Research Article

Evaluation of *In-vitro* Biological and Antibiofilm Activities of Various Leaf Extracts of *Aerva lanata*

M. Abinaya[1], Mahalingam Gayathri[1]*, V. Gopiesh Khanna[1]
[1]Department of Biotechnology, School of Bioscience and Technology, VIT University, Vellore, Tamil Nadu, India.
***Corresponding author's E-mail:** gayathrigopinath@vit.ac.in

Accepted on: 18-03-2016; **Finalized on:** 30-04-2016.

ABSTRACT

The present work was to investigate the phytochemical analysis and to evaluate *in vitro* antioxidant, antibacterial and antibiofilm activities of methanol and petroleum ether leaf extracts of *Aerva lanata*. Dried leaves of *Aerva lanata* were extracted by methanol and petroleum ether solvent. The preliminary phytochemical analysis was performed on the leaf extracts of *Aerva lanata* using standard procedure. Antioxidant activity, total phenolic, and total flavonoid content of the leaf extracts of *Aerva lanata* were determined using spectrophotometric methods. *In-vitro* antibacterial and antifungal activities of leaf extracts of plant against the bacterial pathogens (*E.coli, B.subtilis, P.vulgaris and S.aureus*) and fungal pathogens (*Aspergillus fumigatus* and *Aspergillus niger*) was analysed through well diffusion and MIC methods and compared with the standard drugs. Antibiofilm activity was tested by crystal violet assay. The two extracts was further subjected to GC-MS analysis. The phytochemical analysis carried out for leaf extracts of plant showed the presence of several secondary metabolites. Methanol extract of leaf showed the highest phenolic and flavonoid content and high antioxidant activity. Biological activities of methanol leaf extract showed good inhibitory activity against the bacterial than fungal pathogens. The MIC was recorded in the range of 50-6.25µg/ml and 50-12.5µg/ml against the bacterial and fungal pathogens, respectively. The leaf extracts of *Aerva lanata* for antibiofilm activity against the bacterial pathogens were statistically significant. The leaf extracts of *Aerva lanata* on pharmacological activities like antibacterial, antioxidant, antibiofilm were explored. The pharmocogonostic activity of leaf extracts of *Aerva lanta* has research further.

Keywords: *Aerva lanata,* phytochemical screening, Antioxidant, Antibacterial, GC-MS, and Antibiofilm.

INTRODUCTION

Plants have been used in traditional medicines for more than hundreds of years. Medicinal plants as a group comprise approximately 8000 species and account for about 50% of all the higher flowering plant species in India. There are different types of medicinal treatment in old centuries such as Ayurveda, Unani, and Siddha.

Advantage of using medicinal plants are doesn't causes any side effects when compared with synthetic drugs, because medicinal plants have high content of antioxidant compounds. This gives protective effects against diseases without reducing their therapeutic efficacy. Nowadays herbal drugs have become world important objects, with both medicinal and economic implications.

Aerva lanata belongs to the family *Amaranthacea*. These family consists of about 169 genera and 2300 species. It is one of the important medicinal plants have ever grown throughout the plains of India. *Aerva lanata* is found to be an erect herbaceous weed that is common throughout the hotter parts of India especially all over the plains, this extends up to an altitude of 1000m.

In India, it spreads in the states of Tamil Nadu, Andhra Pradesh and Karnataka. *Aerva lanata* had been used in the Indian folk medicine for the treatment of diabetes mellitus, urinary calculi, hematesis, bronchitis, nasal bleeding, cough, scorpion stings, fractures, spermatorrhea, to clear uterus after delivery and also to prevent lactation (Lakshmi Gaja, 2012). The roots are used in the treatment of headache and fever.

Botanical name : *Aerva lanata*

Family : *Amaranthaceae*

Habitat : Herb

Ayurvedic name : Paashaanabheda, Gorakshayanjaa, Aadaanpaahi, Shatkabhedi

Tamil name : Sirupulai

Traditionally, the plant of *Aerva lanata* is being used as diuretic and anthelmintic expectorant and in the treatment of lithiasis. The leaves of *Aerva lanata* are used as sap for eye complaints, an infusion is given to cure diarrhoea and kidney stone; and the root is used in snake bite treatment. The decoction of leaf is used as gargle for treating sore throat and it is also used in various complex treatments against guinea worm (Krishnan Apai, 2009). The plant is used for arresting haemorrhage during pregnancy, burn healing, as an anti-inflammatory, skin diseases, to dissolve kidney and gall bladder stones, for uterus clearance after delivery and to prevent lactation (Yoga, 1979). The chemical constituents of *Aerva lanata* may be therapeutically active or inactive. The substance that are active in nature are called as the active constituents and inactive ones are called as inert chemical constituents (Iyengar 1995). The plant was also recognised to be useful in the prevention of Cisplatin and

Gentamicin induced acute renal failure. Leaf extract of *A.lanata* was investigated for its effectiveness on the urinary risk factors of calcium oxalate urolithiasis are reported to be very effective (Nevin, 2005).

MATERIALS AND METHODS

Plant collection and extraction

Aerva lanata fresh leaves were collected from local area of Coimbatore. The fresh leaves were washed under running tap water to remove the dust. The plant samples were then air dried for few days and the leaves were crushed into powder by electric blender and stored in air tight container for further use.

The powder leaves were extracted by cold percolation method (Parekh and Chanda 2007) using different organic solvents like petroleum ether and methanol in increasing order of polarity. 10g of powdered leaves was taken in 100ml of petroleum ether and methanol in conical flask plugged with cotton wool and kept in rotary shaker at 120 rpm for 24h. The extracts were filtered by Whatman No 1 filter paper. The residue of various extracts were concentrated using rotary evaporator and the extracts were stored at 4˚C in air tight bottles for further studies.

Phytochemical screening

The preliminary phytochemical screening was carried out using aliquots of both methanol and petroleum ether extracts of *Aerva lanata* leaves were performed by Trease and Harborne (1984) methods.

Determination of total phenolic content

The total phenolic content was determined by using Folin-Ciocalteu's reagent method (Mc Donald, 2001). 50µl of various extracts were mixed with 2.5ml (0.5N) Folin-Ciocalteu's reagent and the mixture was incubated at room temperature for 15 min. Then 2.5ml saturated sodium carbonate solution was added and further incubated for 30 min at room temperature and absorbance was measured at 765 nm. Gallic acid was used as a positive control. Total phenolic content were expressed in terms of gallic acid equivalent (mg g^{-1} of extracted compound). Experiments were performed in triplicates.

Determination of flavonoid content

The flavonoid content was determined by using aluminium chloride method (Chang, 2002). The reaction mixture consisting in a final volume of 3ml, 1.0ml of various extracts 1.0ml of methanol, 0.5ml of (1.2%) aluminium chloride and 0.5ml (120 mM) potassium acetate was incubated at room temperature for 30 min.

The absorbance of all the samples was measured at 415nm. Quercetin was used as positive control (Ghasemi, 2009; Kaneria, 2009).

Flavonoid content is expressed in terms of Quercetin equivalent (mg g^{-1} of extracted compound).

Antioxidant activity

DPPH free radical scavenging activity

The DPPH assay was performed to determine the free radical scavenging potential of various extracts (Azlim, 2010). 1ml of 0.1 mM DPPH in methanol was mixed with different concentrations of various extracts (3ml) and standards. The solution is mixed vigorously and incubated in darkness for 30 min. The DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical which is absorbing at 517nm will be reduced in the presence of antioxidant compounds contained in the extracts. The extracts with more potential shows higher free radical scavenging i.e., the lower absorbance at 517 nm is measured.

The percentage of scavenging was calculated as follows:

% scavenging = (1-A sample/A control) x 100

Where,

A sample – The absorbance measured in the presence of extracts

A control – The one measured in absence of extract.

Reducing power assay

The reducing power assays for the two extracts was determined by the method of Kalaivani (2010). 1ml of different concentrations of various leave extracts was mixed with phosphate buffer (2.5 ml, 2 M, pH 6.6) and potassium ferricyanide (2.5 ml, 1%) and the mixture was incubated at 50ºC for 20 min. 2.5 ml of trichloroacetic acid (TCA, 10%) was added to the mixture which was then centrifuged at 5000rpm for 15min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl3 (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Substances, which have reducing potential, react with potassium ferricyanide (Fe3+) to form potassium ferrocyanide (Fe2+), which in turn react with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm. Thus if the sample has antioxidant properties, the absorption at 700 nm will increase with the concentration of extract.

Biological activities

Microorganism and culture conditions

The bacterial pathogens used for the study such as *Staphylococcus aureus, Bacillus subtilis* for Gram positive, *Escherichia coli, and Proteus vulgaris* for Gram negative microorganisms. The fungal pathogens includes *Aspergillus niger* and *Aspergillus fumigatus.* All the bacterial and fungal pathogens were purchased from Microbial Type Culture Collection (MTCC), Chandigarh, India. All the microbial pathogens were revived from glycerol stocks at 80°C. After bringing it at room temperature, the bacterial and fungal cultures were sub-cultured for activity assay in nutrient broth (NB) and Sabourdaud's dextrose broth (SDB) by incubating at 37°C for 24 h and 48h for fungal strains.

Antibacterial and antifungal activities

Agar well diffusion method

The microbial growth inhibitory potential of two extracts of *Aerva lanata* were determined by using the agar well diffusion method (Sulaiman, 2012). The pre-inoculated bacterial and fungal pathogens were uniformly spread on the Muller Hinton Agar (MHA) and Sabourdaud's Dextrose Agar (SDA) using a sterile cotton swab. Wells were made into the media using a sterile cork borer. Various extracts were dissolved in 10% DMSO to a final concentration of 1mg/ml. 100µl of the various leave extracts were transferred into the well. The standard drugs like Ciprofloxacin for bacteria and Nystatin for fungal were used as positive drug control and 10% DMSO was used as solvent control. The bacterial were incubated at 37°C for 24 h and fungal for 48 h and zones of inhibition were measured in mm after the incubation. The experiment was repeated thrice and average values were recorded for antimicrobial activity.

MIC determination

The minimum inhibitory concentration (MIC) of the extracts was determined for the test organisms on which various leave extracts showed potent antibacterial and antifungal activities (Ali-Shtayeh, 1997). The MIC assay is a technique used to determine the lowest concentration of a particular antibiotic needed to kill bacteria and fungi. 100µl of MHB and SDB was added 1 mL of varying concentration of the extracts and serially diluted to obtain the following final concentrations of extracts: 100mg/L, 50 mg/L, 25mg/L, 12.5 mg/L, 6.25 mg/L, 3.12 mg/L, 1.56 mg/L, 0.78mg/L and 0.39mg/L. Afterwards, 20µl of the test bacterial (*E.coli, B.subtilis, P.vulgaris and S.aureus*) and fungal (*Aspergillus fumigatus* and *Aspergillus niger*) organisms was introduced to the 96 well plates. Growth control (cells+ broth) and media control (only broth). Wells containing bacterial cultures were then incubated at 37°C for 24 hours and fungal for 48 hour. The MIC was defined as the lowest concentration (mg/L) of the various extracts in the wells showing no visible bacterial and fungal growth. The results were measured at 556nm using an ELISA microplate reader.

Antibiofilm activity

Biofilm inhibition assay

The ability bacteria to form biofilms were assayed as described by O'toole and Kolter (1998) with some modifications. In sterile 96-well tissue culture plates containing 50 µl of Mueller–Hinton broth (MHB) per well, a 50 µl of fresh bacterial *E.coli, B.subtilis, P.vulgaris and S.aureus*) suspension was added. Growth control (cells + broth), media control (only broth) and blank control (broth + extract) were included. After incubation at 37 °C for 48 h, the content of each well was gently removed by tapping the plates. The wells were washed with 200 µl of sterile saline to remove free-floating bacteria. Biofilms formed by adherent cells in plate were stained with 0.1% crystal violet and incubated at the room temperature for 20 minutes. Excess stain was rinsed off by thorough washing with deionized water and plates were fixed with 200 µl of 96% ethanol. Optical densities (OD) of stained adherent bacteria were measured at 630 nm using an ELISA microplate reader. All tests were performed in triplicate. The percentage of biofilm inhibition was calculated using the following formula:

% of biofilm inhibition = [(OD growth control – OD sample) / OD growth control] × 100.

The biofilm inhibition concentration (BIC50) was defined as the lowest concentration of extract that showed 50% inhibition on the biofilm formation (Chaieb, 2011).

RESULTS AND DISCUSSION

Phytochemical analysis

Preliminary phytochemical qualitative analysis of methanol and petroleum ether extracts of *Aerva lanata* confirmed the presence of phytoconstituents like saponin, phenolic, flavonoids, alkaloids, terpenoids and reducing sugar (Table 1).

Table 1: Preliminary phytochemical analysis of *Aerva lanata*

Phytoconstituent	Methanol	Petroleum ether
Protein	-	-
Carbohydrates	+	+
Phenols & Tannis	+	+
Flavonoids	+	+
Saponins	+	+
Glyosides	-	-
Alkaloids	+	+
Fats and oils	-	-
Terpenoids	+	+

(+) presence: (-) absence

Determination of total phenolic and flavonoid contents

The total phenolic content for methanol and petroleum ether extracts of plant were determined by Folin Ciocalteu's reagent is expressed in terms of gallic acid equivalent (Standard). The reagent is formed from a mixture of phosphotungstic acid and phosphomolybdic acid which after oxidation of the phenols, is reduced to a mixture of blue oxides of tungsten and molybdenum. The blue coloration produced has a maximum absorption in the region of 750 nm and proportional to the total quantity of phenolic compounds originally present. The plant which shows higher polyphenolic (flavonoids & phenolic) compounds, biosynthesized in the plant sample are responsible for potential free scavenging radical and also effective for the prevention of various diseases (Zhang, 2009).

It shows the presence of total phenolic and flavonoid content of two extracts of *Aerva lanata* found to be higher potential antioxidant activity of the leave extracts (Table 2). The phenolic content are present in both methanol and petroleum ether extracts. The methanol extracts shows the maximum amount of flavonoid content followed by petroleum ether extract.

Among these plant, total flavonoid content is more than the phenolic content.

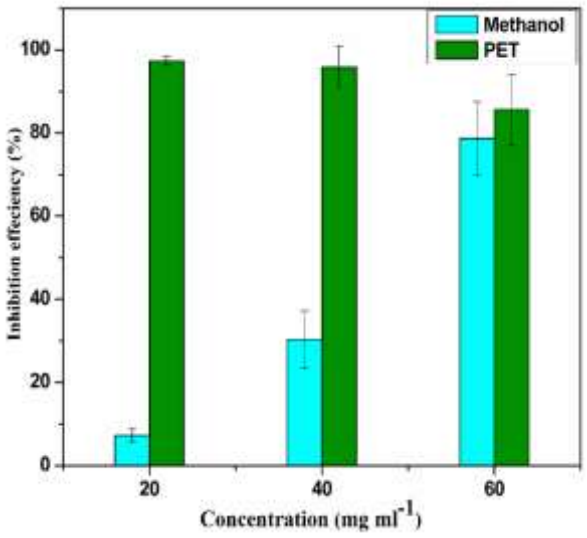
Antioxidant activity

DPPH scavenging assay

Free radicals such as atom or molecules that have at least one unpaired electrons which usually increased the chemical reactivity. DPPH is a stable free radical and that can go and bind to an electron or hydrogen radical to become a stable diamagnetic molecules (Bijaya, 2013). Free radical are unpaired molecules they can react with other molecules and causes cell damage or DNA mutation. These type of molecules are called as antioxidants to protect against the free radical damage and their action permit to ensure a balance between the production and disruption of free radical.

The antioxidant activity of two extracts of *Aerva lanata* is expressed in terms of percentage of inhibition (%). Among the different solvent extracts tested, the methanol extract of *Aerva lanata* were evaluated the strongest capacity for neutralization of DPPH free radical scavenging activity.

The methanol extract of *Aerva lanata* shows the potential antioxidant activity which increases with the increasing concentration of the extract (Fig.1). The moderate capacity to inhibit the DPPH radicals was determined for petroleum ether extract.

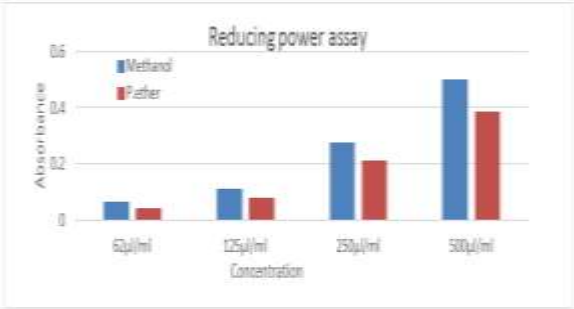


Values are expressed M ± S.E.M in Triplicates

Figure 1: Graphical representation of DPPH free radical scavenging assay of various leaves extracts of *Aerva lanata*

Reducing power assay

In this assay the yellow colour of the test solution changes to various colour green and blue depends upon the reducing power of each compound. The presence of free radical (antioxidant) which have reduction potential, react with potassium ferricyanide (Fe3+) to form potassium ferrocyanide (Fe2+), which then reacts with ferric chloride to form ferrous complex that has an absorption maximum at 700 nm. The methanol extracts of *Aerva lanata* shows increased with the increase concentration of extract than petroleum ether extract (Fig.2). Radicals that increase the reducing power values and percentage of antioxidant activity in DPPH spectrophotometric assay is presumed to have antioxidant activities.



Values are expressed in M ± SD

Figure 2: Graphical representation of reducing power assay for various leaves extracts of *Aerva lanata*

***In-vitro* Biological activities**

Antibacterial Assay

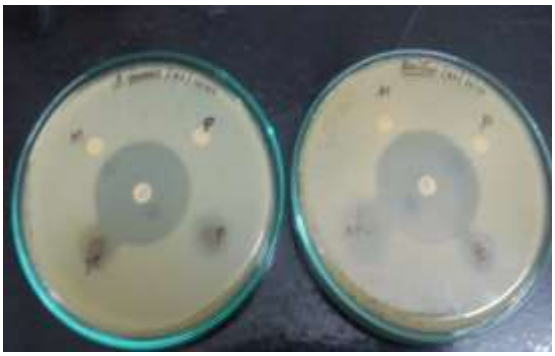
The antimicrobial activity of agar well diffusion method is widely accepted method. Preliminary screening for the antibacterial activity of two leaf extracts of *Aerva lanata* was performed with various pathogens of Gram positive (*S.aureus* and *B.subtilis)* and Gram negative (*P.vulgaris* and *E.coil)* bacterial organisms. The potential of antimicrobial activity shows the methanol and petroleum ether extracts of plant were evaluated according to their zone of inhibition against the various bacterial pathogens and the zone of inhibition were compared with the standard drugs such as Ciprofloxacin (5mcg/disc) (Fig 3 A, B). The result of antimicrobial activity of methanol and petroleum ether leaf extracts of *Aerva lanata* against various bacterial pathogens were tabulated in (Table 3). It was found that both leaf extracts shows high significant zone of inhibition against *S.aureus* and *B.subtilis.* No significant zone of inhibition against the *P.vulgaris* and *E.coil.* Methanol and petroleum ether leaf extracts of *Aerva lanata* showed no zone of inhibition against fungal (*A.niger* and *A.fumigatus*) pathogens.

The various leaf extracts of *Aerva lanata* were evaluated for the antimicrobial and antifungal potential against bacterial and fungal pathogens were study using Minimum Inhibitory concentration (MIC). The MIC values of the methanol extract of *Aerva lanata* ranged against

B.subtilis was found to be 50-0.39µg/ml, while petroleum ether extract of *Aerva lanata* are ranges from 25-0.39 µg/ml against *P.vulgaris* (Table 4). The methanol and petroleum ether extracts of leaf against *A.niger* exhibited good activity in the range of 50 and 12.5µg/ml. However, *Aerva lanata* was observed to be less significant inhibitory against *A.fumigatus* pathogen.

Value M ± SEM: **a**: value expressed in millimetres.

(A)



(B)

M-Methanol: P-Petroleum ether: CIP- Ciprofloxacin

Figure 3: Antibacterial activity of two extracts of *A.lanata*, showing the zone of inhibition against the bacterial (A) *E.coil, P.vulgaris* and *(B) S.aureus, B.subtilis* pathogens

Antibiofilm activity

In-vitro antibiofilm formation is monitored by MTP assay using crystal violet dye and measured by UV spectrophotometrically. Planktonic cells forms biofilms by adhering to each other strong via formation of pili. Crystal violet dye are not only for strains, but also screens a very small amount of adhered molecules that alter biofilm formation. The first stage of biofilm development is by absorption of macromolecules to the surface followed by the attachment of bacteria. The bacteria used for antibiofilm activity have been selected based up on the antibacterial activity. After 72 h of treatment, the bacterial strains are exhibit 86%, 90%, 82%, and 88% biofilm formation at various concentration of methanol and petroleum ether extracts of *Aerva lanata.* The slow growth rate of biofilm formation is associated with organism to minimize the rate of antimicrobial susceptibility. The results shows that both the extracts showed stronger biofilm inhibitory activity against the *B.subtilis* and *S.aureus*. The remaining moderate biofilm inhibitory activity against the *E.coli* and *P.vulgaris.* The cefepime is control showed weak biofilm inhibitory activity against all the pathogens (Fig. 4).

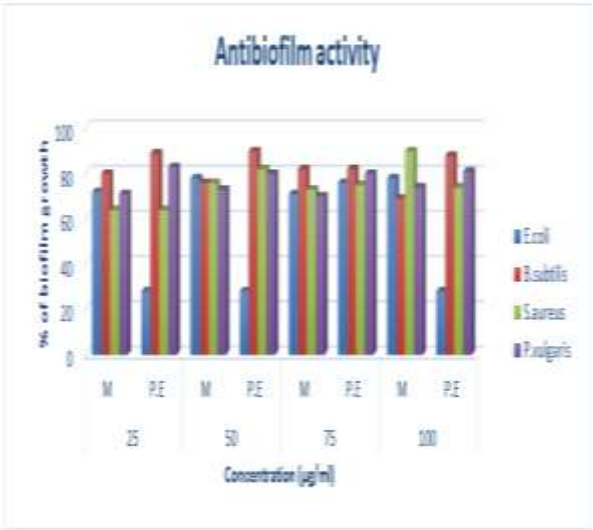


Figure 4: Graphical representation shows that inhibition of biofilm formation of *A.lanata* leaves extracts at various concentration.

GC-MS analyses

GC-MS play an important role for analysis of unknown components of plant origin. The eluted component is detected in the mass detector. The spectrum of the unknown component can be predicated with the help of GC-MS library and also determined the name, molecular weight, structure, molecular formula, retention time and peak area (%). GC-MS analyses of petroleum ether extracts of *Aerva lanata* shown the identification of seven chemical compounds (Table 5) appearances more similarity with the standard mass spectrum in the library (Fig 5). The GC MS outline of methanol leaf extract of *Aerva lanata* (Fig. 6) shown the most abundant phytocompound found to be quinoline (Table 6). GC MS analysis helps towards comprehending the phytocompounds with remedial values in *Aerva lanata.*

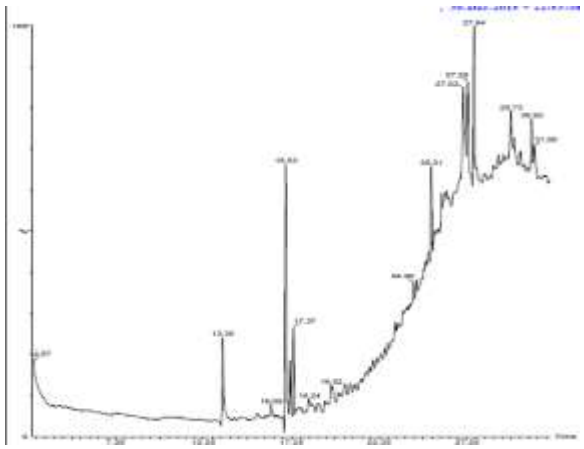


Figure 5: Chromatogram of Petroleum ether leaf extract of *Aerva lanata*

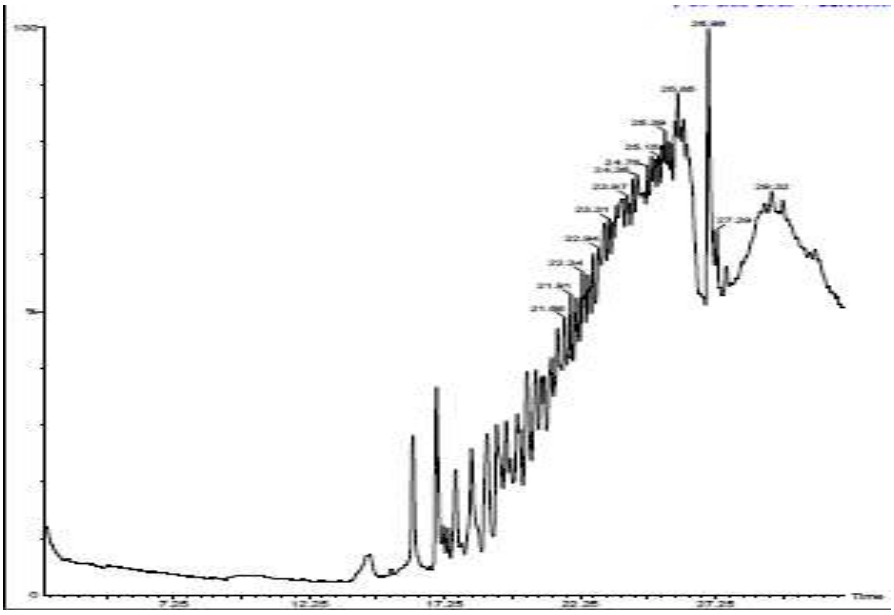


Figure 6: Chromatogram of Methanol leaf extract of *Aerva lanata*

Table 2: Total phenolic content and flavonoid content of two extracts of *Aerva lanata*

Extracts	Total phenolic content (mg eq. of GA/g of extract)	Total flavonoids content (mg eq. of Quercitin/g of extract)
Methanol	0.1763 ± 0.0049	3.0806 ± 0.6526
Petroleum ether	0.183 ± 0.0008	1.1689 ± 0.3286
Standard	3.8435 ± 0.0873	1.7696 ± 0.3051

Values are Mean ± S.E.M in triplicates

Table 3: Zone of inhibition by various leaf extracts of *Aerva lanata* by well diffusion method

Pathogens	Methanol	P.ether	Ciprofloxacin
S.aureus	12.3[a] ± 0.88	14 ± 1	32 ± 1.73
B.subtilis	19.3 ± 2.33	11.3 ± 0.88	35.3 ± 2.02
P.vulgaris	-	-	29 ± 3.17
E.coil	-	-	33 ± 0.57

Table 4: Minimum Inhibitory Concentration (MIC) of various leaf extracts of *Aerva lanata* against bacterial and fungal pathogens

Bacterial pathogens	Methanol	P.ether	CIP
S.aureus	0.80 ± 0.1067	0.71 ± 0.0483	0.80 ± 0.0115
B.subtilis	0.38 ± 0.0764	NG	0.09 ± 0.0237
P.vulgaris	0.60 ± 0.0899	0.64 ± 0.0605	NG
E.coil	0.49 ± 0.077	0.70 ± 0.0476	0.70 ± 0.0476
Fungal pathogens	**Methanol**	**P.ether**	**Nystatin**
Aspergillus niger	1 ± 0.1733	1.00 ± 0.1733	0.19 ± 0.0078
Aspergillus fumigatus	0.65 ± 0.1625	0.06 ± 0.0165	0.14 ± 0.0513

Values are expressed M ± SD of triplicates: NG- Negative: CIP-Ciprofloxacin

Table 5: GC-MS peaks of major volatile bioactive metabolites with molecular structure of petroleum ether leaf extract of *Aerva lanata*

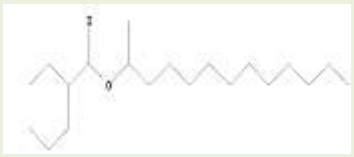
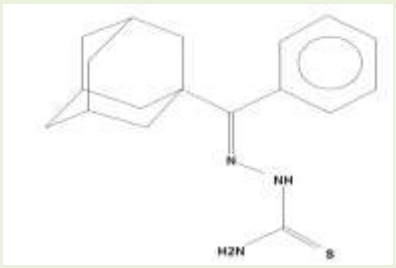
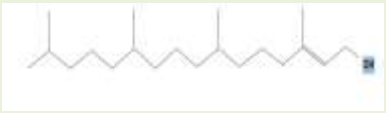
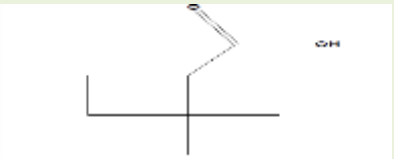
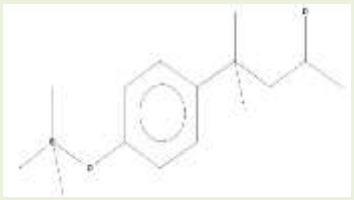
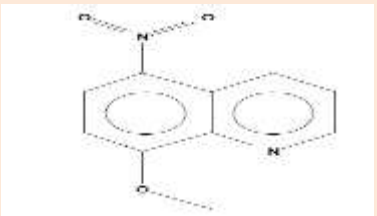
Compound Name	M.W	REV	For	Formula	Structures
Cyclohexanecarboxylic acid, 2-tridecyl ester	310	725	390	C20H38O2	
1-[.Alpha.-(1-Adamantyl)Benzylidene]	313	677	411	C18H23N3S	
3,7,11,15-Tetramethyl-2-hexadecen-1-OL	296	924	887	C20H40O	
Octasiloxane	578	690	338	C16H50O7Si8	
Cyclobutanecarboxylic Acid, 2,2-dimethyl	128	740	329	C7H12O2	
2,6,10,14,18,22 Tetracosahexaene, 2,6,10,15,19,23-Hexamethyl	410	866	616	C30H50	
Trimethyl[4-(2-Methyl-4-Oxo2 Pentyl)Phenoxy]Silane	264	765	301	C15H24O2Si	

Table 6: GC-MS peaks of major volatile bioactive metabolites with molecular structure of methanol leaf extract of *Aerva lanata*

Compound Name	M.W	REV	For	Formula	Structures
Quinoline, 8-Methoxy-5-Nitro	204	632	387	C10H8O3N2	

CONCLUSION

The present work clearly indicated that leaf extracts of *A.lanata* possessed high ability of free radical scavenging assay. The methanol extract of plant are found to be more potential extract compared to the other extract, which may be attributed to the high flavonoid content and phenolic content of the extract. The present GC MS analysis of these leaf extracts correlates with the phytochemical compounds which plays an important role for the evaluation of *in vitro* antioxidant, antibacterial and antibiofilm activities. Further studies are required to isolated and characterize the bioactive compounds and their mechanism of action which may lead to the development of novel compounds for anti-inflammatory activity.

REFERENCES

1. Lakshmi Gaja S, Lakshmi Vijaya S, Rajeswari Devi V. *International research journal of pharmacy*. 3(1), 2012, 28.
2. Krishnan Apai G, Rai VK, Nandy KC, Meena S, Dey PK, Tyagi LK, *International Journal of pharmaceutical sciences and drug research*. 1(3), 2009, 191.
3. Yoga Narasimhan SN, Bhat AV, Togunashi VS. Medicinal plants from Mysore District, Karanataka. *Indian drug Pharmaceut Ind.* 14, 1979, 7-22.2.
4. Iyengar MA. Study of crude drugs. Manipal power press, Manipal, India. 8th ed, 1995, 2.
5. Nevin KG, Vijayammal PL. Effect of *Aerva lanata* against Hepatotoxicity of carbon tetrachloride in rats. *Environmental Toxicol Pharmacol.* 20, 2005, 471-477.
6. Parekh J, Chanda S. *In-vitro* antibacterial activity of the crude methanol extract of *Woodfordia fructicosa* Kurz. Flower (Lythraceae). *Braz. J. Microbial.* 38, 2007, 204-207.
7. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, Chapman and Hall, London, UK, 1984.
8. Trease GE, Evans WC. Pharmcognosy, 15th Ed. London 2002: Saunders Publishers.
9. Mc Donald S, Prenzeler PD, Autolovich M, Robards K. Phenolic content and antioxidant activity of Olive extracts. *Food Chem.* 73, 2001, 73-84.
10. Chang C, Yang M, Wen H, Chern J. Estimation of Total flavonoid content in *propolis* by two complementary colorimetric methods. *J. Food Drug Anal.* 10, 2002, 178-182.
11. Ghasemi K, Ghasemi YD, Ebrahimzadeh MA. Antioxidant activity, phenol and flavonoid contents of 13 citrus species peels and tissues. *Pak. J.Pharm.Sci.* 22, 2009, 277-281.
12. Azlim A, Ahmed JK, Syed Zahir I, Mustapha SK, Aisyah MR, Kamarul RK. Total phenolic content and primary antioxidant activity of Methanolic and Ethanolic extracts of aromatic plants leaves. *International Food Research Journal.* 17, 2010, 1077-1084.
13. Kalaivani T, Lazar M. Free radical scavenging activity from leaves of *Acacia nilotica (L)* Wild. Ex Delile, an Indian medicinal tree. *Food and Chemical Toxicology*. 48, 2010, 298-305.
14. Sulaiman A, Milton W, Khalid A. Antibacterial potential of honey from different orgins: A comparsion with Manuka honey. *Journal of Microbiology, Biotechnology and Food Science.* 5(1), 2012, 1328-1338.
15. Ali Shtayeh MS, Al Nuri MA, Yaghmour RM, Faidi YR. Antimicrobial activity *Micromeria nervosafrom* the Palestine area, *Journal of Ethanopharmacology*. 58(3), 1997, 143-147.
16. Otoole GA, Kolter R. Initiation of biofilm formation in *Pseudomonas fluorescens WCS365* proceeds via multiple, convergent signalling pathways: a genetic analysis. *Mol. Microbiol.* 28, 1998, 449-461.
17. Chaieb K, Kouidhi B, Jrah H, Mahdouani K, Bakhrouf A. Antibacterial activity of Thymoquinone, an active principle of *Nigella sativa* and its potency to prevent bacterial Biofilm formation. *BMC Complement. Altern.med*. 11, 2011, 29.
18. Zhang Y, Wang ZZ. Phenolic compostion and antioxidant activities of two Phlomis species: a correlation study, Comptes rendus Biologies. 332(9), 2009, 816-826.
19. Bijaya LM, Bikash B. Antioxidant capacity and Phenolics content of some Nepalese Medicinal Plants. *Am J Plant Sci.* 4, 2013, 1660-1665.

Source of Support: Nil, **Conflict of Interest:** None.