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RESEARCH ARTICLE



²Comparative Investigation on Antimicrobial Property of Miliusa tomentosa Leaf Oil and Leaf Extract

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8 ABSTRACT

Aqueous extract and volatile oil were obtained from Miliusa tomentosa by using soxhlet extractor and hyodro distillation with a Clevenger-type apparatus respectively. The extract and volatile oil both were iscreened for Antimicrobial activity against different bacteria (Escherichia coli, Staphylococcus aureus, 12 Bacillus subtilis, Klebsiella pncumoniae, Pseudomonas aeruginosa, Bacillus pumilis) and fungi (Candida 13 albicans, Aspergillus niger, Fusarium monoliforme, Trichoderma viridae, Phanerochaete chrysosporium 14 and Pcilomyces species) by cup plate diffusion method. Minimal Inhibitory Concentration (MIC) values of 15 aqueous extract and volatile oil obtained were determined using modified cup plate method. The aqueous 16 extract exhibited weak activity against all the bacteria and one fungi (Candida albicans), while volatile oil 17 showed strong activity against most bacteria including Escherichia coli, Bacillus subtilis, Pseudommonas 18 aeruginosa, Klebsiella pneumoni. Also, a moderate activity was seen against Staphylococcus aureus and 19 Bacillus pumilis. It also showed strong activity against fungi like Candida albicans and Fusarium monoli-20 forme, whereas moderate activity was observed on Aspergillus niger, Trichoderma viridae and the weak 21 activity against the remaining fungi. It can be concluded that Miliusa tomentosa leaf volatile oil finds its 22 use as broad-spectrum antimicrobial agent after extensive investigation, and this may provide a basis for 23 the isolation of constituents of biological interest from Miliusa tomentosa for its potent activity.

24 Keywords: Antimicrobial activity, Antifungal, Miliusa tomentosa, Annonaceae family, Volatile oil, Aqueous 25 extract

27 used in folk medicine as well as to extend the shelf life 48 some parts of India and its tree yields a pale yellow gum 28 of foods, showing inhibition against bacteria, fungi and 49 known as karee gum [3]. But Miliusa balansae is 29 yeasts . Most of their properties are due to essential oils 50 traditionally 30 produced by their secondary metabolism. Essential oils 51 glomerulonephropathy [4]. The plants belonging to 31 and extracts from several plant species are able to 52 family Annonaceae are used as antibacterial, anticancer, 32 control microorganisms related to skin, dental caries, 53 anthelmintic, antiparasitic and pesticidal agents [5]. and food spoilage, including gram-negative and gram- 54 Leaf oil obtained from different species of Miliusa is 34 positive bacteria. Aromatic plants and spices have great 55 also reported and varying amount of constituents are 35 importance for food, cosmetics and pharmaceutical 56 present in it [6]. Miliusa tomentosa oil has been found 36 industries. Their use has taken place since ancient times, 57 to have both antibacterial [7] and analgesic properties 37 and although many of them were substituted by 58[8] In the present study, the in vitro antimicrobial 38 synthetic ones, the demand for natural products is 59 activity of aqueous extract and volatile oil isolated from 39 increasing. The essential oils contents in different 60 leaf of Miliusa tomentosa (Annonaceae) 40 species is influenced by genetic material, culture 61 investigated and compared. 41 conditions and environment, and finally, by crop and 42post-crop processing [1].

The Annonaceae family includes 80 genera and 44 about 850 species distributed in tropical and subtropical 63 Plant material 45 areas of America, Africa and Asia [2]. Since Miliusa 64 46 tomentosa (Roxb.) J Sinclair is one of them, its 65 the month of May from Leghapani (Toranmal hills) of

Higher and aromatics plants have traditionally been 47 traditional uses are not reported but its fruits are eaten in gastropathy used for and was

MATERIALS AND METHODS

Fresh leaves of Miliusa tomentosa were collected in

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66Maharashtra, India and specimen of leaf was119method [9]. Muller Hinton agar was used for the 67 authenticated by Dr. D. A. Patil, HOD, Botany Dept, 120 growth of bacterial strains and Potato Dextrose agar 68SSVPS College, Dhule, Maharashtra, India. The leaves 121 was used for the growth of fungi. In case of spore 69were dried in an oven at 40°C for 24 h, milled and kept at₁₂₂producing organism, sporulated culture was also grown

72 from. leaves.

74 continuous hot extraction using soxhlet extractor. Also, ¹²/₁₂₈microbial suspension having a concentration of 10^s 75500 g of leaves powder was submitted to hydro-129 cells/ml. About 0.1 ml of extract was added to each 76 distillation with a Clevenger-type apparatus according 77 to the European pharmacopoeia and extracted with 3 L 78 of water for 360 min (until no more essential oil was 79 obtained). The essential oil was collected dried under 80 anhydrous sulphate and stored at 4°C until antimicrobial¹³³read based on growth inhibition zone and compared 81 activities were tested. The aqueous extract with greenish¹³⁴ with the standard drug. In order to determine the 82 color yield was 8%, whereas the ultimate yield obtained 135 minimum inhibitory concentration values, which are the 83 for volatile oil was 0.6% with slight brown color.

84 Microorganisms

86 from National Chemical Laboratories (NCL), Pune 140 volatile oil emulsion were then added to each cup. All 87 Maharashtra, India. Escherichia coli NCIM 2110, 141 the tests were repeated in triplicates [10]. 88 Staphylococcus aureus NCIM 2079, Bacillus subtilis 89NCIM 2250, Klebsiella pneumoniae NCIM 2719, 90Pseudomonas aeruginosa NCIM 2036, Bacillus 96 organisms.

97 Preparation of test organism suspension.

Test organism was maintained on slants of medium 99 containing 300 mg of manganese sulphate per liter and 100 was transferred to fresh slant once a week. Then, the slants 150 The aqueous extract exhibited weak activity against all the 101 incubated at temperature 32°C for 24 h. Organism was 151 bacteria and one fungi Candida albicans, whereas no 102 washed by using 3 ml of saline solution from agar slant onto152 activity was seen against the remained tested fungi. 103a large agar surface of medium such as Roux bottle₁₅₃Comparatively volatile oil shows strong activity against 104 containing 250 ml of agar. It was incubated for 24 hour. 154 the tested bacteria Escherichia coli, Bacillus subtilis, 105 Using 50 ml saline solution, the growth from the nutrient 155 *Pseudommonas aeruginosa, Klebsiella pneumonia* and 106 surface was washed. Then organism stored under 156 moderate activity was seen against Staphylococcus 107 refrigeration. Inoculum was adjusted at 530 nm, which 157 aureus and Bacillus pumilis. Volatile oil also showed 108 give transmission equivalent to 10^s cells/ml.

109 Preparation of test samples

110 Aqueous extract was dissolved in DMSO to make a 111 concentration of 100mg/ml. The extracts were diluted in 112a simple dilution manner to make concentrations in the 113 range of 0.15, 0.31, 0.62, 1.25, 2.5 and 5 mg/ml.¹⁶³ weak or no activity against the bacteria and fungi, the volatile 114Emulsion of the oil (20 mg/ml) was prepared in sterile¹⁶⁴oil isolated shown better activity against them this may be 115 distilled water with 10% DMSO.

116 Antimicrobial Assay

118 extracts was determined, using a modified cup plate¹⁶⁹ between the constituents of volatile oil.

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704°Cin dark until use for extraction and isolation of volatile oil. 123 on Potato Dextrose agar. Plant extracts were dissolved 71 Preparation of extracts and collection of volatile oil 124 in DMSO at a concentration of 500 µg/ml and standard 125 antibacterial agent Amoxycillin (10 µg/disc) and 126 antifungal agent Ketoconazole (50 µg/disc) were 500 g of leaves were extracted with 3 L of water by 127 prepared. Each plate was inoculated with 20 ml of ³⁰cup. The plates containing bacteria were incubated at 37 31°C for 24h and those containing fungi were incubated at 3225 °C for 7 days. The positive antimicrobial activity was 136 minimum concentrations of agents showing growth 137 inhibition zone when examined visually, extracts were 138 dissolved in DMSO to make a concentration of 100 Strains, including fungi and bacteria were obtained 139mg/ml. An amount of 0.1 ml of the extract dilution and

142 Phytochemical studies

91 pumilis NCIM 2327 and Candida albicans NCIM 143 Phytochemical investigations of leaf extract revealed 923471, Aspergillas niger NCIM 545, Fusarium the presence of saponin glycosides, alkaloids, tannins 93monoliforme NCIM 1099, Trichoderma viridae NCIM 45 and volatile oils [11]. Whereas volatile oil isolated 941221. Phanerochaete chrysosporium NCIM 1197 and 146 shown presence of [alpha]-pinene, [beta]-caryophyllene 95 Pcilomyces species NCIM 1081 were used as test 147 and cardinene as major components [12] but no cineole 148 as previously reported [13].

RESULTS AND DISCUSSION

158 strong activity against fungi like Candida albicans and 159 Fusarium monoliforme, whereas moderate activity was 160 observed on Aspergillus niger, Trichoderma viridae and the weak activity against the remaining fungi. As shown in ⁵²Tables 1 and 2, Although aqueous extract of leaves showed 165 due to the presence of different terpenes such as 166 monoterpenes and sesquiterpenes which are the constituents 167 of volatile oil and they are known to posses antimicrobial

Antimicrobial activity of the above mentioned 168 activity. This activity might be due to the synergistic effect

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Antimicrobial property of Miliusa tomentosa

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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^{0} C and for fungi: 7 days at 27^{0} C.

Table 2 Minimum Inhibitor	·· · · · · · · · · · · · · · · · · · ·) - f M!!!	1 f +	£ . 11 loss 4. los	
Ladie 2. Minimum Inhibitor	v concentration (mg/m) of <i>Millusa lomeniosa</i>	leaf extract and lea	I OH DV LUDE dH	ution 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	

2005.

-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°C and for fungi: 7 days at 27°C.

The results shows that *Miliusa tomentosa* leaf¹⁸⁷ **REFERENCES** Traqueous extract doesn't revealed the prominent activity¹⁸⁸¹. Duarte MCT, activity of essent activity of essent activity of essent activity of essent transport of the annonaceae for the annon

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