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Physicochemical and phytochemical exploration on non-aerial part of *Curcuma amada*

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Abstract

Curcuma amada rhizome, its extracts and solvent fractionates were subjected to physicochemical and preliminary phytochemical screening using standard tests. The present study deals with phytochemical investigations of non-aerial part (rhizome) of *Curcuma amada* including determination of loss on drying, ash values and extractive values. The qualitative chemical examinations revealed the presence of various phytoconstituents like curcumin, flavanoid, terpenoid saponins, phenolic compounds, carbohydrates, tannins and glycosides in the rhizome of the plant extracts. The presence of various bioactive components confirms the application of *C. amada* for various ailments by traditional practitioners. The study revealed specific identities for the particular crude drug which will be useful in identification and control to adulterate of the raw drug.

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

Introduction

Nature has the vast resource of medicinally active components. Since the primeval age, plants have served as the huge source of raw materials for traditional as well as recent medicine [1-2]. Many countries still depend mainly on medicinal herbs for the treatment of various infectious diseases due to their cost effectiveness and lesser side effects. Traditional tropical medicinal plants could serve as a good supply of new reliable, biodegradable and renewable drugs for the curing of many diseases [3-4]. The medicinal value of plants is mainly due to the presence of some phytochemicals. They are basically plant metabolites, are synthesized in all part of plant body by itself and have some definite physiological action on animals [5-6].

Curcuma amada Roxb. (Mango ginger, Syn. *Curcuma manga* Valetton & Van Zijp) belongs to the family Zingiberaceae. Vernacular name: English- Mango ginger; Assamese-Aam-ada, Am haladhi, Am-ada; Hindi- Am haldi; Bengali- Amada; Manipuri- Yai hanuman. It is a rhizomatous herbs which is found throughout India and other parts of tropical Asia. *Curcuma amada* is an herbaceous perennial with erect to semi-erect plant stature. It is an aromatic herb with a leafy tuft and 60-90cm in height. Leaves are large, long, petiolate, oblong-lanceolate shaped with an acuminate leaf apex, tapering at both ends, glabrous and green on both sides. Lip is semi-elliptic, yellow, 3-lobbed with the mid lobe emarginated. *Curcuma amada* 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. There is a spike/scape/inflorescence with a succession of strong, imbricated, pale-green or straw-colored fertile bracts. These bracts are terminated with a coma or tuft of pale-purple or rose-colored barren bracts, or leaves. Flowers are pale yellow, arranged in spikes in the centre of tuft of the leaves. The flowers are large and long, with 4-5 flowers in each bract. Rhizomes are white or pale yellow, arranged in spikes in the centre of tuft of the leaves. The rhizomes are large and long, with 4-5 rhizomes in each bract. The rootstock or radical bulb is ovoid/conical. The rhizome is large and branched, with a buff-colored external surface. The flesh color is light to pale yellow, with a fragrance of green mango. Sessile/palmate tubers are thick, cylindrical, and fleshy, fingered and arise from the base of the rootstock. Pendulous tubers are present. It has many pharmacological activities like in-vitro anti-inflammatory, anti-diabetic, antioxidant, antimicrobial, analgesic, anti-cancer, antiulcer, antiemetics, antiviral, antitumor, antitubercular, anti hyperglyceridemic, antiangiogenesis, astringent, diuretic, demulcent and antipyretic effects [7-11]. In the present paper, the physicochemical parameters and preliminary phytochemical potential of different solvent extracts of non-aerial part (rhizome) of *Curcuma amada* were done for identification of the drug in dry form and control the adulterants.

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Materials and Methods

Collection of Plant Materials

The rhizome of *curcuma amada* was collected from Achankamar area, Bilaspur area, C.G. in the month of March' 2018. The plant materials were taxonomically identified and authenticated by Botanical Survey of India (BSI), Central Regional centre, Allahabad (U.P.).

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 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 rhizomes 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 [12-15].

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 30-40°C till all the solvent got evaporated. Dried extract was kept in refrigerator at 4°C 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 [16-20].

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. H₂SO₄ 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 underlayered 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. H₂SO₄ 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 (Salkowki'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 colour action indicates presence of oxalates.

Results and Discussion

Results obtained for quantitative determination of proximate analysis and qualitative screening of phytochemicals in rhizome of *C. amada* are presented in Table 1 & 2. Total thirteen phytochemicals were screened in which ten were found present in different solvent extracts. They are cardiac glycosides, flavonoids, phenols, carbohydrates, saponins, tannins, alkaloids, sterols, quinones and terpenoids. Remarkably, carbohydrate, flavonoids, phenols, saponins, tannin, quinones, alkaloids and terpenoids were present in the rhizome of these plants. This suggests that the rhizomes have extensive potentials of phytochemicals.

Physicochemical parameters of the rhizome of *Curcuma amada* Roxb. are tabulated 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 11.08 %. 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 7.68 %. The negligible amount of acid insoluble siliceous matter present in the plant was 4.84 %. The alcohol soluble extractive values indicated the presence of polar constituents like phenols, alkaloids, steroids, glycosides, flavonoids.

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 and terpenoids.

The medicinal value of plants means definite physiological action on the human body due to presence chemical substances. 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 [21].

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

S. N.	Parameters	Results (% w/w)
1	Total Ash	7.68
2	Acid insoluble Ash	4.84
3	Water insoluble Ash	2.47
4	Water soluble extractive value	13.42
5	Alcohol soluble extractive value	5.28
6	Loss on Drying	11.08

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

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 amada* rhizome hold promises as source of pharmaceutically important 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. [22-23].

Conclusion

Proximate analysis is useful in determining authenticity and purity of sample and also these values are important qualitative standards. The screening of a crude drug is necessary for biochemical variation in the drug, deterioration due to treatment, storage and substitution and adulteration. Preliminary Phytochemical screening is a part of chemical evaluation. The qualitative chemical test is useful in detection of adulteration. Phytochemicals found in rhizome extracts of *Curcuma amada* indicates their potentiality as a supply herbal medicine. The results from the ash value, acid insoluble ash and water soluble ash values suggested that the rhizome contains demonstrable quantity of inorganic salts. The phytochemical characterization of the extracts, the isolation of responsible bioactive compounds and their biological activity are necessary for future studies. Standardization of bioactive

extracts obtained from the medicinal plant will be carried out on the basis of the phytochemical compounds present in that plant. Therefore, phytochemical screening of medicinal plants is a crucial step in identifying new and effective sources of therapeutically and industrially important bioactive compounds.

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