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PHYTOCHEMICAL SCREENING AND TOTAL ANTIOXIDANT ACTIVITY OF *Curcuma caesia* (ROXB.)

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ABSTRACT

The present study was carried out in the year 2022 at Department of Botany, Pravabati College, Mayang Imphal, to analyse the phytochemical screening and antioxidant activity of plant-derived products which has greatly influenced by various extraction factors like the method of extraction, temperature and solvent used. Phytochemical screening of *Curcuma caesia* was carried out to determine the phytoconstituents and its antioxidant activity using three different solvent *viz*., Methanol, ethanol and ethyl acetate. Pytochemical screening among the solvent reveals that almost all the important primary and secondary metabolite *viz*., alkaloids, flavonoids, phenolic compounds, tannins etc. were present in methanol and ethanol solvents except ethyl acetate solvent. The total phenolic content in methanol and ethanol rhizome extracts in terms of gallic acid equivalent was 7.27 and 9.40 of extract powder respectively. No phenolic content was observed in ethyl acetate extract. Total antioxidant activity varied among the solvent used in extract with ethanol extract having higher activity than methanol extract. The total antioxidant activity of methanolic and ethanolic extracts of rhizome were found highest (53.4 ± 0.22 µg AAE mg^{-1} and 65.1 ± 0.27 µg AAEmg^{-1} respectively) in highest concentration of 90 µg ml^{-1} of extract. Ethanol was found to be a better solvent for extracting phytochemicals. Total phenolic content, total antioxidant activity and reducing power were highest in ethanolic rhizome extracts. Phytochemical screening showed the presence of almost all the primary and secondary metabolites in methanolic and ethanolic extract of *Curcuma caesia* rhizome except ethyl acetate. The present study indicates rhizome of *Curcuma caesia* possessed antioxidant activity. Ethanol was found to be a better solvent for extracting phytochemicals from *C. caesia.* Total phenolic content was highest in ethanol extract than methanol extract. Total antioxidant activity and reducing power was highest in ethanolic rhizome extract.

(Key words: Phytochemical screening, antioxidant, methanol, ethanol, *Curcuma caesia*)

INTRODUCTION

Approximately, 70% of Indian population relies on traditional medicine to meet primary health care needs (Lavekar and Sharma, 2005). North East India's culture is firmly rooted in traditional medicine. Traditional herbalists Maibas and Maibis preserved medicinal plants *in-situ* in Umanglai (Sacred forests) and *ex-situ* in their personal gardens (Khumbongmayum *et al*., 2005). These have been many reports by different researchers in recent years on folkloric treatment with herbal medicine by the Meitei community in Manipur (Maibam *et al*., 2022; Shyamkiran, 2022; Tomba, 2017 and Yumnam and Tripathi, 2012) reported for folkloric treatment with herbal materials that are being used to cure different diseases by the peoples of Manipur.

With over 1300 species and 52 genera of aromatic perennial herbs with creeping horizontal tuberous or rhizomes, the Zingiberaceae family of flowering plants—commonly referred to as the "ginger family"—is found throughout tropical Asia, primarily in the North Eastern corridor of India. Many species of this family are used as a source of ornamentals, spices or as medicinal plants by the ethnic communities of the region. Members of this families range from small to large herbs with basal sheaths that overlap to form a pseudostem. Both chronic and viral disorders are treated with the bioactive compounds of the Zingiberaceae family. *Curcuma caesia, Alpinia galanga*, *Alpinia nigra*, *Amomum aromaticum*, *Hedychium flavum*, and other members of the Zingiberaceae family are some examples. These natural compounds continue to be sources of cutting-edge medicinal agents for a range of ailments, including infectious disorders. Additionally, due to the effectiveness of many therapeutic applications, developed countries are beginning to adopt herbal medicinal systems. As a result of their preventive mechanisms against conditions like cancer, heart disease, diabetes mellitus, and neurodegenerative disorders, as well as their efficacies in antiviral and antibacterial properties. The herbal medicinal system can also be referred to as "man-friendly medicine"

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because phytochemical compounds treat numerous diseases without harming humans. Additionally, due to their low toxicity, accessibility, and affordability, medicines based on plants have played a significant role in the study and development of novel therapeutics.

Curcuma caesia, a perennial herb belonging to the Zingiberaceae family is native to North-East and Central India. The rhizomes are bluish black in colour and have economic importance due to its high medicinal properties. Fresh rhizomes possessed aromatic properties that has a furious odour and are applied externally to sprain and bruises (Buragohain, 2011). Whole part of the plant, mainly the leaves and rhizome, contained a various chemical constituents, which are used medicinally to fight against various illnesses. Moreover, it is also used traditionally as medicine and in folk cosmetics. Different parts of this plant are used for different purposes. The leaves are freshly eaten or given as a drink to treat cough (Awang-Kanak *et al.,* 2018).

MATERIALS AND METHODS

Collection of plant

Fresh rhizomes of *Curcuma caesia* were collected from the Wabagai, Kakching district, Manipur. The rhizomes were washed thoroughly with tap water followed by distilled water. Then the rhizomes were dried under shaded at room temperature and ground into powder.

Preparation of solvent extracts for qualitative screening

40 g of ground samples were weighed and carried out the process by using 400 ml of methanol, ethanol and ethyl acetate in soxhlet apparatus respectively. The extracts were concentrated by evaporation and stored prior to phytochermical screening.

The extracts were subjected to phytochemical screening to test presence of phytoconstituents such as amino-acids, carbohydrates, proteins, phenol, flavonoids, tannins, steroids and terpenoids, saponins, oils and fats, phlobatanins, etc.

Phytochemical screening

The phytochemical tests were carried out using the standard procedures.

1. Test for alkaloids

Mayer's Test: 2ml of the plant extract in a test tube was treated with a few drops of Mayer's reagent and observed for colour change. An appearance of cream coloured precipitate indicates the presence of alkaloids (Khan *et al*., 2023, Sharma *et al*., 2023).

2. Tests for carbohydrates

Benedict's test: 2ml of extract was treated with Benedict's reagent and heated gently. Formation of orange red precipitate indicated the presence of reducing sugars (Khan *et al*., 2023, Sharma *et al*., 2023).

Fehling's test: 2ml of extract in a test tube was added into 5ml mixture of equal volume of Fehling's A and Fehling's B solutions and heated in a water bath for about 2 mins. Formation of brick red precipitate of cuprous oxide indicated the presence of reducing sugars (Khan *et al*., 2023, Sharma *et al*., 2023).

3. Test for amino acids

Ninhydrin test: 2 drops of 2% ninhydrin solution was added to the plant extract. Appearance of violet or purple colour indicated the presence of amino acid (Khan *et al*., 2023, Sharma *et al*., 2023).

4. Test for proteins

Xanthoproteic test: 2ml of the extract was treated with a few drops of conctrated nitric acid. Formation of yellow colour indicated the presence of proteins (Khan *et al*., 2023, Sharma *et al*., 2023).

Biuret test: To the test solution 4% NaOH solution and few drops of 1% $CuSO_4$ solution were added, appearance of violet colour indicated the presence of protein (Khan *et al*., 2023, Sharma *et al*., 2023).

5. Test for flavonoids

Alkaline reagent test: 2 ml of 2% sodium hydroxide (NaOH) was mixed with 2ml of plant extract. Concentrated or intense yellow colour which turns to colourless by addition of few drops of dilute acetic acid which indicated the presence of flavonoids (Khan *et al*., 2023, Sharma *et al*., 2023).

6. Test for phenolic compounds

Lead acetate test: A few drops of 10% lead acetate solution was added to the test solution. Formation of white precipitate indicated the presence of phenolic compounds (Khan *et al*., 2023, Sharma *et al*., 2023).

7. Test for tannins

Lead acetate test: A few drops of 10% lead acetate solution was added to the test solution. Formation of white precipitate indicated the presence of tannins (Khan *et al*., 2023, Sharma *et al*., 2023).

Ferric chloride test: 2 ml of plant extract, 4 ml of distilled water was added and a few drops of ferric chloride solution were also added. An intense green, purple, blue or black colour indicated the presence of tannin (Khan *et al*., 2023, Sharma *et al*., 2023).

8. Test for steroids and terpenoids

Salkowski's test: 5 ml of the extract was treated with 2 ml of chloroform and filtered. The filtrate was 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 concentrated sulphuric acid to the side carefully (without shaking) indicated the presence of terpenoids (Khan *et al*., 2023, Sharma *et al*., 2023).

9. Test for saponins

Froth test: 3 ml of extract was treated with 10 ml of distilled water in a test tube. The mixture was shaken vigorously for about 5 minutes; it was allowed to stand for 30 minutes and observed for honeycomb froth, which

indicated the presence of saponin (Khan *et al*., 2023, Sharma *et al*., 2023).

10. Test for cardiac glycosides

Keller Killiani test: To the test solution, 2 ml of glacial acetic acid containing a few drops of 2% $FeCl_3$ solution was mixed. 1ml of conc. H_2SO_4 was added along the side of the test tube carefully. A brown ring at the interface indicated the presence of deoxysugar of cardenoloides. A violet ring may appear beneath the brown ring, while in the acetic acid layer, a greenish ring may also form just gradually throughout the layer indicating the presence of cardiac glycosides (Khan *et al*., 2023, Sharma *et al*., 2023).

11. Test for oil and fat

A small quantity of the extract was pressed between the two filter papers. Oil stain on the filter papers indicated the presence of oil (Khan *et al*., 2023, Sharma *et al*., 2023).

12. Test for phlobatannin

Extract was boiled with 2 ml of 1% hydrochloric acid. No red precipitate was formed indicating the absence of phlobatannin (Khan *et al*., 2023, Sharma *et al*., 2023).

Determination of total phenolic content

The Folin-Ciocalteu reagent method was used to determine the amount of phenol content in methanol and ethanol extracts *of Curcuma caesia* rhizome (Bag *et al*., 2016). 2.5 ml of 10% Folin-Ciocalteu reagent and 2 ml of Na_2CO_3 (2% w/v) were added to 0.5 ml of the sample (3 replicates) of rhizome extract solution (1mg ml^{-1}). The resulting mixture was incubated at 45°C for 15 min. The absorbance of sample was measured at 760 nm using UV Visible Spectrophotometer (UV-2700). Gallic acid (50-300 ìg ml^{-1}) was used as a standard compound. The gallic acid standard calibration curve was established by plotting concentration (ìg ml^{-1}) versus absorbance (nm) (y= 0.009675X + 0.004840; R2= 0.9584), where y is absorbance at 760 nm and x is concentration (Figure 1). Total phenolic content in the plant extract was expressed as gallic acid equivalent (mg of gallic acid equivalent g^{-1} of sample) and was calculated by the formula:

$$T = (C \times V)/M$$

Where, T = total content of phenolic compounds, mg g^{-1} plant extract, in GAE; C = concentration of gallic acid established from the calibration curve, ìg ml^{-1}; V = volume of extract, ml; M = weight of the plant (Bhaigyabati *et al*., 2017).

Estimation of reducing power

Various concentrations of the extracts were prepared and mixed with 2.5 ml phosphate buffer and potassium ferricyanide and the mixture was kept at 50°C in water bath for 20 min. After cooling 2.5 ml of 10% trichloroacetic-acid was added and centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and freshly prepared 1% ferric chloride solution (0.5 ml). The absorbance was measured by using UV spectrophotometer at 700 nm. Control was prepared in similar manner excluding samples. Ascorbic acid (0.5 mg ml^{-1}) at various concentrations was used as standard (Khan *et al*., 2023, Sharma *et al*., 2023).

Determination of total antioxidant activity

The total antioxidant activity of the extracts was evaluated by using the phosphomolybdenum method (Saikia *et al.,* 2011). 0.3 ml of the methanolic and ethanolic extract sample (1mg ml^{-1}) as well as ascorbic acid (0.5mg ml^{-1}) was mixed with 3.0 ml of the reagent solution (0.6M sulphuric acid, 28nM sodium phosphate and 4nM ammonium molybdate) separately and the mixture was incubated at 95°C for 90 min under water bath. Absorbance of all the mixtures was measured at 695 nm after cooling. Total antioxidant activity is expressed as the number of equivalents of ascorbic acid in ìg $ml^{-1}$of extract. Total antioxidant activity was calculated by using the formula.

Total antioxidant = O.D. of test x concentration of standard in µg x made up volume of sample (Khan *et al*., 2023, Sharma *et al*., 2023).

Statistical analysis

All sample determinations were conducted in triplicates and the results were calculated as mean ± standard deviation (SD) (Sharma *et al*., 2023).

RESULTS AND DISCUSSION

Phytochemical screening

Phytochemical screening of methanol, ethanol and ethyl acetate extract of *C. caesia* rhizome reveals the presence of phyto-constituents as listed in Table 1.

Almost all the phytochemicals *viz*., amino acids, alkaloids, carbohydrates, proteins, flavonoids, steroids and terpenoids, saponins, cardiac glycosides, phenolic compounds and tannins were present in methanol except oils and phlobatanins and ethanol *viz*., amino acids, alkaloids, carbohydrates, proteins, flavonoids, steroids and terpenoids, saponins, cardiac glycosides, phenolic compounds and tannins were present except oils and phlobatanins extracts of *C. caesia rhizome*. However, most of the important phytochemicals was absent in ethyl acetate extract, only three *viz*., amino acids, carbohydrates, steroids and terpenoids and cardiac glycosides phytochemicals were present. Important phytochemical considered as active medicinal phytochemical were present in the sample that shows a high level of its possible medicinal value (Khan *et al*., 2023, Sharma *et al*., 2023).

Total phenolic content

The phenolic compounds act as free radical terminators and the mechanism of action are through scavenging or chelating process (Platzer *et al*., 2021, Khan *et al*., 2023). Phenolic compounds are having wide bioactivity including antioxidant properties/activity. The antioxidant activity of phenolic compound is due to hydroxyl functional group, however other factors eg., presence of electron withdrawing or releasing group in the aromatic ring having

hydroxyl moiety will increase or decrease the activity. The phenols contain hydroxyls that are responsible for the radical scavenging effect mainly due to redox properties (Bag *et al*., 2016). The present study reveals that the total phenolic content in methanol and ethanol rhizome extracts in terms of gallic acid equivalent was 7.27 and 9.40 of extract powder respectively. Result indicates that ethanol extract of rhizome of *C.caesia* showed higher total phenolic content than methanol. No phenol content was observed in ethyl acetate extract (Table 2). The total phenolic content varies according to the solvent use in extract.

Determination of reducing power

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Figure 2 shows the dose dependent reducing power activity of *Curcuma caesia* methanol and ethanol rhizome extract at different concentration using the potassium ferricyanide reduction method. The concentration of extract ranged from 10-90 µg level. As the concentration of extract is increased the reducing power also increased in both the solvent and attained maximum at 100 µg concentration. Increasing absorbance indicates an increase in reductive ability. Ethanoilc extracts showed higher reducing power than methanolic extracts. The reducing power activity is due to the presence of reductones (phenolics). As reducing power assay measures the electron donating capacity of an antioxidant, it is associated with the presence of reductones. Reductones exhibit antioxidant action by breaking the chain reactions by donating a hydrogen atom and also reported to react with certain precursor of peroxide thereby preventing peroxide formation (Chanda and Dave, 2009, Olayinka *et al*., 2010, Khan *et al*., 2023).

Total antioxidant activity

The data in Table 3 shows that total antioxidant activity varied among the solvent used in extract with ethanol extract having higher activity than methanol extract and also indicate the total antioxidant activity increased with the increase in concentration of extract. The total antioxidant activity of methanolic and ethanolic extracts of rhizome were found highest (53.4 ± 0.22 µg AAE $mg^{-1}$and 65.1 ± 0.27 µg AAE mg^{-1}respectively) in highest concentration of 90 µg ml^{-1} of extract and lowest (30.0 ± 0.076 µg AAE mg^{-1} and 31.5 ± 0.79 µg AAE mg^{-1} respectively) in low concentration of 10 µg ml^{-1} of extract. However, no such activity was observed in ethyl acetate extract which clearly reveals that the total antioxidant activity depends on the solvent type and its concentration used in extraction. The antioxidant quality or activity of plant-derived products has greatly influenced by the extraction factors like the method of extraction, temperature and solvent used. Several studies evidently show that the effectiveness of antioxidant activity of most plant products was significantly increased by using the aqueous mixtures of organic solvents like ethanol, methanol, acetone, isopropanol, or acetonitrile with water rather than using water alone for extraction (Thamizhinyan *et al*., 2019).

Table 1. Phytochemical constituents of *C. caesia* rhizome

Phytochemicals	Test performed	Solvent		
		Methanol	Ethanol	Ethyl acetate
Amino acids	Ninhydrin	+	+	+
Alkaloids	Mayre's test	+	+	-
Carbohydrate	Benedict's test	+	+	-
	Fehling's test	+	+	+
Proteins	Xanthoproteic test	+	+	-
	Biuret test	-	-	-
Flavonoids	Alkaline reagent test	-	-	-
	Lead acetate test	+	+	-
Steroids and terpenoids	Salkowski's test	+	+	+
Saponins	Froth test	+	+	-
Cardiac glycosides	Keller Killiani test	+	+	+
Phenolic compounds	Lead acetate test	+	+	-
	Ferric chloride test	+	+	-
Tannins	Lead acetate test	+	+	-
	Ferric chloride test	+	+	-
Oils	Translucent test	-	-	-
Phlobatanins	HCl test	-	-	-

Key + = presence, - = absence

Table 2. Total phenolic content in *Curcuma caesia* rhizome extract

Solvent	Total Phenolic content (mg GAE g^{-1} of extract)
Methanol	7.27 ± 0.158
Ethanol	9.40 ± 1.175
Ethyl acetate	Not Observed

Assays were performed in triplicate. Values are expressed as means ± SD

Table 3. Total antioxidant of *C. caesia*

Concentration (µg ml^{-1})	Total antioxidant activity in µg AAE mg^{-1} of extract	
	Methanol	Ethanol
10	30.0 ± 0.076	31.5 ± 0.79
30	42.3 ± 0.89	42.3 ± 0.89
50	42.9 ± 0.87	45.9 ± 0.93
70	47.7 ± 2.03	55.5 ± 2.36
90	53.4 ± 0.22	65.1 ± 0.27

Assays were performed in triplicate. Values are expressed as means ± SD

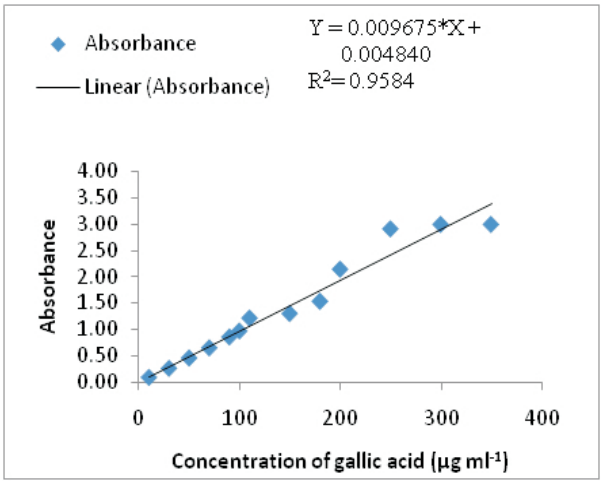

Figure 1. Standard curve of gallic acid (µg ml^{-1})

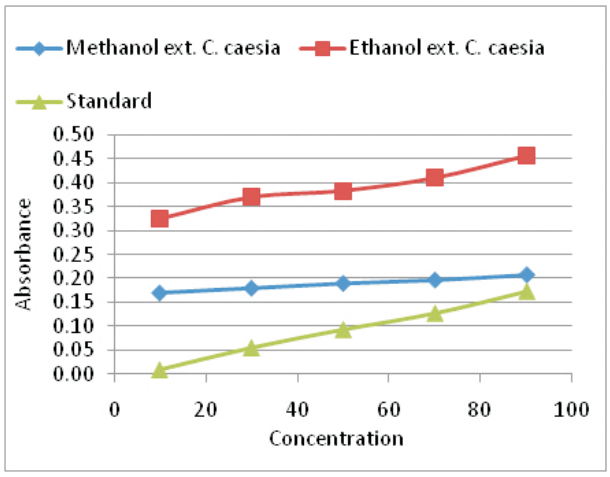

Figure 2. Reducing power of methanolic and ethanolic extracts of *C. caesia*

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