Original Research Paper

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Biochemistry

# ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF ETHANOLIC EXTRACT OF TURMERIC

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**ABSTRACT**

In this study, phytochemical, antioxidant, antimicrobial, anticancer and analysis of curcuma longa were collected from local market of Chennai. The turmeric extract of was extracted with ethanol. The ethanolic extract of turmeric shows great antioxidant activity because of the presence of different phytochemical in curcuma longa steroids, anthroquiones, flavonoids.

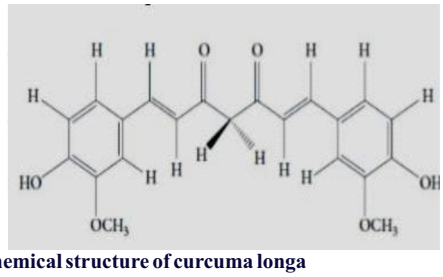
The extract of curcuma longa was used to treat disease which caused by bacteria and fungi.

This study thus summarized as the turmeric extract of are good for health and can be used as pharmacological drugs to treat various diseases like, allergies, Alzheimer disease, cardiovascular disease.

**KEYWORDS :**

## Introduction

Curcuma *longa* or turmeric is a tropical plant native to southern and south eastern tropical Asia. This perennial herb belonging to the ginger family. turmeric measures up to 1 m high with a short stem and tufted leaves. The parts used are the rhizomes. Perhaps the most active component in turmeric is curcumin, which may make up 2 to 5% of the total spice in turmeric. Curcumin is a diferuloyl methane present in extracts of the plant. Curcuminoids are responsible for the yellow colour of turmeric and curry powder. They are derived from turmeric by ethanol extraction. The pure orange-yellow, crystalline powder is insoluble in water.




**(fig.1) chemical structure of curcuma longa**

The structure of curcumin (C21H20O6) was first described in 1815 by Vogel and Pellatier and in 1910 was shown to be diferuloyl methane by (Lampe et al., (1910). Chemical synthesis in 1913 confirmed its identity. Curcumin is not water-soluble, but it is soluble in ethanol or in dimethyl sulfoxide.

Curcumin is found in turmeric, a yellow-coloured spice of the perennial herb Curcuma longa, which has been used widely for centuries not only in cooking but in traditional therapies for various diseases, especially as an anti-inflammatory agent.

Curcumin and its metabolite, tetra hydro curcumin have been extensively investigated as anti- inflammatory and anti-cancer molecule. Curcumin has been widely studied for its anti-inflammatory, anti-angiogenic, antioxidant, wound healing.

Turmeric has been used in traditional medicine as a household remedy to treat various diseases like biliary disorders, anorexia, cough, diabetic wounds, hepatic disorders, rheumatism and sinusitis with simple formulation. Curcumin is the active ingredient of turmeric and is believed to be responsible for its therapeutic activity (Rajeshkumar.,2015).

## CLASSIFICATION

Curcuma species such as:
Curcuma *mangga*,
Curcuma *zedoaria,*
Costus *speciosus,*
Curcuma *xanthorrhiza,*
Curcuma *aromatic,*
Curcuma *phaeocaulis,*
Zingiber *cassumunar*

## SCIENTIFIC CLASSFICATION TURMERIC PLANT

- Kingdom : plantae
- Superdivision : spermatophyta
- Division: : magnoliophyta
- Class: : liliopsida
- Subclass : zingiberales
- Family: : zingiberaceae
- Genus : *curcuma*
- Species: : *curcuma longa*

**( Fig 2) Turmeric plant Cancer cells often spread via three main ways**

1] Local spread: cancer cells grow directly into nearby body tissues.

2] Through the blood circulation: cancer cells break way from the primary tumour and then Slip through the walls of blood vessels into stream until they get stuck Somewhere. Most cancer cells spreading via blood circulation are killed by white cells in the immune system, but some of them stick to platelets to form clumps and give themselves protection, these cells will survive and form metastasis. Through the lymphatic system: cancer cells travel in lymph fluid until they get stuck in small channels inside the lymph node and grow into secondary cancer (metastasis) (Ponnanikajamidin, *et al*.,2015). In this present study obtain the crude extract of turmeric using ethanol solvent to perform total phenol activity using the crude extract of turmeric. The extract were subjected for anti-oxidant activity against DPPH free radical scavenging and FRAP assay. The extract were used for anti-bacterial activity against selected pathogens *Staphylococcus aureus, Bacillus subtilis, and Escherichia coli was performed*

## METHOD AND MATERIALS SOXHLET PROCEDURE

The turmeric was washed with distilled water to remove adherent particles, shade dried and powdered. 25g of sample was weighed and extracted with 300ml of ethanol by continuous hot percolation with the help of soxhlet apparatus for 10hrs of time. On completion the extract was filtered and concentrated using rotary evaporator under reduced pressure and controlled temperature of $50^0C - 60^0C$. The yield obtained weighed 2g**.** The extract was stored for future use.

## PHYTOCHEMICAL ANALYSIS (Bhavani et al., 2017)

### 1.TEST FOR TANNINS

1ml of sample was taken, to that few drops of 0.1 % ferric chloride was added and observed for brownish green or blue black coloration.

**2. TEST FOR SAPONINS**
1 ml of sample was taken, to that 2 ml of water was added. The suspension was shaken in a graduated cylinder for 15 minutes. A layer of foam indicates the presence of saponins.

**3. TEST FOR FLAVONOIDS**
1 ml of sample was taken, to that concentrated hydrochloric acid and magnesium chloride was added and observed for tomato red colour.

**4. TEST FOR ALKALOIDS**
1 ml of sample was taken, to that few drops of drangandoff reagent was added. A prominent yellow precipitate indicates the test as positive.

**5. TEST FOR PROTEIN**
1 ml of sample was taken, to that few drops of Million's reagent was added. A white precipitate indicates the presence of Protein.

**6. TEST FOR STEROIDS**
1 ml of sample was taken, to that two drops of 10% concentrated sulphuric acid was added and observed for brown colour.

**7. TEST FOF ANTHRAQUINONES**
1 ml of sample was taken, to that aqueous ammonia was added and observed for change in colour. Pink, red, or violet colour in aqueous layer indicates the presence of anthraquinoness.

**8. TEST FOR PHENOL**
1 ml of sample was taken, to that 3 ml of 10% lead acetate solution is added a bulky white precipitate indicates the presence of phenolic compounds.

**DPPH ASSAY (Molyneux, 2004)**
DPPH (1,1-diphenyl-2-picrylhydrazyl) is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecules do not dimerise, as would be the case with most other free radicals. The delocalization also gives rise to the deep violet colour, characterized by an absorption band in ethanol solution centered at about 520 nm. When a solution of DPPH is mixed with that of a substance that can donate ahydrogen atom, then this gives rise to the reduced form (Blois,1958) with the loss of this violet colour (although there would be expected to be a residual pale yellow co lour from the picryl group still present).

Representing the DPPH radical by Z and the donor molecule by AH, the primary reaction is Z + AH = ZH + A

where ZH is the reduced form and A is free radical produced in this first step. This latter radical will then undergo further reactions which control the overall stoichiometry, that is, the number of molecules of DPPH reduced (decolorised) by one molecule of the reductant.

**CHEMICALS**
1. 1,1 – diphenyl -2- picrylhydrazyl (DPPH)
2. Dimethylsilphoxide (DMSO)
3. BHT (standard)-1.6mg/ml in methanol
4. Samples desired concentration from 1 mg /ml –max of 5mg / ml (in /DMSO)

**PROCEDURE**
Aliquot 3.7 ml of absolute methanol in all test tubes and 3.8ml of absolute methanol was added to blank.

Add 100µl of BHT to tube marked as standard and 100µl of respective samples to all other tubes marked as tests. 200µl of DPPH reagent was added to all the test tubes including blank. Incubate all test tubes at room temperature in dark condition for 30 minutes. The absorbance of all samples was read at 517nm (pavunraj et al., 2019).

**Table 1: Procedure for DPPH Assay**

| S.NO | REAGENTS | BLANK | STANDARD | TEST |
|---|---|---|---|---|
| 1 | Methanol | 3.8ml | 3.7ml | 3.7ml |
| 2 | BHT | - | 100µl | - |
| 3 | Sample | - | - | 100µl |
| 4 | DPPH | 200µl | 200µl | 200µl |
| Incubation at dark for 30 minutes | | | | |
| O.D at 517 nm | | | | |

**CALCULATION**
% Antioxidant activity = **( Absorbance at blank) - (Absorbance at test)** x100
**(Absorbance at blank)**

**FERRIC ION REDUCTION POTENTIAL (FRAP) ( Benzie and Strain, 1996).** FRAP assay is a simple novel method for assessing "Antioxidant power", Ferric to Ferrous ion reduction at low pH causes a coloured ferrous - tripyridyltriazine complex to form. FRAP values are obtained by comparing the absorbance change at 593 nm test reaction mixtures with those containing ferrous ion in known concentrations.

**Preparation of FRAP Reagents**
- **Reagent A-Acetate Buffer (300mM, pH 3.6)**

16 ml of glacial acetic acid was added to 3.1g of sodium acetate trihydrate; the solution was then made up to 1L using distilled water. The pH of the solution was checked using pH meter.

- **Reagent B-TPTZ (2,4,6 tri [2 pyridyl] s-triazine) Solution**

0.031g of TPTZ was added to 10ml of 40mM HCl.

- **Reagent C-Ferric Chloride Solution**

0.054g of ferric chloride was dissolved in 10ml of distilled water.
Reagents B and C were freshly prepared every time when the assay was performed.

**Preparation of FRAP Reagent**
2.5ml of Reagent B and 2.5ml of Reagent C were added to 25ml of Reagent A to make 30ml of the FRAP reagent. This was placed in a 37ºC water bath for a minimum of 10 minutes. Standard-Ascorbic acid: 1.76 mg of Ascorbic acid was dissolved in 100 ml of distilled water.

**FRAP Assay Procedure**
1. 1ml of distilled water and 80µl of test sample was pipette into the standard 4ml plastic cuvette.
2. 600µl of incubated FRAP Reagent was added to the cuvette, which was briefly inverted to mix the solutions.
3. The reagent blank was also prepared as described above but 80µl of distilled water was added instead of test sample.
4. Change in absorbance at 593nm (as a result of the reduction of the Fe3+ -TPTZ complex at low pH) was recorded at exactly at 4 minutes using spectrophotometer.
5. Each test sample dilution was tested in triplicate to allow a mean absorbance to be calculated.

**Standard Calibration Curve**
A standard solution of 1mM ferrous sulphate was prepared by dissolving 0.139g of FeSO4.7H2O in 580ml of distilled water. Serial dilutions were made and the absorbance at 593nm measured by performing the assay as described above with ferrous sulphate in place of test samples.

**Calculation**
FRAP value of sample (µM) = (Change in absorbance of sample from 0 to 4 minute/change in absorbance of standard from 0 to 4 minutes) x FRAP value of standard (1000µM)

**ANTIBACTERIAL ACTIVITY ASSA**
Number of samples: 01
Number of Microorganisms: 03
*(Staphylococcus* aureus, *Escherichia coli and Bacillus subtilis)*
Standard: Ampicillin (20µl/disc)

**PREPARATION OF INOCULUM**
Stock cultures were maintained at 4°C on Nutrient Agar Slant. Active cultures for experiments were prepared by transferring a loop full of culture from the stock cultures into the test tubes containing nutrient broth, that were incubated at 24hrs at 37ºC. The Assay was performed by agar disc diffusion method.

**AGAR DISC DIFFUSION METHOD**
Antibacterial of extracts was determined by disc diffusion method on Muller Hinton agar (MHA) medium. Muller Hinton Agar (MHA) medium is poured in to the Petri plate. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the bacterial suspension. The disc were placed in MHA

plates and add 20 µl of sample (Concentration: 1000µg, 750µg and 500 µg) were placed in the disc. The plates were incubated at 37ºC for 24 hrs. Then the antimicrobial activity was determined by measuring the diameter of zone of inhibition.

**ANTIFUNGAL ACTIVITY ASSAY**

Number of samples: 01
Number of Microorganisms: 02:*(Aspergillus niger and Rhizophus)*
Standard: Amphotericin-B (20µl/disc)

**PREPARATION OF INOCULUM**

Stock cultures were maintained at 4°C on Sabouraud Dextrose Agar Slant. Active cultures for experiments were prepared by transferring the stock cultures into the test tubes containing Sabouraud Dextrose broth that were incubated at 48 hrs at room temperature. The assay was performed by agar disc diffusion method.

**AGAR DISC DIFFUSION METHOD**

Antifungal activity of the extracts was determined by disc diffusion method on Sabouraud Dextrose agar (SDA) medium. Sabouraud Dextrose agar (SDA) medium is poured in to the petriplate. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the fungal suspension. Amphotericin-B is taken as positive control. Samples and positive control of 20 µl each were added in sterile discs and placed in SDA plates. The plates were incubated at 28ºC for 24 hrs. Then antifungal activity was determined by measuring the diameter of zone of inhibition.

**RESULT**

Antimicrobial, antioxidant and anticancer analysis of turmeric on breast cancer cell line.was carried out. For the study, turmeric powder was extracted with ethanol using soxhlet apparatus.

After condensation of the crude extract was obtained, and it was used for the analysis.

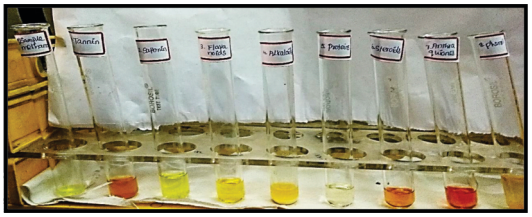
The qualitative phytochemical analysis of the turmeric extract, shows the presence of compound such as: steroides, anthraquiones and flavonoids.

**Table:2 show the result of phytochemical analysis**

| TEST | TURMERIC |
|---|---|
| | *METHANOL* |
| TANNINS | - |
| SAPONINS | - |
| FLAVONOIDS | + |
| ALKALOIDS | - |
| PROTEINS | - |
| STEROIDS | + |
| ANTHROQUINONES | + |
| PHENOL | - |

(+) = Positive
( - ) = Negative

**Figure:5 show the result of phytochemical analysis**



**ANTIMICROBIAL ASSAY**

The crude turmeric extract was subjected to antibacterial assay.The organisms tested were *Staphylococcus aureus ,Bacillus subtilis, and Escherichia coli.*

*staphylococcus aureus* Show the inhibition zone level of 15 mm ,16 mm ,15 mm, at 1000 µg/ml 750 µg/ml500 µg/ml respectively .

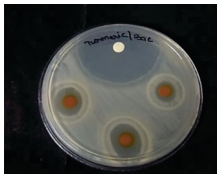
*Bacillus subtilis* the Show the inhibition zone level of 13 mm, 13mm, 10mm.at 1000µg/ml,750 µg/ml,500 µg/ml respectively.

*Escherichia coli* the Show the inhibition zone level of 12 mm, 12mm, 10mm.at 1000 µg/ml,750 µg/ml ,500 µg/ml respectively.

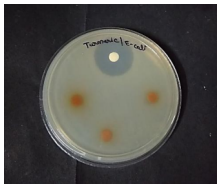
**Table:3 show the antibacterial analysis of turmeric extract**

| ORGANISMS | ZONE OF INHIBITION (mm) CONCENTRATION (µg/ml) | | | ANTIBIOTIC (1MG/ML) |
|---|---|---|---|---|
| | 1000 | 750 | 500 | |
| *Staphylococcus* aureus | 15 | 16 | 15 | 20 |
| *Bacillus subtilis* | 13 | 13 | 10 | 23 |
| Escherichia coli | 12 | 12 | 10 | 20 |

**Figure:6 show the inhibitory zone of tested organism *Bacillus subtilis***



***Staphylococcus aureus***



***Escherichiacoli***

**ANTI-FUNGI ASSAY**

The crude turmeric extract was subjected to antibacterial assay. The organisms tested were. *(Aspergillus niger and Rhizophus).*

*Aspergillus niger* Show the inhibition zone level of 15 mm,16 mm,15 mm,at 1000 µg/ml 750 µg/ml500 µg/ml respectively .

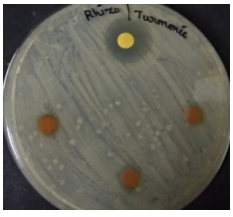
*Rhizophus* the Show the inhibition zone level of 13mm, 13mm, 10mm. At 1000µg/ml,750 µg/ml,500 µg/ml respectively

**Table :4 shows that antifungi analysis of turmeric extract**

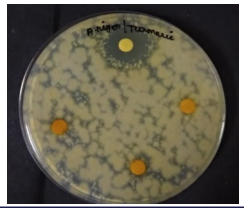
| Organisms | Zone of inhibition (mm) Concentration(µg/ml) | | | Antibiotic (1mg/ml) |
|---|---|---|---|---|
| | 1000 | 750 | 500 | |
| *Aspergillus niger* | 8 | 8 | 6 | 18 |
| *Rhizophus* | 6 | 6 | 6 | 16 |

**Figure:7 shows that anti fungi analysis of turmeric extract**

***Rhizophus.***



***Aspergillus niger***



***DPPH ANALYSIS:***

DPPH analysis was carried out for the turmeric extract. The result shows that 63.6% DPPH activity was observed. The antioxidant analysis of turmeric in FRAP assay shows the value of 630µm .

**Table:5 show the DPPH analysis of turmeric extract**

| S.NO | SAMPLE | Concentration (µg/ml) | O.D | DPPH activity (%) |
|---|---|---|---|---|
| 1 | Sample | 1000 | 0.009 | 63.6% |

**Blank O.D: 0.027**

**FRAP ASSAY:**

the antioxidant activity is also determined on the basic ability of antioxidant in extract to reduce ferric (III) iron to ferrous (II) iron in FRAP reagent. The present study revealed the FRAP value of 630µm

**Table:6 Shows that FRAP assay of turmeric**

| S.No | O.D Value for Sample at | 0 to 4 min Name of the Sample | FRAP(µm) |
|---|---|---|---|
| 1 | Turmeric | 1.914 to 2.040 | 630 (µm) |

**O.D Value for Ascorbic acid at 0 to 4 min = 0.2**

The phytochemical analysis of turmeric extract shows presence of anthroquiones, flavonoids and steroid in the of turmeric extract.

Arutselvi et al., (2012) reported the presence of phytochemical such as flavonoids, phenols, cardiac glycosides in the extracted of curcuma longa the presence study is accordance with the result.

Prashanth, et al., (2013) reported the presence of phytochemicals such as alkaloid, flavonoids, steroid, aminoacid, carbohydrates in the extract of Curcuma longa. The present study is in accordance with the result.

Pawar, et al., (2014) also determined the phytochemical analysis of Curcuma longa.

The antibacterial analysis shows the inhibiting effect on microorganism, The order of Staphylococcus aureus, Escherichia coli, Bacillus subtilis.

The antifungi analysis shows the inhibiting effect on microorganism, The order of Aspergillus niger and Rhizophus

Lee et al., (2013) reported the antibacterial analysis. The results were found to be similar. The DPPH analysis of the present study are recorded 63.6% of scavenging activity.

Maizura, et al., (2011) carried out the DPPH analysis with ethanolic extract of turmeric extract and reported 64.6% scavenging activity. Both results were found to be similar.

In the studies of Wong et al., (2006) the antioxidant activity is also determined on the basic ability of antioxidant in extract to reduce ferric (III) iron to ferrous (II) iron in FRAP reagent. The present study revealed the FRAP value of 630µm. FRAP assay was used for its simplicity and reproducibility. The FRAP value of turmeric is (23.3± 0.9µmol Fe(II)/g.

Curcumin being the active component of turmeric contributes to its anti-cancer activity. Hence, the turmeric extract with its antimicrobial, antioxidant and anti cancer activity can be subjected to further research and aid in pharmological industries and drug development.

## CONCLUSION

The phytochemical analysis of ethanolic of extract turmeric revealed that presence of steroids, anthroquiones, flavonoids. Ethanolic extract of curcuma longa have anti-microbial activity which revealed by using three bacteria, two fungi. The presence of various phytochemicals showed that ethanolic extract of *curcuma longa* have anti-oxidant activity. which was studied by using DPPH and FRAP assay. The free radical scavenging activity of *curcuma longa* showed that the significance anticancer activity. Cancer cell especially in breast cancer cell line using MMT assay.

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