# PHYTOCHEMICAL COMPARISON OF ALCOHOLIC EXTRACT OF FRESH AND DRY *Curcuma longa*

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## ABSTRACT

Curcuma longa *commonly known as turmeric belongs to the family Zingiberaceae which is traditionally used as a spice in Indian food. It is said to have a wide range of biological activities like anticancer, antimicrobial, anti-inflammatory and free radical scavenging activity which is why it is used by Asian countries from ancient period. Phytochemical constituents are non-nutritive plant chemicals that have disease preventive properties. Isolation of bioactive compounds from the plants depends mainly upon the solvents which are used for extraction. In our study, we have used methanol and ethanol as solvents. These extracts were used for qualitative preliminary phytochemical analysis using standard chemical tests. Two types of samples namely dry and fresh sample was used for the study. The study reveals that the sample was more potent than the fresh sample which contained 16 phytochemicals compared to 21 among which the fresh sample contained only 14 bioactive compounds. The phytochemicals includes carbohydrates, proteins, fixed oils, Phlobatannins, alkaloids, flavonoids, gums and mucilage, phenols, glycosides, quinones, cardiac glycosides, steroids, xanthoproteins, steroids, Phytosterols, resins and coumarins.*

**Keyword:** *Curcuma longa*, Phytochemicals, Methanol, Ethanol, rhizome extract.

## INTRODUCTION

Medicinal plants are a gift to us from the nature as they provide a number of health benefits to us. In India these medicinal plants are used for about centuries for their properties and are still used to this date. India has a variety of traditional medicinal systems like Ayurveda, Siddha, Unani and a huge class of ethnomedicine.

Turmeric is a plant that has a very long history of medicinal use, dating back nearly 4000 years. In Southeast Asia, turmeric is used not only as a principal spice but also as a component in religious ceremonies because of its brilliant yellow color. India produces nearly the world's entire turmeric crop and consumes 80% of it. With its inherent qualities and high content of the important bioactive compounds, Indian turmeric is considered to be the best in the world. Erode, a city in the South Indian state of Tamil Nadu, is the world's largest producer of turmeric and is also known as "Yellow City," "Turmeric City," or "Textile City."

As a report by World Health Organization (WHO), over 80% of the people of developing countries are relaying on the traditional medicines that are extracted from the plants for their primary health needs. Use of these traditional medicines for the preparation of modern medicinal preparation is indispensable and thus 'Phytomedicines' are a link between traditional and modern medicine.

The chemicals that are produced by plants are called as phytochemicals. These are produced by the plants primary and secondary metabolism. These phytochemicals are important for the plants and protect them from disease and

damage caused by environmental hazards. The phytochemicals are majorly classified as primary and secondary metabolites. The primary metabolites are responsible for the basic development of the plant which includes the sugars, amino acids, proteins, nucleic acids, chlorophyll, etc.

Secondary metabolites are those which are needed for the survival of the plant in harsh environment. They forms the smell, colour and taste of the plants and secondary metabolites such as flavonoids, tannins, saponins, alkaloids, steroids are found to have other commercial applications like they can be used as coloring agents, as drugs, as flavoring agents, insecticides, pesticides, anti-bacterial and snit-fungal products. Moreover they can also be used to protect humans form many diseases like cancer, diabetes, cardiovascular diseases, arthritis and aging etc.

## MATERIALS AND METHODS

### Sample preparation

The plant sample was brought from the nearby herbal shop at Coimbatore, Tamil Nadu, and India. Two types of samples were used which includes dry and fresh sample. The plant sample was washed and was shade dried until complete removal of moisture and was ground into a fine powder. In case of fresh extraction of *Curcuma longa*, fresh rhizomes of the plant were used (Figure-1, 2).

### Production of plant extract:

The extraction was made in 100% alcohol. Here ethanol and methanol was used for extraction. About 5gram of sample was weighed and added to the conical flasks to which ethanol and methanol was added. In case of fresh *Curcuma longa*, they were descaled, cut into small pieces, grinded in mortar and pestle and about 5gram of the ground paste was taken for extraction. The conical flasks were then kept in an orbital shaker at 120rpm for 24hrs. The content was filtered using filter paper and was stored at 4$^{o}$C until use.

***(Figure – 1:** Curcuma longa)*

*(**Figure – 2:** Curcuma longa, shade dried and powdered)*

## QUALITATIVE ANALYSIS OF PRIMARY METABOLITES

### Test for Carbohydrates

1. Benedict's test: About 0.5ml of the filtrate was taken to which 0.5ml of Benedict's reagent was added. This mixture was heated for about 2 minutes in a boiling water bath. The appearance of red precipitate indicates the presence of sugars.

2. Molisch's test: To about 2ml of the sample, 2 drops of alcoholic solution of @-napthol was added and to the mixture after being shaken well. Few drops of conc.$H_2SO_4$ were added along the sides of the test tube. A violet ring indicates the presence of sugars.

### Test for Proteins

1. Biuret test: 2ml of filtrate was taken to which 1 drop of 2% copper sulphate solution was added. 1ml of 95% ethanol was added. Them it was followed by excess addition of KOH. The appearance of pink colour indicates the presence of proteins.

2. 2ml of extract was mixed with 2ml of water and about 0.5% of conc.$HNO_3$ was added. The appearance of yellow colour indicates the presence of proteins.

### Test for amino acids

1. To 1ml of the extract, few drops of ninhydrin reagent (10mg of ninhydrin in 200ml of acetone) was added. The appearance of purple colour indicates the presence of amino acids.

### Miscellaneous compounds

### Test for resins

1ml of extract was taken and to this few ml of acetic anhydride was added. To this 1ml of conc.$H_2SO_4$ was added. The appearance of orange to yellow colour indicates the presence of resins.

### Test for fixed oils and fats

1. Spot test: Small quantity of the extract was taken and pressed between 2 filter papers. The appearance of spots indicates presence of oils.

2. Saponification test: To the extract, few drops of 0.5N alcoholic KOH and few drops of phenolphthalein was added. This mixture was heated for about 2 hours. The formation of soap or partial neutralization of alkali indicates the presence of fixed oils or fats.

**Test for Gums and Mucilage**

To 1ml of extract, distilled water, 2ml of absolute ethanol was added with constant stirring. White or cloudy precipitate indicates the presence of gums and mucilage.

**Test for Carboxylic acids**

To 1ml of extract a pinch of sodium bicarbonate was added. The production of effervescence indicates the presence of carboxylic acids.

**QUALITATIVE ANALYSIS OF SECONDARY METABOLITES**

**Test for alkaloids**

1. Mayer's test: To a few ml of filtrate, 2 drops of Mayer's reagent was added. A creamy white precipitate shows a positive result for alkaloids.

2. Wagner's test (iodine-potassium iodine reagent): To about an ml of extract few drops of Wagner's reagent were added. Reddish-brown precipitate indicates presence of alkaloids.

3. To 5ml of extract, 2ml of HCL was added. Then, 1ml of Dragendroff's reagent was added. An orange or red precipitate shows a positive result for alkaloids.

**Test for Glycosides**

1. Borntrager's test: To 2ml of filtrate, 3ml of chloroform was added and shaken. The chloroform layer was separated and 10% ammonia solution was added. The pink colour indicates the presence of glycosides.

2. 5ml of extract was hydrolyzed with 5ml of Conc. HCL boiled for few hours in a boiling water bath. Small amount of alcoholic extract was dissolved in 2ml of water and aqueous 10% NaOH was added. The presence of yellow colour was a positive result for the glycosides.

3. 2ml of extract is mixed with about 0.4ml of glacial acetic acid containing traces of $H_2SO_4$ was added. The production of blue colour is positive for glycosides.

**Test for Cardiac glycosides (Killer-Killani test)**

5ml of solvent extract was mixed with 2ml of glacial acetic acid and a drop of ferric chloride solution was added followed by the addition of 1ml of conc.$H_2SO_4$. A brown ring in the interface indicates the presence of deoxy sugars of cardenoloides. A violet ring may appear beneath the brown ring while acetic acid layer a green ring may also form just gradually towards the layer.

**Test for Phenol**

1. Gelatin test: To 5ml of extract, 2ml of 1% solution of gelatin containing 10% of NaCl is added. Appearance of white precipitate indicates the presence of phenol.

2. Lead acetate test: To 5ml of extract, 3ml of 10% lead acetate solution was added and mixed gently. The production of bulky white precipitate is positive for phenols.

**Test for Polyphenols:**

1. To the 3ml of the extract 10ml of ethanol was added and were warmed in a water bath for 15 minutes. To this few drops of ferric cyanide (freshly prepared) was added. The formation of blue – green colour indicates the presence of polyphenols.

2. To 1ml of extract few drops of 5% solution of lead acetate was added. The appearance of yellow precipitate indicates the positive result for polyphenols.

3. To the 5ml of ethanolic extract, 0.1% gelatin solution was added. The formation of precipitate was positive for polyphenols.

**Test for tannins**

To 5ml of extract, few drops of neutral 5% ferric chloride solution were added. The production of dark green colour indicates the presence of tannins.

**Test for flavonoids**

1. To the aqueous solution of the extract, 10% of ammonia solution was added and heated. The production of fluorescence yellow is positive of flavonoids.

2. 1ml of extract was taken and 10% of lead acetate was added. The yellow precipitate is positive for inference for the flavonoids.

3. The extract is treated with conc.$H_2SO_4$ resulting in the formation of orange colour indicates the positive result for flavonoids.

4. To 5ml of dilute ammonia, the plant extract is added and shaken well. The aqueous portion is separated and conc.$H_2SO_4$ was added. The yellow colour indicates the presence of flavonoids.

**Test for Phytosterols**

1. The extract is dissolved in 2ml of acetic anhydride and to which 1 or 2 drops of conc.$H_2SO_4$ was added along the sides. An array of colour change indicates the presence of Phytosterols.

2. The extract was refluxed with alcoholic KOH and saponification takes place. The solution was dilutes with ether and the layer was evaporated and the residue was tested for Phytosterols. It was dissolved in dilutes acetic acid and few drops of conc.$H_2SO_4$ are added. The presence of bluish green colour indicates the presence of polysterols.

**Test for Phlobatannins**

Aqueous extract was boiled with diluted HCL leading to the deposition of reddish precipitate indicates the presence of Phlobatannins.

**Test for saponins**

1. 0.5mg of extract was vigorously shaken with few ml of distilled water. The formation of frothing is positive for saponins.

2. The froth from the above reaction is taken ad few drops of olive oil was added and shaken vigorously and observed for the formation of emulsion.

**Test for Steroids**

To 2ml of the extract, 2ml of chloroform and 2ml of conc.$H_2SO_4$ was added. The appearance of red colour and yellowish green fluorescence indicates the presence of steroids.

**Test for xanthoproteins**

1ml of extract is taken and to this few drops of nitric acid and ammonia are added. Reddish brown precipitate indicates the presence of xanthoproteins.

**Test for Terpenoids (Salkowski test)**

3ml of extract was taken and 1ml of chloroform and 1.5ml of conc.$H_2SO_4$ was added along the sides of the tube. The reddish brown colour in the interface is considered as positive for presence of Terpenoids.

**Test for Coumarins**

To 2ml of the extract, 3ml of 10 aqueous solution of NaOH was added. The production of yellow colour indicates the presence of coumarins.

**Test for quinones**

To 1ml of extract, alcoholic KOH is added the presence of red to blue colour indicates the presence of quinones

## RESULT AND DISCUSSION

In this study, the phytochemicals present in the alcoholic extracts of *Curcuma longa* was analyzed using standard screening methods. Here dry and fresh sample was used. Two types of solvents was used namely ethanol and methanol for the extraction. The samples contain many bioactive compounds like carbohydrates, proteins, alkaloids, flavonoids, glycosides, steroids etc. The study reveals that the dry sample extraction was found to contain 16 phytochemicals among the below mentioned 21 compounds which includes carbohydrates, proteins, fixed oils, Phlobatannins, alkaloids, flavonoids, gums and mucilage, phenols, glycosides, quinones, cardiac glycosides, steroids, xanthoproteins, steroids, Phytosterols, resins, coumarins. The fresh sample on the other side contains only 14 phytochemicals and comparing to dry sample the fresh sample extraction lacks the bioactive compound fixed oils and Phlobatannins. The rhizome extraction of *Curcuma longa* on the whole does not contain carboxylic acids, tannins, saponins and Terpenoids. The qualitative phytochemical analysis of the total extraction of the sample is shown in the table-1.

**Table-1:** *Qualitative Phytochemical Analysis of Rhizome extract of Curcuma longa*

| S. No | Phytochemicals | Dry Methanolic extract | Dry Ethanolic extract | Fresh Methanolic extract | Fresh Ethanolic extract |
|---|---|---|---|---|---|
| 1. | Carbohydrates | + | + | + | + |
| 2. | Proteins | + | + | + | + |
| 3. | Amino Acids | - | - | - | - |
| 4. | Gums and mucilage | + | + | + | + |
| 5. | Resins | + | + | + | + |
| 6. | Fixed oils | + | + | - | - |
| 7. | Carboxylic acids | - | - | - | - |
| 8. | Quinones | + | + | + | + |
| 9. | Alkaloids | + | + | + | + |
| 10. | Glycosides | + | + | + | + |
| 11. | Cardiac Glycosides | + | + | + | + |

| | | | | | |
|---|---|---|---|---|---|
| 12. | Phenol | + | + | + | + |
| 13. | Tannins | - | - | - | - |
| 14. | Flavonoids | + | + | + | + |
| 15. | Phytosterols | + | + | + | + |
| 16. | Phlobatannins | + | + | - | - |
| 17. | Saponins | - | - | - | - |
| !8. | Steroids | + | + | + | + |
| 19. | Xanthoproteins | + | + | + | + |
| 20. | Terpenoids | - | - | - | - |
| 21. | Coumarins | + | + | + | + |

(+)Indicates Presence (-) Indicates Absence

**Qualitative analysis of primary metabolites**

In the qualitative analysis of primary metabolites, the tests are performed for the carbohydrates, proteins, and amino acids, for the qualitative analysis of the carbohydrates, two tests namely Benedict's test and Molisch's test was performed and all the 4 samples were positive for the presence of carbohydrates. For the test of proteins, again two tests were performed namely Biuret test and Conc. $HNO_3$ Test and shows presence of the proteins. The test for amino acids, Ninhydrin test is performed which shows absence of amino acids in all four samples of *Curcuma longa*. The results for Qualitative analysis of primary metabolites are shown in figure-3

**Figure-3** Qualitative analysis of primary metabolites

**Qualitative analysis of miscellaneous compounds**

In qualitative analysis of miscellaneous compounds, the tests for resins, fixed oils and fats, gums and mucilage and carboxylic acids are done. The dry extracts of the *Curcuma longa* shows positive results for the presence of for resins, fixed oils and fats, gums and mucilage and absence of carboxylic acids. Whereas, the fresh extract of the *Curcuma longa* shows positive results for the presence of for resins, gums and mucilage and absence of carboxylic acids and fixed oils and fats. The results are shown in figure-4


**Figure-4:** Qualitative analysis of miscellaneous compounds

**Qualitative analysis of secondary metabolites**

The test for secondary metabolites are done they include the tests for alkaloids, glycosides, Glycosides, Cardiac Glycosides, Phenol, Tannins, Flavonoids, Phytosterols, Phlobatannins, Saponins, Steroids, Xanthoproteins, Terpenoids, Coumarins and Quinone. In the Dry extracts of *Curcuma longa* the alkaloids, glycosides, phenol, flavonoids, Phlobatannins, Steroids, Xanthoproteins, Coumarins and quinones were present and tannins, saponins, terpenoids were absent. Whereas in the fresh extract, Alkaloids Glycosides, Cardiac Glycosides, Phenol, Flavonoids, Phytosterols, Steroids, Xanthoproteins, Coumarins and quinones were present and Tannins, Phlobatannins, Saponins, Terpenoids were absent. The results are shown in the figure 5, 6, 7.

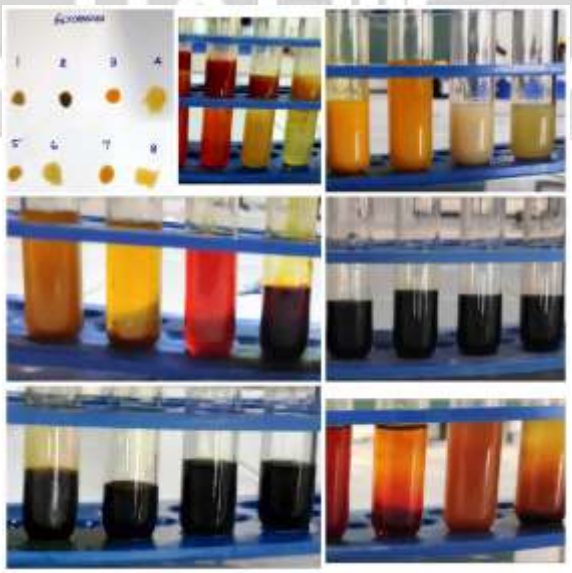

***Figure -5:*** *Tests for glycosides, killer Killani, phenol (gelatin test),*

*Phenol (lead acetate test), phytosterols, quinones and tannins*

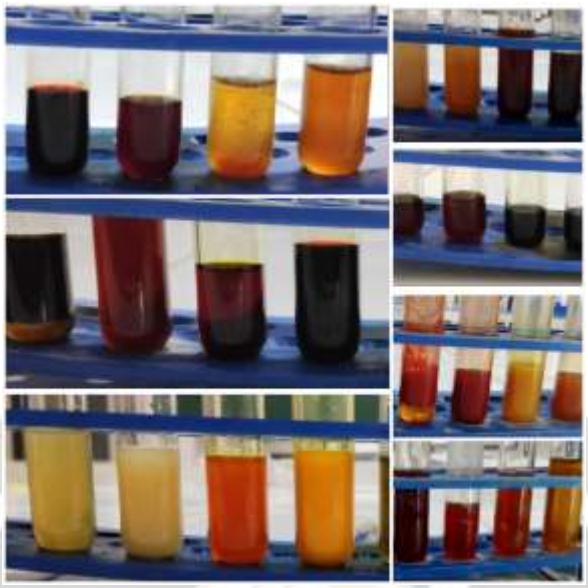

***Figure- 6:*** *Test for Flavonoids, Phlobatannins, saponins, Steroids, xanthoproteins*

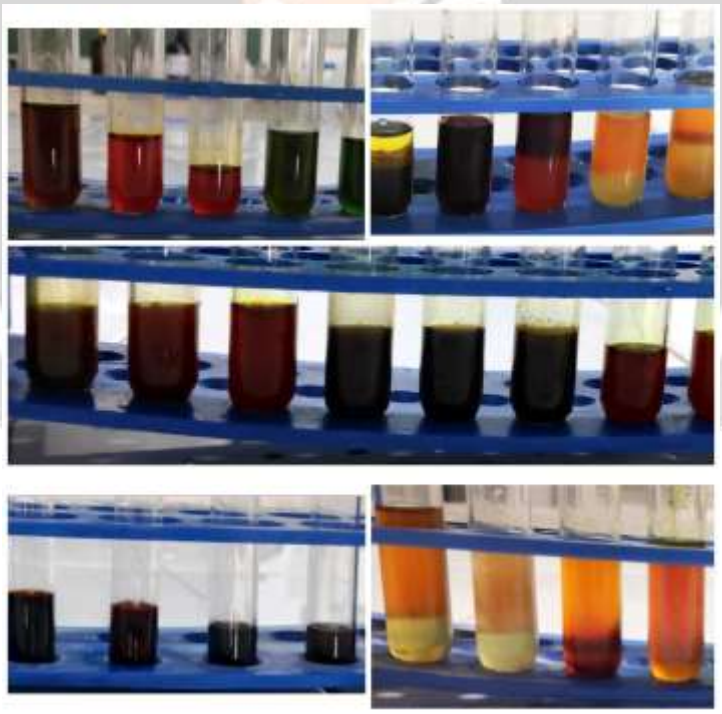

***Figure-7:*** *Test for coumarins and terpenoids, Mayer's test, Borntrager's test, Wagner's test*

## CONCLUSION

*Curcuma Longa* also known as turmeric belonging to the family Zingiberaceae has been widely used from ancient times is now scientifically proven to have many medicinal properties like antimicrobial, anti-inflammatory, anti-cancerous, anti-oxidant and number of phytochemicals. It was evident that the dry sample contained high number of phytochemicals compared to the fresh sample where Methanolic and ethanolic extracts were used. In this study the phytochemical analysis were useful to detect the presence of the bioactive compounds in the plant which subsequently may lead to the discovery and development of medicinal drugs.

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