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RESEARCH ARTICLE

Comparative Investigation on Antimicrobial Property of *Miliusa tomentosa* Leaf Oil and Leaf Extract

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ABSTRACT

Aqueous extract and volatile oil were obtained from Miliusa tomentosa by using soxhlet extractor and hydro distillation with a Clevenger-type apparatus respectively. The extract and volatile oil both were screened for Antimicrobial activity against different bacteria (Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Klebsiella pncumoniae, Pseudomonas aeruginosa, Bacillus pumilis) and fungi (Candida albicans, Aspergillus niger, Fusarium monoliforme, Trichoderma viridae, Phanerochaete chrysosporium and Pcilomyces species) by cup plate diffusion method. Minimal Inhibitory Concentration (MIC) values of aqueous extract and volatile oil obtained were determined using modified cup plate method. The aqueous extract exhibited weak activity against all the bacteria and one fungi (Candida albicans), while volatile oil showed strong activity against most bacteria including Escherichia coli, Bacillus subtilis, Pseudommonas aeruginosa, Klebsiella pneumoni. Also, a moderate activity was seen against Staphylococcus aureus and Bacillus pumilis. It also showed strong activity against fungi like Candida albicans and Fusarium monoliforme, whereas moderate activity was observed on Aspergillus niger, Trichoderma viridae and the weak activity against the remaining fungi. It can be concluded that Miliusa tomentosa leaf volatile oil finds its use as broad-spectrum antimicrobial agent after extensive investigation, and this may provide a basis for the isolation of constituents of biological interest from Miliusa tomentosa for its potent activity.

Keywords: *Antimicrobial activity, Antifungal, Miliusa tomentosa, Annonaceae family, Volatile oil, Aqueous extract*

Higher and aromatics plants have traditionally been used in folk medicine as well as to extend the shelf life of foods, showing inhibition against bacteria, fungi and yeasts . Most of their properties are due to essential oils produced by their secondary metabolism. Essential oils and extracts from several plant species are able to control microorganisms related to skin, dental caries, and food spoilage, including gram-negative and gram-positive bacteria. Aromatic plants and spices have great importance for food, cosmetics and pharmaceutical industries. Their use has taken place since ancient times, and although many of them were substituted by synthetic ones, the demand for natural products is increasing. The essential oils contents in different species is influenced by genetic material, culture conditions and environment, and finally, by crop and post-crop processing [1].

The Annonaceae family includes 80 genera and about 850 species distributed in tropical and subtropical areas of America, Africa and Asia [2]. Since *Miliusa tomentosa* (Roxb.) J Sinclair is one of them, its traditional uses are not reported but its fruits are eaten in some parts of India and its tree yields a pale yellow gum known as karee gum [3]. But *Miliusa balansae* is traditionally used for gastropathy and glomerulonephropathy [4]. The plants belonging to family Annonaceae are used as antibacterial, anticancer, anthelmintic, antiparasitic and pesticidal agents [5]. Leaf oil obtained from different species of *Miliusa* is also reported and varying amount of constituents are present in it [6]. *Miliusa tomentosa* oil has been found to have both antibacterial [7] and analgesic properties [8] In the present study, the in vitro antimicrobial activity of aqueous extract and volatile oil isolated from leaf of *Miliusa tomentosa* (Annonaceae) was investigated and compared.

MATERIALS AND METHODS

Plant material

Fresh leaves of *Miliusa tomentosa* were collected in the month of May from Leghapani (Toranmal hills) of

Maharashtra, India and specimen of leaf was authenticated by Dr. D. A. Patil, HOD, Botany Dept, SSVPS College, Dhule, Maharashtra, India. The leaves were dried in an oven at 40°C for 24 h, milled and kept at 4°Cin dark until use for extraction and isolation of volatile oil.

Preparation of extracts and collection of volatile oil from. leaves.

500 g of leaves were extracted with 3 L of water by continuous hot extraction using soxhlet extractor. Also, 500 g of leaves powder was submitted to hydro-distillation with a Clevenger-type apparatus according to the European pharmacopoeia and extracted with 3 L of water for 360 min (until no more essential oil was obtained). The essential oil was collected dried under anhydrous sulphate and stored at 4^{o}C until antimicrobial activities were tested. The aqueous extract with greenish color yield was 8%, whereas the ultimate yield obtained for volatile oil was 0.6% with slight brown color.

Microorganisms

Strains, including fungi and bacteria were obtained from National Chemical Laboratories (NCL), Pune Maharashtra, India. *Escherichia coli* NCIM 2110, *Staphylococcus aureus* NCIM 2079, *Bacillus subtilis* NCIM 2250, *Klebsiella pneumoniae* NCIM 2719, *Pseudomonas aeruginosa* NCIM 2036, *Bacillus pumilis* NCIM 2327 and *Candida albicans* NCIM 3471, *Aspergillns niger* NCIM 545, *Fusarium monoliforme* NCIM 1099, *Trichoderma viridae* NCIM 1221, *Phanerochaete chrysosporium* NCIM 1197 *and Pcilomyces species* NCIM 1081 were used as test organisms.

Preparation of test organism suspension

Test organism was maintained on slants of medium containing 300 mg of manganese sulphate per liter and was transferred to fresh slant once a week. Then, the slants incubated at temperature 32°C for 24 h. Organism was washed by using 3 ml of saline solution from agar slant onto a large agar surface of medium such as Roux bottle containing 250 ml of agar. It was incubated for 24 hour. Using 50 ml saline solution, the growth from the nutrient surface was washed. Then organism stored under refrigeration. Inoculum was adjusted at 530 nm, which give transmission equivalent to 10^{s} cells/ml.

Preparation of test samples

Aqueous extract was dissolved in DMSO to make a concentration of 100mg/ml. The extracts were diluted in a simple dilution manner to make concentrations in the range of 0.15, 0.31, 0.62, 1.25, 2.5 and 5 mg/ml. Emulsion of the oil (20 mg/ml) was prepared in sterile distilled water with 10% DMSO.

Antimicrobial Assay

Antimicrobial activity of the above mentioned extracts was determined, using a modified cup plate method [9]. Muller Hinton agar was used for the growth of bacterial strains and Potato Dextrose agar was used for the growth of fungi. In case of spore producing organism, sporulated culture was also grown on Potato Dextrose agar. Plant extracts were dissolved in DMSO at a concentration of 500 μg/ml and standard antibacterial agent Amoxycillin (10 μg/disc) and antifungal agent Ketoconazole (50 μg/disc) were prepared. Each plate was inoculated with 20 ml of microbial suspension having a concentration of 10^{s} cells/ml. About 0.1 ml of extract was added to each cup. The plates containing bacteria were incubated at 37 °C for 24h and those containing fungi were incubated at 25 °C for 7 days. The positive antimicrobial activity was read based on growth inhibition zone and compared with the standard drug. In order to determine the minimum inhibitory concentration values, which are the minimum concentrations of agents showing growth inhibition zone when examined visually, extracts were dissolved in DMSO to make a concentration of 100 mg/ml. An amount of 0.1 ml of the extract dilution and volatile oil emulsion were then added to each cup. All the tests were repeated in triplicates [10].

Phytochemical studies

Phytochemical investigations of leaf extract revealed the presence of saponin glycosides, alkaloids, tannins and volatile oils [11]. Whereas volatile oil isolated shown presence of [alpha]-pinene, [beta]-caryophyllene and cardinene as major components [12] but no cineole as previously reported [13].

RESULTS AND DISCUSSION

The aqueous extract exhibited weak activity against all the bacteria and one fungi *Candida albicans,* whereas no activity was seen against the remained tested fungi. Comparatively volatile oil shows strong activity against the tested bacteria *Escherichia coli, Bacillus subtilis, Pseudommonas aeruginosa, Klebsiella pneumonia* and moderate activity was seen against *Staphylococcus aureus* and *Bacillus pumilis.* Volatile oil also showed strong activity against fungi like *Candida albicans and Fusarium monoliforme,* whereas moderate activity was observed on *Aspergillus niger, Trichoderma viridae* and the weak activity against the remaining fungi. As shown in Tables 1 and 2,. Although aqueous extract of leaves showed weak or no activity against the bacteria and fungi, the volatile oil isolated shown better activity against them this may be due to the presence of different terpenes such as monoterpenes and sesquiterpenes which are the constituents of volatile oil and they are known to posses antimicrobial activity. This activity might be due to the synergistic effect between the constituents of volatile oil.

Table 1. Zone of inhibition in diameter (mm) of *Miliusa tomentosa* leaf extracts and leaf oil by agar well diffusion method

	Inhibition Zone in diameter(mm)		
Microorganisms	Aqueous extract	Volatile oil	Standard
Bacteria			
Escherichia coli(NCIM 2110)	8.4	10.5	15.0
Staphylococcus aureus (NCIM 2079)	9.4	11.5	14.3
Bacillus subtilis (NCIM 2250)	5.6	10.4	16.4
Pseudommonas aeruginosa (NCIM 2036)	6.3	9.8	17.0
Klebsiella pneumoniae (NCIM 2719)	3.4	10.1	15.2
Bacillus pumilis (NCIM 2327)	4.7	11.5	16.3
Fungi			
Candida albicans (NCIM 3471)	4.6	10.2	14.8
Aspergillus niger (NCIM 545)	-	9.2	16.7
Fusarium monoliforme (NCIM 1099)	-	8.7	17.1
Trichoderma viridae (NCIM 1221)	-	10.1	18.0
Phanerochaete chrysosporium (NCIM 1197)	8.0	-	14.5
Pcilomyces species (NCIM 1081)	-	4.2	14.2

-Values are inhibition zone (mm), and an average of triplicate.
-Each extract has concentration of 500 µg/ml,
-Standard Drugs: Amoxycillin (10 µg/disc) for bacteria, Ketoconazole (50 µg/disc) for fungi
-Incubation conditions for bacteria: 1 day at 37^0C and for fungi: 7 days at 27^0C.

Table 2. Minimum Inhibitory concentration (mg/ml) of *Miliusa tomentosa* leaf extract and leaf oil by tube dilution method

	MIC (mg/ml)		
Microorganisms	Aqueous extract	Volatile oil	Standard
Bacteria			
Escherichia coli(NCIM 2110)	5	1.25	0.24
Staphylococcus aureus (NCIM 2079)	5	2.5	0.24
Bacillus subtilis (NCIM 2250)	2.5	0.62	0.48
Pseudommonas aeruginosa (NCIM 2036)	5	1.25	0.60
Klebsiella pneumoniae (NCIM 2719)	5	2.5	0.72
Bacillus pumilis (NCIM 2327)	5	2.5	0.96
Fungi			
Candida albicans (NCIM 3471)	5	0.62	0.48
Aspergillus niger (NCIM 545)	-	2.5	0.24
Fusarium monoliforme (NCIM 1099)	-	1.25	0.24
Trichoderma viridae (NCIM 1221)	-	2.5	0.48
Phanerochaete chrysosporium (NCIM 1197)	-	5	0.96
Pcilomyces species (NCIM 1081)	-	5	0.96

-Values are Minimal Inhibitory Concentration (mg/ml), and an average of triplicate.
-Standard Drugs: Amoxycillin for bacteria, Ketoconazole for fungi.
-Incubation conditions for bacteria: 1 day at 37^0C and for fungi: 7 days at 27^0C.

The results shows that *Miliusa tomentosa* leaf aqueous extract doesn't revealed the prominent activity but volatile oil isolated from leaves shows strong and moderate activity against the bacteria and fungi. It can be concluded that Miliusa tomentosa leaf volatile oil finds its use as broad-spectrum antimicrobial agent after extensive investigation. The results obtained in this work are in agreement with recent studies regarding antimicrobial activities of members of the Annonaceae family [5]. These results may provide a basis for the isolation of constituents of biological interest from *Miliusa tomentosa* for its potent activity.

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