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RESEARCH ARTICLE

SYNERGETIC ANTIMICROBIAL POTENTIAL OF AGERATUM CONYZOIDES AND OCIMUM SANCTUM **ESSENTIAL OIL**

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ARTICLE INFO	ABSTRACT
Article History: Received 28 th March, 2024 Received in revised form 25 th April, 2024 Accepted 14 th May, 2024 Published online 25 th June, 2024	There is an increasing interest in scientific research and in industry, for medicinal and aromatic plant because of their potential application in medicine and plant disease control measures. In the present study the petroleum, chloroform, and methanol extract of <i>Ageratum conyzoides</i> showed the antimicrobial activity against <i>Bacillus subtilis</i> , <i>Staphylococcus aureus</i> , <i>E. coli</i> and <i>Pseudomonas</i> and extract of <i>Ocimum sanctum</i> also active against the <i>E. coli</i> , <i>S. aureus</i> , <i>B. subtilis</i> and <i>Pseudomonas</i> sp. While the synergetic study showed that the mixture of extract of these two species also has more

Key words:

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antimicrobial activity against Pseudomonas aeruginosa and S. aureus. These results suggested the synergetic antimicrobial activity against tested bacterial sp. The present study claimed for further study of these two plants and their synergetic effect to treat various diseases and would be useful against infection resulting from Pseudomonas aeruginosa and S. aureus infection.

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INTRODUCTION

Medicinal and aromatic plants produce a wide variety of volatile, aliphatic, and cyclic hydrocarbons and their oxygenated isoprenoid derivatives. Mixture of these substances known as essential oil ^{1,2}. Their essential oil has been widely used since ancient times for therapeutic use and it represent a highly complex class of natural products having well defined role in the economic developments of a country^{3,4}. Thus, the discovery of essential oil preparations that possess antimicrobial activity has been the subject of many research investigations consequence the screening of a wide variety of plant species. Ageratum conyzoides is a tropical source of medicinal and agricultural products. It is an erect herbaceous annual, 35-75 cm tall; stems are covered with fine white hairs, leaves are opposite. The species has great morphological variation and are highly adoptable for different ecological conditions. Ocimum sanctum is one of the commonly known medicinal plants also called Holy basil found throughout the India^{5,6}. Approximately all the part of Ocimum sanctum (Tulsi) plant is using for medicinal purpose specially leaves, stem and seeds. Ocimum sanctum belong to the Lamiaceae family (mint family), is tropical much branch, annual herbs. Different part of the plant has been claimed to be a valuable preparation against several diseases. A tea prepared with the leaves of tulsi is commonly used in cough,

cold and bronchitis^{7,8}. In earlier studies, Ocimum sanctum essential oil was found to posses' anti-inflammatory, antipyretic, analgesic, antiartheritic and antiulcer activity while A. convzoides is widely used to cure wounds; burns, rheumatism, headache, colic, and it have antidysenteric and antilithic activity^{9,10}. In the present study antimicrobial activity of A. conyzoides and Ocimum sanctum has been synergistically investigated against number of microorganisms. However, the synergetic effect of these essential oil still needs to be validated for their biological activities due to the fast-increasing number of multidrug resistance microbes.

MATERIALS AND METHODS

Plant material and preparation of plant extracts: The leaves of A. convzoides and Ocimum sanctum were firstly wash with tap water followed by 0.1% HgCl₂ (Mercuric chloride), then leaves are washed with 70% ethanol for a short interval and then with sterile purified water separately. Process is further followed by drying in an oven at 80°C for 24 hr's and ground to coarse power of both. Accurately weighted powder of both types of leaves (500 mg) was taken in conical flask and extracted with different non-polar solvents for 15 min by sonication. Here I use petroleum ether, chloroform, and methanol solvent for extraction.

Plant part	Extract	Indicator Test: bacteria	Antimicrobial activity			Marginal mean	
			Plant extract (µg/100µl)				
			50	100	150	200	
	Petroleum ether extracts	S. aureus	08.00	08.50	08.95	09.10	08.64
		B. subtilis	07.15	07.55	07.60	07.65	07.49
Leaf of A. conyzoides	Chloroform extracts	S. aureus	11.00	11.40	11.40	11.40	11.30
	Methanol extracts	S. aureus	12.20	12.30	12.35	12.35	12.30
		B. subtilis	11.75	11.75	11.90	12.00	11.91
		E. coli	10.70	10.70	10.80	11.00	10.85
		P. aeruginosa	12.80	12.80	12.80	13.05	12.92

Table 1. Antimicrobial activity of Ageratum conyzoides essential by Well plate method

Antimicrobial activity expressed in terms of mm. diameter of clear zone produced around the well (8 mm) by 100 µl of plant extract at 37 °C for 24 hr's

Plant part	Extract	Indicator	Antimicrobial activity				Marginal
_		Test:					Mean
		bacteria	Plant extract (µg/100µl)				
			50	100	150	200	
	Petroleum ether extracts	E.coli	11.00	11.50	12.67	14.00	12.29
		S.aureus	10.00	11.00	13.00	14.00	12.00
	Chloroform extracts	E.coli	09.33	10.33	11.33	12.33	10.83
Leaf of Ocimum sanctum		B.subtilis	11.67	12.00	12.00	12.00	11.92
		P. aeruginosa	09.00	09.33	13.33	14.00	11.41
	Methanol extracts	S.aureus	13.33	14.00	14.00	14.44	13.94
		P. aeruginosa	12.53	13.00	13.83	14.00	13.34

Antimicrobial activity expressed in terms of mm. diameter of clear zone produced around the well (8 mm) by 100 µl of plant extract at 37°C for 24 hr's

Table 3. Synergetic antimicrobial activity of Ageratum conyzoides and Ocimum sanctum essential oil by Well plate method

Plant part	Extract	Indicator	A	Marginal			
		Test: bacteria	Plant extract (µg/100µl)				mean
			50	100	150	200	
Leaf of Ageratum conyzoides + Leaf of Ocimum sanctum	Petroleum ether	E.coli	10.66	10.75	11.33	11.50	11.06
	extracts	S.aureus	11.50	11.60	13.10	14.20	12.62
		B.subtilis	6.66	7.10	7.25	7.33	7.08
		P.aeruginosa					
	Chloroform	E.coli	08.70	09.20	09.50	10.20	09.40
	extracts	S.aureus	10.66	10.90	11.00	11.10	10.92
		B.subtilis	11.20	11.60	11.80	11.90	11.63
		P.aeruginosa	8.50	08.80	09.10	09.33	08.93
	Methanol extracts	E.coli	10.20	10.50	10.70	10.75	10.54
		S.aureus	15.10	15.40	15.50	15.50	15.48
		B.subtilis	10.90	11.20	11.33	11.33	11.19
		P.aeruginosa	14.66	14.90	15.33	15.66	15.14

Antimicrobial activity expressed in terms of mm. diameter of clear zone produced around the well (8 mm) by 100 µl of plant extract at 37°C for 24 hr's.

The different extracts were filtered through 0.45μ whatman's filter paper in 100 ml volumetric flask and diluted with solvent upto the mark. The final solutions had the concentration of *A. conyzoides* and *Ocimum sanctum* leaves as 5 mg/ml.

Microorganism: The bacterial cultures used as test organism in the study are *E.coli, B.subtilis, S.aureus, P. aeruginosa.* These strains have been used to check the antimicrobial activity of *A. conyzoides* and *Ocimum sanctum* essential oil separately as well as synergistically.

Well Plate Assay: The lawn of each indicator test bacteria was made with the help of spreader on Nutrient Agar plate. Wells (8mm) were punched on the plate with the help of sterile Corkboror. Plates ware incubated at 37° C for 24 hr's. After application of 100μ l of each concentration of plant extracts of both species in the well, both plants were observed for clear zone produced around the well (8mm) at 37° C for antimicrobial activities after 24 hr's of incubation.

RESULT AND DISCUSSION

Antimicrobial activity of Ageratum conyzoides essential oil The petroleum ether extract of A. conyzoides were found to have antimicrobial activity against Staphylococcus aureus and Bacillus subtilis while chloroform extract of A. conyzoides were active against in vitro development of Staphylococcus aureus. Methanol extract of A. conyzoides showed inhibitory activity against Staphylococcus aureus, Bacillus subtilis, E.coli and Pseudomonas aeruginosa.

Antimicrobial activity of Ocimum sanctum essential oil: The petroleum ether extract of Ocimum sanctum were found to have antimicrobial activity against E. coli, Staphylococcus aureus while chloroform extract of Ocimum sanctum were active against E. coli, Bacillus subtilis and Pseudomonas aeruginosa. Methanol extract of Ocimum sanctum showed inhibitory activity against Staphylococcus aureus and Pseudomonas aeruginosa. Synergetic antimicrobial activity of Ageratum conyzoides and Ocimum sanctum essential oil: When we take both the type of leaf extract in equal ratio (1:1) and perform well plate method for analyzing synergetic antimicrobial activity of Ageratum conyzoides and Ocimum sanctum; we analyze that the petroleum ether and methanol extracts of Ageratum conyzoides with Ocimum sanctum resist the growth of S. aureus more strongly than alone extract. While Methanol extract of Ageratum conyzoides with Ocimum sanctum showed more inhibitory activity against Pseudomonas aeruginosa. It may be possible because of the formation of some other chemical compound by reacting, component of both species.

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