

Original Paper

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Phytochemical Screening and Evaluation of Rhizome of *Curcuma Longa* L.

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Abstract

Many plants have a lot of biological effective compounds, that have ability to prevent free radicals and works to reduce the oxidative stress, such as phenolic compounds (phenolic acids, flavonoids and tannins) contributes a lot in preventing the risk of many diseases like cancers, cardiovascular, and neurological diseases. Thus, recently raised focusing on the potential of natural resources in plants especially edible one as phenolic compounds – as natural antioxidant- which is aromatic compounds bearing one or more groups of hydroxylated groups exist in almost all plant parts. Our current study deals with the analysis of rhizome of *Curcuma longa*. Phytochemical screening was carried out in four extracts i.e. petroleum ether, acetone, methanol and chloroform, to identify the bioactive compounds such as alkaloids, carbohydrates, glycosides, steroids, Anthocyanins, Saponins, phenols, triterpenoids.

Keywords: Phenols, Phytochemicals, medicinal properties, extracts, Polyphenols, Antioxidant activities.

Introduction

Plants are majorly needed and used in the pharmaceutical industry because of their wide range of diversity as well as because of their wide variety of medicinal properties. The biologically active compounds present in plants are called Phytochemicals. They can be derived from various plant parts and can be used to produce potential drugs. They are considered as the direct source for the production of drugs and are also used directly in a traditional way. This paper mainly deals with the study of rhizome of *Curcuma longa*, the collection of plant and the isolation of bioactive constituents. *Curcuma longa* L. is the main representative of Zingiberaceae family studied for

variety of medicinal properties, including: antioxidant, anti-inflammatory, antibacterial, analgesic, immune modulatory, pro apoptotic, anti-human immunodeficiency virus properties and anticancer effects (Singh *et al.*, 2003). They are mostly observed in wild and cultivated forms. Mostly distributed throughout the tropics of Asia, Africa and Australia. Turmeric is used as a natural food colorant and as an ingredient in production of various medicinal drugs. Phytochemical Screening was done in four extracts i.e. Petroleum ether, acetone, chloroform and methanol for the identification of wide range of Phytochemical compounds present in the four extracts which are shown below in brief.

Materials and Methods

Plant collection:

The rhizomes of plants species *Curcuma longa* L. were collected from region of Ahmedabad city, Gujarat, India in the month of December-2016

Sample preparation:

The rhizomes of *Curcuma longa* L. were carefully separated, cleaned, washed well to remove all the dirt and are shade dried separately until all the water molecules were evaporated, mechanically ground and coarsely powdered. The powdered material was subjected to solvent extraction with petroleum ether, acetone, methanol and chloroform.

Extraction Technique:

10gm of each leaf powder was added to 100 ml of solvent (petroleum ether, acetone, methanol and chloroform) in a conical flask. They were kept in shaker for 24 hours at room temperature. All the extracts were filtered by using Whatmann No.1 filter paper and the concentrated extracts were collected in the petridishes and allowed to evaporate properly. Petridishes were weighed before and after the evaporation of filtrate to know their weight of extracts and then stored in a cool and dry place.

Phytochemical Screening

Test for alkaloids

Extracts were mixed individually with a small amount of dilute HCl and it is filtered. The filtrate was evaluated carefully with different alkaloidal reagent

Mayer's test: 2ml of Mayer's reagent was added in 1 ml of filtrate. Formation of cream precipitate indicates the presence of alkaloids.

Dragendroff's test: 2ml of Dragendroff's reagent was added in 1 ml of filtrate. Formation of orange brown color indicates the presence of alkaloids.

Hager's test: 2 ml of Hager's reagent is added in 1 ml of filtrate.

Formation of yellow precipitate indicates the presence of alkaloids.

Wagner's test: 2ml of Wagner's reagent is added to 1 ml of filtrate.

Formation of reddish brown indicates the presence of alkaloids

Test for Carbohydrate:

The small amount of extract was dissolved in 5 ml of distilled water and it is filtered. The filtrate was subjected to analysis for the presence of carbohydrates.

Molisch test: The filtrate was mixed with 2 to 3 drops of 1% alcoholic alpha naphthol and along the sides of test tube; 2 ml of concentrated sulphuric acid was added and appearance of purple color ring at the junction of two liquids.

Fehling's test: Fehling A and Fehling B were mixed and few drops of extract were added and boiled. A brick red colored precipitate of cuprous oxide forms if reducing sugars are present.

Benedicts test: 2 to 3 drops of benedicts reagent was added to 1 ml extract and heat almost to boiling. Appearance of red, brown, orange or yellow color indicates the presence of carbohydrates.

Test for glycosides:

Keller-Killiani test: To 2 ml extract, add glacial; acetic acid. 1 drop 5 % FeCl_3 and conc. H_2SO_4 . Brown ring appears indicates the presence of glycosides

Test for proteins and free amino acids:

Small quantity of extracts were dissolved separately in a few ml of water and treated with following reagents:

Millon's test: Few mg of extract is added with Millon's reagent. Formation of red color shows the presence of proteins and amino acids.

Ninhydrin test: 0.1% ninhydrin solution was added to few mg of extract. Appearance of Purple color shows the presence of protein and free amino acids.

Biuret's test: Equal volume of 5 % solution of sodium hydroxide and 1% solution of copper sulphate were added. Appearance of pink color shows the presence amino acids and proteins.

Test for flavonoids:

Few ml of extract is mixed with sulphuric acid. The formation of yellow orange indicates the presence of flavones, formation of yellowish orange color indicates the presence of anthocyanins and the formation of orange to crimson color indicates the presence of flavonones.

Test for steroids:

Salkowski test: To 2 ml of extract add 2 ml chloroform and 2 ml conc. H_2SO_4 and was shaken for few minutes. Chloroform layer appeared red (upper layer) and acid layer showed greenish yellow fluorescence (lower layer) indicates the presence of steroids.

Liebermann-Burchard test: Mix 2 ml extract with chloroform. Add 1 to 2 ml acetic anhydride and 2 drops conc. H_2SO_4 from the side of test tube. First red, then blue and finally green color indicates the presence of steroids.

Test for Triterpenoids:

Liebermann-Burchard test: Mix 2 ml extract with chloroform. Add 1 to 2 ml acetic anhydride and 2 drops conc. H_2SO_4 from the side of test tube. First red, then blue and finally green color indicates the presence of steroids

Test for Tannins:

Gelatin test: To the extract, gelatin (dissolved in warm water immediately) solution was added. Formation of white precipitate indicates the presence of tannins.

Lead acetate test: 10% of lead acetate solution was added in few ml of extract. Formation of white precipitate indicates the presence of tannins.

Tests for Phenol:

Ferric chloride test: Few mg of extract was dissolved in 5 ml of distilled water. 2 ml of 1 % Solution of gelatin containing 10% NaCl was added to it. White precipitates indicate presence of phenolic compounds.

Sodium hydroxide test: Five mg of extract was dissolved in 0.5 ml of 20% sulphuric acid solution. Add few drops of aqueous sodium hydroxide solution. Formation of blue color indicates the presence of phenols.

Ellagic Acid Test: Add few drops of 5% glacial acetic acid and 5% $NaNO_2$ solution was added in few ml of extracts . Muddy or Niger brown precipitate occurs.

Tests for Saponins:

Foam test: Small amount of extract was shaken with little amount distilled water. Formation of foam indicates the presence of saponins. (Prasant Tiwari *et al.* 2011)

Result and Discussion

Alkaloids, carbohydrates, glycosides, steroids are in high amounts in all the extracts, except glycosides which were absent in acetone extract. Flavonoids were absent in chloroform extract. Triterpenoids were found in moderate amount in petroleum ether and acetone extracts, while high amounts were present in chloroform and methanolic extracts. Phenols were present in moderate to very high amount in all extracts (R. S. Sawant *et al.*, 2013)

Table 1: Phytochemical screening of *Curcuma longa* L. rhizome extract

SRNO.	CONSTITUENTS	TESTS	RESULTS			
			PE	AE	ME	CE
A	Alkaloids	Mayer's test	+	+	+	+
		Dragendroff's test	+++	+++	+++	+++
		Hager's test	+	++	++	++
		Wagner's test	-	+	++	-
B	Carbohydrates	Molisch test	+++	++	+++	+++
		Fehling's test	-	-	+	-
		Benedict's test	+	+++	+++	+
C	Glycosides	Keller-Killiani test	++	-	+++	++
D	Proteins and free amino acids	Millon's test	+	+	-	-
		Ninhydrin test	+	+	+	-
		Biurets test	+	+	+	-
E	Flavonoids	H ₂ SO ₄ test	+	++	++	-
		(antho cyanin)	(flavo nes)	(flavo nones)		
F	Steroids	Salkowski test	++	+++	+++	++
		Liebermann- Burchard test	++	++	+++	+++
G	Triterpenoids	Liebermann- Burchard test	++	++	+++	+++
H	Tannins					

H	Tannins	Gelatin test	+	+	-	-
		Lead acetate test	+	-	-	-
I	Phenols	Ferric chloride test	+	++	++	-
		Sodium hydroxide test	+++	++	+++	++
		Ellagic acid test	-	-	-	++
J	Saponins	Foam test	+	+	+	+

Conclusions

Secondary metabolites are used economically as bioactive compounds; they are high value-low volume products (e.g. -Steroids, Quinines, Alkaloids, Terpenoids and Flavonoids) which are used in drug manufacture by the pharmaceutical industries. Plants are rich source of unused drugs that constitute the ingredients in traditional system of medicine, modern medicines and pharmaceutical intermediates. The reason for using plants as medicine lies behind the natural fact of presence of chemical constituents which yield a great medicinal value. The anti-diuretic, anti-inflammatory, anti-analgesic, anti-cancer, anti-viral, anti-malarial, anti-bacterial and anti-fungal activities of the medicinal plants are due to the presence of the above identified secondary metabolites by the phytochemical screening. The Phytochemical screening of the above plants are also important and have commercial interest in both research institutes and pharmaceuticals companies for the discovery of the novel drugs for treatment of wide range of diseases.

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