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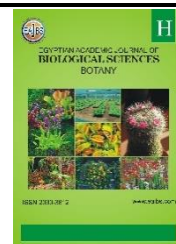
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Phyto-Biochemical Constituents and GC-MS Analysis of *Bidens Pilosa* Leaves and Stems

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ABSTRACT

Alkaloids, phenolics, tannins, glycosides, saponins, sterols, and flavonoids, among other bioactive secondary metabolites, were found in phytochemical analysis. While cardiac glycosides were a lack in aerial parts of the investigated plant and anthraquinones were absent in leaves only. This study identified in *B. pilosa* the total (alkaloids, flavonoids, tannins, saponins, phenolic) %. The non-polar components of *Bidens pilosa* L. were studied. GC-MS analysis of chloroform extracts and crude hexane, as well as other separation chromatographic procedures, were used to identify and characterize ten known chemicals (using MASS). These are the compounds: TRICYCLO[9.3.1.1(4,8)]HEXADE CA-1(15),4,6,8(16),11,13-HEXAE NE-5,14-DIMETHANOL **1**, 1-[(Hexadeuterio)phenyl] Naphthalene **2**, Phthalazine,1,4-diphenyl-, 2-oxide **3**, Pentasiloxane,1,1,3,3,5,5,7,7,9,9-Decamethyl- **4**, Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamet hyl **5**, Hexadecanoic acid, methyl ester **6**, Lamotrigine **7**, 2,5-Dihydroxyacetophenone,2TMS derivative **8**, Acetyl-iso-codeine **9**, Phthalic acid, heptadecyl 2-propylpentyl ester **10**.

INTRODUCTION

Medicinal plants were shown to be a renewable resource that includes substances with nutritional and pharmaceutical characteristics that enable humans to prevent and treat ailments (El Sohaimy, *et al.*, 2015). Plants are important sources of natural antioxidants that can be used to generate novel medications, and medicinal plants have been designated as a repository for numerous bioactive chemical types with various therapeutic qualities. The therapeutic potential of plants was discovered over a long period of time. The great majority of medicinal plant therapeutic benefits include antioxidants, analgesics, anti-inflammatory, antiviral, anti-tumor and antimalarial characteristics (El Sohaimy, *et al.*, 2015). *Bidens pilosa* was from the Asteraceae family, also known as compositae and it is the biggest angiospermic family of dicotyledonous plants, owing to the huge number of individuals (1,620 genus and 23,600 species) which make up this global family in the plant kingdom (Funk, *et al.*, 2005). Attard and Cuschieri, (2009) revealed that Terpenoids (particularly sesquiterpene lactones), flavonoids, alkaloids, polyacetylenes, and numerous phenolic compounds are among the phytonutrients identified in this family. the phytochemical analysis demonstrated that alkaloids, saponins, flavonoids, cardiovascular glycosides and tannins are present in the two

plants (*Bidens pilosa* L. furthermore, *Tridax procumbens* L (asteraceae), while terpenoids, phlobatannin and anthraquinone were absent in both plants. *Bidens pilosa* and *Tridax procumbens* are ethnomedicinal plants that provide an infinite supply of high-value secondary metabolites (Owoyemi and Oladunmoye, 2017). The technology of gas chromatography-mass spectrometry (GC-MS) was developed specifically for examining the many components found in plant extract, as well as their molecular structure. The use of GC-MS to analyze the bioactive components in medicinal plants has two primary advantages. First, the capillary column improves isolation, allowing for a more accurate and precise chemical fingerprint; second, the GC-MS with a connected mass spectrum database could provide quantitative results on the herbs studied, which will be critical for future research into the interaction between phytoconstituents in phyto-medicinals and their bioactivities (Sushma and Arun, 2016). GC-MS is particularly effective in identifying pure compounds in trace levels of less than 1 mg. The GC-MS technique is particularly useful for detecting pure chemicals at extremely low concentrations. As a result, there is no information on a complete GC-MS investigation of the bioactive components of this plant. As a result, a thorough evaluation of the ethanol extract of *Bidens pilosa* leaves and stems was carried out in order to isolate bioactive compounds and therapeutic actions from the more effective ingredients.

MATERIALS AND METHODS

Bidens pilosa plants are collected from two habitats in the northwestern coastal region.

1. Alex-Marsa Matrouh Road,
2. International coast road in Marsa Matrouh, east of Sidi Barni town

Sample Preparation:

The leaves and stems (100 g) of *Bidens pilosa* powdered sample were extracted with a least 70% ethanol and purified depending on the standard technique (Mabry, *et al.* 1970), This extract was utilized to determine the chemical composition of *Bidens pilosa* on a qualitative and quantitative basis, on another hand GC-MASS analysis requires a little quantity of hexane combined with chloroform (v/v) to extract volatile compounds. The slurry was let to still for one day, stirring occasionally, before filtration. The residue was continuously extracted with a % ethanol or hexane to chloroform (v/v) excess volume. The combined filtrates were evaporated at 60° C for a quarter-hour under decreased pressure using a rotavapor apparatus until just a trace of solvent remained.

Preliminary Qualitative Phytochemical Screening;

Using basic phytochemical methods, the ethanolic 70 % crude extracts of *Bidens pilosa* were submitted to preliminary qualitative phytoscreening for the existence of biologically active components (Debiyi and Sofowora, 1978).

Flavonoids Test:

Shinoda Test: The presence of flavonoid was indicated by the pink color when HCl concentrated and segments of magnesium strip were combined with aqueous extract after a few moments.

Glycosides Test: minimal extract quality, mixed with 1 ml water and shaken well, then added a NaOH aqueous solution The presence of glycosides was indicated by a yellow tint.

Cardiac glycosides test: Legal's Test. To treat the extracts, sodium nitroprusside in pyridine, as well as sodium hydroxide, were utilised. A pink to blood-red colour indicates the presence of cardiac glycosides.

Tannins Test:

Lead Acetate Test: Only a few drops of a solution called (10% lead acetate) were combined with 5 ml of extraction. The presence of tannins was detected by the appearance of a yellow

or reddish precipitate.

Sterols Test: 3 ml of the extracts were completely dried by evaporation. After being dissolved in 2 ml chloroform, the remainder was filtered. The filtrate was treated to (Salkowski Reactions), a 1 ml chloroform extract, and concentrated sulphuric acid was poured down the side of the tube. The appearance of a yellow-colored ring that become dark red denoted a positive reaction.

Saponins test: Frothy test:

20 ml of distilled water utilized to dilute the extract by adding and then shaking for a quarter-hour in a measuring cylinder. A 1 cm layer of foam was used to identify saponins..

Phenols test:

Ferric Chloride Test: To the extracts, 3–4 drops of FeCl₃ solution were then added. The existence of phenols is revealed by the blue-black tint when seen it.

Anthraquinone Test:

In a conical flask, for 10 minutes 10 ml of benzene was mixed with 6 g of plant powder sample before filtering. After that, another 10 mL of a 10% ammonia solution was added to the filter and shaken by force for half a minute, the existence of anthraquinones in the ammonia phase was revealed by the existence of (violet, pink, and red) colors.

Total Active Substances Evaluation:

Estimation of Total Phenolic Acids (Li, *et al.*, 2013):

To estimate the quantity of (TPC) total phenolic acids in the extract, the Folin Ciocalteu reagent was utilized. As a standard, gallic acid was utilized, and total phenolics were represented as g/mg gallic acid equal to (GAE). In methanol, gallic acid amounts of 2, 4, 6, 8, and 10 g/ml were made. A plant extract concentration of 1 mg/ml was likewise produced in methanol, The test consisted of mixing 0.5 ml of each sample with 2.5 ml of a 10-fold dilute Folin Ciocalteu reagent and 2 ml of 7.5 % sodium carbonate. After closing the tubes with parafilm and allowing them to sit at room temperature for 30 minutes, the absorbance at 760nm was measured spectrophotometrically. All tests were carried out in triplicate. Reduced substances, such as polyphenols, are responsive to the Folin Ciocalteu reagent. As a result of the reaction, they become blue. This blue colour was spectrophotometrically measured. For the estimate of unknown phenol content, a line of regression from gallic acid (GA) was utilized. From the Gallic acid standard curve, the regression line was shown to be

$$y = 0.03R^2 + 0.0913x = 0.9976$$

The absorbance is denoted by (y), while the g GAE/mg of the extract is denoted by (x). Hence, the goodness of fit for the chosen standard curve was determined to be excellent. By putting the test sample's absorbance (y= absorbance) on the regression line of the previously mentioned (GA).

Total Saponins Estimation: (Okwu and Ukanwa, 2007):

In (200 ml) of 20% ethanol, (20 g) of plant powder was dispersed. At around 55°C, The hot water bath is used to heat the suspension for 240 minutes while being shaken constantly. After filtering the mixture, re-extraction of the residue was performed with (200 ml) of 20% ethanol. Using a 90°C water bath, the full content of the mixture was decreased to 40 ml. The concentration was combined with 20 ml diethyl ether in a 250 ml separating funnel and vigorously agitated. The ether layer was discarded, whereas the aqueous layer was maintained. The purification procedure was repeated with the addition of 60 ml of n-butanol. The mixed n-butanol extract was washed twice with 10 ml of 5% aqueous sodium chloride. A water bath was used to heat the remaining solution. To obtain the desired weight, the samples were dried in an oven after evaporation. As a proportion of total saponins, saponin content was calculated.

Total Tannins Estimation: Gravimetric procedure: (Copper acetate method) (Ali, 1991). This method entails quantitatively precipitating tannin in a copper acetate solution, burning copper tannate to CuO, and estimating the residual CuO. 2g of plant powder were extracted in two different volumes of 100ml acetone-water (1:1) for roughly 60 minutes each and then filtered. In both cases, The mixed extract was poured into a ¼ liter volumetric flask and filled with distilled water to the required volume. In a 500ml beaker, the extraction was heated to boiling then added 30ml of a 15% aqueous copper acetate solution while shaking. The ashless filter paper was used to separate the copper tannate product, which was subsequently burned in a crucible (The crucibles were previously burned at the same temperature and uniform weight). The weight was returned after the residue was treated with a few drops of nitric acid. The following correlation was used to evaluate the weight of Copper oxide and the amount of tannin: 1 g of Copper oxide is equivalent to 1.305 g of tannin.

Total alkaloids Estimation: (Gravimetric method) (Woo, *et al.*, 1977):

90% ethanol was utilized to extract Approximately 10 g of plant powders until exhaustion (as determined by the reagent of Mayer). At a temperature of 40 °C, the alcoholic plant extract was concentrated under reduced pressure until it became dry, then acidified with HCl (3%) and filtered. The acid alkaloid component of the filtrate was extracted using chloroform. Chloroform was used to remove the alkaloid basic component until it was exhausted, and ammonia was used to modify the acidic aqueous layer to an alkaline medium (as determined by reagents of Mayer and Dragendorff). Anhydrous sodium sulphate was used to filter the chloroform extract. It was then evaporated at decreasing pressure until entirely dry, and the percent was estimated by weighing it (w/w).

Total Flavonoid Content (TFC) estimation: (Malla, *et al.*, 2013). Colorimetric analysis was used to evaluate the quantity of total contents of Flavonoids in extracts using an aluminum chloride assay. A 2 ml distilled water was combined with a 0.5ml aliquot of adequately diluted sample solution, then 0.15 ml of a Sodium nitrite 5% solution was added. During 6 min, 0.15 ml of an Aluminum chloride 10% solution was added and allowed to stand for another 6 min before being combined with 2 ml of a Sodium hydroxide 4% solution. Water was immediately poured to raise the definitive volume to 5 ml, following that, the mixture was well-combined once again, then set aside for a quarter-hour. In comparison to a produced water blank, the absorption of the mixture was evaluated at 510 nanometers. For the estimation of total flavonoid, rutin was utilized as a control component. Rutin's calibration curve has been used to calculate the content of total flavonoid in mg rutin/g dry mass (mg rutin/g DW). Three replications of each sample were performed.

$$y = 0.0029x + 0.0034R^2 = 0.9935$$

The absorbance of the extract was (y), while the rutin/mg was (x). so, the fit goodness for a certain standard curve was found to be satisfactory. By inserting the test sample absorbance (y = absorbance) in the line of regression of the rutin previously described.

Qualitative and Quantitative Determination Compounds of *Bidens Pilosa* Plant Using GC-MASS:

GC-MASS was carried out according to Gas chromatography-mass spectro-metry (GC-MS) analysis: By comparing mass spectra and retention times with those of the NIST 11 and WILEY 09 mass spectral databases, the chemical composition of plant extract was separated, purified, and characterized. (Swidan *et al.*, 2020).

RESULTS AND DISCUSSION

The chemical assay is utilized to evaluate the qualitative and quantitative structure of a variety of biochemical substances, allowing for the discovery of active structures that enable the plant to respond biologically. Preliminary phytochemical screening of *Bidens*

pliosa leaves and stems revealed biologically active secondary components such as saponins, flavonoids, tannins, phenolics, glycosides, sterols, and alkaloids. Cardiac glycosides were absent from the studied plant but Anthraquinones, were not found in the leaves of the plant, but they were found in the stems, as shown in the Table (1).

Kayani *et al.*, (2007) revealed that Secondary metabolites are created by secondary reactions coming from primary metabolites such as lipids, carbohydrates, and amino acids. These phytochemical elements are biologically important and have hypolipidemic, anti-tumor, or stimulating effects that can help to minimise the risk of cancer and heart disease. Waititu, *et al.*, (2020), All active compounds (balsams, flavonoids, tannins, phenolic compounds, steroids, terpenoids, alkaloids, glycosides, saponins) were present in the aqueous extract from *Bidens pilosa* excluding anthraquinones. Except for anthraquinones and terpenoids, ethanolic extract from *Bidens pilosa* proved to contain all phytochemicals examined.

Table (2) result shows total active materials in *Bidens pilosa* aerial parts like flavonoids reached (250.6±0.78, 294±0.54) mg/gm rutin and total phenolics reached (338±0.76, 387±0.33) mg/gm gallic acid, as well as its antioxidant and free radical terminator properties, A number of bioactivities, have been revealed in flavonoids and other plant phenolics (Bendini, *et al.*, 2006). As a result, determining the total amount in the aerial regions of the plant is advantageous. Total saponins, tannins, and alkaloids, on the other hand, were (1.51±0.06, 1.76±0.13), (1.38±0.09, and (1.48±0.10, 2.1±0.10) respectively.

GC-MS is one of the most effective methods for determining esters, acids, alcohols, branched-chain hydrocarbons, volatile matter components, long-chain and other chemicals. The phytochemical substances were confirmed using the molecular formula, retention time, and peak area. The active principles, as well as their molecular weight (MW), molecular formula (MF), retention time (RT), and peak area in percent, are provided. The presence of 10 chemicals (phytochemical ingredients) in an ethanolic extract of *Bidens pilosa* leaves and stems revealed the medicinal quality of the plant, according to GC/MS analysis (Table 3).

For the past several years, GC-MS has been utilised to profile metabolites in species of plants. However, only a few plant research facilities have gas chromatography-mass spectrometry. The chemicals discovered have a variety of biological functions, including anti-inflammatory effects (Aparna, *et al.*, 2012), Pesticide, anti-androgenic flavor, antioxidant, hemolytic, 5-Alpha reductase-inhibitor (Kumar *et al.*, 2010), powerful anti-mosquito (Rahuman, *et al.*, 2000).

Gas chromatography-mass spectro-metry investigation of phytochemical substances can be used to identify a plant's therapeutic value. As a result, this type of Gas chromatography-mass spectro-metry investigation is the starting point toward understanding the constitution of therapeutic properties in this natural herb, this kind of investigation will be useful for more detailed study.

Further research is necessary to confirm the pharmacological importance and phytochemistry of *Bidens pilosa*. on another hand biological effect of separation, and compound reflects the important value of *Bidens pilosa* Figures (1,2,3,4).

Table 1. *Bidens pilosa* phytoconstituents.

Bioactive constituents	Leaf	Stem
Flavonoids	+ve	+ve
Phenolics	+ve	+ve
Saponins	+ve	+ve
Glycosides	+ve	+ve
Cardiac glycosides	-ve	-ve
Tannins	+ve	+ve
Alkaloids	+ve	+ve
Sterols	+ve	+ve
Anthraquinones	-ve	+ve

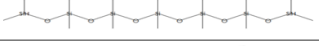

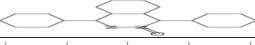

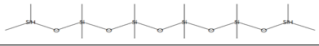


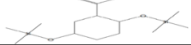
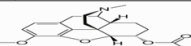

+ve: presence

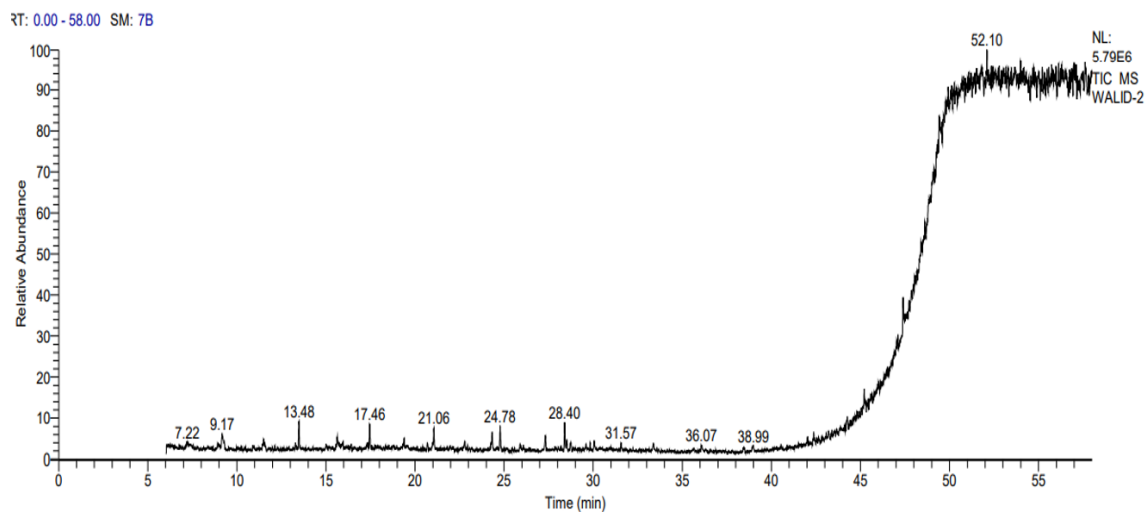
-ve: absence

Table 2. *Bidens pilosa* total active compounds.

Item	Leaf	stem
Total flavonoids (mg/gm rutin)	250.6±0.78	294±0.54
Total phenolic acids (mg/gm Gallic acid)	338±0.76	387±0.33
Total Saponins (%)	1.51±0.06	1.76±0.13
Total Tannins (%)	1.38±0.09	1.26±0.15
Total Alkaloids (%)	1.48±0.10	2.1±0.10

Table 3. GC- MASS analysis of *Bidens Pilosa*.

No.	R.T.	Peak Area (%)	Name of compounds	Molecular formula	Molecular weight	Chemical structure
1	7.22	0.50	TRICYCLO [9.3.1.1(4,8)] HEXADECANE-1(15),4,6,8(16),11,13-HEXAENE-5,14-DIMETHANOL	C ₁₆ H ₁₆ O	224	
2	9.17	2.94	1-[(Hexadeuterio)phenyl] Naphthalene	C ₁₆ H ₈ D ₆	210	
3	13.48	2.66	Phthalazine, 1,4-diphenyl-, 2-oxide	C ₂₀ H ₁₄ N ₂ O	298	
4	17.46	2.42	Pentasiloxane, 1,1,3,3,5,5,7,7,9,9-Decamethyl -	C ₁₀ H ₃₂ O ₄ Si ₅	356	
5	21.06	2.64	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl	C ₁₂ H ₃₈ O ₅ Si ₆	430	
6	24.78	2.43	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270	
7	28.40	3.02	Lamotrigine	C ₉ H ₇ N ₃	255	
8	31.57	0.79	2,5-Dihydroxyacetophenone, 2TMS derivative	C ₁₄ H ₂₄ O ₃ Si ₂	296	
9	36.07	1.07	Acetyl-iso-codeine	C ₂₀ H ₂₃ NO ₄	343	
10	38.99	1.00	Phthalic acid, heptadecyl 2-propylpentyl ester	C ₃₃ H ₅₆ O ₄	516	

**Fig 1.** GC-MS spectral chromatogram of *Bidens Pilosa*

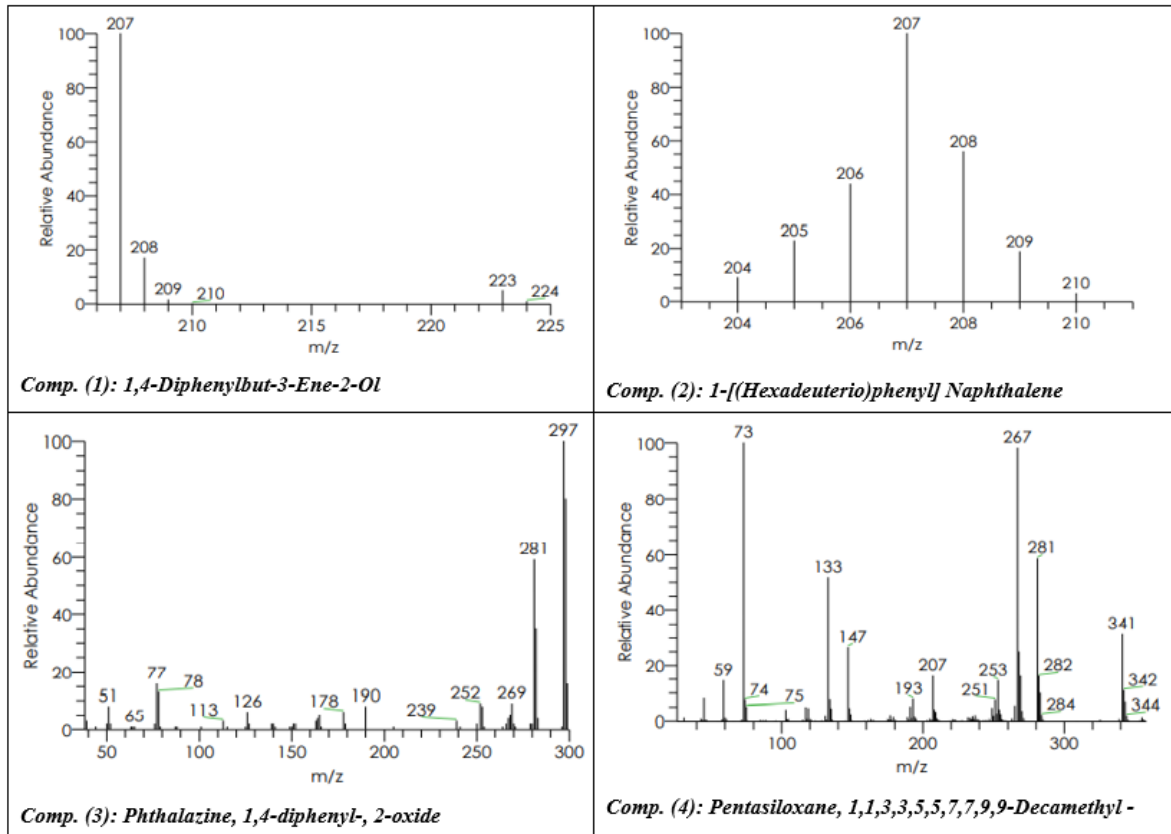


Fig. 2. MASS spectrum for main compounds separation from *Bidens Pilosa*

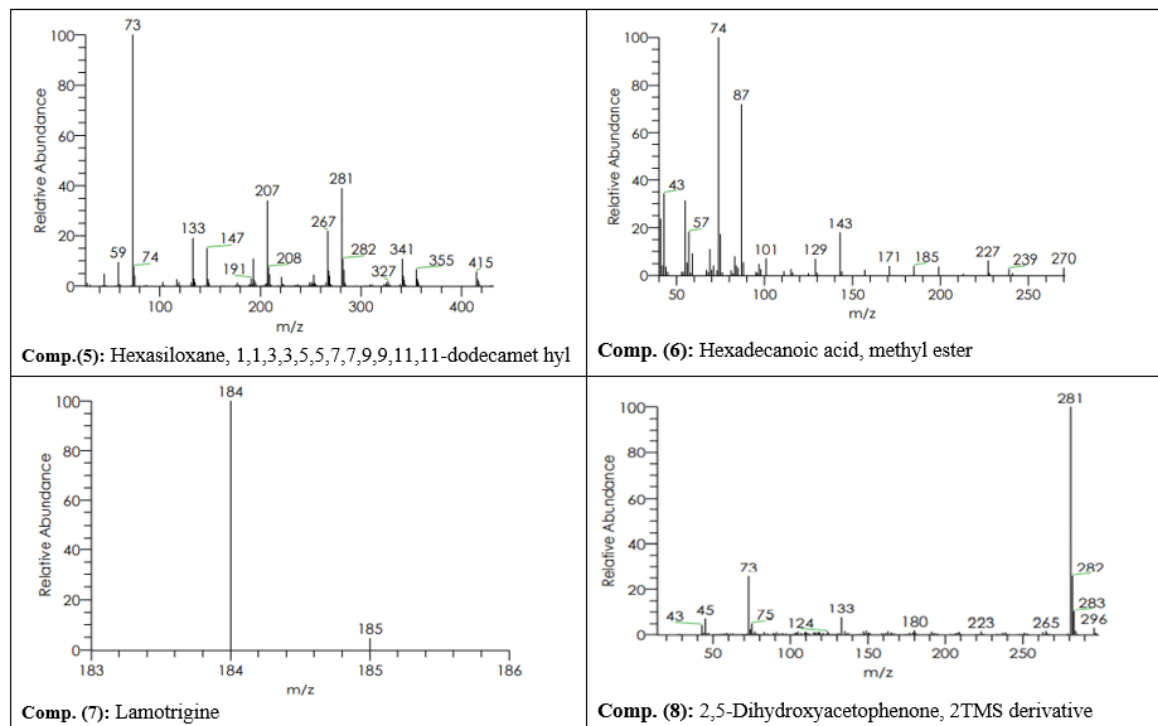


Fig. 3. MASS spectrum for main compounds separation from *Bidens Pilosa*

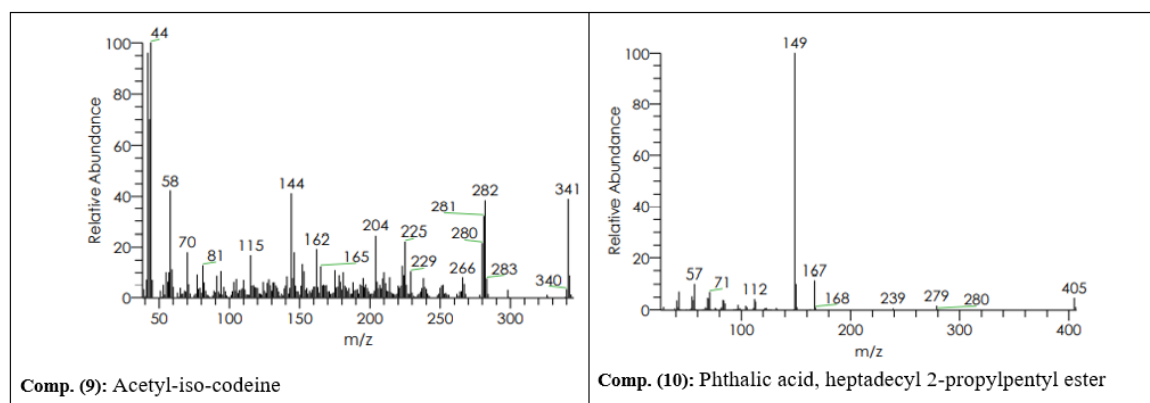


Fig. 4. MASS spectrum for main compounds separation from *Bidens Pilosa*.

Conclusion

Gas chromatography-mass spectrometry analysis of the ethanolic extract of *Bidens pilosa* reveals the presence of medicinally useful bioactive components such as tannins, phenolics, saponins, flavonoids, sterols, and alkaloids. Given the medical benefits of the same components found in many plant extracts, it's no surprise that the components found in *Bidens pilosa* leaves and stems could be just as beneficial. In conventional medicine, research is now conducted to discover its biological activity and optimise its pharmacological profile.

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