Research Article

Study on antibacterial activity of phytochemicals obtained from *Aerva lanata*

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Abstract

Objective: Bioactive compounds of aromatic and medicinal plants were showed remarkable activity against bacteria and fungi. **Material and methods:** *Aerva lanata* was extracted in hot extraction method using low polar to high polar solvents and used to screen the preliminary phytochemicals. The extracts were separated by thin layer chromatography method and identified compounds by NMR techniques. The isolated active compounds and extracts antibacterial activity was screened by disc diffusion assay, minimum inhibitory and minimum bactericidal activity assay methods. **Results**: Among the various extracts studied in the present investigation, the ethanol extracts of *Aerva lanata* (leaves) was found to be different secondary metabolites. The study revealed the polarity of the chemical composition of the leaves of *Aerva lanata.* In the present study, the Rf value of compound isolated from *Aerva lanata* by TLC method is given as; three spots from hexane extract (0.13, 0.17, 0.31) and five spots from butanol extracts (0.03, 0.06, 0.90, 0.12, 0.15. The proton NMR spectrum of the compound gave the following compound 2-Decyl-1-tetradecanol. 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 ± 0.4 mm against *Klebsiella* Sp, 5.1 ± 0.3 mm against *Staphylococus aureus,* 4.5 ± 0.3 mm against *Micrococcus* Sp. and 2.6 ± 0.2mm against *Pseudomonas* Sp. Followed by butanol, hexane extract (150 µg/ml) showed inhibition of *Staphylococus aureus* (4.8 ± 0.3 mm), *Pseudomonas* Sp (3.7 ± 0.2 mm), *Micrococcus* Sp (3.5 ± 0.2 mm) and *Klebsiella* Sp (3.5 ± 0.3 mm). Lower concentration of 2-Decyl -1-tetra decanol (20 µg/ml) also inhibited *Micrococcus* Sp (10 ± 0.3 mm), *Klebsiella* Sp (9 ± 0.3 mm), *Pseudomonas* Sp (8 ± 0.3 mm) and *Staphylococus aureus* (4 ± 0.3 mm). Conclusion: The active compound has good inhibitory effect against the test pathogens and crude extracts. 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.

Keywords: Phytochemical analysis, microbial assay, *Aerva lanata*

Introduction

During the Vedic period itself plants were essential part of human society in different aspects such as construction, furniture manufacture, firewood, medicinal values etc (Manjula et al., 2009; Goyal et al., 2011).Mainly plants were used to cure respiratory infections, diarrhea, malaria, bacterial and fungal infections in rural communities of developing countries (Somchit et al., 2003). In traditional methods were used numerous tropical plants which are cure all kind of diseases (Amarnath et al., 2018). Bioactive compounds of aromatic and medicinal plants were showed remarkable activity against bacteria and fungi. It could be an alternate way to combat against pathogenic microbes (Ramasamy and Charles Manoharan, 2004). Hence in the present study was chosen a test plant *Aerva lanata* for screening their medicinal against pathogens.

Aerva lanata is herb, erect or prostrate with a long tap-root, branched from near the base; branches many, pubescent or wolly- tomentose, striate. Leaves alternate, 2-2 × 1-1.6 cm on the main stem, 6-10 x 5-6 mm on the branches, elliptic or obovate, or subotbicular, obtuse or acute, entire, pubescent above, more or less white with cottony hairs beneath; petioles

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3-6 mm long, often obscure (Goyal et al., 2011). *Aerva lanata* whole plant ethyl acetate and methanol extracts showed interesting antimicrobial activities against *Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Escherichia coli, Shigelladysenteriae, Shigellashiga etc.,* (Chowdhury et al., 2008). In current era plant based drug preparation is very effective to cure the various diseases without side effects (Refaz et al., 2017). Hence the present study programmed to study the therapeutic valuable components obtained from leaves and flowers of *Aerva lanata*.

Materials and methods

Aerva lanata healthy leaves and flowers were collected during early morning period at Ambasamudrum, Tirunelveli District, Tamil Nadu, India. *Aerva lanata* leaves and flowers were collected in bulk, washed, shade dried, macerated and extracted with organic solvent such as hexane, butanol, ethanol, chloroform and water. The extract was filtered and it was finally dried at low room temperature under pressure in a rotary vacuum evaporator (Thermotech, buchi type model th-012). The extracts were concentrated, percentage yield calculated and then subjected to phytochemical screening and TLC profiling studies. The dried extract was properly stored in the desiccators for further experiment and characterization analysis by GCMS.

Phytochemicals Screening

Aerva lanata is extracted in hot extraction method using low polar to high polar solvents and used to screen the following phytochemicals steroids, reducing sugar, alkaloids, phenolic compounds, flavonids, tannins, saponins, aminoacids and ascorbic acids by standard method (Harborne, 1998) and referred with recent techniques.

Thin layer chromatography

A modified form of the thin layer chromatography method described by Farnsworth et al., (1985) was used to determine isolation and enumeration of compounds.

NMR Spectroscopy

The isolated compounds were identified using NMR spectroscopy. 1H NMR spectrum was recorded on a Bruker Advance 300 spectrum operating 300 MHZ 1H Spectral data. The compound -1 obtained by column chromatography method using butanol and petroleum diethyl ether solvent. The active compound was separated in 30-40 fractions.

Antibacterial Assay

The antibacterial activity of *Aerva lanata* (different concentration of extracts and isolated active compound) were screened using disc diffusion method, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) by standard method (Samy and Ignacimuthu, 2001). The pathogenic strains used in the present investigations were *Staphylococcus aureus, Micrococcus sp., Pseudomonas aeruginosa* and *Klebsiella pneumonia.*

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Assay

The extracts and active compounds of *A. lanata* were chosen to determine the MIC and MBC on the *Staphylococcus aureus, Micrococcus sp., Pseudomonas aeruginosa* and *Klebsiella pneumonia.* The MBC value was determined by spreading a loop full of the culture medium from the broth of MIC assay (no showing visible sign of growing) onto fresh MH agar plates. After incubation at 37 °C for 24 hours, the MBC was recorded as the lowest concentration of the test sample showing no bacterial growth on the MH agar plates (Joshua and Takudzwa, 2013).

Results and discussion

Phytochemical screening of *Aerva lanata* leaves was performed and recorded in table 1. Among the various extracts studied in the present investigation, the ethanol extracts of *Aerva lanata* (leaves) was found to be different secondary metabolites. The reducing sugar is present in the hexane, ethanol and aqueous extracts of *A. lanata* and is not present in the extracts of chloroform and butanol. Alkaloids are present in hexane, ethanol, chloroform and aqueous extract except in butanol extract. Saponin is present in butanol, ethanol and aqueous extracts and is absent in remaining solvent extracts. Amino acids present in butanol and ethanol extract of *A. lanata* and are absent in hexane, chloroform and aqueous extracts. Flavonoids are mostly absent in hexane and chloroform extracts. However, it is found to occur in butanol, ethanol and aqueous extracts of the present study. Tannin is

Table 1. Preliminary phytochemical investigation of *A. lanata* leaf extracts

S. No	Phytoconstituents	Extracts of solvents				
		Hexane	Butanol	Ethanol	chloroform	Aqueous
1.	Reducing Sugar	+	-	+	-	+
2.	Alkaloids	+	-	+	+	+
3.	Saponins	-	+	+	-	+
4.	Amino acid	-	+	+	-	-
5.	Flavonoids	-	+	+	-	+
6.	Tannins	+	-	+	+	+
7.	Sterols	+	+	+	+	+
8.	Glycosides	+	+	+	+	+
9.	Phenol	-	+	+	-	+
10.	Sugar	+	-	+	-	-
11.	Terpenoids	-	+	+	-	-

Note: + indicate: Presence, - indicate absence

Table 2. Preliminary phytochemical investigation of *A.lanata* flower extracts

S. No	Phytoconstituents	Extracts of solvents				
		Hexane	Butanol	Ethanol	chloroform	Aqueous
1.	Reducing Sugar	-	-	-	-	-
2.	Alkaloids	+	-	-	-	-
3.	Saponins	-	-	-	-	+
4.	Amino acid	-	-	+	+	-
5.	Flavonoids	+	-	-	-	+
6.	Tannins	-	-	-	-	+
7.	Sterols	+	+	+	+	-
8.	Glycosides	+	+	+	+	-
9.	Phenol	-	-	-	-	+
10.	Sugar	+	+	+	+	-
11.	Terpenoids	+	+	-	-	-

Note: + indicate: Presence, - indicate absence

found to be more in ethanol and chloroform extracts followed by hexane and aqueous extracts and is not found in butanol extracts of *A. lanata*. Sterols and glyocosides were presents in all extracts of *A.lanata*, Phenol present in butanol, ethanol and aqueous extracts but absence in other solvents. Sugar obtained in hexane and ethanol extracts absence in remaining solvents. Terepenoids found in butanol and ethanol extracts of *A.lanata* but not found in hexane, choloroform and aqueous extracts.

Preliminary phytochemical analysis revealed the presence of secondary metabolites in *A. lanata* samples was good. They also having good source of many important minerals such as iron, calcium, manganese and magnesium (Hemalatha and Dhasarathan, 2013). Studies have found certain chemicals, other than nutritional principles, in them have antimutagenic, free radical scavenging and immunity boosting functions, which help to promote health and prevent diseases (Paulsi and Dhasarathan, 2011).

The phytochemical active compounds of *Aerva lanata* were qualitatively analysed for flowers and the results are presented in Table 2. In these screening processes reducing sugar was absence in all extracts of flower sample. Alkaloids and saponins were obtained only in hexane and aqueous extracts respectively. Aminoacid present in ethanol extracts only. Flavonoids found in all extracts of *A.lanata* except hexane and chloroform. Tanin present in aqueous extracts of flower sample. Sterol and glycosides were present in all extracts except aqueous extracts. Phenol found in aqueous extract only and absence in remaining extracts. Sugar presence in hexane and ethanol extracts only. Terpenoids found in ethanol and butanol extracts only. In the present study, flower extracts showed very less phytochemicals compared to leaf extracts. Hence the further compound isolation and characterization performed only in leaf extracts.

The TLC was done for each extract on a precoated silica gel (0.20 mm) using different mobile phases. Toluene-formic acid (95:5) was used as a mobile phase for the hexane and butanol extract, while the chloroform, water and ethanolic extract were run using toluene-ethyl ether-formic acid (5:4:1) as the mobile phase. Chromatograms were viewed under long wavelength UV light (366 nm) and R_F values for different spots were calculated and recorded (Figure 1).

The study revealed the polarity of the chemical composition of the leaves of *Aerva lanata.* In the present study, the Rf value of compound isolated from *Aerva lanata* by TLC method is given as; three spots from hexane extract (0.13, 0.17, 0.31), five spots from butanol extracts (0.03, 0.06, 0.90, 0.12, 0.15), six spots from ethanol extracts (0.03, 0.08, 0.11, 0.13, 0.17, 0.21), four spots from chloroform extracts (0.13, 0.18, 0.21, 0.33) and three from aqueous extracts (0.80, 0.18, 0.34). On comparing the R_f values of the various spots in different solvent system with standard $R_f$values, the various plant extracts may contain the phytoconstituents such as flavonoids and saponin.

The result of the present study was similar to observation of Tullanithi et al. (2010). The various spots obtained with different solvent extracts of *A. Lanata* like hexane, butanol, ethanol, chloroform and water were 3, 5, 4, 3 and 3. *A. frutescens* have 3, 4, 4, 3 and 3 spots. Similar results were reported by Mallikharjuna et al. (2007) in the phytochemical studies on *Strychynos potatorum.*

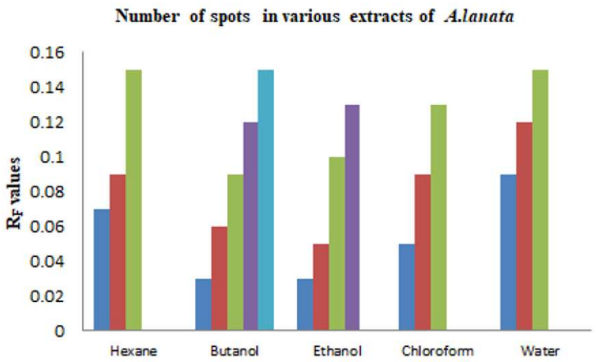

Figure 1. Separation of phyto constituents in different extracts of *A. lanata* by using TLC method

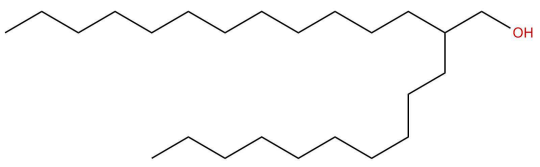

Figure 2. Identified compound of *A. lanata* extract (2-Decyl-1-tetradecanol)

Table 3. The antibacterial effect of *A. lanata e*xtracts against human pathogenic bacterial organisms in disc diffusion method

Solvents	Conc.	Diameter of inhibition excluding disc (mm)			
	(µg/ml)	*S. aureus*	*Micrococcus sp.,*	*Klebsiella sp.,*	*P.aeruginosa*
Hexane	50	4.9 ± 0.3	2.2 ± 0.1	2.4 ± 0.2	2.3 ± 0.1
	75	4.6 ± 0.3	2.2 ± 0.1	2.5 ± 0.2	2.2 ± 0.1
	100	4.7 ± 0.3	2.4 ± 0.1	2.4 ± 0.2	2.2 ± 0.1
	150	4.8 ± 0.3	3.5 ± 0.2	3.5 ± 0.3	3.7 ± 0.2
Butanol	50	4.5 ± 0.2	3.4 ± 0.2	2.4 ± 0.2	2.4 ± 0.1
	75	4.6 ± 0.3	3.7 ± 0.2	2.3 ± 0.2	2.3 ± 0.1
	100	4.5 ± 0.3	3.4 ± 0.3	3.7 ± 0.3	2.2 ± 0.1
	150	5.1 ± 0.3	4.5 ± 0.3	5.8 ± 0.4	2.6 ± 0.2
Ethanol	50	3.4 ± 0.2	2.5 ± 0.2	2.2 ± 0.2	2.2 ± 0.1
	75	3.5 ± 0.2	2.3 ± 0.1	2.3 ± 0.1	3.5 ± 0.2
	100	3.6 ± 0.2	2.5 ± 0.2	2.2 ± 0.1	3.7 ± 0.3
	150	3.9 ± 0.2	3.2 ± 0.3	3.3 ± 0.3	3.9 ± 0.3
Chloroform	50	2.9 ± 0.1	2.5 ± 0.1	2.4 ± 0.1	2.5 ± 0.1
	75	2.6 ± 0.1	2.9 ± 0.2	3.3 ± 0.2	2.4 ± 0.1
	100	3.5 ± 0.2	2.2 ± 0.1	3.5 ± 0.3	2.3 ± 0.1
	150	3.7 ± 0.2	3.2 ± 0.2	3.7 ± 0.4	3.9 ± 0.2
Water	50	2.8 ± 0.2	2.4 ± 0.2	2.3 ± 0.1	2.4 ± 0.1
	75	3.3 ± 0.2	2.6 ± 0.2	2.6 ± 0.1	2.6 ± 0.1
	100	3.7 ± 0.2	2.2 ± 0.2	2.2 ± 0.1	2.2 ± 0.1
	150	3.8 ± 0.2	3.8 ± 0.3	3.8 ± 0.2	3.5 ± 0.2
2-Decyl -1-tetra decanol	20	4 ± 0.3	10 ± 0.3	9 ± 0.3	8 ± 0.3
	40	6 ± 0.3	14 ± 0.4	12 ± 0.4	12 ± 0.4
	60	10 ± 0.4	20 ± 0.5	15 ± 0.4	16 ± 0.5
Streptomycin	50	6.6 ± 0.4	7.5 ± 0.4	8.6 ± 0.4	10.7 ± 0.5

Phytochemical analyses of the present study were in agreement with the earlier reports of Chopra et al. (1969). Each value corresponds to a chemical compounds spot seen on the TLC plate. Similarly R_F values seen in the plant of the *A. esculentus* and *Thespesia populnae* indicating that several compounds have similar polarity and are found throughout a plant (Bevan et al., 2004).

The proton NMR spectrum of the compound gave the following compound 2-Decyl-1-tetradecanol. It is one unusual base pair present in *A. lanata* samples. It is one of the reasons for many of the biological characteristics of the samples (Figure 2). From the findings of phytochemicals obtained in *A. lanata* was showed different active compounds. In this study 2-Decyl-1-tetradecanol is rich source in butanol extracts of *A.lanata.* This phytochemicals were used to prepare plant based drugs against bacterial pathogens.One of the active compounds was isolated and identify by GC-MS method.

In this study, 11 phytochemical screening tests have been carried out included terpenoids, cardiac glycosides, phenols, alkaloids, flavonoids, saponins, steroids, reducing sugars and tannins tests. This study has revealed that terpenoids, cardiac glycosides, phenols, alkaloids, flavonoids, saponins, steroids were present in more than one solvent extracts of *A. lanata*. Terpenoids compounds have been reported that it had antibacterial, antiviral, antidiarrhoeal and antineoplastic effect (Jayashree Dutta, 2013). Steroids also have been reported that it exhibits antimicrobial activity by causing the leakage on liposomes in microorganism, due to its steroidal compounds could be specifically associate with the lipid of membranes. TLC profiling of all 5 extracts gives an impressive result that directing towards the presence of number of phytochemicals. The plant screened for phytochemical constituents seemed to have the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health. 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. Since they are known to be synthesized by plants in response to microbial infection (Dixon et al., 2004), it

should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms (Tsuchiya et al., 2004).

Extract of *A. lanata* leaves showed highest antibacterial activity against chosen gram positive and gram negative organisms (Table 3). 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 (150µg/ml) of *A. lanata* was showed maximum inhibition 5.8 ± 0.4 mm against *Klebsiella* Sp, 5.1 ± 0.3 mm against *Staphylococus aureus,* 4.5 ± 0.3 mm against *Micrococcus* Sp. and 2.6 ± 0.2mm against *Pseudomonas* Sp. Followed by butanol, hexane extract (150µg/ml) showed inhibition of *Staphylococus aureus* (4.8 ± 0.3 mm), *Pseudomonas* Sp (3.7 ± 0.2 mm), *Micrococcus* Sp (3.5 ± 0.2 mm) and *Klebsiella* Sp (3.5 ± 0.3 mm). Ethanol extract (150µg/ml) of *A. lanata* showed inhibition against *Staphylococus aureus* (3.9 ± 0.2 mm), *Pseudomonas* Sp (3.9 ± 0.3 mm), *Klebsiella* Sp (3.3 ± 0.3 mm) and *Micrococcus* Sp (3.2 ± 0.3 mm). Chloroform extract (150µg/ml) also showed inhibition against *Pseudomonas* Sp, *Staphylococus aureus, Klebsiella* Sp and *Micrococcus* Sp as 3.9 ± 0.2 mm, 3.7 ± 0.2 mm, 3.7 ± 0.4 mm and 3.2 ± 0.2 mm respectively. Water extracts (150µg/ml) show their less antimicrobial activity compared to other solvents against test pathogens (*Staphylococus aureus, Klebsiella* Sp and *Micrococcus* Sp was 3.9 ± 0.2 mm and *Pseudomonas* Sp was 3.5 ± 0.2 mm). Extract of butanol reach 90% effect of standard streptomycin drug (50µg/ml). Similar trend of inhibition found in the concentration of 100, 75 and 50µg/ml of all solvent extracts of *A. lanata* (Table 2). Active compound of *A.lanata*, 2-Decyl -1-tetra decanol showed maximum activity against all test pathogens and extracts. 2-Decyl -1-tetra decanol (60µg/ml) inhibited *Micrococcus* Sp (20 ± 0.5 mm), *Pseudomonas* Sp (16 ± 0.5 mm), *Klebsiella* Sp (15 ± 0.4 mm) and *Staphylococus aureus* (10 ± 0.4 mm). In the concentration of 40 µg/ml of 2-Decyl -1-tetra decanol showed inhibition against *Micrococcus* Sp (14 ± 0.4 mm), *Klebsiella* Sp (12 ± 0.4 mm), *Pseudomonas* Sp (12 ± 0.4 mm) and *Staphylococus aureus* (6 ± 0.3 mm). Lower concentration of 2-Decyl -1-tetra decanol (20 µg/ml) also inhibited *Micrococcus* Sp (10 ± 0.3 mm), *Klebsiella* Sp (9 ± 0.3 mm), *Pseudomonas* Sp (8 ± 0.3 mm) and *Staphylococus aureus* (4 ± 0.3 mm). The active compounds have good inhibitory effect against the test pathogens and crude extracts.

The hexane extract showed the least antibacterial activity as it is a non-polar solvent which commonly used to extract essential oils and lipids. This could suggest that the antibacterial compounds that present in this plant are lipid soluble compounds (Arunthathi et al., 2018). All solvent extracts of *A. lanata* did not show any antibacterial activity against Gram negative bacteria, *E. coli*. This is because of the cell wall structure of Gram negative bacteria; contain an extra layer of outer membrane compared to Gram positive bacteria whose only have an inner cell membrane. However, these results are not in accordance with the previous study reported that the methanol extract of *A. lanata* showed antibacterial

Table 4. The Minimum Inhibitory Concentration of various extracts of *A. lanata* against human pathogenic bacterial organisms

Bacterial organisms	Test chemical concentrations (µg/ml)					
	2-Decyl -1-tetra decanol	Hexane	Butanol	Ethanol	Chloroform	Aqueous
S. aureus	15-20	35-40	20-25	35-40	35-40	30-35
Micrococcus sp.,	20-30	30-35	35-40	55-60	45-50	40-45
Klebsiella sp.,	15-20	40-45	20-25	55-60	40-45	35-40
P. aeruginosa	20-25	55-60	30-35	40-45	55-60	40-45

Table 5. 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
S. aureus	35	65	45	60	50	60
Micrococcus sp.,	35	65	50	80	60	70
Klebsiella sp.,	30	80	45	90	75	65
P. aeruginosa	35	90	55	85	80	70

activity against *E. coli* (Sharma et al., 1991). The results showed that the minimum inhibitory concentration range from 20 to 60µg/ml for all extracts of *A. lanata.* The active compound 2-Decyl -1-tetra decanol was showed good results against all test pathogens (Table 4). The results showed that the minimum bactericidal concentration range from 30 to 85µg/ml for all extracts of *A. lanata.* The active compound 2-Decyl -1-tetra decanol was showed good results against all test pathogens (Table 5). 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 (Arunthathi et al., 2018; Sharma et al., 1991 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 - 30µg /ml) and lowest in gram negative bacteria (30 - 40µg /ml). Minimum Bactericidal Concentration was noticed maximum in *Staphylococcus aureus* (20µg /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.

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Conflict of interest

We (Authors) hereby declare that we have no conflict of interest of any form pertaining to this research paper.

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