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RESEARCH ARTICLE

CHARACTERIZATION OF ACTIVE COMPOUND AND EXTRACTS OF *AERVA LANATA* USING ANTIBACTERIAL POTENTIAL PARAMETERS AGAINST PATHOGENS

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ABSTRACT

Medicinal plants have rich sources of therapeutic constituents and shown beneficial therapeutic effects, including anti-oxidant, anti-inflammatory, anticancer, antimicrobial and anti-immune modulator effects. In the present investigated *Aerva lanata* shows predominant amount of phytochemical constituents such as alkaloids, flavones, saponin, terepenoids etc., in butanol extracts. The active compound of 2-Decyl -1-tetra decanol was isolated and confirmed by GCMS method. The active compound inhibited gram positive (*Staphylococcus aureus* and *Micrococcus sp.,*) and gram negative organisms (*Klebsiella* sp. and *Pseudomonas aeruginosa*) at the concentration of 20-35 µg/ml. It shows isolated active compound of 2-Decyl -1-tetra decanol to be used prepare plant based drugs to cure pathogenic bacterial diseases.

INTRODUCTION

Peoples much aware about the side effect of antibiotics and importance of plant based drugs. In addition, many people are interested in having more autonomy over their medical care (Joshi *et al.,* 2009). Chemotherapy induce the cellular permeability and over production of enzymes in short span, it leads to develop resistance of bacterial organisms (Mckeegan *et al.,* 2002 and Reuters, 2005). Plant based antimicrobial have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials (Parekh and Chanda, 2007 and Dhasarathan *et al.,* 2010b). Plant based drugs are efficient as well it induce in controlled manner and degradable with safety manner (Kumar *et al*., 2005). *Aerva lanata* is an indigenous medicinal plant of Asia, South America, and Africa that is commonly used by traditional healers for the treatment of fever, especially malarial fever, dysentery, asthma, hypertension and diabetes. *Aerva lanata* leaves have been assessed for cancer chemo preventive activity (Dhasarthan *et al.,* 2010a). Many kinds of works have been carried out in various medicinal plants as well as in *Aerva lanata.* Reports related to antimicrobial activity of *A.lanata* were scanty in this aspect present study focused to analyze pharmcalogical effect of isolated active compounds to the bacterial pathogens.

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MATERIALS AND METHODS

Pathogenic Strains

The test organisms used were *Micrococcus* sp., *S.aureus., Klebsiella* sp. and *Pseudomonas aeruginosa*. The bacterial strains were collected from clinical laboratory and checked purity with standard screening methods (Yi-Wei Tang *et al.,* 1997). All the bacterial cultures were cultured in nutrient broth (Hi-media) and incubated at 37^0 C for 24 hours.

Antibacterial screening

The antibacterial activity of compound 2-Decyl -1-tetra decanol isolated from *A.lanata* was screened using disc diffusion method, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC).

Disc diffusion method

Filter paper disc diffusion technique in agar was followed to determine antimicrobial activity by the procedure of Garg and Jain (1998.) What mann No.1 filter paper discs of 6-mm diameter, placed in dry Petri plates, were autoclaved. The test compound was dissolved in butanol and made into different concentration (20, 40 and 60 µg/ml). The pathogenic strains were suspended with nutrient broth (Hi-media) by transferring

a loop full of 24 hrs, growth from agar slopes. The suspensions were vortexes and 0.1ml aliquots were spread over respective agar medium plates. The test compound and standard antibiotic, tetracycline (25 µg/ml) loaded discs were then placed over the plates seeded with respective microorganisms. The plates were incubating at 37^0C for 24hrs. The antibacterial activity was determined by measuring the inhibition zone around the discs. The diameter of inhibition zones (including the diameter to the disc) was measured.

MIC and MBC test

Minimum Inhibitory Concentration (MIC) of the compound was determined from the culture plates that had the lowest concentrations and prevented the growth of bacterial strain. Minimum Bactericidal Concentration (MBC) was determined by using the method of Samy and Ignacimuthu (2001). The antibacterial activity of compound 2-Decyl -1-tetra decanol isolated from *A.lanata* was diluted to obtain concentration ranging from 20 µg -60 µg /ml. The test tube containing 3ml of Muller Hinton broth and 0.1 ml bacterial suspensions and 0.1 ml plant extract were incubated at 37^0C for 24h. Bacterial turbidity was measured at 650 nm to determine bacterial inhibition. Tetracyclin at 20 and 40µg /ml was used as a reference for determination of minimum inhibitory and bactericidal concentrations respectively. The tubes containing only the growth medium were used as control. The minimum bactericidal concentration that showed the reduction of the bacterial colony as measured from the turbidity of the culture by OD value.

RESULTS AND DISCUSSION

In the case of butanol extract compound of *A.lanata* showed high antimicrobial activity against all the test pathogens while other extracts showed comparatively moderate activity. The active compound of butanol extracts of *A.lanaata* (60 µg/ml) showed high antimicrobial activity against *Micrococcus sp* (20 mm), *Stabhylococcus aureus* (10 mm) while with *Pseudomonas sp* (16mm) and *Klepsiella* Sp. (15 mm). The chloroform extract of *A.lanata* showed less antimicrobial activity against all the test pathogens. In the case of ethanol extract of *A.lanata* showed moderate antimicrobial activity against all the test pathogens while other extracts showed comparatively. The ethanol extract (60 µg/ml) of showed antimicrobial activity against *Micrococcus sp* (17 mm), *Stabhylococcus aureus* (10 mm) while with *Pseudomonas sp* (12) and *Klepsiella* Sp. (13 mm). The chloroform extract of *A.lanata* showed less antimicrobial activity against all the test pathogens (Table 1). During preliminary screening of A.lanata showed many bioactive chemical constituents such as

Table 1: Screening of antibacterial activity (Disc diffusion method) of test compound and various extracts of A. lanata against pathogenic bacteria organisms

Source	Concentration (µg /ml)	Test organisms			
		Micrococcus Sp	*Stabhylococcus Sp*	*Pseudomonas Sp*	*Klepsiella* Sp.
2-Decyl -1-tetra decanol	20	10	4	8	9
	40	14	6	12	12
	60	20	10	16	15
Hexane extract	20	2	2	2	3
	40	2	3	2	3
	60	2	3	3	4
Butanol extract	20	8	8	8	4
	40	10	10	8	8
	60	12	12	10	10
Ethanol extract	20	12	9	8	9
	40	10	14	10	9
	60	17	10	13	12
Chloroform extract	20	2	3	2	3
	40	2	3	2	3
	60	2	3	2	4
Aqueous extract	20	3	4	4	4
	40	4	5	7	4
	60	6	7	8	7
tetracycline	25	19	12	18	15

Table 2. The Minimum Inhibitory Concentration of various extracts of *A. lanata* against human pathogenic bacteria organisms

Bacterial organisms	Test chemical concentrations (µg/ml)					
	2-Decyl -1-tetra decanol	Hexane	Butanol	Ethanol	Chloroform	Aqueous
Staphylococcus aureus		35-40	20-25	35-40	35-40	30-35
Micrococcus sp.,		30-35	35-40	55-60	45-50	40-45
Klebsiella sp.,		40-45	20-25	55-60	40-45	35-40
Pseudomonas aeruginosa		55-60	30-35	40-45	55-60	40-45

Table 3. The minimum bactericidal concentration of various extracts of *A. lanata* against bacterial pathogens

Bacterial organisms	Test chemical concentrations (µg/ml)					
	2-Decyl -1-tetra decanol	Hexane	Butanol	Ethanol	Chloroform	Aqueous
Staphylococcus aureus	35	65	45	60	50	60
Micrococcus sp.,	35	65	50	80	60	70
Klebsiella sp.,	30	80	45	90	75	65
Pseudomonas aeruginosa	35	90	55	85	80	70

alkaloids, tannins, phenols, terpenoids, quinones and flavonoids. Apart from these, many compounds 2-Decyl -1-tetra decanol as isolated and screened their potential aganst bacterial pathogens. Flavones are phenol structures containing one carbonyl group. The addition of a 3-hydroxyl group yields a flavonol. Flavonoids are also hydroxylated phenol substances but occur as a C6-C3 unit linked to an aromatic ring. Since they are known to be synthesized by plants in response to microbial infection (Dixon *et al.,* 1983), it should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms. More lipophilic flavonoids may also disrupt microbial membranes (Tsuchiya *et al.,* 1996).

The results showed that the minimum inhibitory concentration range from 20 to 60 µg/ml for all extracts of *A,lanata.* The active compound 2-Decyl -1-tetra decanol was showed good results against all test pathogens (Table 2). The results showed that the minimum bactericidal concentration range from 30 to 85 µg/ml for all extracts of *A,lanata.* The active compound 2-Decyl -1-tetra decanol was showed good results against all test pathogens (Table 3). MBC values were found higher than MIC values of the test extracts against test bacterial pathogens. Similar kind of results obtained in different fruits and plant extracts by various workers (Soumya and Nair, 2012, Premalatha Singariya *et al.,* 2012 and Amarnath *et al.,* 2017). Active compound of *A.lanata* and butanol extract required lesser concentration to kill the test bacterial organisms.

Summary and Conclusion

The active compound of *A. lanata* showed highest antibacterial activity; it showed maximum inhibition activity against gram positive bacteria compared to gram negative bacteria. The maximum activity was observed in *Staphylococcus aureus* with 4.5cm as zone of inhibition at 40µg /ml concentration. Minimum Inhibitory Concentration for the isolated compound was found to be highest in gram positive bacteria (20 - 30 µg /ml) and lowest in gram negative bacteria (30 - 40µg /ml). Minimum Bactericidal Concentration was noticed maximum in *Staphylococcus aureus* (20 µg /ml) and minimum in *Klepsiella* sp (30µg /ml). The zone of inhibition caused by active compound of A.lanata was almost equal to standard tetracycline. In this study shows isolated active compound of 2-Decyl -1-tetra decanol to be used prepare plant based drugs to cure pathogenic bacterial diseases without any side effects at cheaper cost.

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