PHYTOCHEMICAL EVALUATION, DETERMINATION OF TOTAL TERPENOID CONTENT ON THE RHIZOME OF CURCUMA AMADA.

Thokchom Sharatchandra Singh¹,², I. Tovishe Phucho¹*, Thokchom Brojendro Singh³.

¹Chemistry Dept., Nagaland University, Lumami, Nagaland, India.
²Chemistry Dept., Thambal Marik College, Oinam, Manipur, India.
³Chemistry Dept., Oriental College (Autonomous), Takyel, Imphal, Manipur, India.

ABSTRACT

Curcuma amada, popularly known as mango ginger is a rhizomatous perennial herb of curcuma genus (zingiberaceae family) having characteristic odour similar to raw mangoes and is used as major ingredient in pickles, candies, salads, sauces and chutneys. Rhizomes of curcuma amada is also used in the treatment of skin disease, diabetes, paralysis, anorexia, dyspepsia, flatulence, colic, wounds, chronic ulcers, fever, gout, bronchitis. Phytochemical ingredients are non-nutritive plant chemical that have disease prevention properties. The aim of the present study deals with the phytochemical ingredient present in the chloroform, ethanol, methanol and distilled water extract of the rhizome of curcuma amada. The extractive value of the rhizome of curcuma amada gave 4.90, 3.99, 3.79 and 2.63% yields of chloroform, ethanol, methanol and distilled water respectively. The phytochemical analysis was performed to detect the the presence of alkaloids, saponin, tannin, flavonoids, coumarin, gum, emodins, phytosterol, anthraquinon, lignand and elagic acid. The present study also deals with the determination of total terpenoid giving 5.89% of the dry rhizome powder.

KEYWORDS: Phytochemicals, Curcuma amada, chloroform, ethanol, methanol, distilled water, terpenoids.

INTRODUCTION

Medicinal plant and herbs are the main resource for synthetic drugs with biological applications, folk medicines, traditional system of medicine and they are also used as food supplements.¹,² Pharmaceutical companies have been using crude plant extracts to produce...
therapeutic drug formulation. The studies of the herbal based drugs formulation are very vast and are the futuristic hope all over the world. About 61% of the new drugs developed in pharmaceutical industry are derived from natural sources; Prevention and treatments of several human diseases in the traditional medicine was successfully controlled by using aromatic medicinal plants. The medicinal property of the plant and herbs is due to the presence of some bioactive constituents such as alkaloids, terpenoids, saponins, cardiac glycoside, flavonoids, tannins, coumarins, and carotenoid and phenolic compounds etc. which produce a definite physiological action on the human beings. Due to the side effects of synthetic drugs, human beings started looking back to the traditional knowledge of plant for their health care in day to day life. Plant and herbs also fulfilled the need of animal kingdom not only the human beings. 80% of the total population of the World use plants and herbs for their basic health care need. In India, Ayurveda and Unani have taken important roles to renew the indigenous system of medicine. Traditionally, single part of a plant or whole plant parts are used as medicine. 95% of the prescription drugs in India were based on traditional system of Unani and Homeopathy. Men have familiar himself with medicinal plants since the beginning of human existence and used them in a variety of way throughout the ages. The number of new plant derived drugs increase likewise the growth of knowledge to cure disease continues at an accelerating space.

*Curcuma amada* popularly known as mango-ginger (Yai-Heinounam, Manipuri name) is having characteristic odour similar to raw mangos and used as major ingredient in the pickles, candies, salads, sauces and chutneys. The Rhizomes of this plant are useful in vitiated condition of pitta, anorexia, dyspepsia, flatulence, colic, bruises wounds, chronic ulcers, skin diseases, pruritus, fever, constipations, hiccough, cough, bronchitis, sprains, gout, halitosis, and inflammations. The biological activities of *Curcuma amada* include antioxidant activity, antifungal activity and antibacterial activity.

The present work was aimed to analyse the phytochemical constituents on Chloroform, Ethanol, Methanol and Distilled water extract for the rhizomes of *Curcuma amada*. This work also analyse the total terpenoid content.

**MATERIALS AND METHODS**

**Collection of plant material**: The mature and healthy rhizomes of *Curcuma amada* were collected from Kangmong village, Imphal west district, Manipur, India in the month of February 2015. The plant samples were brought into laboratory, washed thoroughly in
running tap water to clean the adhering and sand particles and then rinsed in distilled water. The plant species was identified by Prof P. Kumar Singh, Life science dept. Manipur University, Canchipur, Imphal, Manipur, India. The clean rhizomes were cut into thin slice by knife, shaded dried and then converted into fine powder forms with the help of hand grinder and stored in an air tight glass bottle for further use.

**Preparation of extract:** 50gms of course powder of rhizome of *Curcuma amada* was soaked in 250ml each of Chloroform, Ethanol, Methanol and Distilled water respectively at room temperature for 5 days one after another according to their polarity order with occasional shaking. The solvents from the extracts were filtered and evaporated to dryness on water bath. Percentage yield was calculated for each extract (Table No. 1). It was used for the qualitative analysis of plant metabolites.

**Identification test:** The individual extract was subjected to the qualitative phytochemical screening for the presence of some chemical constituents. The phytochemical test was carried out adopting the standard procedure. [15, 16, 17, 18, and 19]

**Alkaloids:** About (3 ml) of concentrated extract was taken in a test tube and about 1ml HCL was added and the mixture was heated gently for about 20 minutes, cooled and filtered and the filtrate was used for the following test of alkaloids.

(a) **Wagner test:** Wagner reagent was prepared by dissolving 2gm of iodine and 6gm of KI in 100ml of water (Altolani Olubunmi, Nigeria). 1ml of the extract was taken in a test tube and then treated with Wagner’s reagent; reddish brown precipitate identified the presence of alkaloid.

(b) **Hagers test:** Preparation of Hager’s reagent. Dissolve 1gm of picric acid in 100ml of water. 1ml of the plant extract was treated with Hager’s reagent, yellow coloured ppt. confirmed the presence of alkaloids.

**Sponin:** 5ml of the plant extract was mixed with 20ml distilled water in a graduated cylinder; shake vigorously for about 15 minute. Formation of foams indicates the presence of saponins.

**Terpenoids:** About 0.2gm of the plant extract was mixed with 2ml of CHCl₃ and conc. H₂SO₄ was carefully added from the side of the test tube. A reddish brown colouration at the interface indicates positive test of terpenoid.
Phlobatannins: Aqueous extract of the plant sample was boiled with 1% aqueous HCl, deposition of red ppt. indicates the presence of phlobatannins.

Cardiac Glycoside: Keller-killani test: Plant extract taken in a test tube were treated with 2ml glacial acetic acid containing a few drop of FeCl₃. A brown colour ring indicates the presence of positive test.

Protein: Xanthoproteic test: A few drops of concentrated HNO₃ were added to the plant extract. Yellow colour indicates the presence of protein.

Tannin: Lead acetate test: 5ml of the plant extract was taken in a test tube and a few drops of 1% lead acetate soln. were added. Formation of yellow ppt. indicates the presence of tannin.

Flavonoid: (a).Alkaline reagent test. To about 1ml of the plant extract taken in a test tube, add a few drops of dilute NaOH. An intense yellow colour indicates the presence of flavonoids.

Coumarin: 2ml of the aqueous extract was taken in a test tube. To this 3ml of 10% NaOH was added; yellow colour formation confirmed the presence of coumarin.

Gum: 2ml of the extract was treated with 2ml of conc. H₂SO₄ it is then treated with Molisch’s reagent. Formation of a reddish violet ring at the junction of the two layers indicates the presence of gum.

Emodins: To the plant extract taken in a test tube, 2ml of NH₄OH and 3ml benzene were added. Appearance of red colour indicates the presence of emodins.

Phytosterol: Salkowski’s test: The plant was treated with chloroform and filtered A few drops of conc. H₂SO₄ was added to this filtered and shakes well, allow to stand, appearance of golden red indicates the positive test

Anthraquinone: 5ml of the plant extract was hydrolysed with dilute H₂SO₄, than add 1ml of benzene and 1ml of NH₃, formation of rose pink colouration suggest the presence of anthraquinone.

Chalcones: 2ml of NH₄OH was added to 0.5 gm. ethanolic extract taken in a test tube, appearance of red colour shows the presence of chalcones.
Cysteine: A few drops of 40% NaOH and 10% lead acetate soln. were added to about 5ml of the plant extract. Boiled for some minute and black ppt. comes out which indicate the presence of amino acid.

Determination of Terpenoids\cite{20}: 100g of the powder sample was taken in a 250ml conical flask and soaked in alcohol for 24hrs. Then, filtered, the filtrate was extracted with petroleum ether; the ether extract was treated as total terpenoids.

RESULT AND DISCUSSION
Table 1: Extractive value of crude extract for different solvents of \textit{Curcuma amada} expressed as percentage

<table>
<thead>
<tr>
<th>Solvents used</th>
<th>% yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform</td>
<td>4.90%</td>
</tr>
<tr>
<td>Ethanol</td>
<td>3.99%</td>
</tr>
<tr>
<td>Methanol</td>
<td>3.79 %</td>
</tr>
<tr>
<td>Distilled water</td>
<td>2.63 %</td>
</tr>
</tbody>
</table>

Table 2: Data showing the preliminary phytochemical screening of the various extracts of the rhizome of \textit{Curcuma amada}

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Solvent used</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chloroform</td>
</tr>
<tr>
<td>a)Wagner’s test</td>
<td>-</td>
</tr>
<tr>
<td>c)Hager’s test</td>
<td>-</td>
</tr>
<tr>
<td>Saponin</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoid</td>
<td>+</td>
</tr>
<tr>
<td>Phlobatanin</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycoside</td>
<td>+</td>
</tr>
<tr>
<td>Protein</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>-</td>
</tr>
<tr>
<td>Coumarin</td>
<td>+</td>
</tr>
<tr>
<td>Gum</td>
<td>+</td>
</tr>
<tr>
<td>Emodin</td>
<td>-</td>
</tr>
<tr>
<td>Phytosterol</td>
<td>-</td>
</tr>
<tr>
<td>Anthraquinon</td>
<td>-</td>
</tr>
<tr>
<td>Chalcones</td>
<td>-</td>
</tr>
<tr>
<td>Cysteine</td>
<td>-</td>
</tr>
<tr>
<td>Lignans</td>
<td>-</td>
</tr>
<tr>
<td>Leucoanthocyanin</td>
<td>-</td>
</tr>
<tr>
<td>Elagic acid</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: - = negative; + = positive
Table 3: Determination of terpenoid of the rhizome of *Curcuma amada* expressed as mg/100g of the powder sample.

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Rhizome of <em>Curcuma amada</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Terpenoids</td>
<td>5.89%</td>
</tr>
</tbody>
</table>

![Graph showing the percentage of crude extract for different solvents.](image1)

**Fig 1:** Graph showing the percentage of crude extract for different solvents.

![Fig. 2: Rhizome of *Curcuma amada*](image2)

**Fig. 2:** Rhizome of *Curcuma amada*

Saxena Jyoti\textsuperscript{[21]} et al (2012) isolated ten phytochemicals (carbohydrate, protein, starch, amino acid, steroid, glycoside, flavonoid, alkaloid, tannin and saponin) from methanolic extract of the rhizome of *curcuma amada*. The present investigation has some contradiction with the finding of Saxena Jyoti et al. They did not give the positive response of flavonoids, tannin and saponin but these three phytochemicals are present in our study. Gayatri Nahak\textsuperscript{[22]} et-al (2011) reported 14 phytochemicals (alkaloid, carbohydrate, glycoside, terpenoids, proteins, amino acids, fats, tannin, saponin, phytosterols, flavonoid, phenol, coumarin, reducing sugar and anthraquinone) from the ethanolic extract of the rhizome. Their finding also found some contradiction with our study as flavonoid, tannin and phytosterols were found absent but these three phytochemicals were found in our analysis. Christina George et al (2013) also
reported 6 phytochemicals (flavonoid, saponin, tannin, carbohydrates, oil and fat and proteins) from methanolic extract of the rhizome. Their report was coinciding with our finding except saponin, in our analysis saponin is present only in distilled water extract.

Table 1 shows the crude extractive value, according to the percentage yield, chloroform extract gives more percentage (4.90%) whereas distilled water extract gives less percentage (2.63 %) while ethanol and methanol gives 3.99% and 3.79 % respectively. Table 2 represent the phytochemical screening for different solvent, in the present investigation chloroform extract shows the presence of 5 phytochemicals, ethanol extract gives 12 phytochemicals, methanol extract shows the presence of 13 phytochemicals and distilled water extract gives 8 phytochemicals respectively. Ethanol and methanol extract shows the presence of similar phytochemicals. Terpenoids content on the dry rhizomes powder of *Curcuma amada* (5.89%) is shown in table 3.

**CONCLUSIONS**

Ethanolic and methanolic extract of the plant rhizome sample shows similar nos. of phytochemicals which are higher than the other solvents used for the study. Thus for further studies these two solvents are pharmacologically more important solvents among the others.

**REFERENCES**


