

INTERNATIONAL JOURNAL OF UNIVERSAL PHARMACY AND BIO SCIENCES

Pharmaceutical Sciences

Research Article.....!!!

Received: 02-03-2013; Accepted: 10-03-2013

PHYTOCHEMICAL ANALYSIS OF METHANOLIC EXTRACT OF *CURCUMA LONGA LINN RHIZOME*

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KEYWORDS:

Curcuma longa,
Phytochemicals, Anti
inflammatory,
Antimicrobial.

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ABSTRACT

Medicinal plants are a rich source of numerous pharmacologically active molecules. Scientists are currently focusing on the Phytochemicals to treat numerous ailments affecting the mankind. *Curcuma longa* (Turmeric) a rhizomatous perennial plant belonging to family Zingiberaceae is widely used as a food additive and as traditional medicine for treating various ailments. It has anti-inflammatory, antifungal, antimicrobial, virucidal, anti mutagenic and antioxidant properties. In view of this phytochemical analysis of Methanolic extract was done. The purpose of the study was to do preliminary phytochemical screening of the Methanolic extract of the turmeric rhizome. 40g of powdered turmeric rhizome was extracted with methanol in the Soxhlet extractor for 36 hours. The extract was concentrated using Rotavapor and dried. The residue yielded a reddish brown sticky mass. The yield was 9% w/w. The preliminary phytochemical analysis of the Methanolic extract showed the presence of tannins, alkaloids, saponins, flavonoids, terpenoids and cardiac glycosides.

1. INTRODUCTION :

Curcuma longa Linn or turmeric is a rhizomatous herbaceous plant, a member of the family Zingiberaceae. It is widely grown in tropical South Asia. It requires temperatures between 20-30°C and good rainfall to grow. Turmeric plants and rhizomes are gathered annually [1]. The plant extracts are known to have anti-inflammatory, antifungal, immunomodulatory, antioxidative, antimutagenic activities, protective effect against AFB₁ induced toxicity, antibacterial activities and anti human immunodeficiency virus activity [2-8].

Turmeric is an important food preparations preservative that preserves freshness and gives a characteristic flavours. *Curcuma* rhizomes have a characteristic dark yellow colour, and it has been found to be a rich source of phenolic compounds, viz. curcuminoids. Curcuminoids contain three different diarylheptanoids Curcumin (diferuloylmethane), Demethoxy curcumin (hydroxyferuloylmethane), and Bisdemethoxycurcumin (di-hydroxycinnamoylmethane) [9-10]. In view of this background we performed the extraction and preliminary phytochemical analysis of the methanolic extract of *Curcuma longa* Rhizome.

2. MATERIAL AND METHODS

2.1 Plant Material

The fresh rhizomes were procured from the Lakshmi Ayurvedic Dispensary, Central Market, Mangalore. The plant and the rhizomes were authenticated by, Dr. Krishna kumar G, Chairman, dept of applied botany, Mangalore University, Mangalore . A voucher specimen (YU/CL/2010) is kept in the department of pharmacology, Yenepoya University, Mangalore, India. The rhizomes were separated, shade dried and grounded into powder. The powder was stored in a clean closed container until further use.

2.2 SOURCE OF CHEMICALS

All the chemicals used in the study were procured from Rajesh chemicals, Mumbai.

2.3 SOXHLET EXTRACTION

The dried powder of turmeric (40g) was placed in the thimble of Soxhlet apparatus. 150 ml of methanol was used as a solvent. The extraction was continued till clear solvent was seen in the thimble. Then the extract was dried in a digital water bath till a dark orange residue was obtained. The percentage yield of the extract was calculated using the following formula

$$\text{Percentage yield} = \frac{\text{Final weight of the dried extract}}{\text{Initial weight of the powder}} \times 100$$

The percentage yield was 9% W/W. The extracts were kept in the refrigerator till further use [11].

2.4 PHYTOCHEMICAL ANALYSIS

The test sample was subjected to phytochemical analysis in order to find out the presence of phytochemical constituents. The phytochemical tests employed for alkaloids and tannins, Cardiac glycosides, saponins and flavonoids and terpenoids [11-14].

TEST FOR ALKALOIDS

Wagner's test

20mg of turmeric was dissolved in 2ml of methanol. Few drops of 1% HCl added to it. Then the mixture was heated, kept in steam and after cooling. Then the mixture was treated with few drops of Wagner's reagent. The sample was observed for turbidity or precipitation.

TEST FOR TANNINS

Lead test

20mg of turmeric was dissolved in 1ml of distilled water in a test tube and 1-3 drops of Ferric chloride were added to the solution. Then the mixture was observed for blue or green colour.

TEST FOR CARDIAC GLYCOSIDES

20mg of turmeric was dissolved in 1ml of glacial acetic acid and 1-2 drops of ferric chloride solution was added. 0.5ml of concentrated sulphuric acid was slowly added along the sides of the test tube. A brown ring at the interface indicated a deoxysugar characteristic of cardenolides.

TEST FOR SAPONINS

Foam test

40 mg of turmeric was dissolved with 5ml of distilled water and shaken vigorously till a stable persistent froth was obtained. The froth was mixed with 3 drops of olive oil and shaken vigorously and then observed for emulsion.

TEST FOR FLAVONOIDS

Ferric chloride test

20mg of turmeric was dissolved in 1ml of distilled water. 0.5ml of dilute ammonia solution was added to it. Conc. Sulphuric acid was added later. A yellow colour indicated the presence of flavonoids. The yellow colour disappeared on allowing the solution to stand.

TEST FOR TERPENOIDS

Salkowaski's test

20mg of turmeric was dissolved in 1ml of chloroform and 1ml of concentrated sulphuric acid was added to it. A reddish brown discolouration at the interface showed the presence of terpenoids.

TEST FOR CARBOHYDRATES

Fehling's test Few drops of extract are heated with Fehling's A and B solution. Appearance of orange red precipitate indicates presence of carbohydrates.

TEST FOR LACTONES

Baljet's test

Treat extract with sodium picrate solution. Appearance of yellow to orange colour indicates presence of lactone ring.

TEST FOR PROTEINS

Biuret's test

Add 2ml of Biuret reagent to 2ml of extract. Shake well and warm it on water bath. Appearance of red or violet colour indicates presence of proteins.

FIXED OILS AND FATTY ACID

Spot test

Prepared spot on the filter paper with the test solution and oil staining on the filter paper indicated the presence of fixed oil & fats.

3. RESULTS AND DISCUSSION

India has about 15% of the 20,000 known traditional medicinal plants in the world. Rural populations depend on the medicinal plants for treatment of the ailments. The results of the preliminary phytochemical screening provide an empirical basis for the use of medicinal plants in traditional therapy. The phytochemical constituents are responsible for the biological and pharmacological actions of these plants. Alkaloids have antibacterial activities. Curcumin, demethoxy curcumin and bis-demethoxy-hydroxycurcumin, are three pharmacologically important Curcuminoids that have been isolated from *Curcuma longa* [15]. They have been shown to possess anti-oxidant, anti-inflammatory, anti-carcinogenic, anti-mutagenic, anti-fungal, anti-viral and anti-cancer properties [16]. Curcumin has also shown to possess radioprotective effect. Curcumin prevents radiation induced damage and help repair cells by anti oxidative, free radical scavenging and anti-lipoperoxidase activity. It increases the level of catalase, SOD glutathione peroxidase along with GSH level. It induces apoptosis in critically damaged cells by activation of caspase 3 and 8. Curcumin up regulates the p53 expression and induces G2/Mitotic cell cycle arrest thereby facilitating the repair and regulation of apoptosis, thus preventing mutagenesis. Curcumin sensitizes squamous cell carcinoma cell lines against radiation [17]. It blocks the synthesis of prostaglandins by inhibition of Cyclooxygenase enzyme, reduces pro-inflammatory leukotriene synthesis by inhibition of lipoxygenase enzyme, reduces the neutrophil infiltration in inflammation and inhibits platelet aggregation. It is also a potent inhibitor of pro-inflammatory cytokines (IL and TNF α). The oxygen radical scavenging activity of curcumin is thought to influence antiinflammatory effect. It is a very potent antioxidant which generates hydroxyl radicals through the Fenton reaction by reducing Fe³⁺ to Fe²⁺ [18].

The major ingredient of turmeric oil was ar-turmerone, curcumin, and ar-curcumenone. The principle constituent ar-turmerone may be responsible for the antioxidant activity of turmeric oil. There are some reports in literature indicating the *in vitro* antioxidant potential of turmeric oil is due to the presence of phenyl ring portion and a 13-unsaturated ketone functions of ar-turmerone. Intraperitoneal administration of oil was found to inhibit PMA-induced superoxide radicals elicited by macrophages. Oral administration of turmeric oil to mice, for a period of one month, increased superoxide dismutase, glutathione, and glutathione reductase enzyme levels in blood and glutathione-S-transferase and superoxide dismutase enzymes in liver. Turmeric oil showed significant reduction in paw thickness in Carrageenan, dextran-induced acute inflammation, and formalin-induced chronic inflammation [19]. Saponins have been reported to have antimicrobial properties and they may act as important precursor for steroidal substances. These steroidal substances have wide range of pharmacological activities [20]. The terpenoids and sesquiterpenes found in the oils also exhibit antiinflammatory and antimicrobial effects. The results of the preliminary phytochemical screening of methanolic extract of Turmeric showed the presence of alkaloids, tannins & phenolic compounds, terpenoids & phytosterols, saponins and flavonoids. (Refer Table 1). Solvents used also determine the phyto-chemical constituents. More polar solvents have lesser components compared to the least polar. Turmeric is widely used in traditional healing. The scientific phytochemical screening can provide a sound scientific rationale for its use in curing numerous ailments.

4. CONCLUSION

The preliminary phytochemical analysis of methanolic extract of *Curcuma longa* showed presence of some important Phytochemicals like alkaloids, tannins, phenolic compounds, phytosterols, terpenoids, saponins and flavonoids (**Table No. 1**). These phytoconstituents have important pharmacological activities like anti mutagenic, anti inflammatory, antibacterial, antiprotozoal, and antioxidant properties. Further Phytochemical analysis of successive extraction using solvents of different polarity is essential.

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TABLE 1: Results of the preliminary phytochemical analysis of Methanolic extract of *Curcuma longa* Rhizome.

Phytochemicals	Test	Observation	Inference
Alkaloids	Wagner's test	Red precipitate	+
Tannins and Phenolic compounds	Lead test	Green colour	+
Terpenoids and Phytosterols	Salkowaski's test	Reddish-brown colour	+
Saponins	Foam test	Presence of emulsion	+
Flavonoids	Ferric chloride test	White precipitate	+
Glycosides		Brown ring	+
Carbohydrates.	Fehling's test	-	-
Test for lactones	Baljet's test	-	-
Test for proteins	Biuret's test	-	-
Fixed oils and fatty acid	Spot test	Presence of spot	+

Presence (+), Absence (-)