Phytochemical characterization and antimicrobial activity of *Curcuma xanthorrhiza* Roxb.

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**ARTICLE INFO**

**ABSTRACT**

Objective: To study the antimicrobial activity and phytochemical characterization of essential oil isolated from the rhizome of *Curcuma xanthorrhiza* against pathogenic bacteria and fungi.

Methods: Fresh rhizomes of *Curcuma xanthorrhiza* were subjected to hydro distillation process to obtain essential oil and characterized by Gas Chromatography– Mass Spectroscopy (GC–MS). The essential oil was evaluated for antibacterial and antifungal activity against thirteen pathogenic bacteria and six fungi by the disc diffusion method.

Results: GC–MS analysis of the essential oil extracted from the rhizome of *Curcuma xanthorrhiza* contained the derivatives of xanthorihizol, camphene and curcumene, monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene, hydrocarbons and other minor compounds. The antimicrobial activity of the oil showed significant inhibitory activity against the human pathogenic bacteria, no activity was observed against the fungi *Aspergillus niger* and *Fusarium oxysporum*.

Conclusions: The findings of the present study indicate that the rhizome extract of *Curcuma xanthorrhiza* possess secondary metabolites and potential to develop antimicrobial drugs.

1. Introduction

The frequency of life–threatening infections caused by pathogenic microorganisms has increased worldwide, becoming an important cause of morbidity and mortality in immuno compromised patients in developing countries [1]. Although a large number of antimicrobial agents have been discovered, pathogenic microorganisms are constantly developing resistance to these agents [2]. Antibiotics are sometimes associated with side effects whereas there are some advantages of using antimicrobial compounds of medicinal plants. The later has fewer side effects, better patient tolerance, relatively less expensive, acceptance due to long history of use and being renewable in nature [3]. Antibacterial constituents of medicinal plants and their use for the treatment of microbial infections as possible alternatives to synthetic drugs to which many infectious microorganisms have become resistant seem to be very much promising [4]. Over the past 20 years, there has been a lot of interest in the investigation of natural materials as sources of new antibacterial agents. Different extracts from medicinal plants were tested and some natural products were approved as new antibacterial drugs [5]. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body [6–10]. The most important of these biologically active constituents of plants are alkaloids, flavonoids, tannins and phenolic compounds [11–15].

In the last few years, a number of studies have been conducted in different countries to prove the antimicrobial efficacy of the bioactive compounds [16–21]. However, there is still an urgent need to identify novel substances active against pathogens with higher resistance. In view of this fact the present study was aimed to evaluate the phytochemical constituents and antibacterial activity of the rhizome extracts of *Curcuma xanthorrhiza*, commonly known as false turmeric.

2. Materials and methods

Rhizomes of *Curcuma xanthorrhiza* was collected from the tropical forests of Bonaccord in the Agastyamala Hills
of Kerala, India. The fresh rhizomes were shade dried and powdered in a mechanical blender. The powdered rhizome was subjected to hydro-distillation using a modified Clevenger–type glass apparatus for 6 hours for isolation of oils separately. The oil samples were stored at 0°C in air-tight containers after drying them anhydrous sodium sulfate and filtered before going to GC–MS analysis.

GC–MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC–20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC–MS) instrument employing the following conditions: column Elite–1 fused silica capillary column (30 × 0.25 mm ID ×1EM df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.5 EI was employed (split ratio of 10:1) injector temperature 250°C; ion–source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C/min, then 5°C/min to 280°C/min, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 550 Da.

Interpretation on mass spectrum of GC–MS was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

Antimicrobial study was carried out by disc diffusion method (Bauer et al., 1966) against the pathogens viz. Bacillus megaterium (MTCC 428), Proteus vulgaris (MTCC 1771), Bacillus amyloliquefaciens (MTCC 2248), Streptococcus thermophilus (MTCC 1938), Xanthomonas campestris (MTCC 2289), Shigella sonnei (MTCC 2957), Enterobacter aerogens (MTCC 290), E.coli (MTCC1), Mycobacterium sp. (MTCC 290), Salmonella typhi (MTCC 734), Klebsiella pneumoniae (MTCC 3040), Staphylococcus aureus (MTCC 3103), Pseudomonas aeruginosa (MTCC 2642), The fungal strains are Aspergillus niger (MTCC 281), Aspergillus flavus (MTCC 2456), Candida albicans (MTCC 3018), Penicillium chrysogenum (MTCC 947), Fusarium oxysporum (MTCC 2480) and Kluveromyces maxianus (MTCC 1389). The microbial strains were procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Sector 39–A, Chandigarh, U.T., 160–056, India.

3. Results

The GC–MS analysis of the essential oil extracted from the rhizome of Curcuma xanthorrhiza showed Xanthorrhizol (64.38%) is the major compound followed by Camphene (8.27%), Curcumin (5.85%), α-Pinene (1.93%), α-thujene (0.16%), β-Pinene (0.14%), Myrcene (0.37%), Linalool (0.27%) and Zingiberene (0.10%) on comparison with the mass spectra of the constituents with the NIST library.

The antimicrobial activity of essential oil extract from Curcuma xanthorrhiza rhizome was tested against thirteen pathogenic bacteria and six fungi. In terms of antibacterial activity, the essential oil showed remarkable antibacterial activity with zone of inhibition of 14mm each against E. coli and Bacillus amyloliquefaciens, followed by Klebsiella pneumoniae (12mm), Shigella sonnei (11mm) and Enterobacter aerogens (10mm). Three bacteria Pseudomonas aeruginosa, Salmonella typhi and Xanthomonas campestris displayed the inhibition zone of 9mm each and Mycobacterium sp., Proteus vulgaris, Streptococcus thermophilus and Staphylococcus aureus showed each 8mm of inhibitory activity, whereas Bacillus megaterium showed 7mm activity against the essential oil isolated from the rhizome of Curcuma xanthorrhiza.

In order to find out the antifungal activity of chemicals present in the rhizome of Curcuma xanthorrhiza six species of fungus were tested. Of these, Candida albicans and Kluveromyces maxianus exposed the maximum and minimum inhibitory zones of 9mm and 7mm respectively. Aspergillus flavus and Penicillium chrysogenum showed the inhibitory zone of 8mm each. However it is evident that, Aspergillus niger and Fusarium oxysporum were resistant to the essential oil extract. The overall inhibitory effect of Curcuma xanthorrhiza extract revealed the better activity against the pathogenic bacteria than fungus.

4. Discussion

Medicinal plants have been used for centuries as remedies for human diseases, because they contain components of therapeutic value [22–26]. Extraction of bioactive compounds from medicinal plants permits the demonstration of their physiological activity. It also facilitates pharmacology study leading to the synthesis of more potent drugs for meeting demand for effective and safe use. In the present study, the plant collected from Western Ghats was identified according to their taxonomical characters as Curcuma xanthorrhiza belongs to the family Zingiberaceae. There are several data in the literature indicating a great variety of pharmacological activities of oil extracted from the members of the family Zingiberaceae, which exhibit antiallergic [27], antimicrobial [28–33], anti-inflammatory [34], anti-hyperlipidaemic [35] anti-noiceptive, anti–psychiatric [36], antioxidant [37, 38], hepatoprotective and immunomodulatory [39] and cytotoxic [40] activities.

The antimicrobial activity of oil extracted from Curcuma xanthorrhiza could be attributed to the broad spectrum of bioactive chemical compounds. On hydrodistillation of fresh rhizomes, about 0.44% of white coloured, pleasant smelling oil was obtained from Curcuma xanthorrhiza. Based on GC/MS analysis the major compound was identified a sesquiterpenoid compound, xanthorrhizol. It is evident that Xanthorrhizol isolated from the methanol extract Curcuma xanthorrhiza showed potent antibacterial [41] and anticandidal activity [42].

Curcumin (diferuyl methane), a yellow pigment is a phenolic compound and a major phytochemical constituent of Curcuma species, has been linked with suppression of inflammation; angiogenesis; tumorigenesis; diabetes;
diseases of the cardiovascular, pulmonary, and neurological systems, of skin, and of liver; loss of bone and muscle; depression; chronic fatigue; and neuropathic pain [43]. Recent literature revealed that curcumin has antioxidant and radical scavenging activity [44]. The bioconjugates having curcumin covalently attached to piperic acid, glycine, glycyl-piperic acid, alanine and acetic acid through its free phenolic groups show better antibacterial and antifungal activities via–α–vis curcumin against some common pathogenic microbes viz. Escherichia coli, Pseudomonas aeruginosa, Pseudomonas pyocynin, Candida krusei GO3 and Candida albicans (yeast). These activities have been found to be equivalent to that of the marketed drugs, Cefepime (antibacterial) and fluconazole (antifungal) [45].

It has been found that curcumin inhibits Bacillus subtilis and Escherichia coli growth by inhibiting FtsZ assembly [46]. It has also shown a wide–spectrum of chemopreventive, antioxidant and antitumor properties. Although its promising chemotherapeutic activity, preclinical and clinical studies highlight curcumin limited therapeutic application due to its instability in physiological conditions [47]. A synthesized curcumin analog, 1,5-diaryl-3-oxo-1,4-pentadiene such as β-diglucoside exhibited higher antibacterial properties but showed lower activity against Bacillus cereus and Staphylococcus aureus and Escherichia coli but showed lower activity against Bacillus cereus and Yersinia enterocolitica than did curcumin. The results clearly demonstrate that conjugation of the phenolic hydroxyl group of curcumin to a sugar moiety rendered it water–soluble whilst retaining/enhancing its in vitro antioxidant, antimutagenic and antibacterial properties [48]. Curcuminoids and other natural and synthetic curcuminoids possess various bioactivities including anti-inflammatory, anti-oxidant, anti-HIV, chemopreventive and anti-prostate cancer effects. Recent studies on curcuminoids, particularly on curcumin, have discovered not only much on the therapeutic activities, but also on mechanisms of molecular biological action and major genomic effects [49].

The mutagenicity studies showed that curcumin, as well as Curcumin–β–diglucoside, afforded high protection against the mutagenicity of sodium azide to Salmonella typhimurium TA 1535 and TA 98. Also, Curcumin–β–diglucoside exhibited higher antibacterial properties against Staphylococcus aureus and Escherichia coli but showed lower activity against Bacillus cereus and Yersinia enterocolitica than did curcumin. The results clearly demonstrate that conjugation of the phenolic hydroxyl group of curcumin to a sugar moiety rendered it water–soluble whilst retaining/enhancing its in vitro antioxidant, antimutagenic and antibacterial properties [49]. Curcuminoids and other natural and synthetic curcuminoids possess various bioactivities including anti-inflammatory, anti-oxidant, anti-HIV, chemopreventive and anti-prostate cancer effects. Recent studies on curcuminoids, particularly on curcumin, have discovered not only much on the therapeutic activities, but also on mechanisms of molecular biological action and major genomic effects [49].

Overall, the present study, along with the previous studies, shows that diverse phytochemical components present in the various species of Curcuma, including the presently studied Curcuma xanthorrhiza are having potent antimicrobial activity. The particular bioactive compounds responsible for antimicrobial activity, whether xanthorrhizol / curcumin or other, has yet to be confirmed.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors are grateful to Rev. Fr. Prem Kumar (Correspondent and Secretary) and the Staff Members of Biotechnology Department, Malankara Catholic College, Mariagiri, Kanyakumari, Tamilnadu, India for their constant encouragement and support.

References
