Phytochemical Profiling and Antibacterial Evaluation of Methanolic Extracts of *Bidens pilosa* and *Bidens bipinnata* Using GC–MS and HPLC

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Abstract

The current study aimed to evaluate the phytochemical profiles and antibacterial activity of the methanolic extracts from Biden pilosa L. and Biden bipinnata L. using gas chromatography-mass spectrometry (GC-MS) and high-performance liquid chromatography (HPLC). The plant parts, leaves, stems, roots, and flowers of both species were collected from natural habitats, dried in the shade, and extracted with methanol under controlled laboratory conditions. The GC-MS analysis detected an extensive range of bioactive compounds, which include flavonoids, alkaloids, phenolic compounds, and their derivatives, with significant qualitative and quantitative differences between plant parts and the species. The key phenolic and flavonoid compounds were verified by an HPLC chromatogram, establishing a distinct chemical fingerprint for every species. The agar well diffusion method was used to assess the extract antibacterial properties against both the Gram-positive and Gram-negative bacteria. The methanolic extract of *Biden pilosa,* especially from leaves and flowers, revealed stronger antibacterial activity than that of , analyze the mechanism of action, to evaluate their therapeutic value for the formulation of plant-*Biden bipinnata.* The combined action of several phytoconstituents, is detected by chromatographic analysis, is responsible for the observed bioactivity. These gaps suggest that the *Bidens* species are a valuable source of natural antibacterial compounds. Further studies are required to isolate particular bioactive constituents of antimicrobial agents.

Keywords: *Bidens pilosa, Bidens bipinnata*, antibacterial activity, phytochemical profiling, methanolic extract, GC-MS, HPLC

1 Introduction

Genus *Bidens(*FamilyAsteraceae) *comprises* approximately 280 species among which important memebers are bidens *pilosa* and *Bidens bipinnata* are the annual herbs. It originated from South America, but it is now widely distributed in most pantropical areas of the world. *Pilosa* refers to the soft hair appearance. Leaves are opposite, petioled, pinnate, with 3–5 sharply serrated ovate leaflets, and are slightly hairy (Xuan *et al.,* 2016 Yang *et al*., 2012). Generally, *B. pilosa* is applied as dry powder for external and as a tincture, maceration, or decoction in internal remedies (Rybalchenko *et al*., 2010), as a whole plant or different parts, has been reported to be useful in the treatment of more than 40 disorders such as inflammation, immunological disorders, digestive disorders, infectious diseases, cancers, metabolic syndrome, wounds, and many others (Tan *et al.,* 2000 & Wiart *et al.,* 2000); it can also be utilized externally in the fresh form to treat snake bites and wounds (Dharmananda *et al.,* 2013); the leaves are used as a treatment for rheumatism, sore eyes, abdominal troubles, ulcers, swollen glands, and toothaches, among others (Hamburger *et al.,*1991). *Bidens bipinnata* is traditionally used to treat a variety of infectious diseases such as; malaria, sore throat, acute nephritis and dysentery and anti-arthritis (Shen *et al.,* 2015); antihypertensive, anti-liver fibrosis and antidiabetic activities (Sul *et al.,* 2022).

A number of flavonoids such as isoquercitrin (Amado *et al*., 2014) and 10 flavonoids have been isolated from aerial parts of the *B. pilosa* plant (Haratifar *et al*., 2014). The phytochemical composition of *B. pilosa* includes 301 compounds of major chemical classes: polyacetylenes, flavonoids, phenolic acids, terpenes (monoterpenes, sesquiterpenes, diterpenes, and triterpene), pheophytins, fatty acids and phytosterols, The major substances identified in *B. pilosa* are polyacetylenes, flavonoids, and triterpenes, and some essential oils. These compounds are considered the main active constituents responsible for the various pharmacological actions of the plant (Xuan *et al.,*2016) (Ullah et al. 2024). About 37 polyacetylenes isolated from different parts of the *B. pilosa* predominantly consist of aliphatic acetylenes consisting of aromatic and glucoside rings or heterocyclic end groups (Xuan *et al.,* 2016). Different compounds of pharmacological importance have been isolated from *B. bipinnata* such as; antidiarrheal compounds (9-Octadecenoic acid (Z)-, methyl ester, and 16-Pregnenolone) (Hoffman *et al*., 2022); using UHPLC–ESI-Q-TOF MS/MS method 12 flavonoids of 14 compounds were successfully identified (Yang *et al*., 2020)(Ullah et al. 2025) A total of 27 compounds including 24 known compounds and 3 new compounds were isolated from *B.*

bipinnata and their structures were established using and NMR, UV, and MS techniques (Yang *et al*., 2012). The spectroscopic (MS and NMR) data of whole plant extracts of *B. bipinnata* revealed the isolation of 38 compounds, including nine ceramides, thirteen flavonoids, five phenylpropanoids, four aliphatics, one pyrimidine, four steroids, one triterpenoid, and one polyacetylene. The listed compounds include five newly reported compounds and 10 known compounds first reported from this plant (Hu *et al*., 2018) (Ba et al. 2024).

The commensal pathogenic bacterium *E. faecalis* commonly found in the gastrointestinal tract of humans. However, the disturbance of this harmless relations with its host prompt the commensal-to-pathogen switch, eventually making it an opportunistic or conditional pathogen (Arias & Murray 2012). This accidental switching causes major problems including UTI (Shaheen *et al*., 2019), high mortality infections such as; pneumonia, meningitis infections, and intra-abdominal infection (Fiore *et al*., 2019). *Methicillin-resistant Staphylococcus aureus* (MRSA) which emerged in England in 1961 has become the main cause of bacterial infection globally (Lee *et al*., 2018). This pathogen is capable of causing soft-tissue and skin infections, endocarditis pneumonia, and sepsis (Mu *et al*., 2020). An agent of multidrug resistance and different health infections *K. pneumoniae* belongs to Gram-negative bacteria (Enterobacteriaceae family). This pathogen produces pneumonia and urinary tract infections (UTIs). They inhibit in the deep organs such as lungs or bladder tissues (Valenzuela-Valderrama *et al*., 2019). The gram-negative pathogen *Pseudomonas aeruginosa* is a common cause of nosocomial infections, particularly cystic fibrosis, pneumonia, and infection in immunocompromised hosts. In recent years studies on the epidemiology of *P. aeruginosa* have identified increasing trends of multi-drug resistant (MDR) isolates and antimicrobials. This is due to the secretions of toxins, formation of biofilms, and quorum sensing which makes it resistant, and severely infectious due to several virulence mechanisms ((Khan et al. 2023)Reynolds & Kollef 2021). *P. aeruginosa* is a common encapsulated, aerobic–facultatively anaerobic, rod-shaped bacterium that can cause disease in plants and animals, including humans (Diggle *et al*., 2020 & Addis *et al*., 2021).

Human health faces significant threats from drug resistance which causes due to inappropriate use of synthetic antibiotics (Zhong *et al*., 2020). Due to the waste of time and money, the phenomenon of developing new antibiotics is very worrying. An annual death rate of 10 million is expected by 2050 due to a lack of adequate and immediate solutions for drug

resistance. Natural antibiotics can provide a way to handle the problem of antibiotic resistance (Nasution *et al*., 2022). Natural products are used as material and conceptual starting points for decades for antibiotics discovery (Mitcheltree *et al*., 2021). Owing to the aforementioned status of the bacterial pathogenic strains it is essential to develop new drug sources to combat their pathogenicity. Natural sources of drugs are more encouraged as compared to synthetic ones as they have no or very few side effects and are naturally supplied in food as well. The current study was, therefore, designed to know the active status of selected species of genus *Bidens* and to pinpoint the responsible active phytochemicals.

2 Methodology

2.1 Collection and identification of plant material

Fresh plant material waere cllected from village Sia warghar district Dir Lower KP pakistan (34º40ˈ8″ N and 72º3ˈ29″ E: Altitude 1700-2800 metre) which were identified by Dr. Ali Hazrat (Plant taxonomist) Associate Professor Department of Botany University of Malakand KP Pakistan. The voucher specimen (*Bidens bipinnata*: H.UOM. BG.858 and *Biden pilosa*: H.UOM. BG.859) were deposited to the herbarium of Department of Botany University of Malakand KP Pakistan.

2.2 Sample extraction

The procedure of Zaman *et al*. (2022), were followed for extraction of crude extracts with minor modification. The plants were washed after collection throughly with tap water to remove dust and soil. The collected palnts were divided into different plant parts (leaves, stem, roots, and flower) and then were shade dried. The dried plant materials were crushed into fine powder with the help of grinder. The fine powder were immersed into the analytical grade methanol (sigma-Aldrich). The plant materials (powder plants) were kept soaking in methanol for 10 days. The details of quantity of plant powder, solvent, and The process was repeated till the desired amount was achieved.

2.3 Phytochemical analysis

2.3.1 GC-MS

The chemical investigations of the extracts were carried out through gas chromatography mass spectrometry (GC/MS) using techniques adapted by Ahmad & Zafar (2021) and Yaşar *et al*. (2005). The gas chromatograph (Shimadzu) hyphenated to a mass spectrometer QP 2010 plus (Tokyo, Japan) having automatic sampler (AOC-20S) and injector (AOC-20i). Helium gas was

used as a carrier medium. The chromatographic separations were carried out in capillary column (TRB-FFAP; Technokroma) with 30 m length; 0.35 mm i.d.; 0.250 µm thickness, treated with polyethylene glycol. Other GC-MS conditions include: 250 ºC temperature of ion source (EI), 240 °C interface temperature, 100 KPa pressure and 1.8 minutes cut time for solvent. Sample and standard of 1 µl were injected into column of GC. The injector operation was done in split mode having a splitting ratio of 1:50 with 240 °C temperature of injection. The temperature program of column started for one minute at 50 °C that changed at a rate of 15 °C/min to 150°C. The temperature was increased at the rate of 2.5 °C/min up to 175 °C for five minutes. The temperature was then further increased at a rate of 2.5 °C/minute to 220 °C holding constant for three minutes. The total time for elution was noted to be 43 minutes. The scanning of MS was done from mass/charge ratio of 85 to 380.

The software for GC-MS solutions was used for controlling the system and for getting the data. GC-MS was performed by using Thermo Scientific (GC-MS) DSQ; model Thermo Scientific GC Focus Series DSQ at the Central Resource Laboratory (CRL) Department of Chemistry University of Peshawar. The instrument runs with software version of 2.0.71. The identification of each compound with their particular peak position and retention time (Rt) in chromatogram were identified with spectrum of the known components stored in the library. Interpretation of mass spectrum of GC-MS was done using data base of library having more than 75,000 compounds. The name, molecular weight and structure of components were then ascertained. The relative percentage was calculated by comparing its average peak area to the total area.

2.3.2 HPLC

HPLC-UV characterization and quantification were carried out according to Zeb (2015). 1 gram powdered sample was dissolved in 20 ml of methanol and water in the ratio of 1:1 (v/v). The mixture was then heated at 70˚C for 1 hour in water bath followed by centrifugation for 10 minutes at 4000 rpm. After that sample (2 mL) was filtered into HPLC vials through Whattman filter paper. HPLC (high performance liquid chromatography) Agilent-1260 infinity system was used, with basics parts like UVAD (ultraviolet array detector), a quaternary pump, degasser and auto-sampler. The separation was carried out by Agilent-Zorbax-Eclipse column (XDB-C18). Column gradients system consists of solvent B (deionized water: methanol: acetic acid in the ratio of 180: 100: 20; v/v) and solvent C (water: methanol: acetic acid in the ratio of 80: 900: 20; v/v). The gradient system was started with solvent B 100%, 85%, 50% and 30% at 0, 5, 20

and for 25 minute and finally solvent C (100%) for 30-40 minutes. Elution occurred after 25 minutes. The ultraviolet array detector (UVAD) was set at 280 nm for the analysis of phenolic compounds and the chromatogram were recorded from 190-500 n. Identification of phenolic compounds was done using their retention times, UV spectra and available standards while quantification was done taking the percent peak area.

2.3.3 Antibacterial assay

Four pathogenic bacterial strains, two Gram positive (*Enterococcus faecalis, Methicillin-resistant Staphylococcus aureus* and two Gram negative (*Klebsiella pneumoniae*, *Pseudomonas aeruginosa*) were used in the current assay. The bacterial samples were collected in screwed test tubes and all the samples were transferred to laboratory and different growth media were prepared (Abiala *et al.,* 2016). Bactericidal activity was determined by using disc diffusion method as described by (Ruparelia *et al*., 2008). Bacterial cultures were refreshed in nutrient broth for 24 h at 35 °C. Nutrient agar medium was used for the growth of bacterial strains. All chemicals used were of commercial grade. Briefly, about 0.75 mL of the broth culture containing 10^8 colony forming units (CFU) per ml of the test strain was added to 75 mL of nutrient agar medium at 45 °C, mixed well, and then poured into a 14 cmsterile petri plate. The CFU (10^8) were confirmed by counting the colonies at different dilution of the original sample. Filter paper discs of 6 mm in size ware prepared from whatman no. 1 filter paper. Media, filter paper discs along with other apparatus required in this assay were autoclaved for sterilization. The experiment was performed in microbiological safety cabinet. Bacterial strains were streaked on solidified labeled nutrient agar plates. Cephalosporins (Ceftoxaime-USP Reference standard CAS644885-4 sigma aldrich) was used as standard drug. Then 7.5 µg, 15 µg and 30 µg of samples were loaded per disc. The discs were placed on respective places in petri plate and incubated for 48 h at 35 °C for antibacterial activity. the efficacy of antibacterial activity was measured by observing the zone of inhibition around the dsics. The zones of inhibitions were measured in milli metre (mm) after 48.

3 Results

3.1 Gas chromatography mass spectrometry of leaves (GC/MS):

GC–MS analyses of the leaves of Bidens pilosa and Bidens bipinnata 3 biological active compund from bidens pilosa and 1 from bidens bipinnata were identified showen in (Fig. 1 and table .1)

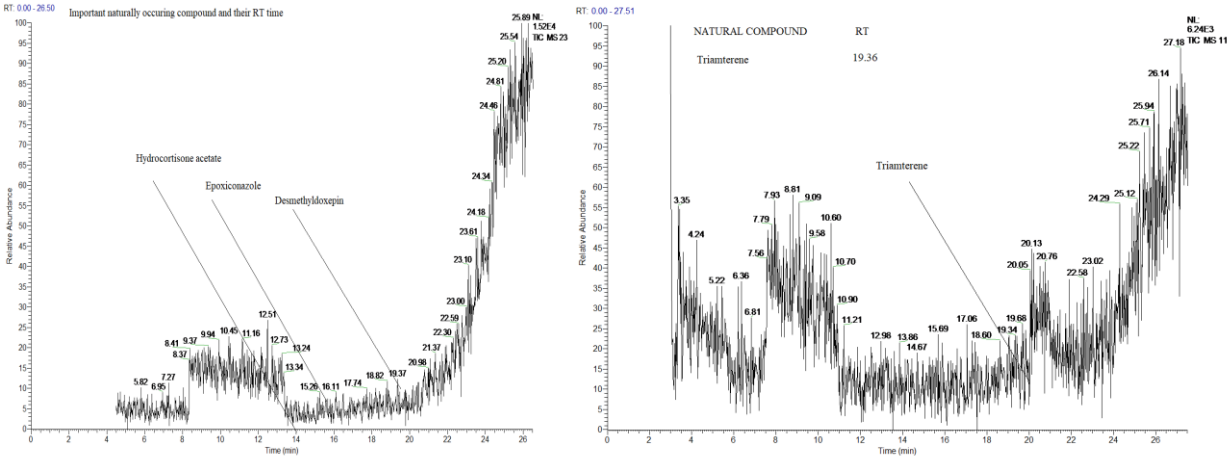
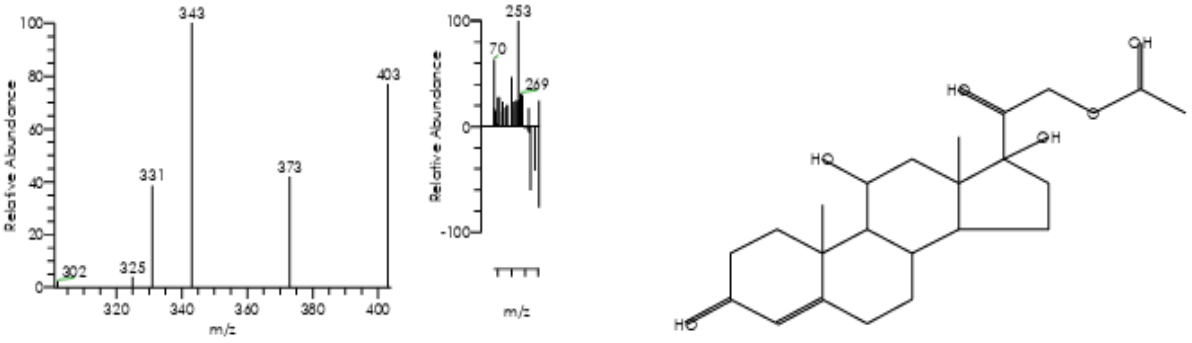


Fig no: 1 chrmatoghram analyses of leaves of *bidens pilosa* and *bidens bipinnata*

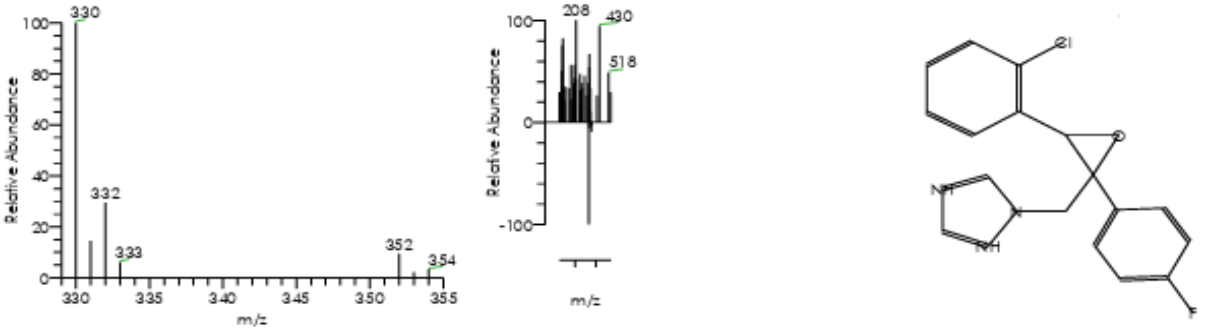
Table 1.1 GC-MS spectral analysis of Methanolic extract of leaves of *bidens pilosa* and *bidens bipinnata*

S. No.	Bidens pilosa					Bidens bipinnata				
1	Area %	RT (min)	Compound	Formula	Mol. Weight	Area %	RT (min)	Compound	Formula	Mol. Weight
2	0.21	14.6	Hydrocortisone acetate	$C_{23}H_{32}O_6$	404	2.96	19.36	Triamterene	$C_{12}H_{11}N_7$	253
3	0.59	15.58	Epoxiconazole	$C_{17}H_{13}ClFN_3O$	329	—	—	—	—	—
4	0.25	19.74	Desmethyldoxepin	$C_{18}H_{19}NO$	265	—	—	—	—	—

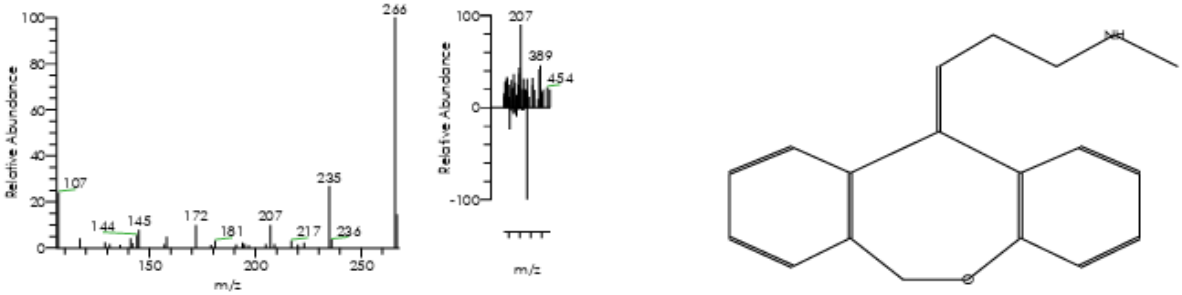
Structures of natural compund from leaves of Bidens pilosa



Hydrocortisone acetate

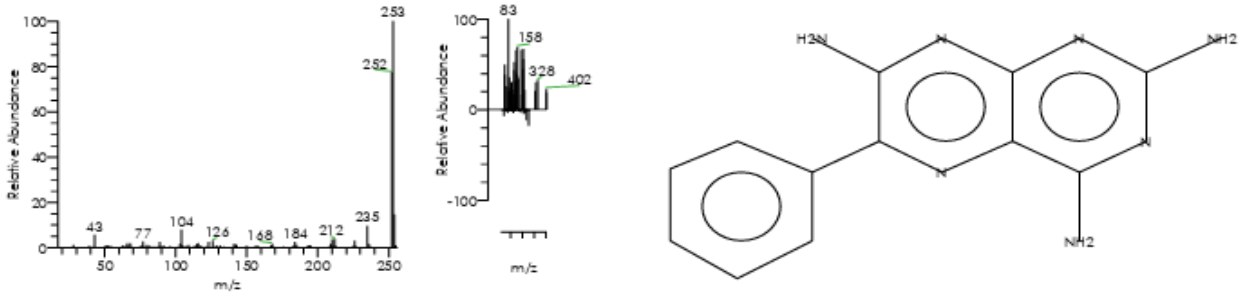


Epoxiconazole



Desmethyldoxepin

Structures of natural compund from leaves of Bidens bipinnata



Gas chromatography mass spectrometry of stem

GC–MS analyses of the stem of Bidens pilosa and Bidens bipinnata 1 biological active compund from bidens pilosa and 3 from bidens bipinnata were identified showen in(Fig. 1.1 and table .1.1)

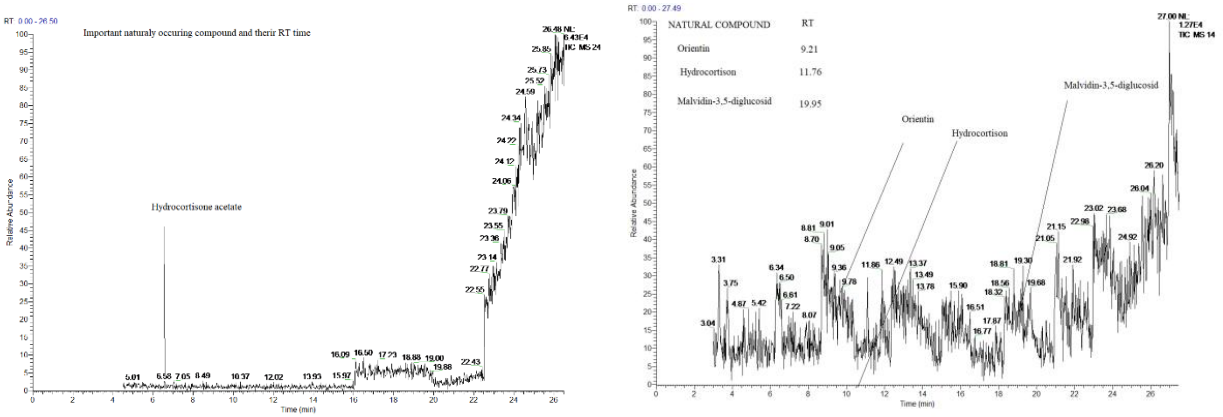
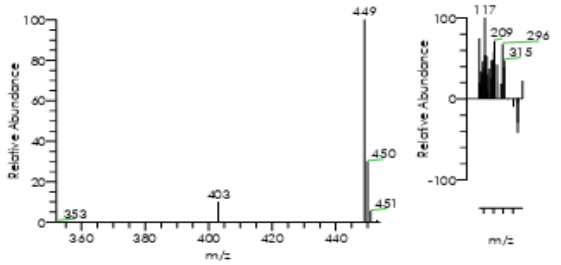


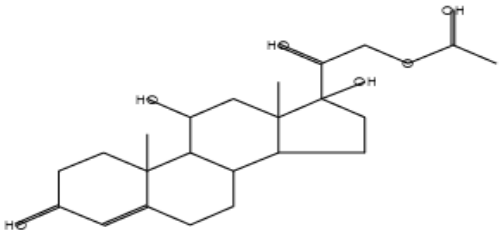
Fig no: 1.1 chrmatoghram analyses of stem of *bidens pilosa* and *bidens bipinnata*

Table 1.1 GC-MS spectral analysis of Methanolic extract of stem of *bidens pilosa* and *bidens bipinnata*

S. No.	Bidens pilosa					Bidens bipinnata				
1	Area %	RT (min)	Compound	Formula	Mol. Weight	Area %	RT (min)	Compound	Formula	Mol. Weight
2	0.14	6.58	Hydrocortisone acetate	C23H32O6	404	1.06	9.21	Orientin	C21H20O11	448
3	—	—	—	—	—	1.18	11.76	Hydrocortison	C21H30O5	362
4	—	—	—	—	—	2.45	19.95	Malvidin-3,5-diglucosid	C3H2Cl4O	194

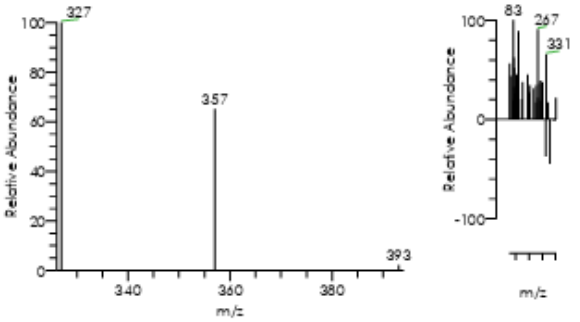
Structures of natural compound from stem of *Bidens pilosa*

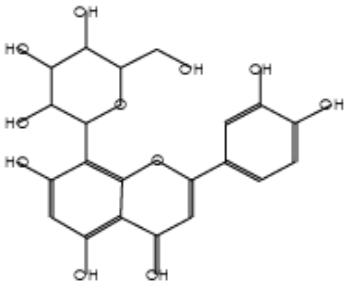




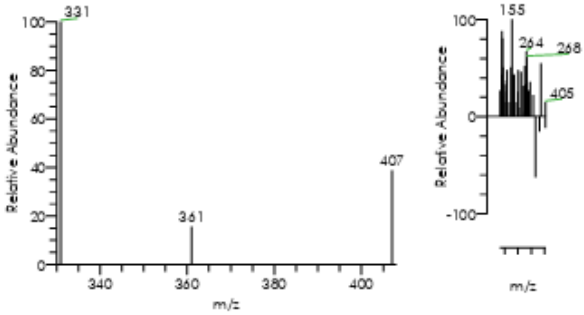
Hydrocortisone acetate

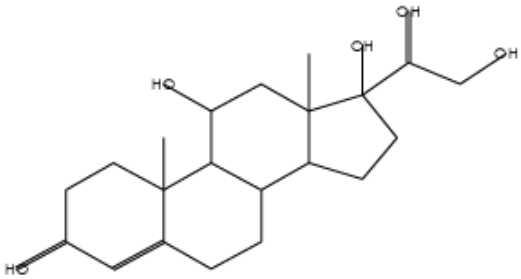
Structures of natural compund from stem of Bidens bipinnata



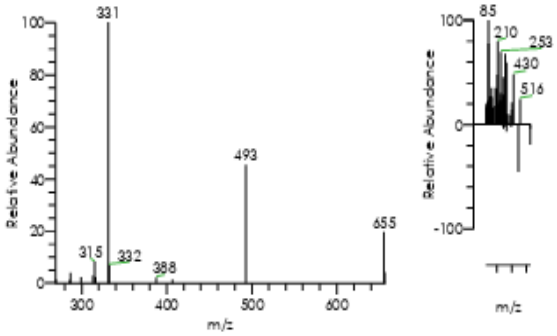


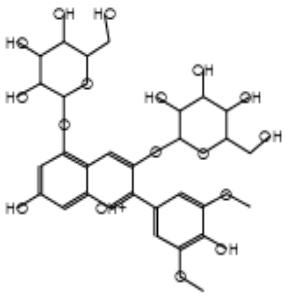
Orientin





Hydrocortison





Malvidin-3,5-diglucosid

Gas chromatography mass spectrometry of Flowers

GC–MS analyses of the stem of Bidens pilosa and Bidens bipinnata 4 biological active compund from bidens pilosa and 4 from bidens bipinnata were identified showen in(Fig. 1.2 and table .1.2)

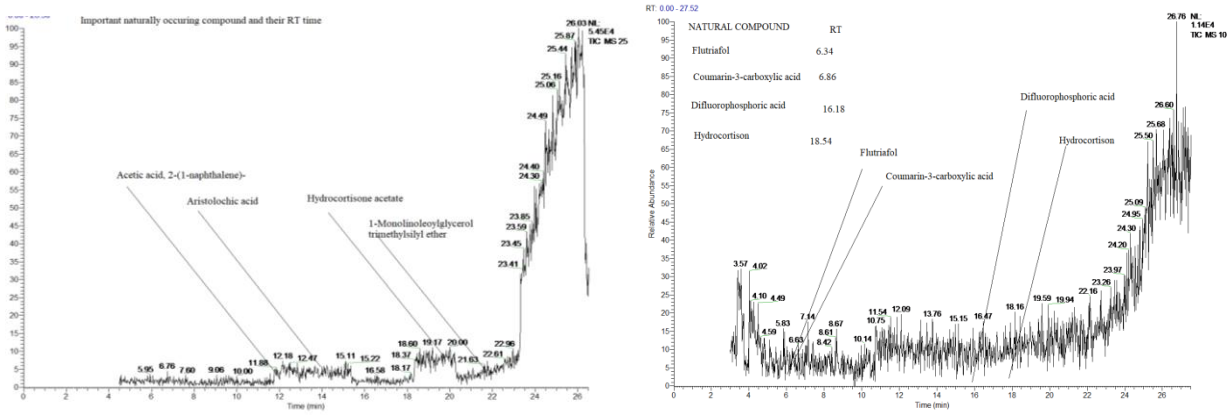
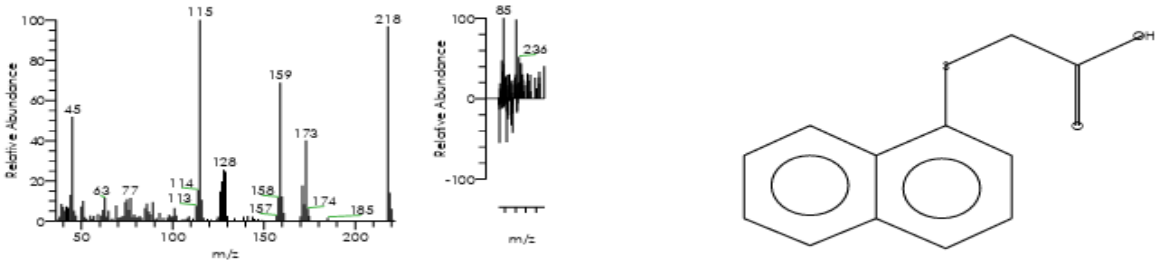


Fig no: 1.2 chrmatoghram analyses of flowers of *bidens pilosa* and *bidens bipinnata*

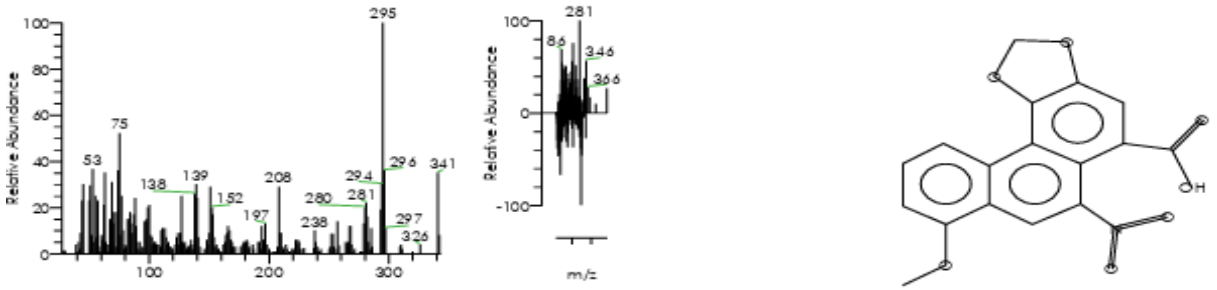
Table 1.2 GC-MS spectral analysis of Methanolic extract of flowers of *bidens pilosa* and *bidens bipinnata*

S. No.	Bidens pilosa					Bidens bipinnata				
1	Area %	RT (min)	Compound	Formula	Mol. Weight	Area %	RT (min)	Compound	Formula	Mol. Weight
2	0.32	11.85	Acetic acid, 2-(1-naphthalene)-	C12H10O2S	218	2.99	6.34	Flutriafol	C18H23NO3	301
3	0.2	13.38	Aristolochic acid	C17H11NO7	341	3.17	6.86	Coumarin-3-carboxylic acid	C10H6O4	190
4	1.76	19.98	Hydrocortisone acetate	C23H32O6	404	0.29	9.68	Difluorophosphoric acid	F2HO2P	102
5	0.2	21.61	1-Monolinoleoyl glycerol trimethylsilyl ether	C27H54O4Si2	498	1.52	18.54	Hydrocortison	C21H30O5	362

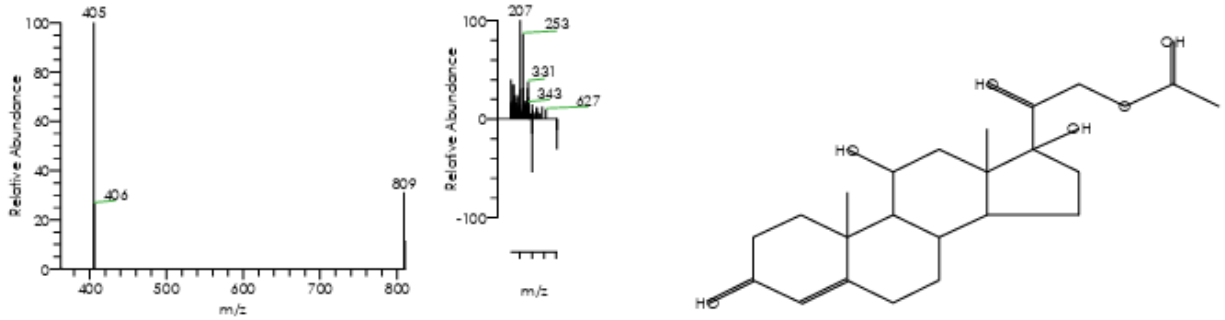
Structures of natural compund from flowers of Bidens pilosa



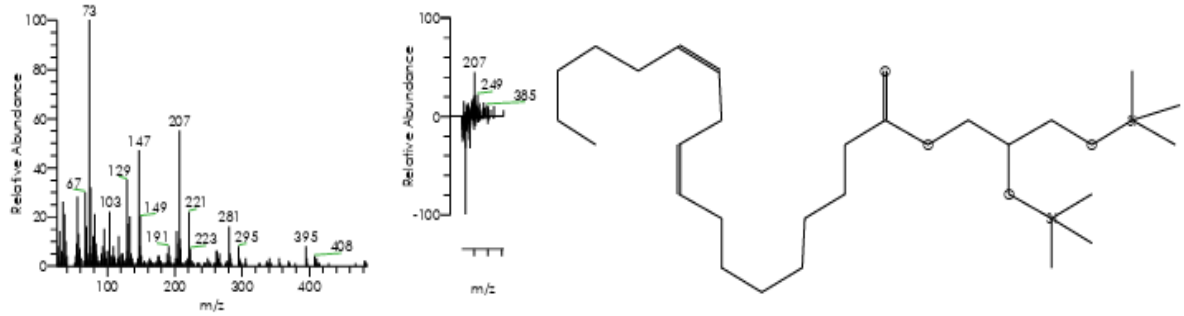
Acetic acid, 2-(1-naphthalene)-



Aristolochic acid

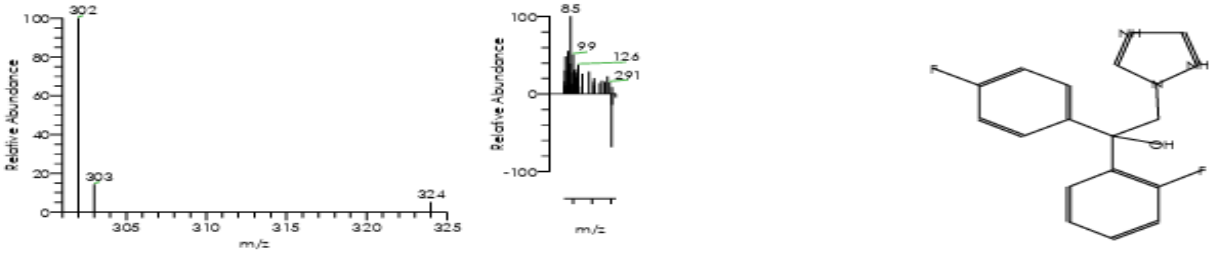


Hydrocortisone acetate

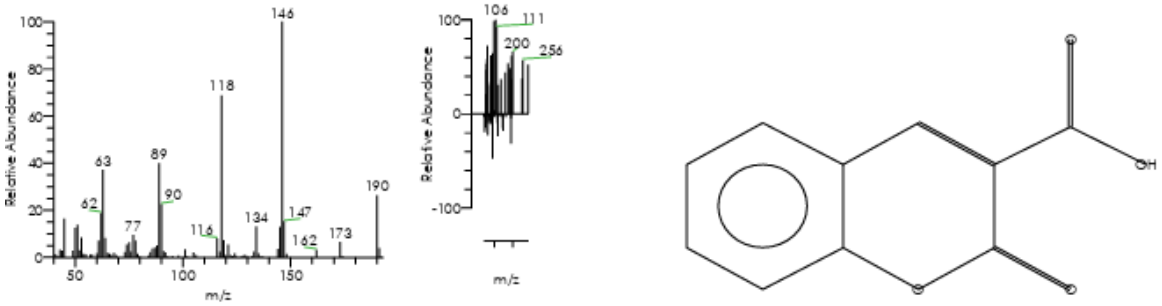


1-Monolinoleoylglycerol trimethylsilyl ether

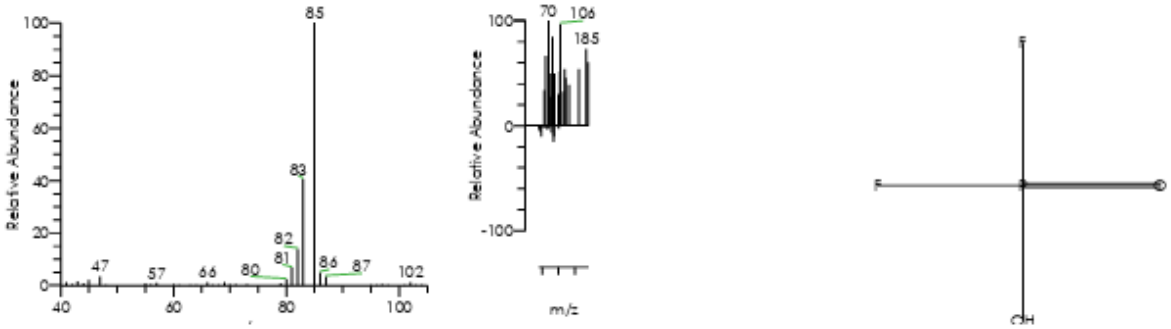
Structures of natural compund from flowers of Bidens bipinnata



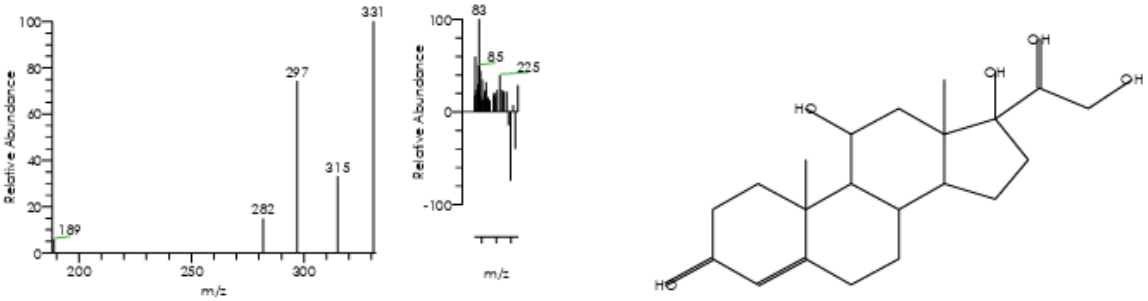
Flutriafol



Coumarin-3-carboxylic acid



Difluorophosphoric acid



Hydrocortison

3.2 Gas chromatography mass spectrometry of Root

GC–MS analyses of the stem of Bidens pilosa and Bidens bipinnata 3 biological active compund from bidens pilosa and 5 from bidens bipinnata were identified showen in(Fig. 1.3 and table .1.3)

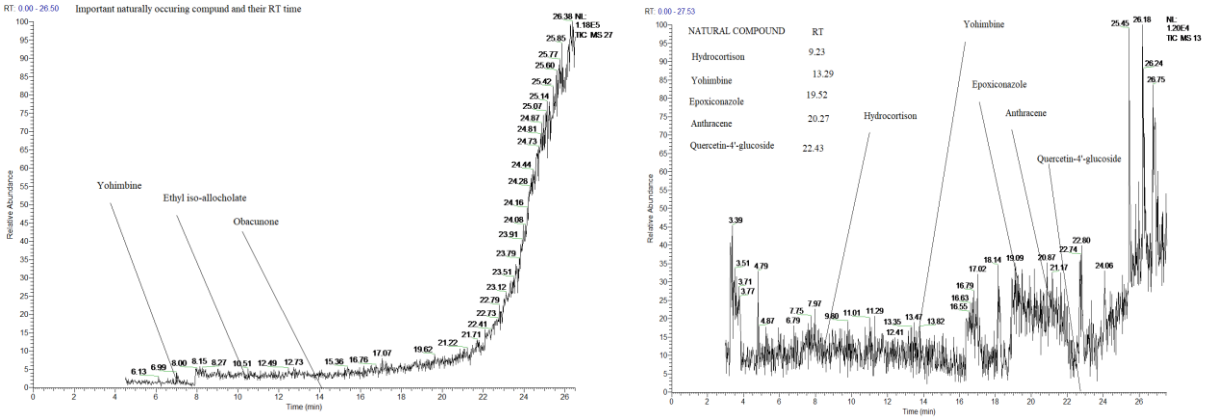
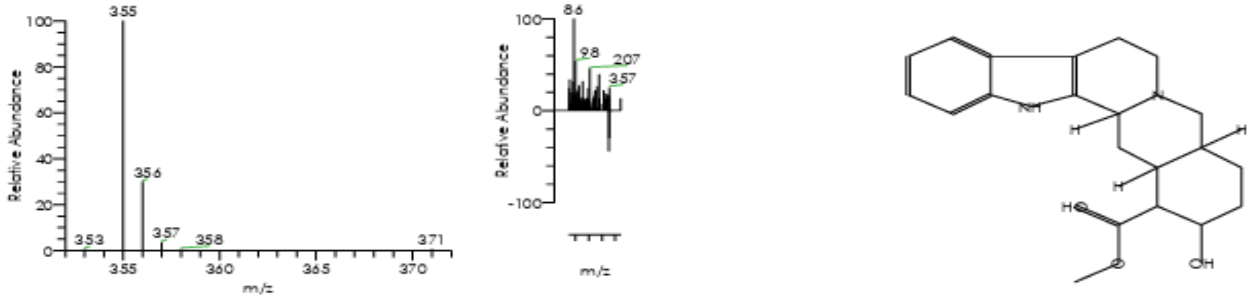


Fig no: 1.3 chrmatoghram analyses of Roots of *bidens pilosa* and *bidens bipinnata*

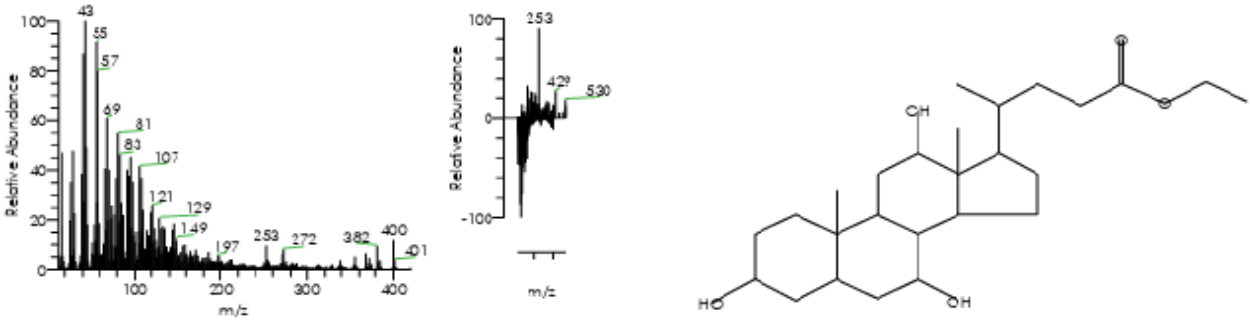
Table 1.3 GC-MS spectral analysis of Methanolic extract of Root of *bidens pilosa* and *bidens bipinnata*

S. No.	Bidens pilosa					Bidens bipinnata				
1	Area %	RT (min)	Compound	Formula	Mol. Weight	Area %	RT (min)	Compound	Formula	Mol. Weight
2	0.5	5.48	Yohimbine	C21H26N2O3	354	0.71	9.23	Hydrocortison	C3H2Cl4O	194
3	0.94	10.22	Ethyl iso-allocholate	C26H44O5	436	3.7	12.27	Yohimbine	C21H26N2O3	354
4	0.21	14.5	Obacunone	C26H30O7	454	0.21	19.52	Epoxiconazole	C17H13ClFN3O	329
5	—	—	—	—	—	0.73	20.27	Anthracene	C26H20	332
6	—	—	—	—	—	1.68	22.43	Quercetin-4'-glucoside	C21H20O12	464

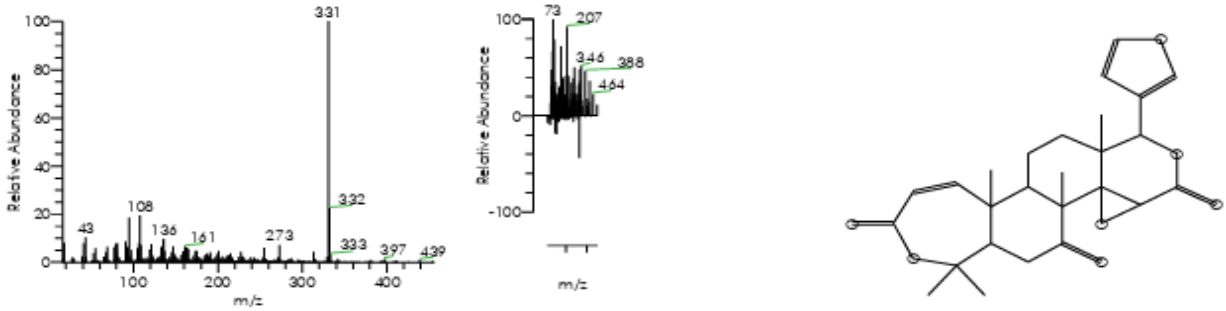
Structures of natural compund from root of Bidens pilosa



Yohimbine

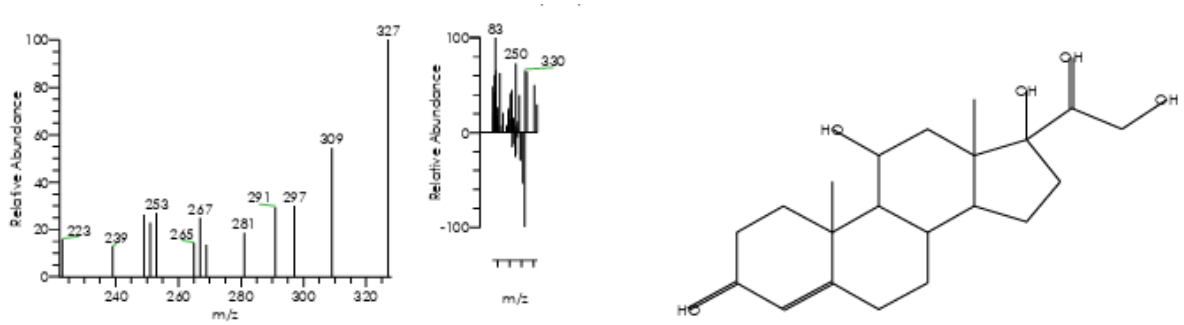


Ethyl iso-allocholate

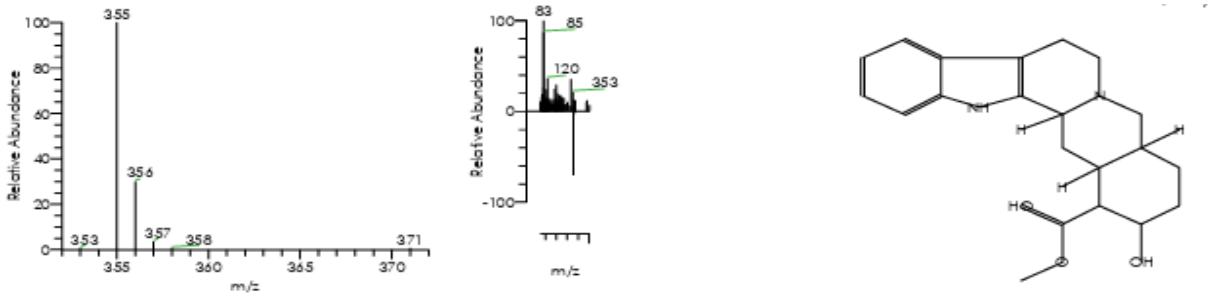


Obacunone

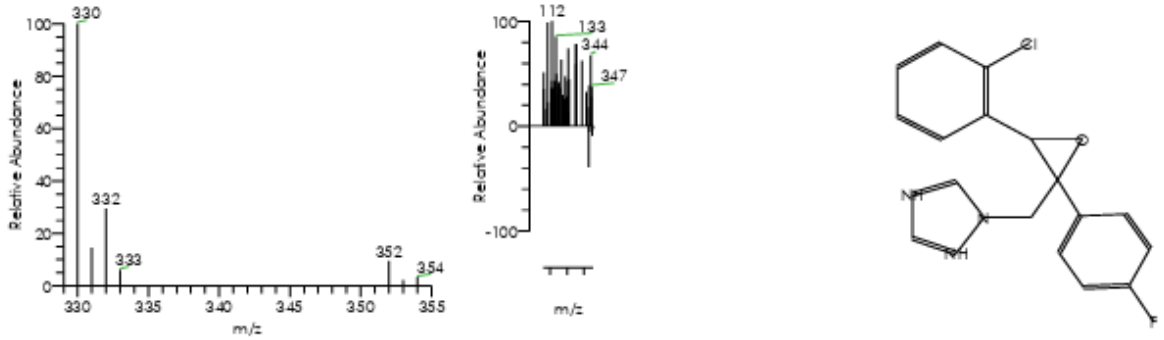
Structures of natural compund from root of Bidens bipinnata



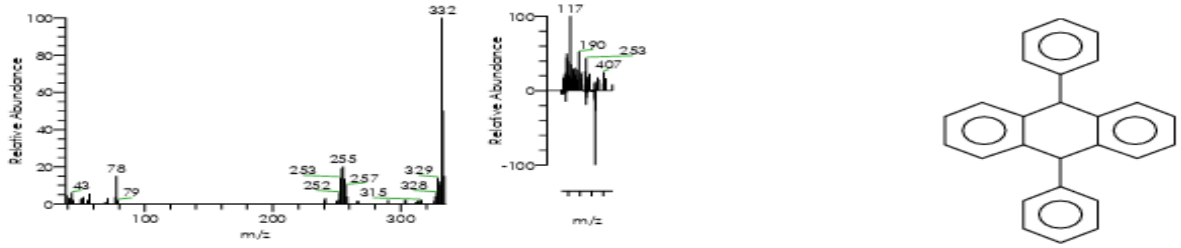
Hydrocortison



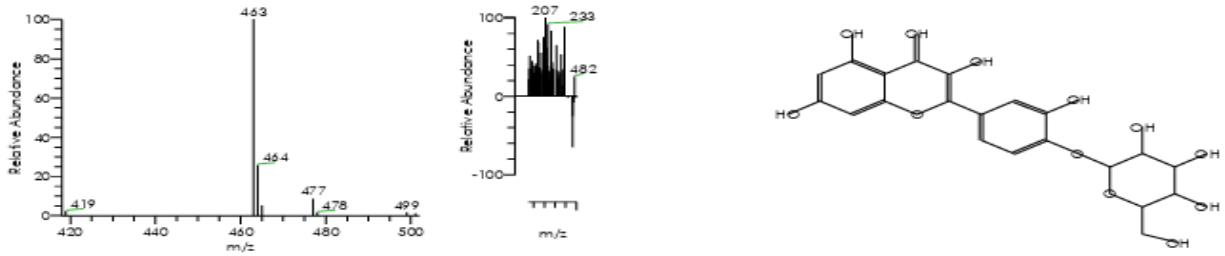
Yohimbine



Epoxiconazole



Anthracene



Quercetin-4'-glucoside

3.3 HPLC Analyses

Two major factors play a vital role in natural product research. These are the separation and purification of bioactive constituents in crude plant samples or their fractions obtained from different natural sources, and their accurate structural identification. HPLC is a robust analytical technique mainly used for the qualitative analysis of non-volatile classes of compounds such as phenolic, terpenoids, and alkaloids . It is highly efficient and provides rapid and better analytical separation with higher sample loading capacity (Kumar, 2017). According to The recent HPLC analyses the fowling compound has been identified from the leaves, stem, flowers and root of Bidens pilosa and Bidens bipinnata shown in the figures and tables

HPLC analyses of leaves

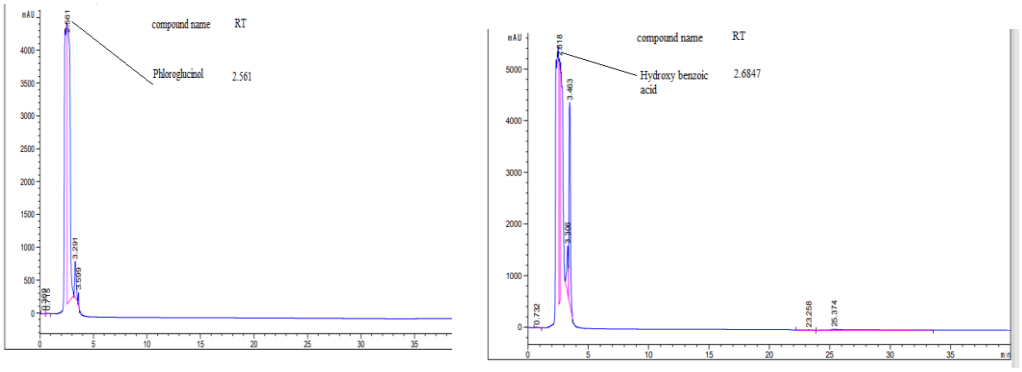


Figure no .2 *HPLC and UV analyses of leaves of Bidens pilosa and Bidens bipinnata*

Table no.2 phenolic compound from leaves of bidens pilosa and bidens bipinnata

S.No	Sample	No. of Peaks	Retention Time (min)	Phenolic Compound Identified	HPLC–UV λmax (nm)	Concentration (µg/mL)	Identification Reference
1	*B. pilosa* (Leaves)	1	2.523	Phloroglucinol	250	48,454.40	Reference Standard
2	*B. bipinnata* (Leaves)	1	2.523	Phloroglucinol	250	48,454.40	Reference Standard

HPLC analyses of stem

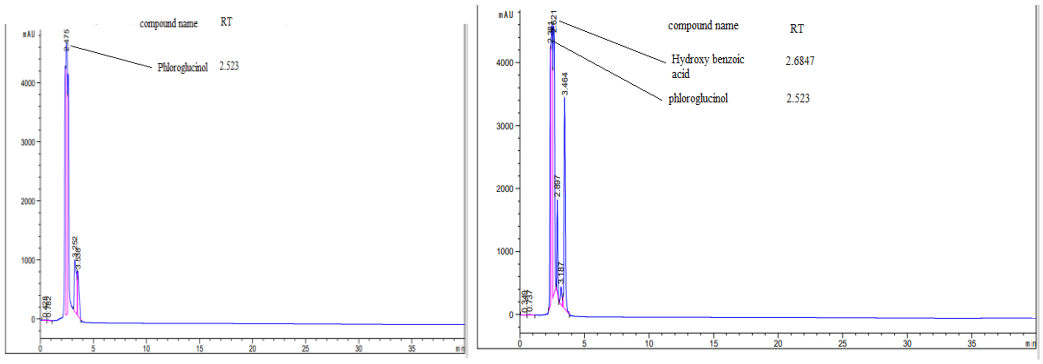


Figure no: 2.1 *HPLC and UV analyses of stem of Bidens pilosa and Bidens bipinnata*

Table no: 2.1phenolic compound from stem of bidens pilosa and bidens bipinnata

S.No	Sample	No. of Peaks	Retention Time (min)	Phenolic Compound Identified	HPLC–UV λmax (nm)	Concentration (µg/mL)	Identification Reference
1	*Bidens pilosa* (stem)	1	2.523	Phloroglucinol	250	48454.4	Reference Standard
2	*Bidens Bipinnata* (stem	1	2.523	Phloroglucinol	250	48454.4	Reference Standard
3	//	2	2.6847	Hydroxy benzoic acid	250	6233.6	Reference Standard

HPLC analyses of flowers

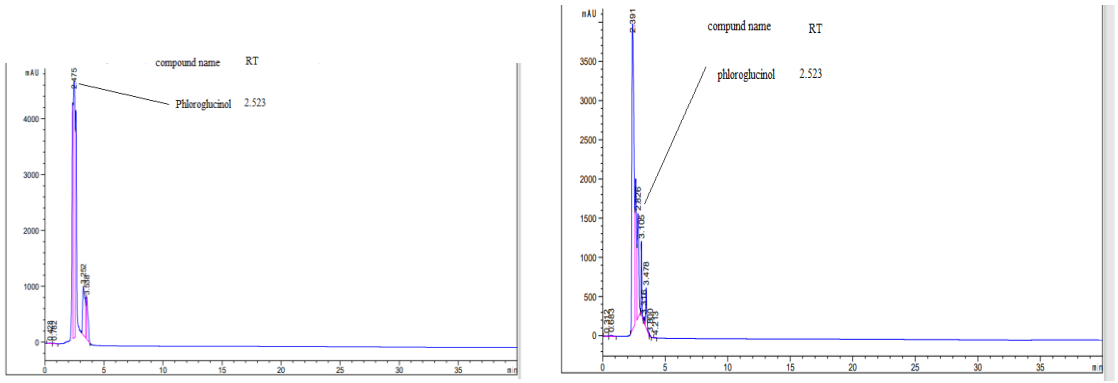


Figure no: 2.2 *HPLC and UV analyses of Flowers of Bidens pilosa and Bidens bipinnata*

Table no: 2.2 phenolic compound from *Flowers* of bidens pilosa and bidens bipinnata

S.No	Sample	No. of Peaks	Retention Time (min)	Phenolic Compound Identified	HPLC–UV λmax (nm)	Concentration (µg/mL)	Identification Reference
11	*Bidens. pilosa* (flower)	1	2.523	Phloroglucinol	250	48454.4	Reference Standard
22	*Bidens.bipinnata* (flower)	1	2.523	Phloroglucinol	250	48454.4	Reference Standard

HPLC analyses of flowers

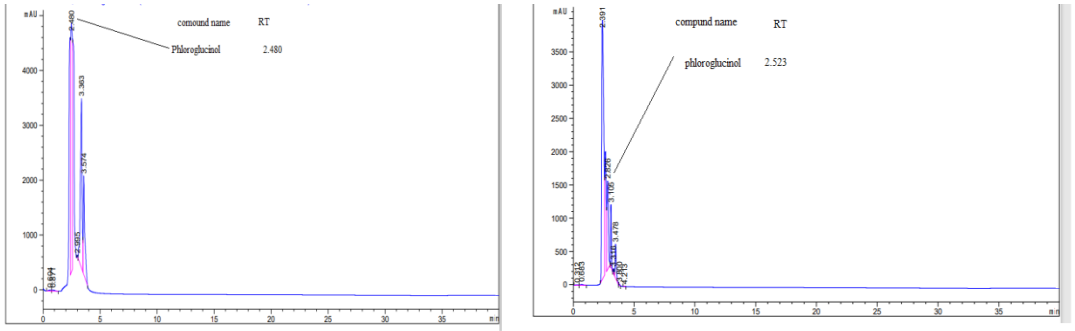


Figure no: 2.1 *HPLC and UV analyses of root of Bidens pilosa and Bidens bipinnata*

Table no: 2.1phenolic compound from *root* of bidens pilosa and bidens bipinnata

S.No	Sample	No. of Peaks	Retention Time (min)	Phenolic Compound Identified	HPLC–UV λmax (nm)	Concentration (µg/mL)	Identification Reference
	Bidens. pilosa (root)	1	2.523	Phloroglucinol	250	48454.4	Reference Standard
	//	2	2.6847	Hydroxy benzoic acid	250	6233.6	Reference Standard
	Bidens.bi pinnata (root)	1	2.523	Phloroglucinol	250	48454.4	Reference Standard

3.4 Antibacterial activites

Antibacteral assay was performed againest the four selceted bacterail strain and three concentartion viz. 7.5 µg, 15 µg and 30 µg of each sample were used the result figure 3 and 3.1 (table: 3, 3.1, 3.2, 3.3)revealed that all fuction were found to inhabit the growth of selected bacteral strain however methanloic fraction showed excelent activites.

Figure no 3 show the frowth of inhibation against *Enterococcus faecalis*

Table :3 Antibacterial activities of various fractions of Bidens pilosa and bidens bipinnata against Strain 1-*Enterococcus faecalis*

Plant samples	Strain 1-*Enterococcus faecalis*			
	Plant Parts	Concentration-a	Concentration-b	Concentration-c
		Mean+SD	Mean+SD	Mean+SD
B. pilosa	Leaves	10.1±0.28	7.5±0.5	6.43±0.51
	Stem	7.16±0.76	6.33±0.28	6.86±0.35
	Flowers	7.93±0.40	8.06±0.11	6.3±0.43
	Root	7.33±0.30	7.9±0.36	6±0.1
B. pinnata	Leaves	7.26±0.25	6.4±0.17	6.86±0.15
	Stem	7.23±0.25	7.63±0.32	6.16±0.20
	Root	6.66±0.28	6.54±0.11	5.95.±0.1
	Flowers	7.23±0.25	6.7±0.2	6.66±0.15
Control	Positive control leaves	6.03±0.05	6.06±0.11	6.06±0.11
	Pc stem	6.6±0.1	6.6±0.1	6.6±0.1
	Pc flowers	6.53±0.15	6.53±0.15	6.53±0.15
	Pc root	6.4±0.1	6.4±0.1	6.4±0.1

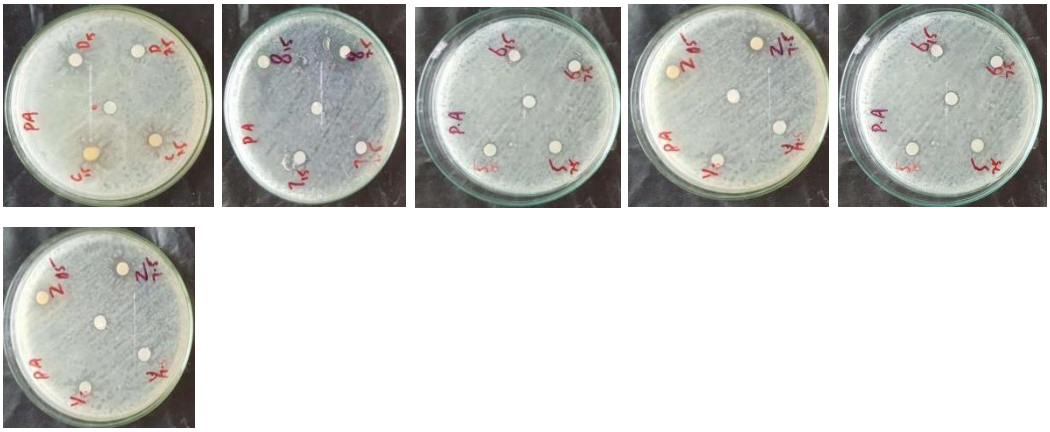


Figure no 3 .1 show the Growth of inhibation against *Pseudomonas aeruginosa*

Table :3.1 Antibacterial activities of various fractions of Bidens pilosa and bidens bipinnata against Strain 2- *Pseudomonas aeruginosa*

Plant samples	Strain-*Pseudomonas aeruginosa*			
	Plant Parts	Concentration-a	Concentration-b	Concentration-c
		Mean+SD	Mean+SD	Mean+SD
B. pilosa	Leaves	9.26±0.25	7.66±0.57	6.3±0.60
	Stem	7.33±0.76	7.5±0.5	6.26±0.60
	Flowers	8.9±0.36	9.36±0.55	6.33±1.52
	Root	7.53±0.45	8.5±0.5	5.53±0.15
B. pinnata	Leaves	8.53±0.35	9±1	7.73±0.47
	Stem	7.5±0.5	7.7±0.60	7.56±0.51
	Root	7.2±0.26	8.06±0.11	7.23±0.32
	Flowers	9.3±0.36	9±0.26	8±0.1
Control	Pc leaves	7.5±0.5	7.5±0.5	7.5±0.5
	Pc stem	8.06±0.11	8.06±0.11	8.06±0.11
	Pc flowes	7.63±0.55	7.63±0.55	7.63±0.55
	Pc root	8.16±0.15	8.16±0.15	8.16±0.15

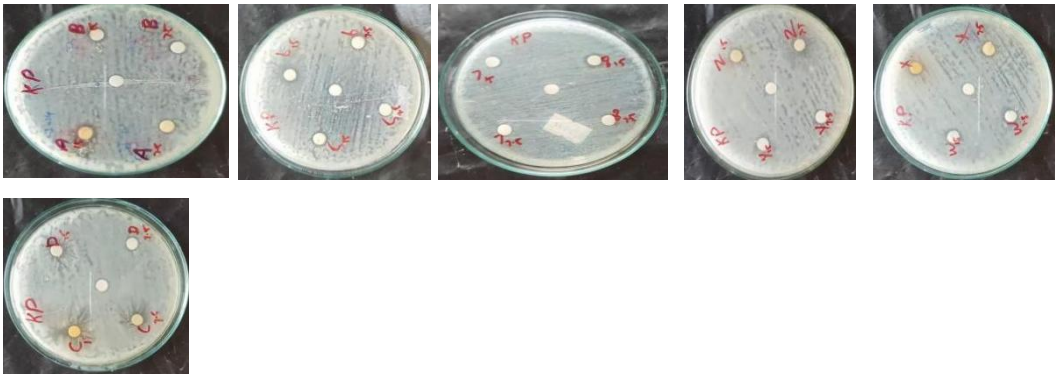


Figure no 3 .2 show the Growth of inhibation against *Klebsiella pneumoniae*

Table :3.2 Antibacterial activities of various fractions of Bidens pilosa and bidens bipinnata against Strain 3- *Klebsiella pneumoniae*

Plant samples	Strain-*Klebsiella pneumonia*			
	Plant Parts	Concentration-a	Concentration-b	Concentration-c
		Mean+SD	Mean+SD	Mean+SD
B. pilosa	Leaves	8.33±0.76	7.33±0.28	6.13±0.23
	Stem	8.16±0.28	7.2±0.26	5.3±0.26
	Flowers	8.33±0.57	8.16±0.28	7.13±0.15
	Root	8.33±0.57	6.93±0.11	6.16±0.20
B. pinnata	Leaves	8.16±0.28	7.5±0.5	6.23±0.25
	Stem	8.5±0.5	7.66±0.28	7.23±0.20
	Root	7.66±0.28	6.83±0.15	6.2±0.26
	Flowers	9.33±0.28	8.5±0.5	8.4±0.36
Control	Pc leaves	6.56±0.11	6.56±0.11	6.54±0.11
	Pc stem	6.66±0.28	6.66±0.28	6.66±0.28
	Pc flowers	6.4±0.17	6.4±0.17	6.4±0.17
	Pc root	6.63±0.23	6.63±0.23	6.63±0.23

Figure no 3 .3 show the Growth of inhibation against MRSA

Table :3.3 Antibacterial activities of various fractions of Bidens pilosa and bidens bipinnata against Strain 3-MRSA

Plant samples	Strain-MRSA			
	Plant Parts		Concentration-b	Concentration-c
		Mean+SD	Mean+SD	Mean+SD
B. pilosa	Leaves	7.23±0.25	8.33±0.57	6.5±0.70
	Stem	0±0	0±0	0±0
	Flowers	0±0	0±0	0±0
	Root	0±0	0±0	0±0
B. pinnata	Leaves	6.66±0.28	6.5±0	5.53±0.15
	Stem	7.4±0.17	7.33±0.57	6,5±0
	Root	6.33±0.28	7.1±0.17	6.53±0.20
	Flowers	6.93±0.40	6.66±0.28	7.33±0.57
Control	Pc leaves	6.46±0.57	6.46±0.05	6.46±0.05
	Pc stem	6.66±0.15	6.66±0.15	6.66±0.15
	Pc flowes	6.43±0.11	6.43±0.11	6.43±0.15
	Pc root	6.6±0.17	6.6±0.17	6.6±0.17

Table :3.3 Antibacterial activities of various fractions of Bidens pilosa and bidens bipinnata against Strain 3-MRSA

4 DISCUSSION

4.1 Overview of Findings: Function Specialization in *Bidens* Species

This study compares and assesses the phytochemicals and the antibacterial properties of *Bidens pilosa* and *Bidens bipinnata* which together, manifest the uniqueness of each. One of the most important observations in this study is the labeling of the *B. pilosa* leaf extract as a high-potency "specialist" antimicrobial agent. It was the most active against Enterococcus faecalis (10.1 ± 0.28 mm) and the only extract able to inhibit Methicillin-resistant Staphylococcus aureus (MRSA). However, *B. bipinnata* was shown to be a "generalist"; its antibacterials were moderate

and world-wide and even though it had a wider phytochemical diversity in the roots and stems—differentiated by various flavonoid and alkaloid profiles—the extract from this plant was rather weak. Salt-tolerant *B. pilosa* leaves were provided with the chemical that could be responsible for targeted pathogen defense at the next level. The findings bolster the hypothesis that *B. pilosa* leaves have an innate chemical defense which is entirely made up of the compounds evolved specifically for the high-level pathogens defence.

4.2 Phytochemical Profiling and Structural Analogues

HPLC analysis revealed Phloroglucinol as the major phenolic compound in all parts of both species. Phloroglucinol is a powerful bacteriostatic agent that inhibits bacteria by disturbing the cell membrane. However, the steady occurrence of Phloroglucinol is at odds with the stark contrast in antibacterial activity that was noted between the species. This indicates that Phloroglucinol is a base-level defense agent, whereas the increased activity of *B. pilosa* extract is due to the presence of other particular secondary metabolites determined by GC-MS.

Significant qualitative differences were disclosed in the GC-MS profiling. *B. bipinnata* roots and stems had Orientin (a flavone glycoside) and Coumarins, substances that are recognized for their anti-inflammatory effects (Yang et al., 2012). Another fact to be noted is that the GC-MS library search yielded high probability matches for synthetic pharmaceutical compounds (e.g., Triamterene, Hydrocortisone acetate, Epoxiconazole). It is not biologically possible that plants produce these exact synthetic compounds. Instead, these peaks probably stand for the natural analogues with similar structures, such as pteridine derivatives (relating to Triamterene) and phytosteroidal esters (comparable to Hydrocortisone), which exhibit similar mass fragmentation patterns. The intricate scaffolds that characterize *B. bipinnata* contribute to the plant's chemical diversity and support its traditional application for various diseases.

4.3 Antibacterial Efficacy and Comparative Potency

The study's most critical finding is that the crude plant extracts very often surpassed the positive controls, which is rarely the case in the natural product research area. Above all, the *B. pilosa* leaf extract was the one that presented a 10.1 mm inhibition zone (±0.28) for E. faecalis, and this was nearly twice as much as the positive control (6.03 mm ±0.05) in terms of effectiveness. Hence, this implies that the extract is of synergistic potency that is beyond the standard monotherapy for this strain.

In addition, the findings on MRSA indicate a very strict tissue-specific defense mechanism. The *B. pilosa* leaves were the only part of the plant that possessed the ability to inhibit MRSA (8.33 mm), while the stem, flower and root extracts of the same plant had no activity at all. Looking at it from an ecological point of view, this is in agreement with the Optimal Defense Theory, which suggests that plants direct their most potent chemical defenses to the most photosynthetically valuable tissues (leaves) and reproduction organs (flowers) to ensure survival.

Regarding the Gram-negative bacteria which are usually resistant because of their outer lipopolysaccharide membrane; *B. pilosa* flower extract has proved to be highly effective against P. aeruginosa (9.36 ± 0.55 mm) compared to the control (7.63 mm). This leaves the door open for the identification of specific amphiphilic compounds in the flowers that could penetrate the Gram-negative cell wall.

4.4 Mechanism of Action and Safety Consideration

It is very likely that the observed bioactivity is not due to only one compound, but rather the phytoconstituent synergistic action. Phloroglucinol and the fatty acid derivatives are probably the ones that first weaken the bacterial lipid bilayer, thus facilitating the entry of other alkaloids and flavonoids that interfere with intracellular processes.

Detection of Aristolochic acid in *B. pilosa* flower extracts, however, poses major safety concerns. Aristolochic acids are classified as potent nephrotoxins and carcinogens. This probably accounts for the strong antimicrobial activity of the flowers but at the same time, it makes internal use for therapy very high-risk. Thus, *B. pilosa* leaf extract, which revealed the highest anti-MRSA activity and did not contain aristolochic acid, has become the more reliable and hopeful drug development candidate.

4.5 Limitations and Future Directions

This study reveals a significant potential for antimicrobial effect, but certain limitations must be acknowledged. The limitation that was mainly mentioned is the insecure identification of metabolites through the usage of single-quadrupole GC-MS. As it was pointed out earlier, the detection of "drug-like" analogues (for instance, fungal azoles and synthetic steroids) is based on the erroneous matching of natural isomers in libraries. The results highlight the richness of the *Bidens* metabolome but are not sufficient to give a conclusive proof of the structures.

The future research directions will be three-fold: (a) The use of advanced spectroscopic techniques, in particular High-Resolution LC-MS/MS and NMR (1H and 13C), will be paramount in unambiguously determining the structures of the compounds that have been detected as synthetic drugs in the current analysis. (b) The detection of Aristolochic acid in the flowers and the potentially bioactive alkaloids in the roots of necessitate acute toxicity and cytotoxicity assays (e.g., against fibroblast or kidney cell lines) to find out the therapeutic index of these extracts. (c) Future studies should not have to work on crude extracts, as the *B. pilosa* leaves have already shown being specific for anti-MRSA activity. Fractionation will be the next step, where the exact synergistic partner(s) of Phloroglucinol will be isolated that are actually responsible for the targeted inhibition of resistant Staphylococcal strains.

Conclusion

To sum up, the research corroborates the medicinal use of the *Bidens* genus in traditional medicine and the distribution of its bioactive parts is understood better. Although *B. bipinnata* is a chemically diverse "generalist" plant with many traditional uses, *B. pilosa* leaves are the ones that contain the best antibacterial agents for a specific purpose. The extract's ability to resist E. faecalis growth and inhibit MRSA, has put it forward among the best candidates for plant-based antibiotics for new drug development.

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