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THE POSSIBLE USE OF BIDENS PILOSA IN THE FIELD OF PHARMACOLOGICAL RESEARCH

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ABSTRACT

Bidens pilosa is the common name for an annual plant that is native to the humid forests of Central America. Historically, many different civilizations have used the edible herb Bidens pilosa L. as a therapy for a broad variety of illnesses. All of the plant's biologically active components have been successfully isolated by the research community and characterised. Polyacetylenes and flavonoids make up a significant component of these substances. Studies of B. pilosa using pharmacognosy and phytochemistry have uncovered bioactive chemicals. Some examples of these compounds are terpenes, essential oils, tannins, polysaccharides, phenols, amino acids, and ascorbic acid. Diseases of the respiratory system are among those that benefit from the ingestion of these herbs in the form of decoctions, teas, or juice preparations. Based on a review of published literary sources, this investigation seeks to provide comprehensive data on the chemical components, biological and pharmaceutical effects, and toxicity of this plant. Preparations, extracts, and isolated chemicals from this plant have been shown to have a broad range of therapeutic benefits, including those against malaria, allergies, hypertension, inflammation, diabetes, bacteria, and fungi. Infected wounds and burns may also be treated topically using a poultice produced from juice mixtures. The benefits of the weed may exceed the threats it causes to the environment, despite the fact that it is designated an invasive species in many nations. As a result of its potential therapeutic effects, some individuals may choose to utilise this herb in place of, or in addition to, more traditional medical therapies.

KEYWORDS: Bidens Pilosa, phytochemicals, flavonoids, polyacetylenes, phenolic compounds, pharmacology.

1. INTRODUCTION

Originally from the Asteraceae family, B. Pilosa is a widespread annual weed found throughout the tropics and subtropics of the globe. In certain areas, people consume the plant, while in others, it is used in traditional medicine. It is also known to be effective against a wide variety of health problems.[16] This plant has long been used in traditional ethnomedicine for the treatment of malaria, skin infections, digestive issues, and liver disorders. This plant has been proven to have hepatoprotective, anti-inflammatory, and cytotoxic properties against a wide variety of cancer cells. Phenylpropanoids, polyacetylenes, polyphenols, triterpenes, saponins, and alkaloids were all found in B. pilosa by means of phytochemical investigation.

The plant's supposed medicinal properties seem to be linked to the presence of bioactive phytochemical compounds. Some of these compounds have been found to be useful in halting the spread of disease-causing bacteria and fungus; two that come to mind are polyacetylenes and sesquiterpene lactones.[7] On the other hand, flavonoids have been demonstrated to successfully lower inflammation levels. The phytochemicals and essential oil of B. pilosa have been shown to have free radical scavenging action due to the presence of phenolic compounds. In reaction to osmotic stress and autoxidation, it is physiologically normal for an organism to produce an excess of reactive oxygen species.[11] Many diseases, including cancer, are thought to have their origins in an excess of reactive oxygen species in the human body.

Although under normal conditions natural antioxidants are able to give one electron to neutralise these free radicals, under extreme conditions they are unable to do so. Antioxidants assist the body's own antioxidant defence mechanisms in neutralising harmful free radicals and preventing DNA from being damaged by the constant oxidative stress the body experiences.[13] Nonetheless, resistance to therapy is a big defeat in the war against illness. Since just a small percentage of bacteria have developed resistance to the many antimicrobial treatments on the market, this has

presented serious problems for public health. Herbal medicine has come a long way in the previous 56 years, becoming an effective help to health and a viable hope for novel pharmaceuticals produced from plants.[6] Previous research has indicated that some isolated chemicals derived from B. pilosa are effective, leading scientists to suspect that the plant itself may have anticancer characteristics.

However, the precise polyphenols and flavonoids present in B. pilosa remain unknown, despite claims that it includes caffeoylquinic acid, luteolin, and quercetin, among others. Based on these results, we tested whether an extract of B. pilosa leaves had any antioxidant, antibacterial, anticancer, or mosquitocidal activities in vitro.[9] The phenolic, anticancer, and volatile compounds were identified and quantified using UHPLC-QqQLIT-MS/MS and GC MS, respectively, further proving the plant's potential for use in healthcare systems.

2. METHODS

Plant collection and extract preparation

The leaves of B. pilosa were gathered in December of 2022 at the [Botanical Garden at Mizoram University in Mizoram, India]. The sequence of a rRNA gene found in the plant's internal transcribed spacer (ITS) was submitted to the NCBI genbank under the accession number MF440588. The ITS rRNA genes were also used to distinguish plants. The voucher specimen (MZU/BT/26) is available in the collection of the [Department of Biotechnology at Mizoram University]. The healthy leaves were blended into a powder after being dried in the shade for three days at a temperature of 30 degrees Celsius plus or minus 2 degrees Celsius. For 48 hours, while stirring occasionally, we were able to remove 50 grams of powder from 750 millilitres of methanol. The extract was made in a [Buchi, India], rotating evaporator at 40 degrees Celsius and low pressure. As soon as the crude extract was ready, it was placed in the fridge and kept at 4 degrees Celsius.

Reagents

The following chemicals were used: gallic acid monohydrate, L-ascorbic acid A.R., acetic acid glacial A.R., ferric chloride hexahydrate, ferrous sulphate, sodium acetate trihydrate ACS, 2,2-azinobis-3-ethylbenzothiazoline-6-sulphonic acid disodium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), dimethyl Purchases were made at Fluka and Sigma-Aldrich for the acetonitrile, methanol (of LC-MS quality), and formic acid (of analytical grade), respectively (St. Louis, MO, USA). The Direct-Q 8 UV water purification system was used to provide the highest possible grade water (EMD Millipore Corporation, Billerica, MA, USA). Solvents and other reagents were purchased from Hi-Media in Mumbai, India, and were of analytical quality.

Phytochemical analysis

Total phenolic content (TPC) determination

We used spectrophotometry using the Folin-Ciocalteu technique to gain a feel for TPC. From 10 to 100 mg/mL of extract and from 10 to 500 mg/mL of gallic acid, respectively, were serially diluted. Ten millilitres of extract was combined with ninety millilitres of folin reagent (1:10 v/v in water) and one hundred millilitres of 15% Na2CO3 to make a solution with a volume of two hundred millilitres, the exact amount needed for a 96-well microplate. Later, a microplate holding the mixture was placed inside. After incubating the mixture for a whole hour in the dark, the absorbance was checked at 725 nm using a UV/Vis microplate spectrophotometer (Multiskan GO, Thermo Scientific, Massachusetts, United States). This study's findings were presented in terms of gallic acid equivalent (GAE) per gramme of extract, which was calculated using a gallic acid standard curve.

Determination of total flavonoids

We used a variant of the aluminium colorimetric technique to quantify the flavonoids present in the plant extract. After incubating the mixture of 150 uL of methanol extract and 150 uL of ethanolic AlCl3 at a concentration of 2% in the dark for one hour, the absorbance at 420 nm was determined. Micrograms of quercetin equivalent (QE) per milligramme of plant extract were reported as the total flavonoids content.

Determination of antioxidant potential

By using DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay

Using the DPPH test the antioxidant capacity of a methanolic extract of B. pilosa leaves was determined. To sum up, various concentrations of plant extracts (10-100 g/ml) were added to a newly made 200 l DPPH methanolic solution. It's possible that the concentrations might range from 10 to 100. (0.1 mM). After incubating the reaction mixture at room temperature for 30 minutes, the absorbance at 517 nanometers was taken. Methanol + DPPH served as a blank, whereas ascorbic acid concentration served as the standard. Scavenging capability of DPPH radicals may be calculated using the following formula: % decolorization = [1-(OD Sample/OD Control] X 100. The procedures were run three times, and the mean results were recorded. Specifically, the concentration at which 50% of the DPPH colour was removed was used to determine the IC50 concentration.

Antimicrobial assays

Sample preparation for antimicrobial assay

Once the dimethyl sulfoxide solution was prepared, 10 mg of the B. pilosa leaf crude methanolic extract was added (DMSO). To evaluate the antibacterial efficacy against all of the recommended test species, the final concentration was raised to 10 mg/mL, and then further diluted to achieve varied concentrations of 1, 5, 7, and 10 mg/mL.

Test strains

The agar well diffusion test was used to examine the antibacterial activity of a methanolic extract of B. pilosa leaves. Several strains of Gram-positive bacteria were used in the study. These included Staphylococcus aureus (MTCC-96), Bacillus subtilis (MTCC-2097), and Micrococcus luteus (MTCC-2070). E. coli (MTCC-739), Pseudomonas aeruginosa (MTCC-2453), and the yeast pathogen Candida albicans were also used (MTCC-3017).

Antimicrobial assay by using agar well diffusion method

The antibacterial medicines were first tested using an agar well diffusion test. The organisms were concentrated to provide optical densities of 0.5 McFarland and 108 cfu/ml, and then plated out on agar for further examination. We drilled 6-millimetre-diameter holes in sterilised cork and filled them with 50-microliter samples of extract at varying concentrations. The disc containing the antibiotic tetracycline was used as a positive control, while dimethyl sulfoxide (DMSO) was used as a negative control. The antibacterial properties were seen as a distinct halo zone surrounding the full wells. Three individuals were used in the experiment.

3. RESULTS

Total phenolics and flavonoids contents

B. pilosa leaves extract total phenolic content (TPC) was determined using the Folin-Ciocalteu technique and represented as mg/GAE equivalent. The phenolic concentration of the extract was measured to be 72 g of GAE per mg of DW, which is rather high. After doing the maths, we found that there were 123.33 micrograms of quercetin per milligramme of dry weight (DW) of total flavonoids (Fig 1).

Antioxidant potential

The IC50 value was used to determine the antioxidant capacity of the plant extract examined using DPPH and ABTS as the two different radical scavenging assays. We were successful because of our adherence to these beliefs. The antioxidant capacity (IC50) of B. pilosa leaves was determined to be 80.45 g/ml for the DPPH scavenging test and 171.6 g/ml for the ABTS scavenging test (Fig 2). A more powerful plant extract will have a lower IC50 value when assessing antioxidant capacity.

Antimicrobial assay using agar well diffusion method.

A crude extract of B. pilosa leaves in methanol possesses antibacterial action, as shown in Table 1. The extract was very effective against bacteria, with a zone of inhibition spanning 9.1-18.2 mm. B. pilosa's antibacterial activity was shown to be highest against E. coli, with an inhibition zone of 18.2 millimetres at a dosage of 10 mg/mL, in comparison to the typical amount of ampicillin (30 g/mL). At a dosage of 10 mg/mL, the extract significantly reduced the growth of S. aureus, M. luteus, and P. aeruginosa, with values of 15.66, 14.66, and 14 mm for each organism, respectively. When tested against C. albicans at the same concentration, the extract only demonstrated 9.1 mm inhibition. Contrarily, when evaluated at greater concentrations, the extract's efficacy was relatively modest.

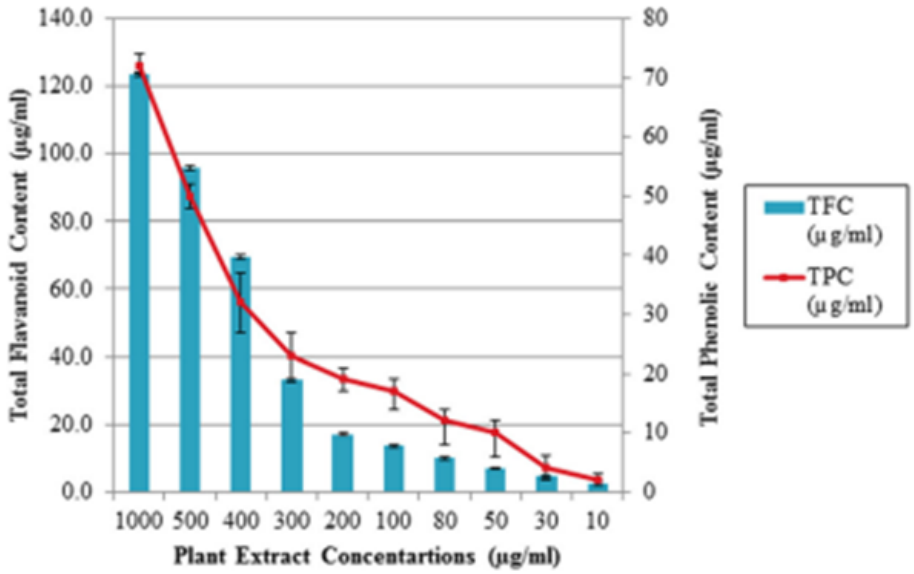

Figure 1: Total phenolic contents and total flavonoids content determined in the leaves extract of B. pilosa. Bar represents the means ± SD of triplicate experiments.

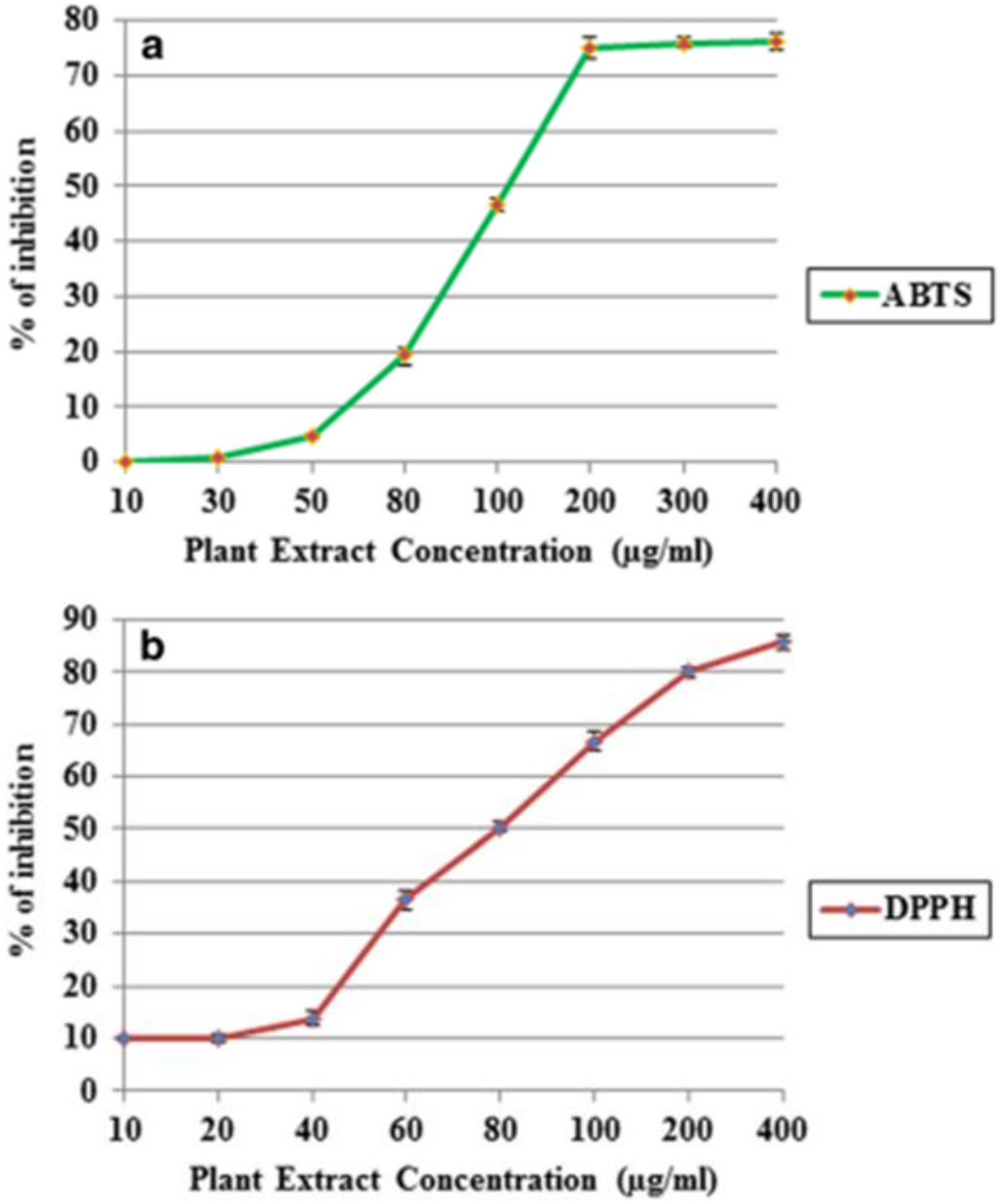

Figure 2: Antioxidant potential of leaves extract of B. pilosa. a ABTS assay (b) DPPH assay.

Table 1: Antimicrobial activity of methanolic extract of Bidens pilosa leaves using agar well diffusion method.

Test Organisms	Diameter of zone of inhibition (in mm)		ANOVA
	Methanolic extract (Zone of inhibition ± SE)	Ampicillin (30 µg/mL) (Zone of inhibition ± SE)	
P. aeruginosa	13.97 ± 0.61	15 ± 0.35	P < 0.05
C. albicans	9.0± 0.32	30 ± 0.31	P < 0.05
E. coli	18.3 ± 0.36a	15 ± 0.11	P < 0.05
S. aureus	16.11 ± 0.25	15 ± 0.01	P < 0.05
B. subtilis	3.1 ± 0.24	10 ± 0.35	P < 0.05
M. luteus	15.12 ± 0.18	15 ± 0.00	P < 0.05

Mosquitocidal bioassay

In Table 2, we can see the MR of C. quinquefasciatus third-instar larvae after they were treated to methanolic extracts of B. pilosa. The maximum MR of B. pilosa was seen 24 and 48 hours after exposure to 1000 ppm (P 0.05). (Table 3). After incubation for 12 hours, the methanol extract of B. pilosa demonstrated the greatest larvicidal activity, at 100%. After being exposed to larger quantities, the larvae struggled for a while, but ultimately succumbed to their wounds and perished.

Table 2: Time dependent mortality check of larvicidal activity of crude methanolic extract of B. pilosa till 48 h at different concentrations.

Plant extract	Concentration in PPM	% Mortality ± SE (Time in h)									
		1	3	6	12	18	24	30	36	42	48
Methanolic leaf extract of B. pilosa	50	0	0	0	4.1 ± 0.25	12.6 ± 0.12	21.5 ± 0.25	38.2 ± 0.17	47.3 ± 0.10	55.7 ± 0.20	68.1 ± 0.20
	100	0	0	6.4 ± 0.11	11.7 ± 0.25	22.5 ± 0.10	39.3 ± 0.10	48.6 ± 0.17	60.2 ± 0.10	68.4 ± 0.25	84.4 ± 0.27
	200	0	0	15.1 ± 0.18	29.5 ± 0.12	43.1 ± 0.27	56.2 ± 0.25	68.0 ± 0.10	77.2 ± 0.05	86.4 ± 0.15	98.1 ± 0.10
	300	0	11.3 ± 0.10	20.1 ± 0.06	28.7 ± 0.15	40.1 ± 0.25	60.5 ± 0.15	75.4 ± 0.17	87.5 ± 0.25	100.0 ± 0.00	-
	400	16.3 ± 0.17	28.1 ± 0.25	42 ± 0.05	59.1 ± 0.15	75.2 ± 0.10	90.8 ± 0.15	100.0 ± 0.00	-	-	-
	500	24.3 ± 0.10	46.9 ± 0.05	70.1 ± 0.15	91.1 ± 0.17	100.0 ± 0.00	-	-	-	-	-
	1000	24.2 ± 0.28	43.1 ± 0.11	68.3 ± 0.24	100 ± 0.00	-	-	-	-	-	-
	Control	0	0	0	0	0	0	0	0	0	0

Table 3: Log probit and regression analysis of third larval instars of C. quinquefasciatus in different concentrations of methanolic extract of B. pilosa for 24 h and 48 h.

Plant extract	Time	Chi Square	LC50 (ppm)	95% confidence limits		df	R^2 Value	Slope ± SE	Intercept ± SE	F value	P value
				Lower limit	Upper limit						
Methanolic leaf extract of B. pilosa	24 h	0.00	149.2	90.3	248.5	4	0.96	0.169 ± 0.015	16.92 ± 4.69	119.1	0.0004
	48 h	0.676	102.3	94.3	110.3	4	0.99	0.064 ± 0.022	75.67 ± 6.69	8.31	0.045

Table 3: Log probit and regression analysis of time dependent larvicidal efficacy of methanolic extract of B. pilosa at different concentrations against third instar larvae of C. quinquefasciatus.

Plant name	Concentration	Chi Square	LT50 (h)	95% confidence limits		df	R^2 Value	Slope ± SE	Intercept ± SE	F value	P value
				Lower limit	Upper limit						
Methanolic extract of Bidens pilosa	50	2.8	27.61	25.74	30.18	8	0.96	1.491 ± 0.097	−8.48 ± 2.63	232.9	0.0001
	100	0.8	26.36	22.09	28.76	8	0.99	1.789 ± 0.057	−5.74 ± 1.566	959.1	0.0001
	200	0.8	20.17	15.74	22.5	8	0.98	2.089 ± 0.083	0.76 ± 2.25	628.8	0.0001
	300	0.8	19.85	16.67	21.67	8	0.98	2.215 ± 0.101	3.196 ± 2.76	471.3	0.0001
	400	5.4	11.17	8.43	12.4	4	0.86	1.816 ± 0.258	30.91 ± 7.0	49.45	0.0001
	500	10.0	5.62	3.82	5.14	7	0.99	1.264 ± 0.367	55.43 ± 9.95	11.8	0.008

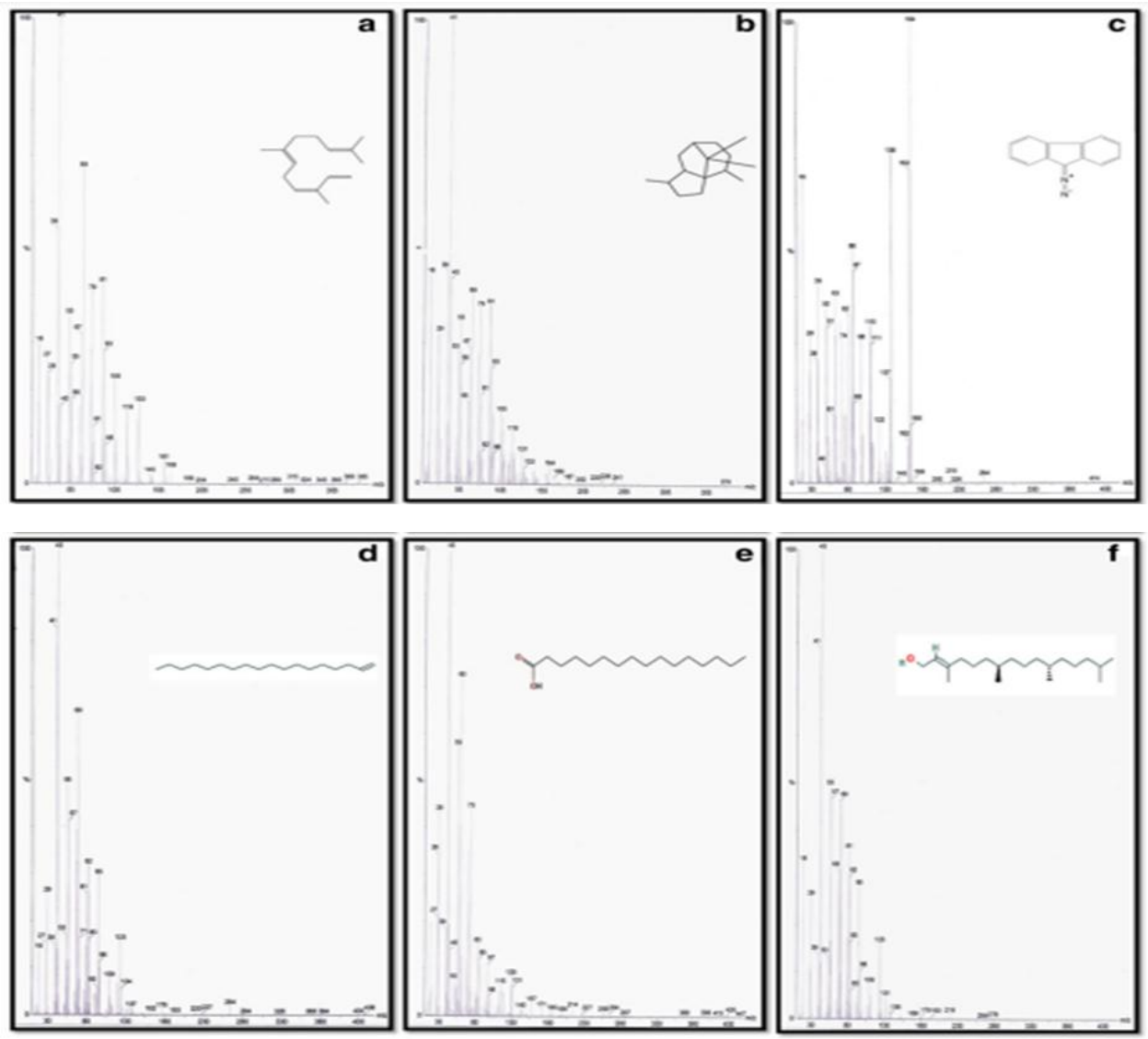

Figure 3: GC-MS Chromatogram detected six volatile compounds from methanolic extract of the Bidens pilosa plant compared with the NIST library. a 1,3,6,10-Dodecatetraene, 3,7,11-trimethyl-(Z,E); (b) 1H-3A, 7-Methyl Azulene, Octahydro-1,4,9,9-tetramethyl; (c) 9H–Fluorene, 9-Diazo; (d) 1-Octadecyne; (e) N-Hexadecanoic acid and (f) 3,7,11,15-Tetramethyl-2-Hexadecen-1-ol.

Dose-response (LC50) and time-response (LT50) larvicidal bioassay

The findings of the larvicidal assay for B. pilosa are shown in Table 3, including the fatal concentration (LC50) after 24 and 48 hours of testing. At 24 hours, the methanolic extract of B. pilosa was most efficient against larvae (LC50= 148.7) while at 48 hours, it was least effective (LC50= 101.7). The Chi-square value for the B. pilosa plant extract varied substantially from 0.045 to 0.0004. There were also statistically significant changes in larval mortality when exposing B. pilosa methanolic extract to the same population at different doses (50-1000 ppm) and exposure durations (24 and 48 h) (P 0.0004). The LC50 values for the methanolic extract of B. pilosa were 0.168 0.015 at 24 hours and 0.063 0.022 at 48 hours at the 95% confidence level (89.3–247.7 ppm at 24 h; 94.4–109.5 ppm at 48 h). It was shown using regression analysis that there was a positive relationship between exposure levels (X) and mortality rates (Y), with R2 values of 0.96 and 0.99 for X and Y, respectively. A larvicidal bioassay using methanolic extract of B. pilosa at various doses was performed on C. quinquefasciatus for 48 hours to determine the time required for the extract to kill the larvae (50–1000 ppm). For C. quinquefasciatus, the LT50 for B. pilosa methanolic extract at 500 ppm was six hours (Table 3). Statistical study showed a link between LT50 values and death rates; for the B. pilosa methanolic extract, these values were 4.28 at 500 ppm and 29.98 at 50 ppm, with a 95% confidence level of UL50 of 2.212 0.101 at 300 ppm. The Chi-square test also yielded a significant result (P 0.008). (4.28 at 300 ppm).

4. DISCUSSION

When it comes to plants' secondary metabolites, phenolics are among the most prevalent. Rose and

Kasum's research suggests that phenolic chemicals may help humans stay healthy by warding off certain illnesses. Additionally, flavonoids are phenolic compounds with systemic antioxidant effects. TPC values of 72 g of GAE/mg of DW were discovered in this investigation. Dry weight (DW) analysis revealed one of the highest flavonoid concentrations ever measured, with 123.3 g of Quercetin per milligramme. More phenolic and flavonoid components were formed, which improved the antioxidant potential of the evaluated extract. When comparing the TPC and TFC concentrations of various B. pilosa parts, Cortés-Rojas and colleagues observed that TPC and TFC levels were highest in the leaves and flowers.[4] In plants, flavonoids mainly defend against UV radiation and eliminate free radicals that may otherwise cause damage. Thus, it should come as no surprise that TFC is most abundant in the regions of the plant that get direct sunshine.

It is widely documented that free radicals play a role in the onset of clinical symptoms. Antioxidants protect the body from free radicals, which may cause a wide variety of ailments. Reducing ROS production and protecting cells with antioxidant defence systems are also viable options.[45] Antioxidant capacity of B. pilosa methanol extract was determined using DPPH and ABTS methods. The DPPH IC50 value of the methanolic extract of B. pilosa was calculated to be 80.45 g/ml over the course of our research. Researchers reported an IC50 for DPPH of 94.2 mg/mL, however we were able to find a value that is lower.[2] The essential oil of B. pilosa's leaves and flowers had an IC50 value of 47 and 50 g/ml for antioxidant activity, respectively. This demonstrated that the selected plants' leaves were the best source of antioxidants. If you want to test for decolorization, ABTS is the better choice since it creates the radicals in a stable form very instantly, well before they interact with putative antioxidants. In contrast to the IC50 value of 0.75 mg/mL determined, we discover that the IC50 value of ABTS is 171.6 g/ml.

Human bacterial pathogens that contribute to a variety of food-borne diseases were tested, and the results showed that B. pilosa had high antibacterial activity against S. aureus, P. aeruginosa, M. luteus, and E. coli. Comparison of the methanolic extract of B. pilosa to the conventional antibiotic ampicillin (50 g/disc) revealed that the extract was much more effective against gram-negative bacteria (18.1 mm diameter zone of inhibition) than ampicillin was (14.6 mm diameter zone of inhibition). It was revealed that E. coli has the biggest inhibitory zone (18.2 mm). These findings contrast with those of a previous research that observed that a methanolic extract of B. pilosa demonstrated a zone of inhibition against E. coli, but found that it was smaller (16.0 mm). Results from our research show that the B. pilosa leaf extract significantly inhibits the growth of S. aureus (15.6 mm). In a similar vein, Ashafa and Afolayan discovered that a methanol extract of B. pilosa inhibited the development of the Gram-positive bacteria S. aureus (5.0 mm). The B. pilosa methanolic extract has been shown to be ineffective against P. aeruginosa and S. aureus in certain prior studies. One theory is that the cell walls of these bacteria account for their resistance to the extracts. Some plant extracts may not be absorbed by the body because cellular barriers prevent their entry.

5. CONCLUSION

The bioactive potential of a methanolic extract of B. pilosa leaves is further corroborated by our results, which also highlight the ecological significance of human well-being. The information gathered might be used to improve future research of the plant under consideration, which could lead to its eventual use in the treatment of cancer, oxidative stress, and antimicrobial infections.

Around the last 40 years, scientists all over the world have studied the pharmacological and phytochemical qualities of B. pilosa, lending credence to its usage in traditional forms of folk medicine. There are not enough well-designed human clinical studies of B. pilosa to make any clear conclusions regarding the plant's usefulness as a herbal treatment, despite the plant's interesting potential. The great variety of pharmacological and biological effects attributed to this plant is due to the many preparations, extraction techniques, and single chemicals that may be obtained from its several components. Polyacetylenes and its derivatives are among the most physiologically active chemicals that have been isolated in large quantities. PHT (compound 1) is the key pharmacological agent responsible for these dramatic results. Other components in this plant, including flavonoids, phenolic acids, terpenes, phytosterols, and fatty acids, have also been related to pharmacological effects. The medicinal properties of the plant have been related to these compounds.

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