ANTIBACTERIAL ACTIVITY OF CURCUMIN EXTRACTED FROM TURMERIC

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ABSTRACT
Turmeric (Curcuma longa L.) is rhizomatous perennial plant belongs to Zingiberaceae family having phytochemicals such as Curcumin I (94%), II(0.6%), III(0.3%), fatty acids(5.1%), protein(6.3%), carbohydrate(69.4%), minerals (3.5%), moisture(13.1%), essential oil having suggestive therapeutic effect. The present work is to examine antimicrobial activity of Curcumin extracted from turmeric rind (kachchi haldi) against gram negative bacteria E. Coli and gram positive bacteria Bacillus subtilis respectively by using agar dilution test and it was observe that it impart an adequate antimicrobial activity against the tested microorganism.

KEYWORDS: Curcumin, Turmeric (Curcuma longa L.), extraction, antimicrobial activity.

INTRODUCTION
Turmeric (Curcuma longa L.) is important medicinal plant belongs to Zingiberaceae family and genus Curcuma.[1] Turmeric is commonly used for condiment & food coloring in Asian cuisine & has wide range of application from tropical to in-vivo applications.[2] Turmeric is rhizomatous perennial plant grows in both tropical & subtropical regions of Southern Asia with warm humid climates & completely flourish temperature above 29.8°Celsius.[3] Turmeric have tuberous roots (fragment aroma, bitter, taste resembling of ginger) of 5-10cm long & 2.54cm wide and tapered at each end & its exterior can be yellow tan or olive green in color with interior of root is hard, firm & deeply rust colored with parallel rings & give saliva a yellow color with leaving warm sensation in mouth.[4] This root contains CaCl2, starch, gum, wood fiber & yellowish coloring material called Curcumin. The leaves extended upward from erect, thick stems arising from roots, are divided into lance shaped & narrow at each end (61cm) & flower arise from leaves with pale yellow color grouping of 3 to 5.[5]

Curcumin is yellow color compound having molecular formula C15H24O6 & molecular weight 368.385g/mol is a vital phytochemical of turmeric which is responsible for its yellow color & many biological activities.[1] Curcumin structure with several functional groups was first identified in 1910,[6] having aromatic ring system (phenols) are connected by 2 α-β unsaturated carbonyl groups which is good Michael acceptor & undergoes nucleophilic addition.[7] Curcumin is easily soluble in acetone, ketone, ethanol, and chloroform but not soluble in water indicates lipophilic in nature.[8] Curcumin is used for detection of Boron by reacting with boric acid, it forms red color compound rasocynine. It is poorly absorbed from the gut, when administered orally 75% of Curcumin was excreted through feces & negligible amount through urine. Curcumin is most bioactive part of turmeric & powerful antioxidant by protecting cells damage by free radicals.[9] Curcumin has numerous medicinal/pharmaceutical/therapeutic properties such as anti-inflammatory, anti-depressant, anti-bacterial, anti-cancer, anti-cholesterol, anti-Alzheimer wound healer, anti-diabetic, anti-viral actions.[10][11]

The present studies include
- Extraction of Curcumin from turmeric using soxhlet apparatus method.
- Characterization & Analysis of Curcumin by using UV spectrophotometer, Thin Layer Chromatography (TLC) & IR spectroscopy.
- Examine Antimicrobial Activity against gram positive bacteria & gram negative bacteria strains respectively.

MATERIALS AND METHODS
Extraction of Curcumin from turmeric using Soxhlet Apparatus method:
MATERIALS REQUIRED: Turmeric, acetone & Soxhlet apparatus.

SOXHLET EXTRACTION: Turmeric rind (kachchi haldi) was taken dried in oven for 2 days at 50-60°Celsius then powdered it. 20g of powder was weighed & placed in soxhlet and 200ml of acetone was added to it &
refluxed until the yellow color of extraction faded away. Final extract was collected & stored in freezer & analyzed.

**EVALUATION & CHARACTERIZATION OF CURCUMIN**

![Curcumin structure](image1)

1. **PHYSICAL & CHEMICAL PROPERTIES OF CURCUMIN**
   - Molecular formula: C_{21}H_{20}O_{6}
   - Molecular weight: 368.385 g/mol
   - Melting point: 183°C, 361°F, 456K
   - Powder form: Bright yellow orange color powder
   - Solubility: acetone, ketone, ethanol, chloroform
   - Sensitivity: Light sensitive

2. **IDENTIFICATION OF CURCUMIN**
   a) Thin Layer Chromatography (TLC) Analysis
   - Sample detail: Curcumin
   - Adsorbent: Per coated silica gel
   - Solvent system: n-hexane: ethyl acetate (7:3)
   - Sample preparation: Isolated Curcumin (liq) was applied on TLC plate with the aid of capillary tube.
   - Detection: Color & R_f value were recorded using spraying the plates with alcoholic KOH solution (R_f value= 0.62)

![TLC plates](image2)

b) Ultra Violet Spectrophotometer Analysis
One drop of isolated Curcumin was mixed with the 95% acetone λ_{max} was determined.

<table>
<thead>
<tr>
<th>λ_{max}(nm)</th>
<th>Absorbance</th>
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<tbody>
<tr>
<td>423</td>
<td>2.082</td>
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![UV analysis](image3)
Fig.2 UV spectra of extracted curcumin

c) Identification by IR Spectroscopy

<table>
<thead>
<tr>
<th>Frequency (cm⁻¹)</th>
<th>Functional group</th>
</tr>
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<tbody>
<tr>
<td>3437.89</td>
<td>O-H stretching (alcoholic)</td>
</tr>
<tr>
<td>3007.63</td>
<td>C-H aromatic stretching vibration</td>
</tr>
<tr>
<td>2917</td>
<td>-CH₃ asymmetrical stretching</td>
</tr>
<tr>
<td>2845.49</td>
<td>-CH₂ asymmetrical stretching</td>
</tr>
<tr>
<td>1720</td>
<td>-C=O stretching</td>
</tr>
<tr>
<td>1554.43</td>
<td>C=O aromatic stretching</td>
</tr>
<tr>
<td>1424.17</td>
<td>CH₂ bending</td>
</tr>
<tr>
<td>1361.16</td>
<td>CH₃ bending</td>
</tr>
<tr>
<td>1122.1092</td>
<td>C-O stretching</td>
</tr>
</tbody>
</table>

ANTIBACTERIAL ACTIVITY
Anti-bacterial activity was studied against gram negative bacteria E. coli & gram positive bacteria Bacillus subtilis.

PROCEDURE
The in vitro antibacterial activity of isolated Curcumin was carried out by miller agar dilution method.

- **PREPARATION OF LB AGAR & LB BROTH**
  4gm of LB Agar was measured and added to the 100ml distilled water in a conical flask & 2.5gm of LB Broth

- **ANTIBACTERIAL ACTIVITY SHOWN BY CURCUMIN AGAINST E. coli & Bacillus subtilis AS SHOWN FOLLOWS**

were taken and added to the 100ml of distilled water then both are autoclaved, LB Agar & LB Broth was autoclaved for 3hrs then LB Agar was used for plating of autoclaved petri plates where microorganism was added to the LB Broth in a shaking chamber at 37º Celsius for 24hrs for microorganism growth.

- **DETERMINATION OF ANTIBACTERIAL ACTIVITY**
  Agar coated petri plates were coated by 100µl of LB Broth containing gram positive Bacillus subtilis & gram negative E. coli respectively for determination of culture growth. Then plates having coating of LB broth containing microorganism was loaded by 10µl of extracted Curcumin to determine its antibacterial activity.
DISCUSSION AND CONCLUSION
Curcumin was extracted out from turmeric rind (kachi haldi) using soxhelt extraction method which is observed a best method for maximum recovery of Curcumin. Extracted Curcumin is further identified and characterized by UV spectrophotometer, IR spectroscopy & TLC. Curcumin have shown good antibacterial activity against gram positive bacteria (*Bacillus subtilis*) & gram negative bacteria (*E. coli*) respectively.

REFERENCES