2005) for the detection of the presence of flavonoid, tannin, alkaloid, phenol, terpenoid, saponin, quinone, sterols, protein and carbohydrate etc.

Test for tannin:

i) To 0.5 ml extract solution, added 1 ml distilled water and 1-2 drops of ferric chloride solution to it and observed for blue black coloration which indicates presence of tannin ii) 10% lead acetate solution was added to 0.5 ml extract solution and observed for white precipitation which indicates presence of tannin.

Test for saponin:

0.2 g of the extract was shaken with 5ml of distilled water and then heated to boil. Frothing shows the presence of saponin.

Test for flavonoid:

0.2 g of the extract was dissolved in 10% NaOH solution, yellow coloration indicates the presence of flavonoid.

Test for phenol:

To 2ml of extract solution, added 2ml of alcohol and few drops of ferric chloride solution and observed for coloration.

Test for cardiac glycoside:

To the plant extract few ml of glacial acetic acid, ferric chloride and concentrated H2SO4 were added. Green color indicates the presence of cardiac glycosides

Test for alkaloid:

Extracts were dissolved individually in diluted hydrochloric acid and it is filtered. Mayer'sreagent was added separately to about 1ml of the filtrate in a different test tube.The formation of a faint turbidity or precipitation on the addition of the above reagents indicates the presence of alkaloids.

Test for terpenoid and steroid:

5ml of extract solution was mixed in 2ml of chloroform, and 3ml of conc. sulphuric acid was added to form a layer. A reddish-brown colouration of the interface was formed to show positive results for the presence of terpenoid. Red colour at the lower surface indicates presence of steroid.

Test for Amino acids: Two drops of Ninhydrin Reagent was added to the plant extract. Purple colour indicates the presence of amino acids.

Quantitative phytochemical analysis:

Determination of Total Phenolic Content:

Estimation of total phenol content in the selected plant extract was measured spectrophotometrically by Folin–Ciocalteu colorimetric method, (Nabavi et al., 2008, Kametkar et al., 2014, Sahu and Saxena, 2013), using Gallic acid as the standard and Total phenol value is expressed in terms of gallic acid equivalent (GAE) as mg/g of of sample. For this purpose, the calibration curve of gallic acid was drawn (FigureII). 1ml of standard solution of concentration0.01, 0.02, 0.04, 0.06, .08 and 0.1 mg/ml of gallic acid were prepared in methanol. Concentration of 0.1 and 1mg/ml of plant extract were also prepared in methanol and 0.5ml of each sample were introduced into test tubes, Folin-Ciocalteu reagent 5ml (1:1 diluted with distilled water) and mixed thoroughly. After five minutes 5ml of 10% Na2CO3 solution was added. The solution was warmed for one minute, and then cooled. The absorbance of the reaction mixtures were measured at 760 nm with UV Visible spectrophotometer.

Determination of the Total Flavonoid:

Aluminum chloride method was used for flavonoid determination **(***Olajire* and Azeez, 2011, Sahu and Saxena, 2013). In this method Quercetin was used as standard and flavonoid contents were measured as quercetin equivalent. For this purpose, the calibration curve of quercetin was drawn (Figure II). 1ml of standard solution of concentration0.01, 0.02, 0.04, 0.06, .08 and 0.1 mg/ml of quercetin were prepared in methanol. Concentration of 0.1 and 1mg/ml of plant extract were also prepared in methanol. 1ml of standard or extract solution (concentration0.01, 0.02, 0.04, 0.06, .08 and 0.1 mg/ml) was taken into 10ml volumetric flask, containing 4ml of distill water. 0.3ml of 5%NaNO2 added to the flask. After 5min, 0.3ml 10%AlCl3 was added to the mixture. At the 6th min add 2ml of 1M NaOH was added and volume made up to 10ml with distilled water. The absorbance was noted at 510nm using UV-Visible spectrophotometer.

TLC analysis of methanolic extract of *Curcuma caesia* and *Curcuma aromatica*:

Preparation of Extracts: In brief, 10 grams of each plant material was weighed, mixed with 100 ml of extraction solution and agitated for 48 h at 30 °C and 150 rpm. After incubation the extract was collected and fresh extraction solution (50 ml) was added to the flask and incubated in same conditions for another 24 h. Extracts were pooled and filtered through Whatmann no. 1 filter paper. The extracts ware dried in Soxhlet apparatus and dissolved in suitable amount of solvents.

Preparation of TLC Plates:

The TLC plates were prepared by using Silica gel 'G' as 30 gm of silica gel was weighed and made to a homogenous suspension with 60 ml distilled water for two minutes, this suspension was distributed over the plate which was air dried until the transparency of the layer disappeared. The plates were dried in hot air oven at 110°C for 30 mins and then stored in a dry atmosphere and used whenever required. Samples were prepared by diluting the crude extracts with solvent and then applied usually 5 µl volumes to the origins of a TLC plate 2cm above its bottom with the help of capillary tubes.

Development of Chromatogram:

After the spot loaded on TLC plates it was allowed to dry. The TLC plates were placed in the containers having different solvents listed below (SI to SIV) and left to run the solvent till upper end. TLC was performed for

alkaloids, flavanoids, tannins and phenols adopting the method of Sonam et al., 2017.

Solvent I: Ethyl acetate: Chloroform: water (5:3:1) for Alkaloids

Solvent II: n-Butanol: Ethyl acetate: water (5:10:15) for Flavonoids

Solvent III: Chloroform: water: (6:4) for Tannins.

Solvent IV: Methanol: water (6:3) for Phenols.

RESULT AND DISCUSSION:

Vertical sections of fresh rhizomes were made to analysis the odour and colour of the rhizome. The colour of the rhizome of *C. caesia* is Light bluish – black with Camphoraceous, Pungent smell and the colour of the rhizome of *C. aromatica* is orange yellow with Camphoraceous smell moisture content is 16.63% and 14.21% repectively, (Table – 1).

Table: 1. Odour, colour and moisture content of the rhizome of *Curcuma caesia and Curcuma aromatica*

Name of the species	Odour	Colour	Moisture Content
Curcuma caesia	Camphoraceous, Pungent smell	Light bluish - black	16.63 %
Curcuma aromatica	Camphoraceous	Orange yellow	14.21 %

Table- 2: Qualitative Phytochemical screening of rhizome extracts of *C. caesia* and *C. aromatica.*

Plants	Solvents	Phytochemicals							
		Tan.	Sap.	Phen.	Flav.	Alka.	Terpa.	Glyc.	Amin.
C. caesia	Petroleum Ether	+	_	+	_	+	+	_	_
	Chloroform	+	_	+	+	+	+	+	_
	Ethyl acetate	+	_	+	+	+	+	_	_
	Ethanol	+	_	+	+	+	+	+	_
	Methanol	+	+	+	+	+	+	+	+
C. aromatica	Petroleum Ether	+	_	+	_	+	_	_	_
	Chloroform	_	_	+	+	+	+	+	_
	Ethyl acetate	_	_	+	+	+	_	_	_
	Ethanol	+	+	+	+	+	+	+	+
	Methanol	+	_	+	+	+	+	+	+

Tan. - Tanin; Sap. - Saponin; Phe. – Phenol; Flav.- Flavnoid; Alk.- Alkaloid Terp.- Terpenoid Glyc.-Glycoside
Amin. - Amino acid S. '+' Positive; '-' Negative.

Figure - I: Standard calibration curve of Gallic acid

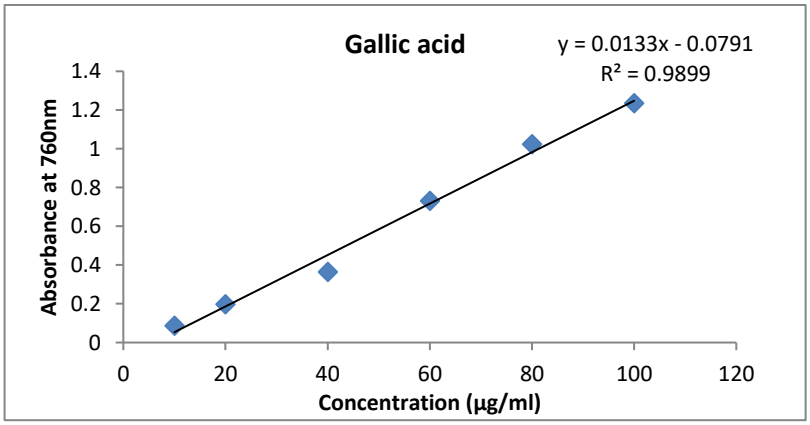


Figure - II: Standard calibration curve of Quercetin

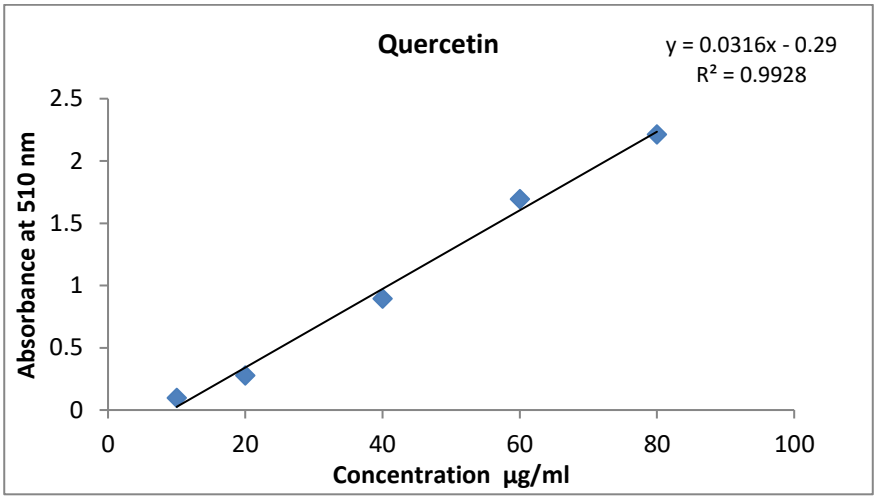


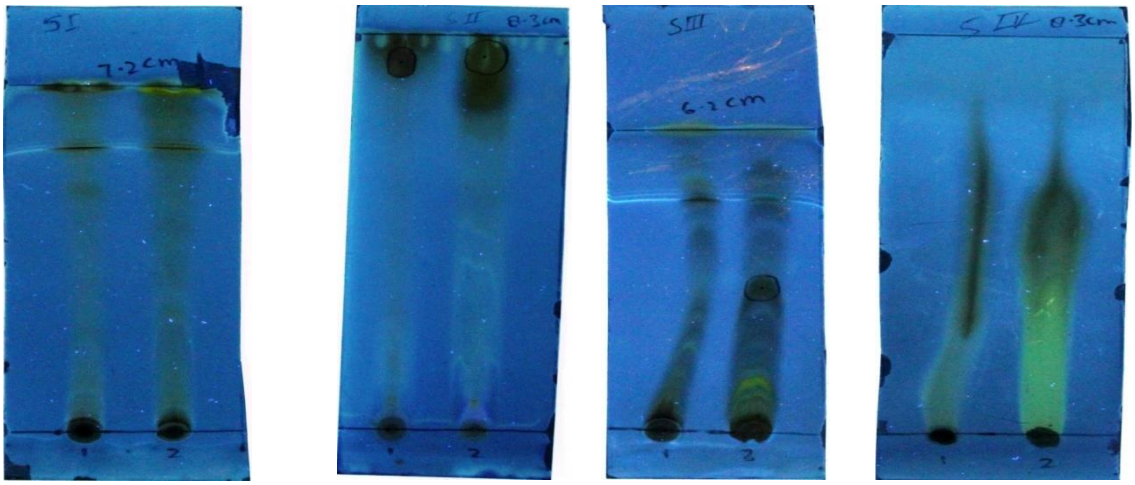
Table- 3: Total amount of Phenol and Flavonoid content.

contentsName of the species	Plant part/ Extract name	Total phenol (in mg/g, gallic acid equivalent)	Total flavonoid (in mg/g, quercetin equivalent)
Curcuma caesia	Rhizome/ Methanol extract	63.42	32.87
Curcuma aromatica		59.8	32.47

Table – 4: Rf value in Methanolic extract:

Solvent system	Rf Value	
	C. caesia	***C. aromatica***
Solvent I: Ethyl acetate: Chloroform: water (5:3:1) for Alkaloids	6/7.3=0.822	6/7.3=0.822
Solvent II: n-Butanol: Ethyl acetate: water (5:10:15) for Flavonoids	7.8/8.4=0.929	8/8.4=0.952
Solvent III: Chloroform: water: (6:4) for Tannins	4.9/6.3=0.777	2.9/6.3=0.46
Solvent IV: Methanol: water (6:3) for Phenols	4.4/8.3=0.530	4.8/8.3=0.578

Figure – III: Thin layer chromatography of methanol extract of *Curcuma caesia* (1) and *Curcuma aromatica* (2)



Alkaloid **Flavonoid** **Tannin** **Phenol**

The preliminary phytochemical screening of the rhizome of *Curcuma caesia* Roxb and *Curcuma aromatica* Salisb was found to exhibit the positive tests various phytochemicals viz. tannin, phenols, flavonoids, alkaloidas , terpanoids and negative test for saponins, amino acids (Table – 2).

Total amount of phenol and flavonoid contents: Total amount of phenol and flavonoid contents were

calculated from gallic acid (y = 0.013x – 0.079, R2 = 0.989) and quercetin (y = 0.031x – 0.029, R2 = 0.992) standard curves (Figure I & II). The total phenol and flavonoid contents in Curcuma caesia is 63.42 mg/g, gallic acid equivalent and 32.87 mg/g, quercetin equivalent respectively. Similarly, in *Curcuma aromatica* it is 59.8 mg/g, gallic acid equivalent and 32.47 mg/g, quercetin equivalent respectively (Table-3)

Results of TLC Analysis: TLC analysis of Methanol extract confirmed the presence of alkaloids, flavonoids, tannins and phenols with Rf value shown in (Table – 4).

CONCLUSION

Curcuma caesia Roxb. and *Curcuma aromatica* Salisb. are two important medicinal plants of Zingiberaceae. Both the species are widely used by different ethnic communities of study site like, Ahom Deuris, ,Kaibartta, Kalita, Mishing, Deuri, Chutiya,Tea tribes etc as phytoremedies to cure various ailments including dysentery, stomach ache, indigestion, constipation, worm infection, gastric, toothache, skin diseases, itching, sprains etc.. *Curcuma caesia* is a critically endangered and *C. aromatica* is a threatened species as per the IUCN Red List of threatened species. Both the species are found in the wild habitats of the study site, but their status in the study site is very rare.

Phytochemical screening exhibit positive test for different phytoconstituents like tannin, phenols, flavonoids, alkaloidas, terpanoids, saponins, amino acids etc in both the plants. Presence of important phytocostituents in both phytochemical and TLC analysis clearly indicates the medicinal value of both the plant species, which validates the traditional knowledge of different ethnic communities if the study site regarding the application of these plants as phytomedicines against different ailments. Therefore, in depth study on these plants from the study site have a positive prospect.

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