Antimicrobial activity Of *Curcuma longa* Along With Its Total Polyphenolic AndCurcuminoid Content

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Abstract-Turmeric or Haldi, a spice derived from the rhizomes of the plant *Curcuma Longa*. *Curcuma longa* is a member of the Zingiberaceae family or the ginger family. The bright yellow color of turmeric comes mainly from compounds known as Curcuminoids. Curcuminoid is a group of fat-soluble polyphenolic compounds includes curcumin, desmethoxycurcumin, and bisdemethoxycurcumin. Curcumin is the primary curcuminoid in turmeric, it has powerful anti-inflammatory effects and is a very strong antioxidant and is approximately 77% of the curcuminoid content. Turmeric is also a source of polyphenols, which is a category of plant compounds that offers various health benefits. They can act as antioxidants, meaning they can neutralize harmful free radicals that would otherwise damage cells, regularly consuming polyphenols is thought to boost digestion and brain health, as well as protect against heart disease, type 2 diabetes, and even certain cancers. Plants show medicinal properties because of the phytochemical present in them. In order to extract these essential phytochemicals out of the rhizomes of turmeric, Acetone, Methanol, Ethanol, and Chloroform solvents were used and further analysis were done by Qualitative Phytochemical Screening, Analysis of Polyphenolic component using UV-Visible Spectrophotometer, analysis of curcuminoid content using HPLC (High-Performance Liquid Chromatography) and anti-microbial testing via well diffusion method on *S.aureus* (Gram +ve) and *E.coli* (Gram -ve).

Keywords: Phytochemicals, Polyphenols, Curcuminoids-HPLC.

Introduction

Curcuma longa L. (turmeric) of ginger family (Zingiberaceae) is one of oldest cultivated spice plants in the south-east Asian countries. For many years rhizomes of the plant has been used as a safe and active drug for the treatment of various chronic diseases. Curcuma longa is a perennial herb with orange, tuberous pulpy roots that grow to about 60 cm in length. India produces about 400,000 tons per year or about 80% of the world's supply of commercial turmeric[1].

Figure-1 Plant of Turmeric

Figure-2 Diagram of Plant

Figure-3 Fresh Rhizomes

Figure-4 Dried Rhizomes

The yellow color, which is characteristic of the turmeric rhizome, is due to the presence of 3–5% of curcuminoids. The curcuminoids include curcumin, demethoxycurcumin, bis-demethoxycurcumin, and curcumin of which curcumin is the major bioactive constituent[2,3.]

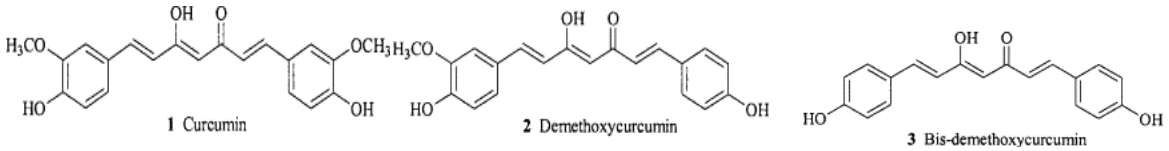

Figure-5 Different Curcuminoids

It is recommended for treating high cholesterol, abdominal pains, Wounds, eczema, psoriasis, Jaundice, menstrual disorder, Inflammations, diabetes, Cancerous Symptoms, and as a blood purifying activity[4]. Many species of Curcuma are traditionally used for their medicinal properties, Anti-inflammatory Antibacterial, and Antifungal activity has been reported for species such as *C.longa, C. zedoria, C. aromatica, and C. amada*[5].Curcuminoids exhibit properties like free-radical scavenging, antioxidant activity and also act as inhibitors of human immune deficiency virus type 1 (HIV-1) integrase enzyme.

Plant extracts and oils have been used for a wide variety of purposes since ancient times. One of their purposes is as a source of medicine as they contain a range of organic compounds with therapeutic values. The majority of people depend on traditional medicine as their primary healthcare. About 80% of people in this world depend on herbs for health. According to WHO, medicinal plants will be the best source to obtain a variety of drugs. Herbal products are highly effective for treating a wide range of diseases and infections[6].

Besides serving medicinal purposes, plant extracts are also used as herbs and spices. These spices and herbs are considered effective and safe against certain ailments. Long-term consumption of these substances is also guaranteed not to cause any side effects. Herbs like Ashwagandha, Triphala, and Shatavari have been used as medicine in traditional Indian medicine or Ayurveda. Ayurvedic medical systems have wide uses for both fresh and dried preparations. The dried powders are used to treat distinctly different ailments via using them as pastes or plant juices[7].

There is a common concept among people that herbal medicines being natural in origin have no side effects and are safe. Herbal remedies used in traditional medicine provide an interesting and still largely unexplored source for the creation and development of potential new drugs for chemotherapy which might help to overcome the growing problem of resistance and also the toxicity of the currently commercially available antibiotics. Several studies have reported the broad-spectrum antimicrobial activity for curcumin including antibacterial, antiviral, antifungal, and ant malarial activities[8,9].

Plant phenolics are important constituents that contribute to functional quality, color, and flavor and have significant roles both as singlet oxygen quenchers and free radical scavengers, helping to minimize molecular damage[10].These compounds may be classified into different groups as a function of the number of phenol rings that they contain and of the structural elements that bind these rings to one another. Distinctions are thus made between the phenolic acids, flavonoids, stilbenes, and lignans[11,12].

The objectives of the research were to extract the phytochemicals out of the *Curcuma Longa* rhizomes using organic solvent (Acetone, Chloroform, Ethanol, Methanol). Qualitative screening of the Solvent extract for presence of different phytochemicals, to test the microbial activity of the extracts against *E. coli* (Gram Negative) and *Staphylococcus aureus* (Gram Positive) , analysis of poly phenolic compounds and analysis of curcuminoid compound using High-Performance Liquid Chromatography (HPLC).

Material and Methods

Plant Material: Dried turmeric rhizomes were collected from the quality assurance department of The Himalaya Drug Company (Dehradun) which was then further dried under shade for 5 days. It was then grounded

into a coarse powder. The powder was then kept in an air tight jar for further usage.

Crude Extraction: 20 grams of coarse powder is soaked in 100 ml of Acetone, Chloroform, Ethanol and Methanol at room temperature for 3 days inside an Iodine Flask with occasional shaking. The solvents were then filtered using Whatman ™ no.41 filter paper and concentrated using a water bath. The extracts were then kept in glass bottles for further usage.

Identification Test: The individual extract was subjected to the qualitative phytochemical screening forth presence of some chemical constituents. Phytochemical tests were carried out adopting standard procedures[13,14,15]. All of the reagents were made by adopting standard procedures[16].

Alkaloids

Mayer's Test: In which the alkaloid solution produces white yellowish precipitate when a few drops of Mayer's reagents are added.

Flavonoids: Themethanolic extract was warmed with metal Mg and added 5-6 drops of conc. hydrochloric acid. The red color was observed for Flavonoids.

Tannins: In which 0.5 ml of extract solution, 1ml of water and 5-8 drops of Fehling's solution was added. Blue color was observed for gallic tannins and green black for catecholic tannins.

Steroids:1 ml of extract was dissolved in 3 ml of Chloroform Equal volume of Concentrated H_2SO_4 was added into the test tube (from the side of the test tube slowly). The appearance of green fluorescence in the acid layer and pink colour in chloroform layer confirms the presence of steroids in the extract.

Saponins: 2ml of extract was taken in a test tube and 8 ml of distilled water was added into it.The test tubes were then shaken vigorously for 5 minutes.Formation of stable foam confirms presence of saponins in the extract.

Phenols:1 ml of sample was taken in a test tube. A few drops of Alcoholic $FeCl_3$ were added into the extract.Appearance of blue-black colour indicates presence of phenols in the extract.

Method

Test Solution

Pipette out 2.0 ml of solution in 25 ml volumetric flask

Phytosterol

Salkowski's Test: 2 ml of extract was taken in a test tube then 2 ml of chloroform was added and filtered.The filtrate was treated with a few drops of Conc. H_2SO_4,Shaked and allowed to stand. Appearance of golden red colour indicates presence of phytosterols in the extract.

Carbohydrates

Iodine Test: 2 ml of extract was taken into a test tube and few drops of iodine solution were addedinit.Appearance of blue colour indicates the presence of carbohydrates in the extract.

Reducing Sugar

Fehling Test: 2 ml of extract was taken in a test tube. The extract was hydrolysed with a few drops Dil. HCl and neutralized with a few drops of Dil. NaOH.

1 ml of Fehling A was added following by 1 ml of Fehling B. The test tube was then gently heated on a water bath. Formation of brick red ppt indicates the appearance of reducing sugars in the extract

Total Polyphenol Content Estimation

Reagent Preparation

Sodium Carbonate Solution: Add 29 gm of Sodium carbonate in 100 ml of water

Phosphomolybdotungstic Reagent: Mix Folin&Ciocateu's Phenol reagent (2N) (Make LobaChemie) with water in ratio 1:1

Standard Solution Preparation

Stock Pyrogallol Standard Solution: Add 50 mg of pyrogallol in 100 ml water.

Working Pyrogallol Standard Solution: Add 5 ml of pyrogallol standard solution in 100 ml of water.

Test Solution Preparation: Take 1 gm of sample in 250 ml flat bottom flask. Add 150 ml water in it and place it on the water bath at 97^0C for 30 minutes**.** Cool the flask under running water and allow the residue to settle**.**Transfer the supernatant in a 250 ml volumetric flask. Repeat the process with 50 ml and 25 ml respectively**.** The whole extract was transferred after the last cycle.The volume was then bought up to 250 ml**.** The extract was then filtered **.**The filtrate was then kept for further processing.

Add 1.0 of Folin&Ciocateu's Phenol Reagent
Add 10.0 ml of water
Bring the volume to 25 ml with Sodium Carbonate solution

After exactly 30 minutes take absorbance standard at 760 nm using water as compensation liquid

Standard Solution

Pipette out 2.0 ml of working standard in a 25 ml volumetric flask

Calculation

$$\frac{A_1}{A_2} * \frac{W_1}{V_1} * \frac{V_2}{W_2} * \frac{25}{100} * \text{Purity of Standard}$$

A_1 = Sample Absorbance
A_2 = Standard Absorbance
W_1 = Weight of Standard in mg
W_2 = Weight of Sample in mg
V_1 = Volume of Standard made (100 ml)
V_2 = Volume of Sample made (250 ml)

HPLC analysis of Curcuminoids content

HPLC Instrument

The methanolic extract was concentrated and analyzed using HPLC of *Curcuma longa* was analysed as per standard method[17]with some modifications. The extract was filtered through membrane syringe filter (0.20 m) and
20 µl of filtrate was used for analysis in the HPLC.

Preparation of standard solution
Standard solution: Standard Curcumin was dissolved in methanol in a 50 ml volumetric flask to a final concentration of 1.0 mg/ml and sonicated for 10 minutes in an ultrasound bath and completed to the final volume.
Working Solution: (0.01 mg/ml) This solution was prepared by adding 1 ml of standard solution in 100 ml of volumetric flask and

Add 1.0 of Folin&Ciocateu's Phenol Reagent
Add 10.0 ml of water and bring the volume to 25 ml with Sodium Carbonate solution
After exactly 30 minutes take absorbance standard at 760 nm using water as compensation liquid.

HPLC conditions

Column : C18 phenomenexluna (250×4.6 mm 5µ)
Mobile phase : Orthophospheric acid (0.1%) in water : Acetonitrile (50:50)
Flow rate - : 1 ml/minute
Wavelength : 420 nm
Injection : 20 µl
Temperature : 35°C
Run time : 20minute

Procedure

Stabilized the instrument with the mobile phase till the baseline is satisfactory then injected the standard solution of three times and then recorded the chromatogram. The % RSD between the results should be less than 2 % and then injected the sample solutions and recorded the chromatogram.

make the volume up to the mark with methanol.
Sample preparation (5.0mg/ml)
Test Solution was prepared by adding 100 mg of sample in 50 ml of methanol in a flat bottom flask. The solution was then heated on a water bath for 30 minutes at 800C. The solution was then cooled down to room temperature and makes the volume up to the mark with methanol.

Calculation of Curcuminoid

$$\frac{\text{Area of Sample}}{\text{Area of Standard}} \times \frac{\text{Concentration of Standard}}{\text{Concentration of Sample}} \times \text{Purity of standard}$$

Anti-bacterial Assay

Microbial Samples: The microbial samples used for the procedure were obtained from the microbiology unit of department of quality assurance and quality control, The Himalaya Drug Company. Microorganisms used were *Staphylococcus aureus* (Gram Positive) and *Escherichia coli* (Gram negative) They were subculture in recommended media purchased from Hi-Media, India private Ltd, Mumbai and stored in 4°C for further use.

Culture media and antibiotics: Nutrient Agar was used for the culture of the bacteria. Ciprofloxacin was used as a standard antibiotic for bacteria.

Screening for anti-bacterial activity: Antibacterial activity of all the extracts was tested by well diffusion agar method. Culture plates were prepared by first preparing the stock bacterial culture by mixing a loop full of *S.aureus* in autoclaved nutrient agar. Stock culture was then incubated at 37^0C for 48 hours. 2 ml of stock culture was then added in autoclaved working culture at 40^0C to 60^0C. 20 ml of working culture was poured in sterile plates and kept aside for solidifying. After the media in the plated solidifies a sterile cork

borer was used to make wells of 5 mm diameter in the centre of the plate. The wells were filled with sample extracts. The same procedure was used for E.coli. For positive control Ciprofloxacin antibiotic was used and negative controls were made with acetone, chloroform, ethanol and methanol instead of sample extracts. The anti-bacterial assay plates were then incubated at 37^0C for 24 hours. The diameter of the zone of inhibition around each well was taken as a measurement for antibacterial activity.

Results and Discussions

Results of Phytochemical Screening

The various Secondary metabolites that are present in the rhyzome of *Curcuma longa* are responsible for this therapeutic effect. Presences of phytochemicals were analyzed by the qualitative tests which are shown in the table-1. According to the tests conducted Acetone extract showed presence 8 phytochemicals (Alkaloids, Flavonoids, Saponins, Tannins, Steroid, Phenols, Carbohydrates, Reducing Sugars) Chloroform (Alkaloids, Flavonoids, Saponins, Phytosterols, Steroid, Phenols, Carbohydrates, Reducing Sugars), ethanol (Alkaloids, Flavonoids, Saponins, Tannins, Phytosterols, Steroid, Phenols, Reducing Sugars) and methanol(Alkaloids, Flavonoids, Saponins, Tannins, Steroid, Phenols, Carbohydrates, Reducing Sugars) extract showed presence of 9 phytochemicals in each of them.

Table-1Phytochemical Screening in Different extract of *Curcuma long*

Phytochemical	Acetone	Chloroform	Ethanol	Methanol
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	+	+	+
Tannins	+	-	+	+
Phytosterols	-	+	+	-
Steroid	+	+	+	+
Phenols	+	+	+	+
Carbohydrates	-	+	-	+
Reducing Sugars	+	+	+	+

Results of Polyphenolic Content

Table-2Polyphenolic Content of *Curcuma longa* at 760nm

Sample	**Absorbance**	**% Polyphenolic content**
Curcuma longa	0.326	0.611%
Standard	0.331	

Results of HPLC- Curcuminoid Content

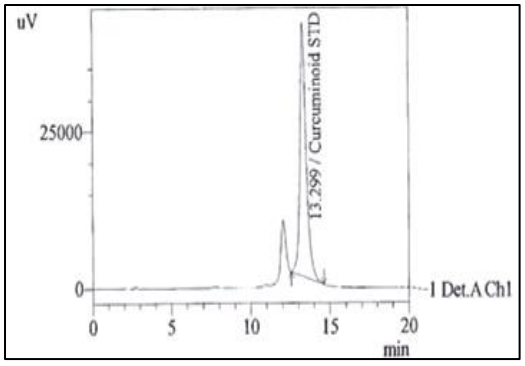

Figure-6 Curcuminoid Standard

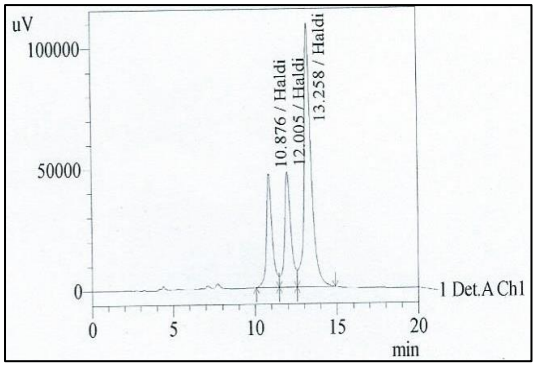

Figure-7*Curcuma longa* Sample

1st peak of Bis demethoxy curcumin,
2nd of Demethoxycurcumin,
3rd of Curcumin

Table No-3HPTLC Results of Haldi (*Curcuma longa*)

Curcuminoid Name	Amount Present
Bisdemethoxycurcumin	0.9622 % (9622 ppm)
Demethoxycurcumin	1.0804 % (10804 ppm)
Curcumin	2.7705 % (27705 ppm)
Total Curcuminoid	4.8166 % (48166 ppm)

The most active component of turmeric is curcumin which makes up to 2-5% of the spice, and is responsible for most of the therapeutic effects. Turmeric contains a wide variety of phytochemical including curcumin, demethoxycurcumin, bisdemethoxycurcumin, zingiberene, curcumenol, eugenol. The characteristic yellow color of turmeric is due to Curcuminoid first isolated by Vogel in 1842[18].According to above studies out of the total curcuminoid content Beside methoxy curcumin is .9622 %, Demethoxycurcumin is 1.0804 % and Curcumin is 2.7705%.

Anti-Bacterial Assay

Table-4Anti- Bacterial Activity of *Curcuma longa*

Solvents	***Staphylococcus aureus***	***Escherichia coli***
Acetone	13 mm	12 mm
Chloroform	15 mm	13 mm
Ethanol	20 mm	17 mm
Methanol	25 mm	13 mm
Ciprofloxacin	30 mm	28 mm

The antibacterial activity of Acetone, chloroform,ethanol,and methanol extract of Curcuma longa determined against S.aureus and E.coli by well agar diffusion method. The growth inhibitory activities off all the extracts against the tested bacteria are summarized in Table-4. The results of antibacterial activity assay clearly show that all the extracts have antibacterial activity against the S.aureus.

Bacterial growth inhibition results shown in Figure-8 clearly indicate that Methanol extract has given the best anti-microbial activity against *S. aureus* (Gram +ve) and Ethanolic extract has given the best anti-microbial activity against *E. coli* (Gram -ve). This antimicrobial activity of rhizome of Curcuma longa is due to the presence of phytochemical constituents, viz. alkaloids, terpenoids,

flavanoids, phenolics, tannins,Saponins that are known for their antimicrobial properties.

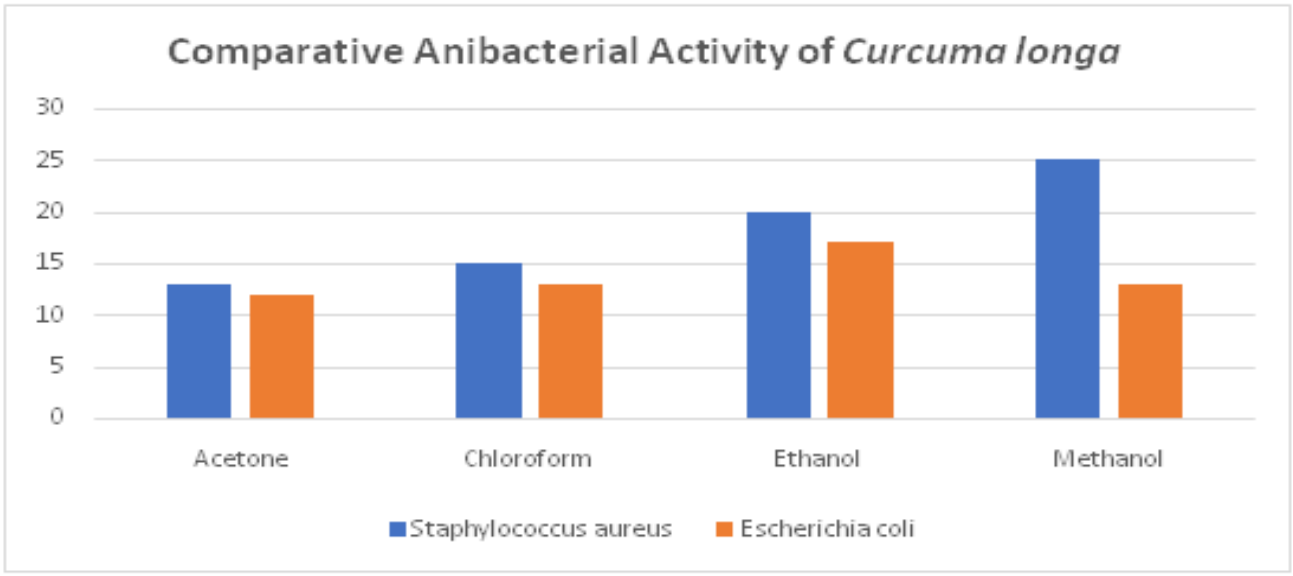

Figure-8 Different solvents in *Staphyllococcus aureus*

Figure-9 Different solvents in *Staphyllococcus aureus*

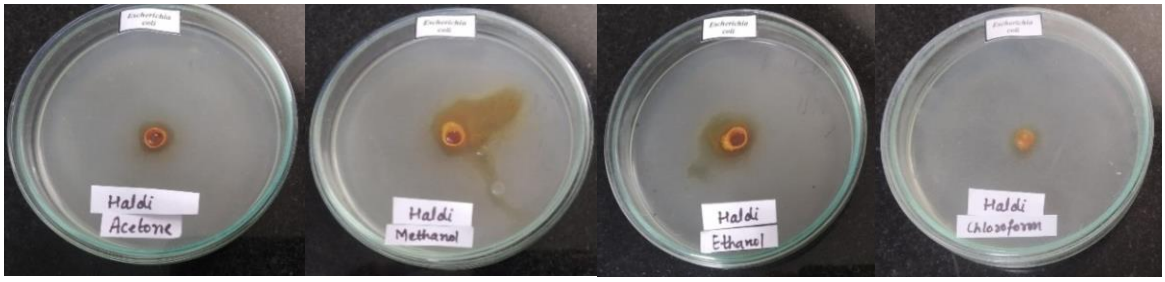

Figure-10 Different Solvents in *Escherichia col.*

Conclusion

In the light of above data ,it can be concluded that a simple chemical profiling and semi quantitative method for natural products using analytical method might be applied to diverse field related quality control of medicinal plants. It is also clear that turmeric has lots of potentials and has broad spectrum pharmacological actions and is beneficial for long term and daily usage. Turmeric is regarded as one of the best drug in many diseases like Diabetes, Skin diseases etc, which is in use since ages owing to its multiple pharmacological activities. Turmeric is enrichedwith manyusefulphytoconstituents which are responsible for its efficacy. Curcumin is one such phytoconstituents, a nutraceutical substance with numerous pharmacological activities. It can also be stated that many of these phytochemicals can be used safe substitute to many of the chemical drugs which are present in the market presently as these won't cause any side effect it taken for a longer time. These phytochemicals are also easy to obtain as they need no artificial synthesis.

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