IN VITRO ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL OF ERIGERON FLORIBUNDUS

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ABSTRACT
The essential oil of Erigeron floribundus was screened against ten human pathogenic bacteria and fungi. The oil was found active against Staphylococcus aureus, Escherichia coli, Candida albicans, Aspergillus niger, Saccharomyces cerevisiae and Penicillium chrysogenum with minimum inhibitory concentration (MIC) of 0.41±0.18, 0.72±0.47, 0.36±0.23, 0.45±0.28, 0.57±0.59 and 0.88±0.63 mg/ml, respectively. The essential oil of E. floribundus was found more active against the tested fungal strains.

KEYWORDS: Essential oil, Erigeron floribundus, antimicrobial activity

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INTRODUCTION
There is an increasing interest in the medicinal plants as a natural alternative to synthetic drugs1. The spread of drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases2. Over the years, essential oils and plant extracts have evoked interest as sources of natural products. Essential oils produced by plants is believed to be predominantly a defense mechanism against pathogens and pests3. Essential oils and their compositions are gaining increasing interest because of their relatively safe status, wide acceptance by consumers and their exploitation for potential multi-purpose functional use4.

Erigeron floribundus (Kunth) Sch. Beep. Syn. Conyza sumatrensis (Retz.) (Asteraceae) known as a reputed medicinal plant, traditionally used for the treatment of skin disorders by the rural populace as well as those from the urban areas in South Africa5. This species is widely distributed in temperate regions of India6. The extracts of E. floribundus have been reported antifungal7, antipyretic and anti-inflammatory activities8. However, volatile constituents and antifungal activity of the essential oil of E. floribundus has been previously investigated9. The aim of present study was to evaluate the antimicrobial efficacy of the essential oil extracted from E. floribundus collected from Western Ghats region of North Western Karnataka, which has not previously investigated.

MATERIALS AND METHODS
Plant material
The aerial parts of E. floribundus were collected from the campus of Regional Medical Research Centre (RMRC), (ICMR), Belgaum, Karnataka at a height of 800 m. The plant was identified and authenticated in RMRC, Belgaum, where a voucher specimen (No. 421) is deposited.

Isolation of essential oil
The aerial parts (1 kg) of E. floribundus were steam distilled using copper still fitted with spiral glass condensers for 3 h and water distillate was extracted with n-hexane and dichloromethane. The organic phase was dried over anhydrous sodium sulphate and the solvent was distilled off using thin film rotary vacuum evaporator at temperature range 25°-30° C. The oil yield was 0.01% (v/w).

Microbial strains
The microbial strains namely Bacillus subtilis (NCIM 2063), Micrococcus flavus (NCIM 2379), Staphylococcus aureus (NCIM 2079) (Gram-positive...
bacteria), Escherichia coli (NCIM 2574), Klebsiella pneumoniae (NCIM 2957), Salmonella typhimurium (NCIM 2501) (Gram-negative bacteria), Aspergillus niger (NCIM 620), Candida albicans (NCIM 3471), Penicillium chrysogenum (NCIM 733) and Saccharomyces cerevisiae (NCIM 3090) (Fungi) were obtained from the National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune.

**Screening of antimicrobial activity**

The antimicrobial activity was tested using disk diffusion method. The inocula of microbial strains were prepared from 18 hours old culture and suspensions were adjusted to 0.5 McFarland standard turbidity (∼10⁴ CFU (colony forming unit)/ml for bacteria and ∼10⁵ CFU/ml for fungi)⁹. Petri plates were prepared by pouring 20 ml of nutrient agar (HI MEDIA, India) for bacterial strains and YPG agar (HI MEDIA, India) for fungal strains and allowed to solidify for half an hour. The inoculum (0.05 ml) was spread on the top of the solidified media and allowed to dry for 10 min. The essential oil was dissolved in 10% dimethyl sulfoxide (DMSO) which is reported to be non toxic to microorganisms at this percentage¹¹ with Tween 80 (0.1% v/v) as solvent control. Streptomycin (Nicholas Piramal India Limited, Gujarat, India) and fluconazole (Shwarde Pharmaceuticals (I) Pvt. Ltd., Goa, India) (20 µl of 1 mg/ml) were used as positive reference standards for bacterial and fungal strains, respectively. After delivery, the essential oil and reference drugs, the plates were left for 30 min at room temperature to allow the diffusion of essential oil. The plates were than incubated for 24 h and 48 h at 37 °C for bacteria and fungi, respectively. The diameters of inhibition zones were measured in millimeters¹². Assay was carried out in triplicate and mean values are reported (Tables 1).

**Determination of the minimum inhibitory concentration**

The minimum inhibitory concentration (MIC) was determined by using the tube dilution method¹³. Bacteria and fungi were grown in peptone water. The inocula of microbial strains were prepared from 18 hours old culture and suspensions were adjusted to 0.5 McFarland standard turbidity (∼10⁴ for bacteria and ∼10⁵ for fungi colony forming unit (CFU) per milliliter)⁹. After this, a different concentration of the essential oil of E. floribundus was prepared at a concentration range of 5.0-0.009 mg/mL. The reference antibiotics 1 mg/ml of streptozotocin for bacteria and fluconazole for fungi were used at a concentration range 1.02-0.001 mg/ml. Serial two fold dilution was prepared for different concentrations of the essential oil and reference antibiotics. The tubes were incubated for 24 and 48 hours at 37 °C, for bacteria and fungi, respectively. The lowest concentration of test drugs that inhibits the visible microbial growth considered as MIC¹⁴. All determinations were performed in triplicate and mean ± SEM is reported (Table 1).

**RESULTS AND DISCUSSION**

The essential oil of E. floribundus showed qualitative (diameter of inhibition zone) and quantitative (minimum inhibitory concentration) in vitro antimicrobial property against tested pathogens summarized in Table 1. The qualitative antimicrobial activity evaluated by using disk diffusion method. The diameters of inhibitory zones (IZ) recorded in millimeter. It observed that the oil was effective against all tested microbial strains. The essential oil of E. floribundus was found active against the tested microorganisms, with average IZ of 18.7 mm. The maximum IZ of the oil showed against C. albicans followed by A. niger, E. coli and S. cerevisiae with IZ of 26.6, 20.3, 21.0 and 18.0 mm, respectively. All tested pathogens were effective at a concentration of 1.25 mg/ml except P. chrysogenum. Moreover, the oil was effective at a lower concentration of 0.62 mg/ml against A. niger and C. albicans (Table 1).

The quantitative in vitro antimicrobial activity of the essential oil of E. floribundus is reported as minimum inhibitory concentration (MIC) in mg/ml (Table 1). The oil was found more susceptible against the fungi C. albicans with MIC value of 0.36±0.23 however, the oil showed slightly less effective against A. niger and S. cerevisiae with MIC values of 0.45±0.28 and 0.57±0.59, respectively. The oil was less effective against the P. chrysogenum with MIC value of 0.88±0.63. The oil was found more susceptible against Gram-positive bacteria like S. aureus with MIC value of 0.41±0.18, while very less effective against M. flavus and B. subtilis with MIC value of 1.66±0.72 and 2.91±1.09, respectively. The oil was also moderately susceptible against E. coli with MIC value of 0.72±0.47, which is higher than S. aureus. Other microorganisms K. pneumoniae and S. typhimurium were found less effective with MIC value of 0.83±0.36 and 1.35±1.09, respectively. The essential oil of E. floribundus possessed lesser antimicrobial activity than that of standard reference antibiotics. The finding of antimicrobial activity of the essential oil of E. floribundus attributed the traditional use as antifungal infections but this plant can be use for external application as antibacterial contagion also, however, is less potent towards bacteria.
REFERENCES


Table 1: Zone of inhibition (mm) and MIC (mg/ml) of the essential oil of Erigeron floribundus

<table>
<thead>
<tr>
<th>Sl.no.</th>
<th>Microbial strains</th>
<th>Zone of inhibition (mm)</th>
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<tr>
<td></td>
<td></td>
<td>5 mg</td>
<td>2.5 mg</td>
<td>1.25 mg</td>
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<td></td>
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<tr>
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<td>2</td>
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<td>8.3</td>
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<tr>
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<tr>
<td></td>
<td>Fungi</td>
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<td>9.1</td>
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</table>

RA, reference antibiotics (streptomycin for bacteria, fluconazole for fungi). Values are mean±standard deviation of six experiments in replicate. - no inhibition.

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