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RESEARCH ARTICLE

SCREENING OF SECONDARY METABOLITES IN *AERVA LANATA* AND THEIR EFFICIENCY AGAINST BACTERIAL PATHOGENS

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ABSTRACT

Aerva lanata is consists different kind of secondary metabolites such as steroids, alkaloids, flavanoids, saponind etc., that act as antimicrobial substance to control pathogens. The butanol extracts of *Aerva lanata* leaves was found all kinds of secondary metabolites. The butanol extract (150 µg/ml) of *A.*lanata was showed maximum inhibition 5.8 mm against *Klebsiella* Sp, 5.1 mm against *Staphylococus aureus,* 4.5 mm against *Micrococcus* Sp. and 2.6 mm against *Pseudomonas* Sp. Followed by butanol, hexane, ethanol, chloroform and water showed their antimicrobial activity against test pathogens. *A. lanata* may be useful to control different antibiotic resistant strains due to presence of active bioactive compounds.

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INTRODUCTION

Aerva lanata is commonly called as Polpala, in English it is stone breaking plant. It is a bisexual, plant with alternate leaves, an indigenous medicinal plant of Asia, South America, and Africa. *A.lanata* is popular in treatment of diseases like malarial fever, dysentery, asthma, hypertension and diabetes etc., (Rajesh *et al.,* 2011). Worldwide now a day's peoples are preferred, plant based drugs for any kinds of treatment due to lower side effects. Usage of plant based drugs increased every year as per WHO report (Aruna *et al.,* 2013). *Aerva lanata* leaves have been assessed for cancer chemo preventive activity also in traditional treatment process (Chakrabortya *et al.,* 2002). All these kind of activities observed due to the presence of secondary metabolites such as glycosides, betulin, α-amyrin etc., (Joshi, 2007). Many kinds of phytochemical such as alkaloid, terepenoids, flavanoids, steroids etc., were responsible for inhibit growth of pathogens. *Aerva lanata* is comprised all the above phytochemical that act as antimicrobial substance to control pathogens (Chowdhury *et al.,* 2000). This plant is having mineral such as calcium, silicon, magnesium, carbon etc., helpful to recover various kind of diseases (Ragavendran *et al.,* 2012). Clinical microbiologists have two reasons to be interested in the topic of antimicrobial plant extracts. First, it is very likely that these phytochemicals will find their way into the arsenal of antimicrobial drugs prescribed by physicians; several are already being tested in humans. Antioxidant, anti diuretic, hepatoprotective, hypoglycemic works have been carried out in various medicinal plants as well as in *Aerva lanata* also, but mechanism of antimicrobial and immunomodulation studies are scanty. Hence, in the present investigation the phytochemical screening and their impact against human pathogen are analysed.

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MATERIALS AND METHODS

In the present study the plant *Aerva lanata* was collected in early morning without much disturbance in Ambasamudram, Tirunelveli District, Tamil Nadu, India using sterile polythene bag and knife and immediately transferred to the laboratory for further analysis. The plant material was shadow dried for one week, then it powdered with the help of mixer grinder and used for preparation of plant extraction.

Preparation of extracts

Ten gram of powdered plant material was taken in clean sterile Soxhlet apparatus and extraction was done with 100 ml of different solvents (low polar to high polar) like as hexane, butanol, ethanol, chloroform and water. After extraction the

extracts were dried in room temperature until extract reach into solid form. From the solid extract suitable concentrations (50, 75, 100 and 150µg/ml) were made using Dimethyl sulfo-oxide (DMSO) for further analysis.

Preliminary phytochemical screening (Harborne, 1998)

The extracts of *Aerva lanata* were used for the analysis of phytochemical screening. The plant extract mixed with minimum quantity of chloroform, then add few drops of acetic anhydride and one drop of concentrated sulphuric acid. After addition of sulphuric acid the contents give purple colour changing to blue to green, it indicates presence of sterol. The plant extract mixed with 2ml of Fehling's reagent and 3ml of water. The test content was boiled, develop red orange colour Presence of reducing sugars. The plant extract mixed very small quantity of anthrone, few drops of concentrated sulphuric acid and heat the contents. The content colour change into green to purple colour indicates presence of sugar. The plant extract taken with 2N hydro chloric aqueous, layer formed, decanted and to which are added one or two drops of Mayer's reagent. White turbidity or precipitate formed in the presence of alkaloids. A bit of magnesium add into plant extract and one drop of neutral ferric chloride. It develops intense colour presence of phenolic compounds. A bit of magnesium adds into plant extract and one /two drops of concentrated hydrochloric acid, then it was heat. The contents produce red or orange colour in the presence of flavanoids. Plant extract mixed with water and lead acetate. It gives white precipitate in the presence of tannins. Plant extract mixed with water and shake it develop foamyleather in the presence of saponins. Plant extract mixed with ninhydrin. It develop blue or violet colour in the presence of amino acids.

Pathogenic Strains

The test organisms used were *Micrococcus* Sp, *Staphylococus aureus, Pseudomonas aerogenosa and Klepsiella* Sp. The bacterial strains were collected and checked purity with specific measures. All the bacterial cultures were cultured in nutrient broth (Hi-media) and incubated at 370 C for 24 hours. The antibacterial activity of *Aerva lanata* was screened using disc diffusion method.

Disc diffusion method

Filter paper disc diffusion technique in agar was followed to determine antimicrobial activity by the procedure of Garg and Jain (1998.) Whatmann No.1 filter paper discs of 6-mm diameter, placed in dry Petri plates, were autoclaved. The test extracts in measured quantities were dissolved in minimum amount of acetone. Sterilized filter paper No.1 discs were loaded with the extracts of *A.lanata* using different solvents. The amount of extracts loaded in each disc was in the concentration *viz*., 50µg/ml, 75µg/ml and 100µg/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 extracts and tetracycline 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.

Table 1. Preliminary phytochemical screening of different extracts of the *A.lanata*

S.No	Tests	Extracts				
		Hexane	Butanol	Chloroform	Ethanol	Aqueous
1	Reducing sugar	+	+	-	-	+
2	Tannins	-	+	+	-	-
3	Alkaloids	+	+	+	-	+
4	Saponins	-	-	-	-	-
5	Aminoacids	-	+	+	-	-
6	Flavonoids	-	+	-	-	+
7	Phenolic compounds	+	+	+	+	+
8	Sugars	-	+	-	+	+
9	Sterols	+	+	-	-	-

Table 2. The antibacterial effect of *A lanata e*xtracts against human pathogenic bacterial organisms in disc diffusion method

Solvent	Conc.(µg/ml)	Diameter of inhibition (mm)			
		Staphylococcus aureus	*Micrococcus sp.,*	*Klebsiella sp.,*	*Pseudomonas aeruginosa*
Hexane	50	4.9	2.2	2.4	2.3
	75	4.6	2.2	2.5	2.2
	100	4.7	2.4	2.4	2.2
	150	4.8	3.5	3.5	3.7
Butanol	50	4.5	3.4	2.4	2.4
	75	4.6	3.7	2.3	2.3
	100	4.58	3.4	3.7	2.2
	150	5.1	4.5	5.8	2.6
Ethanol	50	3.4	2.5	2.2	2.2
	75	3.5	2.3	2.3	3.5
	100	3.6	3.5	2.2	4.9
	150	3.9	5.2	5.3	5.9
Chloroform	50	2.9	2.5	2.4	2.5
	75	2.6	2.9	3.3	2.4
	100	3.5	2.2	4.5	2.3
	150	3.7	5.2	5.2	3.9
Water	50	2.8	2.4	2.3	2.4
	75	3.3	2.6	2.6	2.6
	100	3.7	2.2	2.2	2.2
	150	3.8	3.8	3.8	3.5
Streptomycin	50	6.6	7.5	8.6	10.7

RESULTS AND DISCUSSION

Pharmaceutical drugs are prepared from a potent source of medicinal plants. Various studies in *A. lanata* proved the plant have bioactive compounds to prepare commercial drugs. In this study, among the various extracts of *A.lanata* was studied and observed preliminary phytocemicals in Table 1. The butanol extracts of leaves of *Aerva lanata* was found to be different secondary metabolites. The reducing sugar is present in the hexane, butanol and aqueous extracts of *Aerva lanata* and is not present in the extracts of chloroform and ethanol. Alkaloids are present in all solvent extracts of *A.lanata* except in ethanol extracts. Saponin is present in butanol, ethanol and aqueous extracts and is absent in remaining solvent extracts. Amino acids are one of the secondary metabolites and are present in butanol and chloroform extract of *Aerva lanata* and are absent in hexane, ethanol and aqueous extracts. Flavonoids are mostly absent in hexane, ethanol and chloroform extracts. However, it is found to occur in butanol and aqueous extracts of the present study. Tannin is found to be more in butanol and chloroform extracts and is not found in hexane, ethanol and aqueous extracts. Pervykh *et al.* (1992) isolated flavanoids from *A.lanata* for identificationof natural compounds in different plants. The *A.lanata* flowering and fruiting parts also composed various kind of secondary metabolites. The root of *A.lanata* showed maxiumum alkaloid (0.58%) and lower flavanoid (0.25%) were reported by Aruna *et al.*, 2013). In the present investigation also proved leaf part of *A.lanata* also having essential secondary metabolites. It helps to prepare various kinds of drugs to cure the diseases. Extract of *A. lanata* leaves showed highest antibacterial activity against chosen gram positive and gram negative organisms (Table 2). In the case of butanol extract of *A.lanata* showed high antimicrobial activity against all the test pathogens while other extracts showed comparatively moderate activity. The butanol extract (150 µg/ml) of *A.*lanata was showed maximum inhibition 5.8 mm against *Klebsiella* Sp, 5.1 mm against *Staphylococus aureus,* 4.5 mm against *Micrococcus* Sp. and 2.6 mm against *Pseudomonas* Sp. Followed by butanol, hexane, ethanol, chloroform and water show their antimicrobial activity against test pathogens. Extract of butanol reach 90% effect of standard streptomycin drug. Flavones are phenol structures containing one carbonyl group (as opposed to the two carbonyls in quinones) (Marjorie, 1999). 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. Presence of steroids, falvonoids, terpenes in *A.lanata* was showed maximum anti fungal activity against *A,niger, C. albicans, R. oligosporum* etc (Chowdhury *et al.,* 2000). Aerial part of *A.lanata* showed antimicrobial activity reported by Muthukumaran *et al.,* (2011). Similarly leave extracts of *A.lanata* showed inhibition against both gram positive and gram negative organisms. In nutshell the present study suggested to prepare plant based drugs from *A. lanata* may be useful to control different antibiotic resistant strains due to presence of active bioactive compounds.

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