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# Phytochemical characterization and Antimicrobial activity of oil and solvent extracts of *Curcuma longa*

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## ABSTRACT

Volatile oil from the rhizome of *Curcuma longa* was isolated, characterised by Gas Chromatography-Mass Spectroscopy. In *Curcuma longa*, 240% of yield was obtained from methanol extract, 180% of acetone extract, 140% yield of hexane rhizome extract and 1.4% of essential oil. GC–MS Analysis indicated that the essential oil of *Curcuma longa* 30 peaks. Major constituents were identified as α-turmerone (38.24%), Camphor (32.3%) and β–turmerone (22.25). The antimicrobial activity of oil was tested against human and plant pathogenic bacteria and fungi. The oil showed significant inhibitory activity against the pathogenic bacteria and fungi. No activity was observed against the fungi *Aspergillus* niger.

**Keywords:** *Curcuma longa*, Phytochemical, Antimicrobial activity, GC/MS Analysis.

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## INTRODUCTION

Plants and plant based medicaments are the basis of many of modern pharmaceuticals we use today for various ailments. The phytochemical research based on ethno-pharmacological information is generally considered an effective approach on the discovery of new anti-infective agents from higher plants [1].

Curcuma is a rhizomatous herb belonging to Zingiberaceae family. The plants belonging to this family are found to be a rich source of substances of phytochemical interest. Number of plants from this family is used in traditional system of medicine [2]. Turmeric powder, curcumin and its derivatives and many other extracts from the rhizomes were found to be bioactive [3]. The main activities of essential oils and plant extracts have been found to be antimicrobial, anti-inflammatory, anticancer and antiviral [4].

*Curcuma longa* is a perennial plant having a short stem with large oblong rhizomes, which are often branched and brownish yellow in colour [3]. Various experiments proved the presence of bioactive compounds from curcuma and show that it has antimicrobial, anti-inflammatory, anticancer and antiviral activities [4].

The present study involves the use of various techniques like Gas chromatography, Mass spectrometry analysis for phytochemical characterization of the herbal drug preparations. The antimicrobial activity can be tested using agar diffusion technique. By using this work, the antibacterial drugs can be developed or can be used for the improvement of the existing medicines.

## MATERIALS AND METHODS

### PLANT MATERIAL:

Curcuma longa rhizome was collected from Bonacaud forest area on Western Ghats situated at an altitude of 850m (Plate-1).

### OIL EXTRACT OF *CURCUMA LONGA* RHIZOME:

150g of *Curcuma longa* was cut in to small pieces and were distilled using Clevenger type of distillation apparatus with sterile water. Distillation process was done for 24 hours and the oil was collected and dried over anhydrous sodium sulphate and was refrigerated at $4^{o}$C for further use.

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**Plate-1: *Curcuma longa* plant with rhizome**

**METHANOL, ACETONE AND HEXANE EXTRACT OF *CURCUMA LONGA* RHIZOME:**

5 gram of Curcuma longa rhizome were ground with 10 ml of methanol, acetone and 15ml hexane by using mortar and pestle. Then the homogenized mixture was centrifuged at 7000rpm for 15minutes. After centrifugation the supernatant were collected filtered through a layer of Whatman No.1 filter paper. The extracts were sterilized by UV and then stored at 4oC for further use.

**GC-MS ANALYSIS:**

Mass Spectrometry Analysis was Performed on a Shimadzu GC 17A QP 5000 MS coupled with a mass detector, fitted non-polar DB-5 (Di Phenyl Di Methyl siloxane) and capillary column of length 25mm x 0.25 mm [5]. GC MS operation was carried out with the following conditions: initial temperature 60$^{o}$C, programmed from 60$^{o}$C – 300$^{o}$C with the injection temperature at 260$^{o}$C and detector temperature at 300$^{o}$C. The injection volume was 0.1µl with helium as carrier gas at the flow rate of 0.6ml per minute. Relative Retention times (RRts) of constituents were determined using C5-C30 straight chain alkanes as standards.

**MICROBIAL STRAINS:**

The antimicrobial activity was carried out by disc-diffusion assay [6, 7]. The Gram positive, Gram negative bacterial and strains from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Sector 39-A, Chandigarh, U.T., 160-036, India. The bacterial strains used are *Bacillus megaterium* (MTCC 428), *Proteus vulgaris* (MTCC 1771), *Bacillus amyloliquefaciens* (MTCC 2248), *Streptococcus thermophilus* (MTCC 1938), *Xanthomonas compestris* (MTCC 2289), *Shigelli sonnei* (MTCC 2957), *Enterobacter aerogens* (MTCC 2990), *E.coli* (MTCC1), *Mycobacterium* (MTCC 290), *Salmonella typhi* (MTCC 734), *Klebsiella pneumoniae* (MTCC 3040), *Staphylococcus aureus* (MTCC 3103), *Pseudomonas aeruginosa* (MTCC 2642) and the following fungal strains has also been used for this study, *Aspergillus niger*

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(MTCC 281), *Aspergillus flavus* (MTCC 2456), *Candida albicans* (MTCC 3018), *Penicillium chrysogenum* (MTCC 947), *Fusarium oxysporum* (MTCC 2480), *Kluveromyces maxianus* (MTCC 1389).

**ANALYSIS OF ANTIMICROBIAL ACTIVITY:**

The oil, methanol, acetone and hexane extracts were subjected to the antimicrobial assay followed by Kirby Bauer method [8-11]. The filter paper discs of 5mm size were prepared and the extracts of *Curcuma longa* rhizome was applied over the filter paper discs. The extract was evaporated after each addition and allow to dry for 30 minutes. Ten bacterial culture (Microbial Type Culture Collection- MTCC Collection) were maintained as pure cultures in Nutrient Agar slants with periodic sub-culturing was done every 4 – 5 days. Three fungal strains (MTCC Collection) were maintained as pure culture in Rose Bengal Agar slants and Potato Dextrose Agar slants with periodic sub-culturing was done every 7 -8 days. The plates were incubated at room temperature for three days. After three days inhibition zones including the diameter of the disc were measured using digital vernier caliper.

## RESULTS AND DISCUSSION

**PHYTOCHEMICAL CONSTITUENTS IN *CURCUMA LONGA* RHIZOME EXTRACT:**

In the present study, GC–MS Analysis indicated that the essential oil of *Curcuma longa* contained about 30 peaks. Major constituents were identified as α-turmerone (38.24%), Camphor (32.3%) and β–turmerone (22.25%). The compounds present in peaks are given in the figure -1 and table – 1. The same compound α-turmerone, β–turmerone was identified as the major compounds in the *Curcuma longa* rhizome extract [12].

**Figure 1: Chromatogram for oil extract of *Curcuma longa* rhizome**

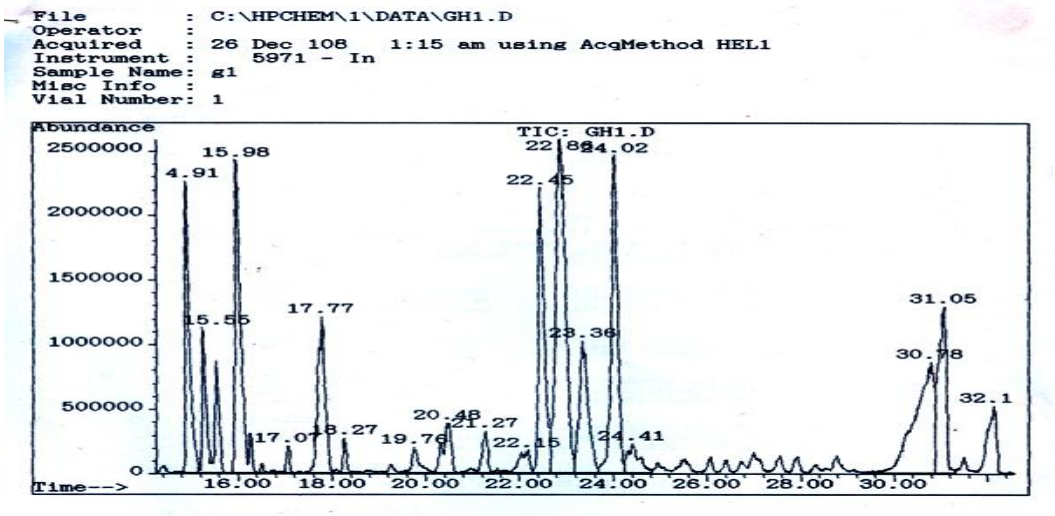

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**Table-1: Essential oil composition of *Curcuma longa* rhizome extract with its relative retention time, percentage**

| Sl. No | RRt | Percentage | Compound |
|---|---|---|---|
| 1 | 14.78 | 2.88% | α-pinene |
| 2 | 14.78 | 2.36% | β-pinene |
| 3 | 17.12 | 38.24% | α-turmerone |
| 4 | 18.48 | 22.25% | β-turmerone |
| 5 | 18.76 | 0.92% | Hexadiene |
| 6 | 19.83 | 6.7% | α-Phellandrene |
| 7 | 19.89 | 16.89% | Cyclohexene |
| 8 | 20.43 | 0.92% | Octadiene |
| 9 | 20.83 | 6.3% | Methyl hexyne |
| 10 | 24.42 | 32.3% | Camphor |
| 11 | 31.05 | 5.5% | 1,8-Cineole |

**ANTIMICROBIAL ACTIVITY:**

In vitro studies in this work, the antimicrobial activity of methanol, acetone, hexane, oil extract from *Curcuma longa* rhizome were tested against thirteen bacteria and six fungi. *Curcuma longa* show highest activity against *Salmonella typhi* and low activity against *Xanthomonas campestris*. Unlike antibacterial activity, Curcuma species exhibit moderate activity against fungi. Most of the plants extracts and oil have no activity against tested fungi. The extracts show good inhibitory effect on *Candida albicans* and no activity against *Aspergillus niger*. Among these 4 extracts, oil extracts shows higher activity than other solvent extract. In this experiment the oil extracted compounds may act as the antimicrobial agent when compared with other extracts, these showed weak activity against these particular microbial organisms. Antimicrobial activity of *Curcuma longa* rhizome extract was showed in table-2.

Various evidences reveal that *Curcuma longa* can inhibit the growth of a number of bacteria namely *E. coli, Pseudomonas aeruginosa, Staphylococcus aureus, Shigella* etc [13,14]. In case of oil, lot of experiments show that essential oil of *Curcuma longa* and *Curcuma aromatica* can inhibit the growth of *Bacillus cereus, E.coli, Staphylococcus aureus, Salmonella* etc [15]. The present study states that the essential oil collected from all the species exhibit high activity against all these organisms. *Curcuma longa* has low activity against E.coli, *Pseudomonas aeruginosa* and some *Mycobacterium* species [16].

In the Present study, *Curcuma longa* has significant antifungal activity against *Aspergillus flavus, Fusarium oxysporum and Penicillium chrysogenum*. A group of scientists proved that *Curcuma longa* oil has antifungal activity against *Aspergillus flavus, Penicillium digitalum, Fusarium moniliforme* [17]. Various scientists proved that *Curcuma longa*-ether, ethanol and chloroform extracts possess antifungal activity against a number of fungi [18,19].

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The present study predicted that methanol, acetone and hexane extracts of *Curcuma longa*, showed significant antifungal activity.

**Table-2: Antimicrobial Activity of *Curcuma longa* rhizome oil and solvent Extracts against bacterial and fungal pathogens:**

| Sl. No | Bacteria | Zone of inhibition (mm) | | | |
|---|---|---|---|---|---|
| | | Oil | Methanol | Acetone | Hexane |
| 1 | *Bacillus megaterium* | 7 | 11 | 6 | 0 |
| 2 | *Proteus vulgaris* | 10 | 10 | 6 | 0 |
| 3 | *Bacillus amyloliquefaciens* | 11 | 9 | 8 | 7 |
| 4 | *Streptococcus thermophilus* | 7 | 9 | 6 | 10 |
| 5 | *Xanthomonas campestris* | 10 | 8 | 0 | 0 |
| 6 | *Shigella sonnei* | 8 | 8 | 6 | 7 |
| 7 | *Enterobacter aerogens* | 9 | 8 | 13 | 0 |
| 8 | *Ecoli* | 20 | 8 | 0 | 0 |
| 9 | *Mycobacterium* | 8 | 9 | 13 | 6 |
| 10 | *Salmonella typhi* | 9 | 11 | 0 | 10 |
| 11 | *Klebsiella pneumoniae* | 13 | 8 | 13 | 0 |
| 12 | *Staphylococcus aureus* | 10 | 8 | 0 | 7 |
| 13 | *Pseudomonas aeruginosa* | 8 | 10 | 9 | 8 |
| | **Fungi** | | | | |
| 14 | *Aspergillus niger* | 0 | 0 | 0 | 0 |
| 15 | *Aspergillus flavus* | 9 | 9 | 11 | 9 |
| 16 | *Candida albicans* | 9 | 8 | 8 | 10 |
| 17 | *Penicillium chrysogenum* | 8 | 0 | 0 | 0 |
| 18 | *Fusarium oxysporum* | 0 | 0 | 9 | 0 |
| 19 | *Kluyveromyces maxianus* | 13 | 0 | 0 | 9 |

## CONCLUSION

From this study it can be concluded that the extracts of *Curcuma longa* possess antimicrobial activity. The use of medicinal plants to cure diseases has been extensively applied by people. *Curcuma longa* is a spice, which is a natural ingredient of our daily food. Thus it was confirmed that *Curcuma longa*, a natural ingredient of our daily food can provide protection to a certain extent against our natural enemies like bacterial pathogens. Also it was showed that *Curcuma longa* in the Western Ghats truely have medicinal values. Data from the literature as well as our results reveal the great potential of plants for the therapeutic treatment and have not been completely investigated. Additional studies would be needed further to evaluate the potential of this oil as antimicrobial agents. So as a result it could be estimated that the plant chosen for study has a wide range of activity against various pathogens.

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