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Research Article

Evaluation of Antimicrobial Activity and Characterization of Bioactive Compound from Leaf and Inflorescence Extract of *Lavandula Bipinnata* by FT-IR And 1H-NMR

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ABSTRACT
Fresh leaf, inflorescence and stem of *Lavandula bipinnata*, collected from Rajpipla of Gujarat, India and were screened for antimicrobial activity. All the collected tissue were extracted sequential by using four different solvent n-Hexane, ethyl acetate, methanol and distilled water to study their antibacterial activity against selected six gram positive and gram-negative bacteria. The n-Hexane extract of Inflorescence and Leaf produced a maximum zone of inhibition against selected Gram positive and Gram-negative bacteria compared to ethyl acetate, methanol and D/W extract. Based on the antibacterial activity of Inflorescence n-Hexane exhibit highest antibacterial activity against PS (30mm) and BC (26mm) and n-Hexane Leaf extract (13mm) against PS.MIC was observed is in the range of 0.5mg/ml to >8mg/ml whereas MBC values is in range the of 0.5mg/ml to 8mg/ml against selected bacterial strains. Based on the FTIR result *Lavandula bipinnata* have high amount of phenolic and terpenoids compound and have pronounced antimicrobial activity. The FTIR and 1H-NMR results clearly show that leaf and inflorescence extract of *Lavandula bipinnata* is an interesting source for biologically active compounds i.e. cortisol acetate, Ethyl(R)-3-(6-amino-9H-purin-9-yl)-2-hydroxypropionate.
Keywords: Antimicrobial**,** MIC, MBC, FTIR and 1H-NMR

INTRODUCTION

Natural products from medicinal plants, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. Due to an increasing demand for chemical diversity in screening programs, seeking therapeutic drugs from natural products, interest in plant chemistry has grown throughout the world. Research studies leading to extraction, isolation, identification and biological study of plant constituents have now formed the major field of the study. Pharmaceutical industries started to use crude extracts of medicinal plants for manufacturing drugs[1]. The acceptance of traditional medicine as an alternative form of health care couple with development of microbial resistance to the available antibiotics has led researchers to investigate the antimicrobial activity of medicinal plants[2-4]. Presence of tannins, flavonoids, terpenoids, saponins, steroids, cardiac glycosides, volatile oils, alkaloids, anthraquinones and other phenolic have been reported to have antimicrobial activities[5-7].

Lavandula bipinnata O.Ktze**.** Synonym *L. burmanni* Benth. belongs to family Lamiaceae it is distributed in tropical and subtropical region. It was found in review literature that *Lavandula bipinnata* is useful in stings or bites of poisonous animals act as an antidote against poison and essential oil obtained also have antibacterial property. The paste of roots is applied over the sting of wild animals, the grounded leaves are given for inhalation to the person who has been stung by a serpent in order to prevent him from falling into sleep[8]. In combination with other herbs, it is used internally in treatment of rheumatism. Leaf paste applied on decayed tooth to reduce pain[9]. Paste of roots are used externally for stings and bites of poisonous animals. Furthermore, they exhibit antibacterial, antifungal, antispasmodic, and antioxidant activity, and also regulate digestive activity. Due to the biologically active substances present in them, herbs have antimicrobial, antioxidant, and therapeutic properties, and may be extensively used, as they are effective as synthetic drugs. The plant belongs to this genus have a number of beneficial properties for the human body. Besides its application in herbal treatment, lavender is widely used in the cosmetic, perfume, food, and aroma therapeutic industries[10-11].

Therefore, the organisms used in this study are known to cause dysentery, fever, diarrhoea, wound, tooth decay, ulcers, typhoid fever and various stomach related problems [12,13]. The purpose of this study is to identify and characterize the bioactive compound (s) from the leaves and inflorescence of *Lavandula bipinnata*. In this paper, we report the isolation and characterization of known compounds from the plant namely cortisol acetate, Ethyl(R)-3-(6-amino-9H-purin-9-yl)-2-hydroxypropionate.

MATERIALS AND METHODS

Plant material

The fresh leaf, stem, and Inflorescence of *Lavandula bipinnata* Lam. were collected from Rajpipala, Gujarat, India and identified by referring "Flora of Gujarat state" [14] and confirmed by Dr. A.S. Reddy (Plant Taxonomist) and Dr. Sandip Patel Department of Biosciences, Sardar Patel University, Vallabh Vidyanagar, Gujarat.

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Extract Preparation

The fresh leaf, stem, and Inflorescence were collected and washed thoroughly with running tap water to remove dirt particles. All the materials were dried at room temperature and powdered with grinder. Extract was prepared by infusion extraction method [15]. For sequential extraction 50 gm of dry powdered or fresh material of each sample was soaked in 250 ml n-Hexane at room temperature for 24 hours. Extracts were filtered through Whatman's filter paper no.1 and the filtrates were centrifuged at 3000 rpm for 10 minutes to remove solid debris. The supernatant was collected and concentrated by solvent recovering assembly (J-sil, India) and dried completely at room temperature and stored it in a refrigerator until further use.

The filtrate collected on filter paper was completely dried and resuspended sequentially into each of 250 ml ethyl acetate, methanol and distilled water at room temperature for 24 hours. The extract was filtered, and the filtrate was centrifuged at 3000 rpm for 10 minutes and the supernatants were collected. All the fractions were stored in a refrigerator until further use. The crude hot plant extracts were also prepared by soxhlet extraction method. About 20gm powdered plant material was uniformly packed into a thimble and extracted sequentially with 250ml of different solvents separately i.e. ethyl acetate, methanol and distilled water. The extraction process continued for 24 hrs or till the solvent in siphon tube of an extractor became colourless. The extract was then transferred to evaporating plate for drying completely at room temperature and stored it in a refrigerator until further use.

Selected microorganisms

12 bacterial strains used in the study, among these were six Gram-positive namely *Bacillus cereus* (ATCC 11778), *Bacillus subtilis* (ATCC 6051), *Staphylococcus aureus* (Isolated), *Staphylococcus epidermidis* (ATCC 155), *Micrococcus luteus* (ATCC 4698), *Enterococcus faecalis* (Isolated) and six Gram-negative bacteria *Escherichia coli* (ATCC 25922), *Salmonella typhi* (NCTC8394), *Salmonella paratyphi* (MTCC 735), *Pseudomonas aeruginosa* (ATCC 25668), *Klebsiella pneumoniae* (ATCC 15380), *Serratia marcescens* (Isolated). All the tested strains are reference strains and were collected from MTCC (Microbial type culture collection, Chandigarh), ATCC (American type culture collection, Manassas, Virginia) and NCTC (National collection of type culture). The bacterial cultures were grown on nutrient agar medium (Hi Media, pH 7.4) at 37ºC and were maintained at 4°C.

Antibacterial assay

In the present study, the antibacterial activities of leaf, stem and inflorescence crude extracts prepared in different solvents were screened by agar well diffusion method [16]. An inoculum size of 1×10 CFU/ml of bacteria which compared with 0.5 McFarland turbidity in a refrigerator for 30 minutes for pre-diffusion of plant extract and turbidity standards was used. Each extract of 100 µl (stock solution 100 mg/ml) was added in a previously marked sterile nutrient agar petriplates and the wells were punched with sterile cork borer and filled with each plant extract. Plates were placed then incubated at 37ºC for 24 hours. After incubation all the plates were examined and zone of inhibition (excluding well diameter in mm) was measured as a property of antimicrobial activity. Antibiotic such as Ciprofloxacin and Doxycycline (20µg/ml) as a positive control and 100% DMSO and solvents i.e. hexane, ethyl acetate and methanol as a negative controls.

Minimum inhibitory concentration (MIC)

In the present study, minimum inhibitory concentration (MIC) was evaluated by serial broth dilution method for the plant extracts showing more than 7mm to 30mm of inhibition. Density of bacterial suspension was maintained uniformly throughout the experiment at $1x10^8$ CFU/ml by comparing with 0.5 Mc Farland turbidity standards. 40µl of plant extract from stock solution (100mg/ml) was taken into the first dilution tube and added 960µl of nutrient broth and mixed well. 500µl of solution from first dilution tube was taken and added 500µl of nutrient broth into second tube, this step was repeated 5times and from last tube 500µl solution was discarded. Final volume was made upto 1ml by adding 500µl of test organism in each tube. The MIC was tested in the concentration range between 8mg/ml to 0.250mg/ml. Tubes were incubated at 37ºC for 24 hours in an incubator. 100µl (0.1%) 2,3,5 – triphenyl tetrazolium chloride solution as a growth indicator was incorporated in each tube to find out the bacterial inhibition and tubes were further incubated for 30 minutes at 37ºC. Bacterial growth was visualized when colorless 2, 3, 5-triphenyl tetrazolium chloride was converted into red color formazon in the presence of live bacteria. MIC assay was repeated thrice by using DMSO and nutrient broth as controls.

Minimum Bactericidal Concentration (MBC)

To determine the minimum bacterial concentration (MBC), l00µl of broth was collected from those tubes tested for determination of MIC which did not show any growth and spread on sterile nutrient agar plate for any bacterial growth. Plates were incubated at 37ºC for 24 hours. After incubation the concentration at which no visible bacterial growth was observed considered as the minimum bactericidal concentration [17].

Phytochemical Screening

Each fraction of the column eluted sample was subjected to TLC to find out the separation of single compound and confirmation from the fraction. Thin Layer Chromatography was performed on prepared plates with Silica gel F254 grade (Merck, Germany) as stationary phase. A one-dimensional ascending development technique was used to detect the constituents of an extract on TLC plate. Visual detection was done in daylight and under UV light at a wavelength of 254nm and 344 nm depending on the nature of compounds separated.

Column Chromatography

The silica gel (60-120 mesh, Chiti Chem, India) column was prepared in column (30x450 mm size) and dead space was packed with glass wool. Silica gel (75g) was equilibrated in mobile phase to form approximately 72cm column length and 4cm breadth. The packed column was allowed to settle for 12hr and then the column was washed twice with mobile phase. The ethyl acetate extract was loaded on the top of silica gel column and eluted with

increasing polarity of toluene: ethyl acetate and chloroform: methanol (100:0, 80:20, 60:40, 20:80, 0:100). Twenty fractions, each of 25ml were collected at flow rate of 1ml/min. The collected fractions were then concentrated using rotary vaccum evaporator under reduced pressure for spectral analysis.

Spectroscopic analysis

The isolated compounds were dissolved in deuterated methanol CD_3OD and 1H NMR spectra was recorded using a Bruker Avance 400 spectrometer (Bruker, Illinois, USA) operating at 100 MHz. Tetramethylsilane (TMS) was used as an internal standard. The chemical shift values were reported in ppm (δ) unit and the coupling constants (J) are in Hz. FT-IR spectra of the compounds were measured using IR grade potassium bromide (KBr). The compounds were separately mixed with 200mg KBr to obtain round disc with the help of hydraulic press. Round disc was later subjected to FT-IR in the range of 4000-400 cm^{-1} using Perkin Elmer spectrophotometer, spectrum instrument (Germany) with FT-IR paragon 1000 PC software.

RESULT AND DISCUSSION

Results of antimicrobial activity of *Lavandula bipinnata* extracts against Gram positive and Gram-negative isolates by the agar well diffusion method were shown on Table (1-4) respectively. The microbial susceptibility was collectively summarized in Table (5-12).

The n-Hexane inflorescence extract presented the highest antimicrobial activities, as it inhibited most 11 isolates (out of 12 isolates used) of the bacteria under the study whether Gram positive or Gram negative with inhibition zone ranging from (14-30mm) Fig (1) except *Salmonella para-typhi* which showed no any susceptibility to this extract On the other hand, the ethyl acetate, extracts from the inflorescence show low antimicrobial activity (5mm to 9mm) zone of inhibition only 11 isolates (out of 12 isolates used), Differences in polarity among the various solvents are perhaps responsible for the differences in solubility of plant active principles, hence variation in degree of activity. The methanol inflorescence extract showed no any activity against Gram-positive bacteria & Gram-negative bacteria but inhibited only 4 isolates (out of 8 isolates used) while the distilled water extract showed no any activity against Gram-negative bacteria but inhibited 1 Gram-positive bacteria (out of 6 isolates used). The n-Hexane inflorescence activities were more in comparison with the reference antibiotics used in the study.

Drastically, n-Hexane leaf extract produced a good antimicrobial activity against selected bacterial strains with inhibition zones range (6 mm -13 mm) Fig (2) against Gram-positive or Gram-negative bacteria with not exhibited any activity against *Salmonella paratyphi*. Ethyl acetate and methanol leaf extract displayed least to no activity against all bacterial strains whereas no activity was observed in distilled water leaf extract against all tested organisms. Whereas Stem n-Hexane, ethyl acetate, methanol and distilled water extracts exhibited least or no activity against other test organisms. Antimicrobial activity in *Lavandula stoechas* essential oils showed similar results against SA, EC, *Proteus mirabilis*, KP, PS, SE, EN, BS [18]. Antibacterial efficacy examined using distilled water, methanol, ethanol, acetone, chloroform whole plant extract against SA, PS and EC similarly ethanol, acetone, methanol extracts showed antibacterial activity against SA and EC except PS[19]. Among the different extracts tested n-Hexane inflorescence extract showed better results against most of the organisms tested in this study.

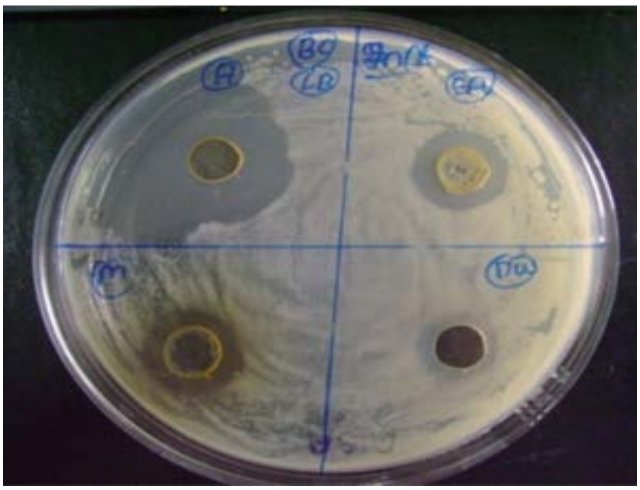


Figure 1: Antibacterial activity of *Lavandula bipinnata* inflorescence extract against tested bacteria *Bacillus cereus*

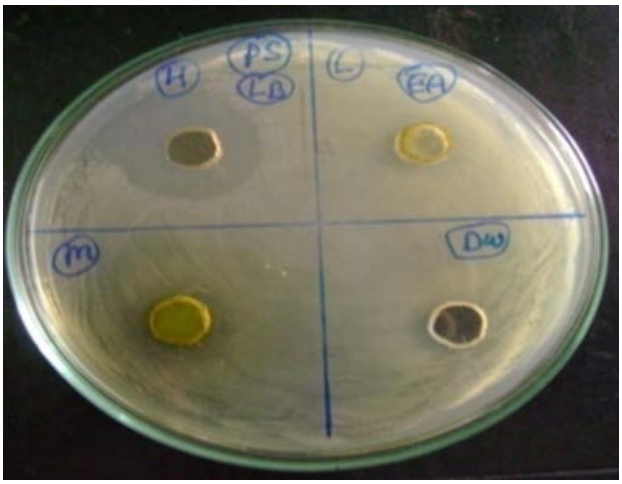


Figure 2: Antibacterial activity of *Lavandula bipinnata* leaf extract against tested bacteria *Pseudomonas aeruginosa*

The MIC values of n-Hexane leaf extract was observed at 0.5 mg/ml against SP, 1mg/ml against SM, SA, 2mg/ml against BC, 4mg/ml against KP, 8mg/ml against ST, EC, >8mg/ml against BS, SE and EN (Table 5). The n–Hexane inflorescence extract also exhibited similar MIC values at 1mg/ml against SM and PS, 2mg/ml against ML and BC, 4mg/ml against SE, SA, EC, ST and SP, 8mg/ml against KP, >8mg/ml against BS and EN (Table 5). n- Hexane stem extract exhibited MIC values at 4 mg/ml against KP and 8 mg/ml against ST and >8 mg/ml against SP (Table 5). Ethyl acetate inflorescence extract showed MIC values

at against 0.5 mg/ml against KP, 2 mg/ml against BS and ST, 4 mg/ml against BC and PS, 8mg/ml against SE and SM, >8 mg/ml against ML and EN. Ethyl acetate stem extract also showed MIC value at 2 mg/ml against ST (Table-6). Methanol leaf extract exhibited MIC value at 4 mg/ml against BC (Table 7). Methanol inflorescence extract showed MIC values at 4 mg/ml against BC and PS (Table 7).

The MBC was performed for n-Hexane, ethyl acetate, methanol and distilled water extract among all this extract susceptibility was observed in n-Hexane leaf extract at 4 mg/ml against SM (Table 9) and ethyl acetate Inflorescence extract at 2 mg/ml against PS & 8mg/ml against BC (Table 10) whereas most selected bacterial strains are resistant to selected methanol and distilled water extract. The MBC value is lower than MIC value where bacteria are susceptible against selected bacterial strains. Among the different extracts test hexane leaf and ethyl acetate inflorescence extracts have shown lower MIC and MBC values than other extracts against the tested bacterial strains.

The present study showed that Gram-negative bacteria are more susceptible than Gram-positive bacteria. This is due to the fact that leaf and inflorescence extract and their components exhibits hydrophobicity, which enables them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering it more permeable [20-23]. Probably, extensive leakage from bacterial cells or the exit of critical molecules and ions led to bacterial death [24]. Demonstration of low MIC and MBC value is an indication that the phytoconstituents of the plant have therapeutic potential

Antibacterial compounds of n-Hexane and ethyl acetate extracts may be non-polar in nature because they were detected at 254 nm and 546nm for the presence of terpenoids and phenols respectively Fig (3A-F). This specific compound can be separated by suitable mobile phase for specific active principles successive column chromatography but quantitatively only three compounds were isolated, purified and characterized from Inflorescence extract of *Lavandula bipinnata* by column chromatography. Isolated components were further purified by recrystallization. Compounds were further identified and confirmed by 1 H-NMR and IR, and spectra of the compounds. The compounds were characterized on the basis of spectroscopic analysis and compared with reported data in literature.

Identification of the bioactive compounds isolated from selected plant extracts was carried out by FT-IR and ^{1}H NMR spectroscopy. The FT-IR spectrum identifies functional groups and bonding present in the molecule. NMR spectra were recorded using a Bruker 400 NMR Spectrometer. Isolated compounds were dried, weighed (5-10mg) and dissolved in (2ml) deutrated solvent (Merck) for NMR. ^{1}H NMR spectrums of compounds were recorded at 25ºC to confirm its structure Fig (4-7). Compounds were dissolved in deutrated methanol for the purpose. Chemical shifts were reported as δ values relative to an internal reference tetramethylsilene (TMS), and coupling constants were reported in Hertz.

Table 1: Antibacterial activity in crude n-Hexane extract of selected plant species

Plant Name	**Part used**	**Extract**	**Zone of Inhibition**											
			Gram positive						**Gram negative**					
			BC	BS	EN	ML	SA	SE	EC	KP	PS	SM	SP	ST
Lavandula bipinnata	L	CH	9	9	8	6	10	9	8	10	13	12	-	10
	INF		26	16	18	19	17	17	18	16	30	14	7	14
	ST		6	6	-	-	-	-	-	5	7	7	-	7
Ciprofloxacin (20 µg/ml)			11	10	12	9	14	11	7	10	9	22	8	14
Doxycycline (20 µg/ml)			14	12	9	8	11	5	15	13	4	20	11	19

Table 2: Antibacterial activity in the crude ethyl acetate extract of selected plant species.

Plant Name	**Part used**	**Extract**	**Zone of Inhibition**											
			Gram positive						**Gram negative**					
			BC	BS	EN	ML	SA	SE	EC	KP	PS	SM	SP	ST
Lavandula bipinnata	L	CEA	-	-	-	-		5	-	4	-	4	-	-
	INF		9	7	9	7	6	8	5	8	9	8	-	7
	ST		-	-	-	-	-	-	-	-	4	-	-	7
Ciprofloxacin (20 µg/ml)			11	10	12	9	14	11	7	10	9	22	8	14
Doxycycline (20 µg/ml)			14	12	9	8	11	5	15	13	4	20	11	19

Table 3: Antibacterial activity in crude methanol extract of selected plant species.

Plant Name	**Part used**	**Extract**	**Zone of Inhibition**	
			Gram positive	**Gram negative**

			BC	BS	EN	ML	SA	SE	EC	KP	PS	SM	SP	ST
Lavandula bipinnata	L	CM	-	7	-	-	-	-	-	4	4	2	-	-
	INF		8	-	-	-	-	4	-	6	7	-	-	-
	ST		7	-	-	-	-	-	-	-	8	5	-	-
Ciprofloxacin (20 µg/ml)			11	10	12	9	14	11	7	10	9	22	8	14
Doxycycline (20 µg/ml)			14	12	9	8	11	5	15	13	4	20	11	19

Table 4: Antibacterial activity in the crude distilled water extract of selected plant species.

Plant Name	**Part used**	**Extract**	**Zone of Inhibition**											
			Gram positive						**Gram negative**					
			BC	BS	EN	ML	SA	SE	EC	KP	PS	SM	SP	ST
Lavandula bipinnata	L	CD	-	-	-	-	-	-	-	-	-	-	-	-
	INF		-	-	-	-	-	4	-	-	-	-	-	-
	ST		-	-	-	-	-	-	-	6	-	-	-	7
Ciprofloxacin (20 µg/ml)			11	10	12	9	14	11	7	10	9	22	8	14
Doxycycline (20 µg/ml)			14	12	9	8	11	5	15	13	4	20	11	19

BC-*Bacillus cereus; BS-Bacillus subtilis***; EN**-*Enterococcus faecalis***; ML**- *Micrococcus luteus***; SA**- *Staphylococcus aureus***; SE**-*Staphylococcus epidermidis***; EC**-*Escherichia coli***; KP**-*Klebsiella pneumoniae***; PS**-*Pseudomonas aeruginosa***; SM**-*Serratia marcescens***; SP**-*Salmonella paratyphi;* **ST**-*Salmonella typhi* **L**-Leaf; **INF**-Inflorescence; **ST**-Stem **CH**-Cold n-Hexane; **CEA**- Cold ethyl acetate; **CM**-Cold methanol; **CD**- Cold distilled water

Table 5: Minimum inhibitory concentration of effective n-Hexane plant extracts.

Plant Name	**Part used**	**Extract**	**MIC (mg/ml)**											
			Gram positive						**Gram negative**					
			BC	**BS**	**EN**	**ML**	**SA**	**SE**	**EC**	**KP**	**PS**	**SM**	**SP**	**ST**
Lavandula bipinnata	L	CH	2	>8	>8	>8	1	>8	8	4	-	1	0.5	8
	INF		2	>8	>8	2	4	4	4	8	1	1	4	4
	ST		-	-	-	-	-	-	-	4	-	-	>8	8

Table 6: Minimum inhibitory concentration of effective ethyl acetate plant extracts

Plant Name	**Part used**	**Extract**	**MIC (mg/ml)**											
			Gram positive						**Gram negative**					
			BC	**BS**	**EN**	**ML**	**SA**	**SE**	**EC**	**KP**	**PS**	**SM**	**SP**	**ST**
Lavandula bipinnata	L	CEA	-	-	-	-	-	-	-	-	-	-	-	-
	INF		4	2	>8	>8	-	8	-	0.5	4	8	-	2
	ST		-	-	-	-		-	-	-	--		-	2

Table 7: Minimum inhibitory concentration of effective methanol plant extracts

Plant Name	**Part used**	**Extract**	**MIC (mg/ml)**											
			Gram positive							**Gram negative**				
			BC	**BS**	**EN**	**ML**	**SA**	**SE**	**EC**	**KP**	**PS**	**SM**	**SP**	**ST**
Lavandula bipinnata	L	CM	-	4	-	-	-	-	-	-	-	-	-	-
	INF		4	-	-	-	-	-	-	-	4	-	-	-
	ST		-	-	-	-	-	-	-	-	1	-	-	-

Table 8: Minimum inhibitory concentration of effective distilled water plant extract

Plant Name	Part used	Extract	MIC (mg/ml)											
			Gram positive						Gram negative					
			BC	BS	EN	ML	SA	SE	EC	KP	PS	SM	SP	ST
Lavandula bipinnata	L	CD	-	-	-	-	-	-	-	-	-	-	-	-
	INF		-	-	-	-	-	-	-	-	-	-	-	-
	ST		-	-	-	-	-	-	-	-	-	-	-	>8

BC-*Bacillus cereus; BS-Bacillus subtilis***; EN**-*Enterococcus faecalis***; ML**- *Micrococcus luteus***; SA**- *Staphylococcus aureus***; SE**-*Staphylococcus epidermidis***; EC**-*Escherichia coli***; KP**-*Klebsiella pneumoniae***; PS**-*Pseudomonas aeruginosa***; SM**-*Serratia marcescens***; SP**-*Salmonella paratyphi***; ST**-*Salmonella typhi* **L**-Leaf; **INF**-Inflorescence; **ST**-Stem*;* **CH**-Cold n-Hexane; **CEA**- Cold Ethyl acetate; **CM**-Cold methanol; **CD**- Cold distilled water

Table 9: Minimum bactericidal concentration of effective n-Hexane plant extracts.

Plant Name	Part used	Extract	MBC (mg/ml)											
			Gram positive						Gram negative					
			BC	BS	EN	ML	SA	SE	EC	KP	PS	SM	SP	ST
Lavandula bipinnata	L	CH	R 2	-	-	-	R 1	-	R 8	R 4	-	S 4	R 4	R 8
	INF		R 2	-	-	R 2	R 4	R 4	R 4	R 8	R 1	R 1	R 4	R 4
	ST		-	-	-	-	-	-	-	R 4	-	-	-	R 8

Table 10: Minimum bactericidal concentration of effective ethyl acetate plant extracts

Plant Name	Part used	Extract	MBC (mg/ml)											
			Gram positive						Gram negative					
			BC	BS	EN	ML	SA	SE	EC	KP	PS	SM	SP	ST
Lavandula bipinnata	L	CEA	-	-	-	-	-	-	-	-	-	-	-	-
	INF		S 8	R 2	-	-	-	R 8	-	R 0.5	S 2	8 R	-	2 R
	ST		-	-	-	-		-	-	-	-		-	2 R

Table 11: Minimum bactericidal concentration of effective methanol plant extracts

Plant Name	Part used	Extract	MBC (mg/ml)											
			Gram positive							Gram negative				
			BC	BS	EN	ML	SA	SE	EC	KP	PS	SM	SP	ST
Lavandula bipinnata	L	CM	-	R 4	-	-	-	-	-	-	-	-	-	-
	INF		R 4	-	-	-	-	-	-	-	R 4	-	-	-
	ST		-	-	-	-	-	-	-	-	R 1	-	-	-

Table 12: Minimum bactericidal concentration of effective distilled water plant extract

Plant Name	Part used	Extract	MBC (mg/ml)											
			Gram positive						Gram negative					
			BC	BS	EN	ML	SA	SE	EC	KP	PS	SM	SP	ST
Lavandula bipinnata	L	CD	-	-	-	-	-	-	-	-	-	-	-	-
	INF		-	-	-	-	-	-	-	-	-	-	-	-
	ST		-	-	-	-	-	-	-	-	-	-	-	-

BC-*Bacillus cereus; BS-Bacillus subtilis***; EN**-*Enterococcus faecalis***; ML**- *Micrococcus luteus***; SA**- *Staphylococcus aureus***; SE**-*Staphylococcus epidermidis***; EC**-*Escherichia coli***; KP**-*Klebsiella pneumoniae***; PS**-*Pseudomonas aeruginosa***; SM**-*Serratia marcescens***; SP**-*Salmonella paratyphi* **; ST**-*Salmonella typhi;* **L**-Leaf; **INF**-Inflorescence; **ST**-Stem**; CH**-Cold n-Hexane; **CEA**- Cold Ethyl acetate; **CM**-Cold methanol; **CD**- Cold distilled water

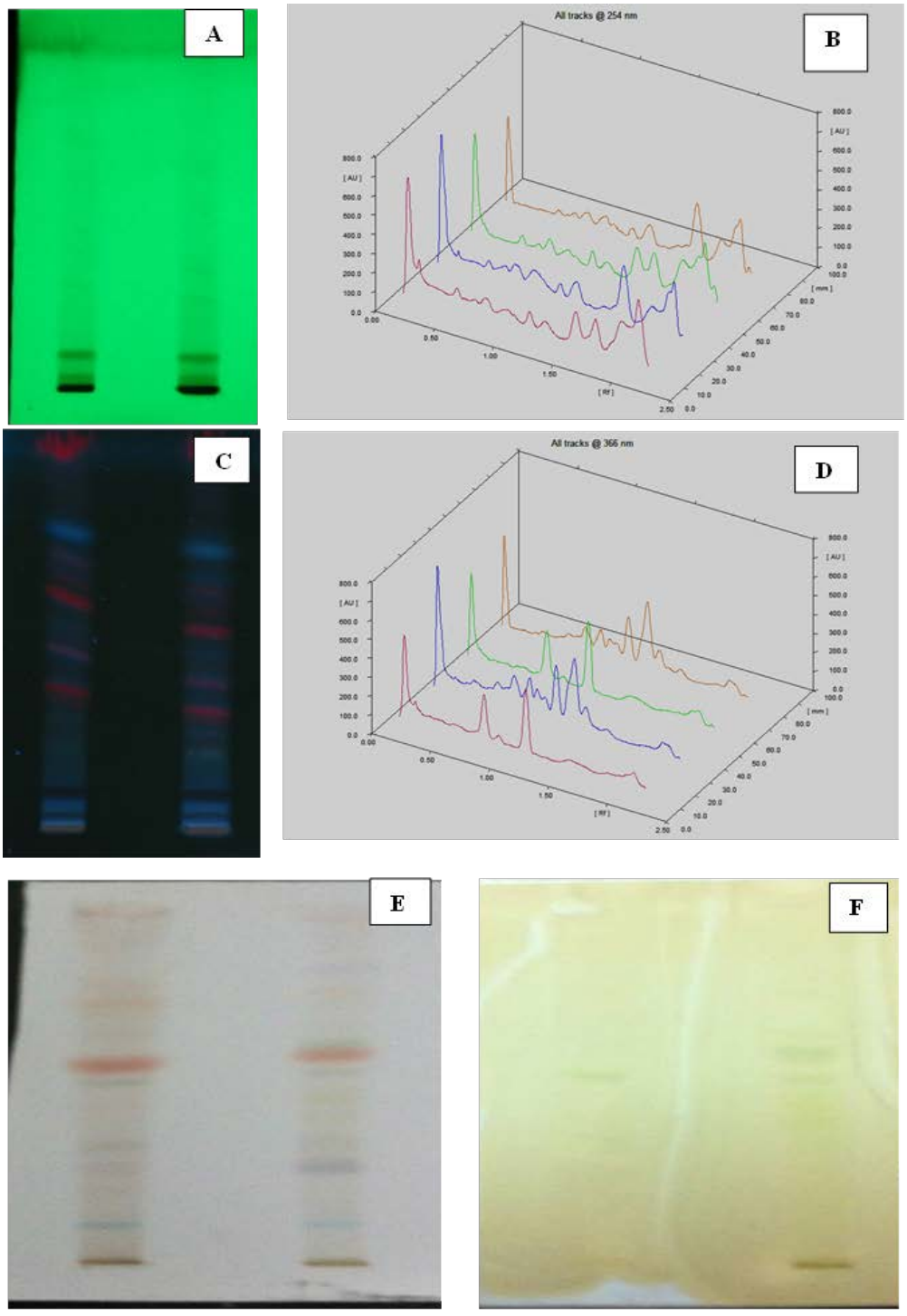


Figure 3: A&B-HPTLC fingerprinting graph of crude n-hexane extract scanned at 254 nm; C&D- HPTLC fingerprinting graph of crude n-hexane extract scanned at 366 nm; E&F-Detection of presence of secondary metabolites in n-hexane crude extract (Lane 1- leaf extract; Lane 2-Inflorescence extract)

For structural elucidation *Lavandula bipinnata* leaf and inflorescence crude extract was subjected to ^{1}H NMR and IR spectroscopy directly to find out the active compounds. The following probable compounds were identified.

Cortisol acetate

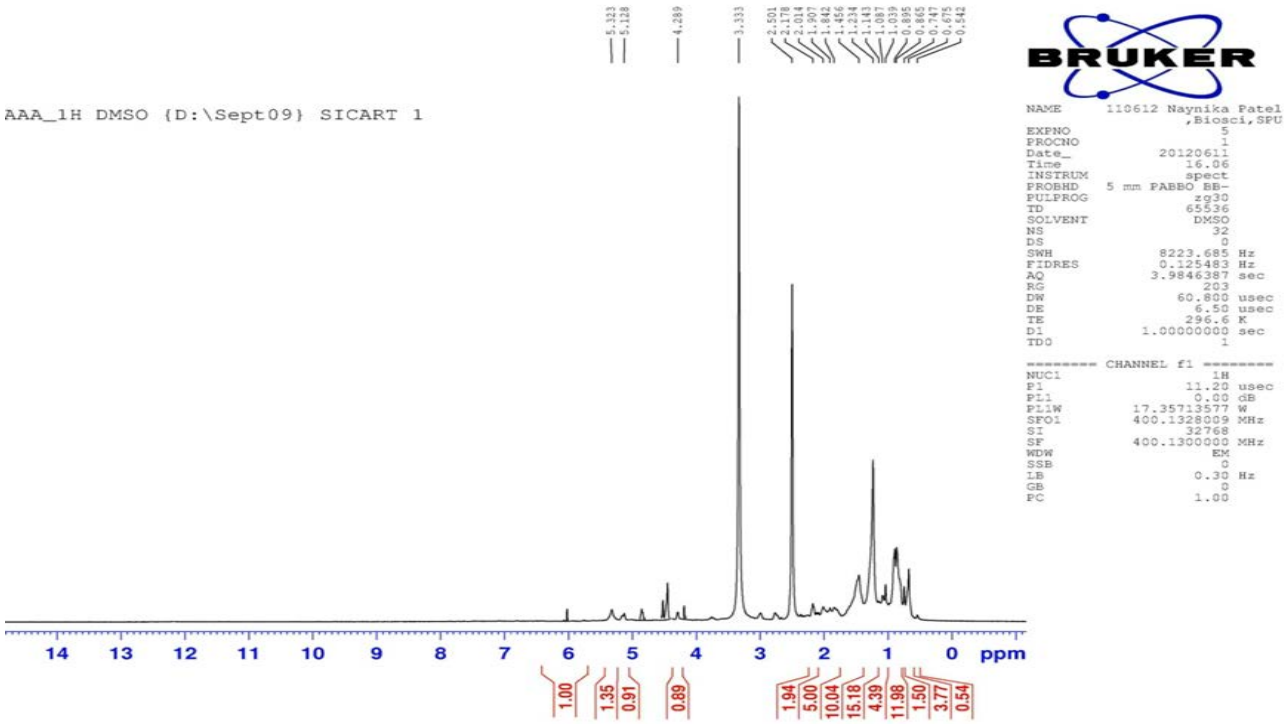


Figure 4: ^{1}H NMR of isolated compound

^{1}H NMR (MeOD) figure 4: δ ppm 0.74 (3 H, s, CHs), 1.23 (3 H, d, 19-CHs), 2.50. (3 H, s, al-acetate), 3.33 (1 H, m, 6&-H), 4.21 (1 H, m, 1 la-H), 4.36 (2 H, d, 0.CH2 .CO), 4.84 (2 H, AB system, CHz) and 5.12 and 5.32 (1 H, d, 4-H). The singlet obtained for three equivalent protons at δ 3.33 suggested the presence of a methoxy (OCH_3). Two protons of methylene (CH_2) group resonate at δ 2.50 ppm. Saturated CH_2 protons appeared between 1.62 and 1.48 ppm. Signal at δ 6.04 indicates the presence of hydroxyl proton of hydroxyl group. The three methoxy protons resonated at δ 2.01. The three protons of benzylic CH_3 resonated at δ 2.17.

FT-IR Spectroscopy

FT-IR spectrum of *Lavandula bipinnata* leaf and inflorescence extracts showed presence of three major compounds in different extracts, which are identified as follows.

Cortisol acetate

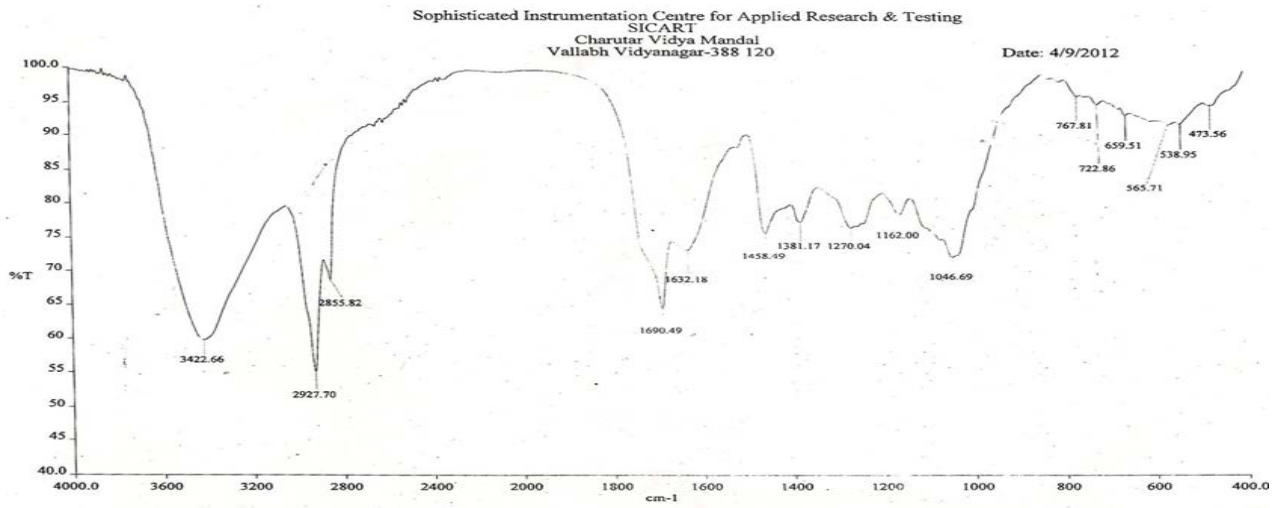
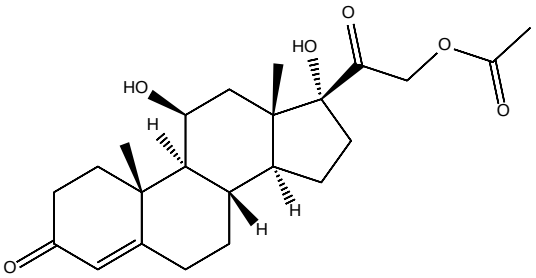


Figure 5: FT-IR spectrum of isolated compound

The Infrared (IR) spectrum of the Ethyl acetate (inflorescence) extract figure 5 shows various absorption bands in the region of 4000 – 400 cm-^{1}. The major absorption bands present in the spectrum confirms the presence various functional groups and bonding in the

isolated compound are as follows. Presence of hydroxyl group (OH) in the structure of compound is precisely confirmed by the presence of strong and broad band at 3422.66 cm^{-1}. The sharp band present at 2927.70 and 2855.82 cm^{-1} for C-H stretching indicates that there is a presence of methylene (CH_2) and methyl (CH_3) in the structure. Sharp and strong band t 1690 cm^{-1} clearly indicates the presence of C=O stretching of carbonyl group. The C-H banding band of alkanes is observed at 1458.19 cm^{-1}. Banding band of O-H is observed at 1381.17 cm^{-1}. The band observed at 1046 cm^{-1} is due to the presence of C-O stretching in the structure. Various absorption frequencies observed in the spectrum were compared with standard data base of compounds and from the data base the most probable compound predicted is Cortisol acetate. Similar results were obtained in *Lavandula sps.* essential oil extract [25].



17-Hydroxy Corticosterone 21-Acetate (**Cortisol acetate**)

Ethyl(R)-3-(6-amino-9H-purin-9-yl)-2-hydroxypropionat

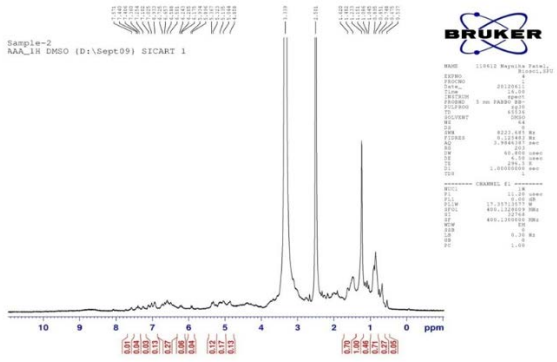


Figure 6: ^{1}H NMR of isolated compound

^{1}H NMR (MeOD) figure 6: δ ppm 1.62 (s, 3H), 3.33 (s, 3H), 4.85 (d, *J*= 8.6 Hz, 1H), 4.04 (br s, 1H), 5.13 (d, *J*= 8.6 z, H), 7.02–7.25 (m, 2H), 7.36–7.40 (m, 3H); 5.04 (s, 2 H, CH_2N); 6.17 (s, 1 H, CHN_2); 7.25 (br s, 2 H, NH_2); 7.44 (s,1 H, H2); 7.57 (s, 1 H, H8). The signal at δ 5.93 indicates the presence of hydroxyl proton of hydroxyl group. The doublet appeared in the range of δ 7.25 indicates the presence of amine (NH_2). The singlet obtained for two equivalent protons at δ 5.04 suggested the presence CH_2 attached to nitrogen atom. The saturated CH_2 protons appeared between δ 1.62 and 3.71 ppm. Two methylene protons resonated as doublets and appeared at δ 4.85 with J values of 8.6 Hz. The two singlets appeared at δ 5.04 and 6.17 clearly indicates the presence of proton attached to nitrogen atom.

Ethyl(R)-3-(6-amino-9H-purin-9-yl)-2hydroxypropionate

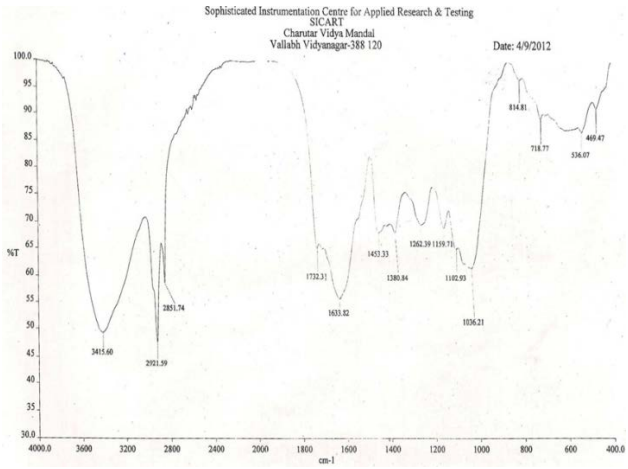
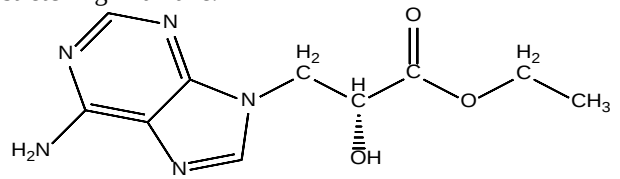


Figure 7: FT-IR spectrum of isolated compound.

The FT-IR spectrum of Hexane (leaf extract) figure 7 showed various bands which were compared with standard IR- absorption frequencies and the structure for extracted and separated compound was confirmed. The major peaks are as follows. The broad appeared at 3415. 60 cm^{-1} indicates the presence of hydroxyl (OH) group in the structure. Sharp peaks at 2921.59 and 2851.74 cm^{-1} are of C-H stretching due to the presence of CH_2 and CH_3 respectively. Band at around 1732 cm^{-1} clearly indicates the presence of C=O stretching in saturated aliphatic chain. The presence of strong band at 1633.82 cm^{-1} is of N-H bending band which clearly confirms the presence of NH_2 in structure. The band at 1453.33 cm^{-1} is due to the C-H stretching in alkane.



Ethyl (R)-3-(6-amino-9H-purin-9-yl)-2-hydroxy propionate

CONCLUSION

In present study the qualitative composition of *Lavandula bipinnata* showed broad spectrum of antimicrobial activity against a selected bacterial strain responsible for the most common bacterial diseases. These promissory extracts support modulation of new therapeutic drugs. Further, additional in vivo studies and clinical trials would be needed to justify.

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New Vallabh Vidyanagar, Gujarat, India) for identification of chemical constituent

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