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Preliminary phytochemical screening and inorganic element analysis of rhizomes of *curcuma leucorhiza roxb.*

Th.Sharatchandra Singh^{1*}, Th.Brojendro Singh², Tovishe Phucho¹

1.Department of Chemistry, Nagaland University, Lumami, Nagaland.

2.Department of Chemistry, Oriental College, Imphal, Manipur.

ABSTRACT

The present paper deals with the phytochemical screening and Inorganic elemental analysis of the plant materials. *Curcuma leucorhiza* belongs to the family zinziberacea and has been used in folklore medicine to treat various ailments. . The rhizomes of *curcuma leucorhiza* are used for the treatment of enlarged liver spleen and stomach ulcer. The decoction of its rhizomes is used in the treatment of diabetes and cancer in local traditional medicines .The biological activities of *curcuma leucorhiza* include antioxidant activity, antibacterial activity and antifungal activity. The rhizome parts of *curcuma leucorhiza* was slice,dried,grind into powder and then extracted with acetone, chloroform, methanol and ethanol solvents giving 9.06,12.90,7.30 and 13.20% yield respectively. Phytochemical studies of the extracts showed the presence of terpenoids, alkaloids, saponins, flavonoids, glycosides, carbohydrates, phenolic, tannins, phytosterols, anthocyanin, proteins and amino acids etc. Quantitative determination of alkaloid was done by Harborne (1973) method. Inorganic elements are determined by a Graphite Furnace Atomic Absorption Spectrometer. Rhizome of *curcuma leucorhiza* contains minerals such as Fe, Zn, Cu, Mo, Cr, Mn and were found to be 0.971, 0.414, 1.70, 0.013, 5.091 and 0.015 ppb respectively The results obtained shows that the extracts contain bioactive compounds and also trace element present in the plant extracts were within the permissible limits. The study will provide referential information for the treatment of various diseases.

Keywords: *Curcuma leucorhiza*, Phytochemicals, extract, element Analysis.

*Corresponding Author Email: thokchomsharat@gmail.com

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INTRODUCTION

Since time immemorial, herbal medicines have been used by mankind. The oldest traditional system of India, Ayurveda reveals that ancient Indians had a rich knowledge of medicinal value of diff. plants. With advent in science many of the crude drug used in traditional system have been investigated scientifically¹. The use of the medicinal plants is increasing in many countries where 39% of drugs contain natural products². According to World Health Organization medicinal plants would be the source to obtain a variety of drugs, provided it is proven to be efficacious, safe, less toxic, available and reliable natural resource³. About 80% of individual populations from developed countries used traditional medicine, which has compounds derived from medicinal plants⁴. The phytochemical research based on ethno pharmacological information is generally considered on effective approach on the discovery of new anti-infective agents from higher plants⁵. There has been a revival of interest in herbal medicines. This is due to increased awareness of the limited ability of synthetic pharmaceutical products to control major diseases and the need to discover new structures as lead compounds from the plants kingdom⁶. Herbal products are suitable for treating a wide range of infection and diseases⁷. This interest in medicinal plants all over the worlds reflects recognition of the validity of many traditional claims regarding the value of natural products in health care⁸. Medicinal plants are part of human medicine since the dawn of civilization. These plants are making backbone of traditional medicinal system of India (Nayak 1994). The therapeutic basis of herbal medication are by the presence of diverse bioactive compounds like Alkaloid, terpenoid, flavonoid, phenolic compounds and Proteins etc. in plants and also for the treatment of diseases which are still incurable, medicinal plants can serve as a source of novel therapeutic agent⁹.

The human body requires both metallic and non-metallic elements within certain permissible limits growth and good health. A number of minerals essential human nutrition are accumulated in different parts of plants as it accumulates minerals essential for growth from the environment¹⁰. Trace elements play a vital role in the formation of bioactive chemical constituents in medicinal plants and are therefore for both their medicinal and toxic properties¹¹. Trace elements play both curative and preventive role in combating diseases. Deficiency of trace elements in human beings subjects can occur under the most practical dietary conditions and in mans can occur under the most practical dietary conditions and in many disease statuses¹². The plants belonging to curcuma genus (family zinziberacea) has a lot of potential in terms of medicinal properties. Literature reveals its anti-inflammatory, cholagogue, hepatoprotective, blood purifier, carminative properties

and regenerator of liver tissue ¹³. *Curcuma leucorrhiza* Roxb (Manipuri Yaigou) is a rhizomatous perennial herb growing wildly for about 4 feet in height. The rhizomes of *curcuma leucorrhiza* are used for the treatment of enlarged liver spleen and stomach ulcer ¹⁴. The decoction of its rhizomes is used in the treatment of diabetes and cancer in local traditional medicines ¹⁵.

MATERIAL AND METHOD:

Plant materials:

Fully mature and healthy rhizomes of *curcuma leucorrhiza* Roxb were collected from Kangmong area, Imphal west district, Manipur during the month Jan.-Feb.2014. The collected fresh rhizome were washed many times by tap water and slice into 1mm thin by knife, shaded dried about ten days and ground into fine powder with the help of a hand grinder and then kept into a air tight container still further use.

Crude Extraction:

250gm. of fine rhizome powders of *curcuma leucorrhiza* were extracted in 500 ml each of acetone, chloroform, methanol and ethanol in cold condition for 7 days with occasional shaking. The solvent from the total extract were filter through ordinary filter paper and then dried under shaded. Percentage of yield was calculated from each extract (Table 1). It was used for the qualitative analysis of phytochemical metabolites.

Identification Test:

The individual extract was subjected to the identification test for various active chemical constituents. Phytochemical identification test were carried out adopting standards protocols (Trace *Et-al* 1983, Kokate *et-al* 1997, Hegde *et-al* 2010, Mohammad Nisar *et-al* 2011. R.S.Sawant *et-al* 2013).

Alkaloid:

About (3 ml) of concentrated extract was taken in a test tube and about 1ml HCL was added the mixture was heated gently for about 20 min. cooled and filter, 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, it is then treated with Wagner's reagent; reddish brown precipitate identified the presence of alkaloid.

(b) Hager's test. Preparation of Hager's reagent. Prepared a cold saturated solution of picric acid in water. 1ml of the plant extract was treated with Hager's reagent, yellow coloured ppt. confirmed the presence of alka

(c) Mayer`s test: Preparation of Mayer`s reagent. In 60 ml of distilled water 1.36gm of mercuric chloride was dissolved and 5gm of KI in 10ml of water. The two soln. were mixed and diluted to 100ml with distilled water. About 1.2 ml of the plant extract was taken in a test tube to this 0.2 ml of dilute HCL and 0.1 ml Mayer`s reagent was added. Yellowish buff coloured precipitate confirmed the presence of alkaloid.

Anthocyanin:

To 2ml of the aqueous extract taken in attest tube, 2 ml of 2N HCL and NH_3 was added, the appearance of pink red turns blue violet indicates the presence of Anthocyanin

Anthraquinone:

5ml of the plant extract was hydrolysed with dilute H_2SO_4 , than add 1ml of benzene and 1ml of NH_3 , formation of rose pink colouration suggest the presence of anthraquinone.

Amino acid:

A few drop of 40% NaOH and 10% lead acetate soln. were added to about 5ml of the plant extract. Boiled some minute black ppt. comes out indicate the presence of amino acid.

Coumarin:

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

Chalcones:

2ml of NH_4OH was added to 0.5 gm. ethanolic extract taken in a test tube, appearance of red colour shows the presence of chalcones.

Cardial Glycoside:

Keller-killani test: Plant extract taken in a test tube were treated with 2ml glacial acetic acid containing a few drop of FeCl_3 . A brown colour ring indicates the presence of positive test.

Carbohydrates:

Molish test: Plant extract was dissolved in 5ml of distilled water and filtered. The filtrate was treated with 2 drops of 10% alcoholic soln. of alpha naphthol soln., presence of carbohydrate was indicated by the formation of violet ring at the junction.

Emodins:

To the plant extract taken in a test tube 2ml of NH_4OH and 3ml benzene were added appearance of red colour indicate the presence of emodins.

Flavonoid:

(a).Shinoda`s test: 0.5 ml of plant extract taken in attest tube,5-10 drops of dilute HCl and small piece of ZnCl_2 or MgCl_2 were added and the soln. was boiled few minutes. Reddish pink colour

indicates the presence of flavonoids.

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

(c). NH₄OH test: 10% NH₄OH were added to 3ml of the plant extract taken in attest tube, formation of yellow fluorescence indicates the presence of flavonoids.

Glycoside:

Plant extract was treated with glacial acetic acid, few drops of 5% ferric chloride and concentrated sulphuric acid were added, observation of reddish brown colouration at the junction of two layers and bluish green colour in the upper layer ¹⁶.

Protein:

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

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.

Phenol:

Ferric chloride test: About 4 drops of alcoholic FeCl₃ soln. was added to the plant extract. Bluish black colour indicates the presence of phenol.

Phlobatannins:

Aqueous extract of the plant sample was boiled with 1% aqueous HCl, deposition of red ppt. indicates the presence of phlobatannins.

Sponin:

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

Steroid:

10ml of chloroform was mixed with 1ml extract of the test sample. To this solution equal volume of conc. H₂SO₄ was pure carefully from the side of the test tube. The upper layer turns red and H₂SO₄ layer showed yellow with green fluorescence indicates the presence of steroid.

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 redished brown colouration at the interface indicates positive test of terpenoid.

Triterpenoid:

Liebermann Burchard test: 10mg plant extract was dissolved in 1ml CHCl₃; 1ml acetic anhydride was added following the addition of conc. H₂SO₄ slowly from the side of the test tube. Reddish violet colour indicates the presence of triterpenoid.

Tannin:

(a) FeCl₃ test: 4ml plant extract and 4ml FeCl₃ was treated together green colour formation indicates the presence of condensed tannin.

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

Quantitative Phytochemical Analysis:**Total alkaloid content¹⁷.**

5gm of the plant sample was weighed into a 250 ml beaker and 200ml of 10% acetic acid in ethanol, was added, covered and allowed to stand for 48 hours. After filtration the extracts were concentrated on a water bath to ¼th of the original volume. Concentrated ammonium hydroxide was added in drops to the extract until the precipitation was complete. The whole soln. was collected and filtered, washed with dilute ammonium hydroxide and then filtered. The residue obtained was dried and weighed.

RESULTS AND DISCUSSION:

Langoljam Rena Devi et-al has reported antimicrobial and antifungal activity of curcuma leucorrhiza. They also reported 49 compounds from the extracted oil of curcuma leucorrhiza Roxb. According to the percentage of yield ethanolic extract gives more percentage whereas methanolic extract gives less yield. The extractive value of acetone, chloroform, methanol and ethanol are given in Table 1.

In the present screening test of acetone, chloroform, methanol and ethanol shows the presence of 8 phytochemicals, 15 phytochemicals, 19 phytochemicals, and 11 phytochemicals respectively. The results of phytochemical investigation are given in Table 2.

Table. 1:- Percentage Yield of various Solvents

Solvent	%
Acetone	9.06
Chloroform	12.90
Methanol	7.30
Ethanol	13.20

Present investigation reported, curcuma leucorrhiza will be useful in screening for medicines like alkaloids, flavanoids, phenolics, phenolics and other chemicals. The results are encouraging but

before being put in practice scientific scrutiny are absolutely necessary.

Table 2: Phytochemical Analysis of Rhizomes of *Curcuma leucorrhiza* Roxb.

Phytochemicals	Names of extract			
	Acetone	Chloroform	Methanol	Ethanol
Alkaloids				
(a). Wagner`s	-	+	+	-
(b). Hager`s	+	+	+	+
(c). Mayer	+	-	+	-
Saponin	+	+	+	+
Tannin				
(a). FeCl ₃ test	-	-	+	-
(b).Lead acetate test.	+	-	+	+
Steroid.	+	+	+	+
Anthocyanin.	+	-	-	-
Coumarin.	+	+	+	+
Protein.	-	+	+	-
Amino acid.	-	+	+	-
Terpenoids	+	+	+	+
Triterpenoid	+	+	+	+
Phytosterol	-	+	+	-
Flavonoids				
(a). Shinoda`s test	-	+	-	+
(b). NH ₄ OH test	+	+	+	+
Phenol	-	+	+	-
Phlobatanins	-	+	-	-
(c). Zn test		-	+	-
Leucoanthocyanin		-	-	+
Anthraquinone		-	+	+
Chalcones		-	-	+
Glycoside		+	+	+
Cardial glycoside		-	+	+
Carbohydrate		-	-	+

Note: + = Present; - = Absent

Quantitative Phytochemical Analysis:

Total alkaloid contain: The result of alkaloid content is given in Table 3. The alkaloid content was found to be 2.4 % w/w in the plant powder.

Elemental Analysis:

The result obtain shows that rhizome of *curcuma leucorrhiza* contains inorganic element of Fe, Zn, Cu, Mo, Cr, Mn were found to be 0.971, 0.414, 1.70, 0.013, 5.091 and 0.015 ppb respectively. Elemental analysis were done by graphite furnace Atomic Absorption Spectrophotometer. Elemental analysis results are shown on Table 3.

Table 3: Alkaloid content of *curcuma leucorrhiza*

Weight of rhizome powder	Result
5 gm.	2.4% w/w

Table 4: Inorganic element content in rhizome of curcuma leucorhiza

Element	Result(ppb)
Iron	1.030 +_0.010
Zinc	0.4408 +_0.034
Copper	1.92 +_0.08
Molybdenum	0.012 +_0.002
Chromium	4.336 +_0.679
Manganese	0.014 +_0.008

CONCLUSION:

From the above analysis Methanolic extract contained more nos. of bioactive compound. Thus methanolic extract is more important than the other extract.

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