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Physicochemical and phytochemical evaluation on non-areal part of *Curcuma caesia*

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Abstract
Curcuma caesia rhizome extracts and its solvent fractionates were subjected to physicochemical and preliminary phytochemical screening using standard tests. The present study deals with phytochemical explorations of non-aerial part (rhizome) of *Curcuma caesia* comprising determination of loss on drying, ash values and extractive values. The qualitative chemical investigations exposed the existence of different phytoconstituents like flavanoids, terpenoids, saponins, phenolic compounds, steroids, alkaloids, quinones, proteins, carbohydrates, tannins and glycosides in the rhizome of the plant extracts. The occurrence of many bioactive constituents affirms the utilization of *C. caesia* for several disorders by traditional practitioners. The study disclosed specific individualities for the particular crude drug which will be suitable in recognition and control to adulterate of the raw drug.

Keywords: *Curcuma caesia*, physicochemical analysis, extraction techniques, phytochemical screening

Introduction
Nature has the huge reservoirs of therapeutically active constituents. Since the primitive age, plants have functioned as the enormous origin of raw ingredients for customary as well as modern remedy [1, 2]. Many countries still rely mainly on medicinal herbs for the curing of several contagious infections due to their low cost and slighter side effects. Orthodox tropical medicinal floras could help as a good source of novel consistent, ecofriendly and sustainable medicines for the remedial of many ailments [3, 4]. The therapeutic value of plants is mainly due to the occurrence of some phytochemicals. They are essentially plant metabolites, are produced in all part of plant organism by itself and have some specific functional operation on animals [5, 6].

Curcuma caesia Roxb. (Black Turmeric, Syn. *Curcuma kuchoor* Royle) belongs to the family Zingiberaceae. Vernacular name: English- Black zedoary; Hindi- Kali Haldi, Nar Kachura, Krishna Kedar; Sanskrit- Rajani, Nishaa, Nishi, Raatri; Bengali- Kala haldi; Assamese- Kala haladhi; Telugu- Nalla Pasupu, Manupasupu; Marathi- Kala-haldi; Manipuri- Yaingang Amuba or yaimu; Mizo-Aihang, Ailaihang. It is a rhizomatous herbs which is found throughout India and other parts of tropical climate around the world. *Curcuma caesia* is an herbaceous perennial with erect to semi-erect plant stature. It is a rhizomatous aromatic herb with a leafy bunch and 30-60 cm long. Leaves are large, long, petiolate, oblong-lanceolate shaped with an acuminate leaf apex, tapering at both ends, glabrous and green on both sides. Corolla is long tubular, pale yellow lip-3 lobe semi- elliptic. *Curcuma caesia* has a lateral or central inflorescence on a long erect peduncle, covered with 5–6 sheaths, and hidden by the sheathing bases of the leaves. Inflorescence is a spike, about 15 cm long or altogether about 30 cm high on basal peduncle. Flowers are pale yellow, reddish at the outer border and shorter than their bracts. Petiole and sheath are about as long as the blade. Spikes appear before the leaves. Flowers appear in June and July, while fruits mature in September and October [7-12].

The rhizome is tuberous with camphoraceous sweet odor, about 2–6 cm in diameter, the shape and size is often variable. It is sessile, laterally flattened, and covered with adventitious roots, root scars, and warts; moreover, it shows longitudinal circular wrinkles on the surface giving the look of nodal and internodal zones to the rhizome. The surface (cork) of rhizome is dark brown, bluish black, or buff in color; it shows circular arrangements of remnants of scaly leaves, which gives a false impression of growth rings. The branching is more or less sympodral. It has many pharmacological activities like *in-vitro* anti-diabetic, antioxidant, antimicrobial, analgesic, anti-cancer, antiulcer, antiemetics, antiviral, antitumor,

anti-inflammatory, anti-tubercular, anti-asthmatic, anti-hyperglyceridemic, astringent, anti-diarrhoetic and antipyretic effects [7-14]. In the present paper, the physicochemical parameters and preliminary phytochemical potential of different solvent extracts of non-aerial part (rhizome) of *Curcuma caesia* were executed for identification of the drug in dry form and control the adulterants.

Materials and Methods

Collection of plant materials

The rhizome of *curcuma caesia* was collected from Indra Gandhi Krishi Viswavidyalaya area, Raipur (C.G.) in the month of February' 2019. The plant materials were taxonomically identified and authenticated by Principal Scientist, Centre of Excellence on Medicinal and Aromatic Plants, Indra Gandhi Krishi Viswavidyalaya, Raipur (C.G.).

Processing of plant materials

The plant Materials were cleaned, washed with fresh water and shade dried until all the water molecules evaporated, cut into pieces, and the shed dried plant materials (rhizome) was taken and grinded into coarse powder. The powdered samples were stored in a clean and air tight glass container with proper labeling for analysis.

Preliminary physicochemical characteristics

Air dried rhizomatous materials were used for quantitative determination of proximate analysis e.g., loss on drying, total ash, acid insoluble ash, alcohol soluble extractive values. These physicochemical studies were done according to standard procedure of Indian Pharmacopoeia and WHO guidelines [15-18].

Preparation of plant extracts

Solvent extraction

Crude plant extract was prepared by Soxhlet extraction method. About 50 gm of powdered plant material was uniformly packed into a thimble and extracted with 250 ml of different solvents separately. Solvents used were petroleum ether, chloroform, ethyl acetate, acetone methanol, ethanol and water as per solvent polarity. The process of extraction continues for 24 hours or till the solvent in siphon tube of an extractor become colorless. After that the extract was taken in a beaker and kept on water bath and heated at 30-40ºC till all the solvent got evaporated. Dried extract was kept in refrigerator at 4ºC for their future use in phytochemical analysis.

Qualitative phytochemical analysis

The extracts were tested for the presence of bioactive components by using following standard methods [19-22].

Phytochemical Screening:

Test for Alkaloids (Wagner's test)

A fraction of extract was treated with 3-5 drops of Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 100 ml of water) and observed for the formation of reddish brown precipitate (or colouration).

Test for Carbohydrates (Molisch's test)

Few drops of Molisch's reagent were added to 2 ml portion of the various extracts. This was followed by addition of 2 ml of conc. H_2SO_4 down the side of the test tube. The mixture was then allowed to stand for two-three minutes. Formation of a red or dull violet colour at the interphase of the two layers was a positive test.

Test for cardiac glycosides (Keller Kelliani's test)

5 ml of each extract was treated with 2 ml of glacial acetic acid in a test tube and a drop of ferric chloride solution was added to it. This was carefully underlayed with 1 ml concentrated sulphuric acid. A brown ring at the interface indicated the presence of deoxysugar characteristic of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may form.

Test for flavonoids (Shinoda test)

To the extract, a few magnesium turnings and a few drops of concentrated hydrochloric acid were added and boiled for five minutes. Red coloration identifies the presence of flavonoids.

Test for phenols (Ferric chloride test)

A fraction of the extracts was treated with aqueous 5% ferric chloride and observed for formation of deep blue or black colour.

Test for phlobatannins (Precipitate test)

Deposition of a red precipitate when 2 ml of extract was boiled with 1 ml of 1% aqueous hydrochloric acid.

Test for amino acids and proteins (1% Ninhydrin solution in acetone).

2 ml of filtrate was treated with 2-5 drops of ninhydrin solution placed in a boiling water bath for 1-2 minutes and observed for the formation of purple colour.

Test for saponins (Foam test)

To 2 ml of extract was added 6ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

Test for sterols (Liebermann-Burchard test)

1 ml of extract was treated with drops of chloroform, acetic anhydride and conc. H_2SO_4 and observed for the formation of dark pink or red colour.

Test for tannins (Braymer's test)

2 ml of extract was treated with 10% alcoholic ferric chloride solution and observed for formation of blue or greenish colour solution.

Test for terpenoids (Salkowski's test)

1 ml of chloroform was added to 2 ml of each extract followed by a few drops of concentrated sulphuric acid. A reddish brown precipitate produced immediately indicated the presence of terpenoids.

Test for Quinones

A small amount of extract was treated with concentrated HCl and observed for the formation of yellow precipitate (or colouration).

Test for oxalate

To 3 ml portion of extracts were added a few drops of ethanoic acid glacial. A greenish black colouration indicates presence of oxalates.

Results and Discussion

Results obtained for quantitative determination of proximate analysis and qualitative phytochemical screening of *C. caesia* rhizome is exhibited in Table 1 & 2. Total thirteen phytochemicals were screened in which eleven were found present in different solvent extracts. They are cardiac glycosides, flavonoids, phenols, carbohydrates, saponins, tannins, alkaloids, sterols, quinones, proteins and terpenoids. Remarkably, carbohydrate, flavonoids, phenols, saponins, tannin, alkaloids, quinones, amino acids and terpenoids were present in the rhizome of these plants. This indicates that the rhizomes have large possibilities of phytochemicals.

Physiochemical parameters of the *Curcuma Caesia* Roxb rhizome are shown in Table 1. Different extracts of the powdered rhizome were prepared for the study of extractive values. Percentage of extractive values was calculated with reference to the air dried drug. The results are shown in Table 1. Deterioration time of the plant material depends upon the amount of water present in plant material. If the water content is high, the plant material can be easily deteriorated due to fungus. The loss on drying at 105°C in rhizome was found to be 10.02 %. Total Ash value of plant material indicated the amount of minerals and earthy materials attached to the plant material. Analytical results showed total Ash value content was 9.00 %. The negligible amount of acid insoluble siliceous matter present in the plant was 4.49 %. The alcohol soluble extractive values indicated the presence of polar constituents like phenols, alkaloids, steroids, glycosides, flavonoids etc.

In these screening process alkaloids, tannins, saponins, flavonoids and terpenoids, glycosides and phenols shows different types of results in different solvents. From the rhizome, water extract showed the presence of carbohydrate, alkaloids, saponins and tannins. However, ethanol and acetone had the presence cardiac glycosides, carbohydrates, flavonoids, phenols, saponins, proteins, alkaloids and terpenoids. The methanol extract had the presence of cardiac glycosides, carbohydrate, alkaloids, flavonoids, phenol, tannins, saponins, phenols, sterols, quinones, proteins and terpenoids.

Different phytochemicals have been found to possess a wide range of activities, which may help in protection against diseases. Alkaloids protect against chronic diseases. Saponins protect against hypercholesterolemia and antibiotic properties. Steroids and triterpenoids show the analgesic for central nervous system activities [23].

Table 1: Physicochemical analysis of rhizome of *Curcuma caesia*

S.N.	Parameters	Results (% w/w)
1	Total Ash	9.00
2	Acid insoluble Ash	4.49
3	Water insoluble Ash	2.56
4	Water soluble extractive value	13.53
5	Alcohol soluble extractive value	6.08
6	Loss on Drying	10.02

Table 2: Result of phytochemical evaluation of rhizome of *Curcuma caesia*.

S.N.	Phytochemicals/ Solvent Extracts	Pet. Ether	Chloroform	Ethyl acetate	Acetone	Ethanol	Methanol	Water
1	Alkaloids	-	-	+	+	+	+	+
2	Cardiac Glycosides	-	+	+	+	+	+	-
3	Carbohydrates	-	+	+	+	+	+	+
4	Flavonoids	-	+	+	+	+	+	-
5	Phenols	-	+	-	+	+	+	+
6	Phlobatannins	-	-	-	-	-	-	-
7	Proteins	+	+	-	+	+	+	-
8	Saponins	+	+	-	+	+	+	+
9	Sterols	+	+	+	-	-	+	+
10	Tannins	-	-	-	-	-	+	+
11	Terpenoids	+	+	-	+	+	+	-
12	Quinones	+	+	-	+	-	+	-
13	Oxalates	-	-	-	-	-	-	-

+ = present; - = absent.

The result indicates that *Curcuma caesia* rhizome carry potentials as source of pharmaceutically significant phytochemicals. Flavonoids present in non-areal parts like rhizomes play some metabolic role and control development in living system. Tannins are known to inhibit pathogenic fungi. The flavonoids and phenolic compounds in plant have been reported to exert multiple biological effects including antioxidant, free radical scavenging abilities, anti-inflammatory, anti-carcinogenic, astringent, anti-diabetic, anti-tubercular, antipyretic effects etc. [24, 25].

Conclusion

Proximate analysis is beneficial for checking reality and clarity of sample and also these values are significant for qualitative standards. The screening of a crude drug is essential for biochemical alteration in the drug, degradation due to handling, storage and substitution and contamination. Preliminary Phytochemical screening is a part of chemical exploration. The qualitative chemical test is useful in exposure of contamination. Phytochemicals found in rhizome extracts of *Curcuma caesia* indicates their prospect as a resource herbal remedy. The results from the ash value, acid insoluble ash and water soluble ash values implied that the rhizome holds noticeable amount of inorganic salts. The phytochemical characterization of the extracts, the isolation of important bioactive composites and their biological activity are inevitable for future studies. Standardization of bioactive extracts procured from the medicinal plant will be conceded on the basis of the phytochemical components exist in that plant, which is a vital step in recognizing novel and potent sources of medically and industrially significant constituents.

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