*Journal of Harbin Engineering University*
*ISSN: 1006-7043*
Vol 45 No. 9
September 2024

# Bioassay-Guided Antimycobacterial Activity and Identification of Ethyl Linoleate from *Bidens pilosa* Linn.

***Ajanaku C.O[1], Akinduti P.A.[2], Mordi R.C.[3], Osamudiamen P.M[4], Dada O.A.[1], Egharevba G. O.[1] Oluyori A.P.[1] Ajanaku K.O.[1]**

[1]Department of Chemistry, Landmark University, Landmark University, Omu-aran, Kwara State, Nigeria
[2]Department of Biological Sciences, Covenant University, Ota, Ogun State, Nigeria
[3]Department of Chemistry, Chrisland University, Ajebo Road, Abeokuta, Ogun State, Nigeria
[4]Bells University of Technology, Ota, Ogun State

**Abstract**

**Introduction**: The plant, *Bidens pilosa* has wide applications ethno-botanically as an anti-inflammatory, antimalarial, antioxidant, as well as antimicrobial tool for several decades. As an anti-bacterial, it has been employed in the treatment of mouth and stomach ulcers in South Africa; treat hypertension and diabetes in Brazil as well as infections in China. One of the major public health concern is the spread of infectious diseases such as Tuberculosis (TB). Infectious diseases like TB, pose serious threats to human health, particularly in developing countries. Therefore, there is the need for continuous development of new TB drugs and vaccines.

**Objectives**: This study was aimed to study the antimycobacterial activity of the extracts and constituents of the extracts of the roots of *Bidens pilosa* (BP) plant.

**Methods**: Bioassay activity of the root extracts of *Bidens pilosa* led to its further purification. The root extracts which exhibited anti-mycobacterial activity against the mycobacterium species were purified using column chromatography in order to isolate the individual components and tested again with the mycobacterial isolates. The crude, hexane, chloroform and methanol fractions of BP roots were tested against mycobacterial species.

**Results**: The anti-mycobacterial screening identified the fraction of hexane showing strong anti-mycobacterial sensitivity against all the species used. The study led to the isolation of a compound identified as 9, 12-octadecadienoic ethyl ester, (Ethyl linoleate).

**Conclusions**: The results from this study show the isolated compound from *Bidens pilosa* could form prospective framework for drug development effort levelling *M. tuberculosis*.

**Keywords**: *Bidens pilosa*, hexane extract, antimicrobial activity, infectious diseases, medicinal plants

## 1. Introduction

One of the highly infectious diseases demanding urgent global attention as recognized by the World Health Organization is Tuberculosis. Records indicate that 30% of the universal populace is being latently affected with *mycobacterium tuberculosis*[1]. Treatment requires an intensive approach for a duration of six months using prescribed drugs such as: Isoniazid, Rifampicin, Ethambutol, Pyrazinamide, Fluoroquinolone. The inability of patients to complete dose within stipulated time, inadequate facilities and resulting side effects have resulted in resistance to these drugs. The resistance to these drugs has required the need for unceasing exploration for better drugs with no side effects.

Natural products have played vital roles in advancing synthetic and biosynthetic chemistry,

and medicine. Research has shown that the major source of medicine is mostly from natural products, depending on the collaboration from different scientific disciplines [2]. Herbal medicine is the preferred primary health care option for over 80% of the population around the world [3]. *Bidens* (Asteraceae), a genus with about 240 species and a reputation for invasiveness, is one of the widely distributed plants; largely found in China, America, African and Japan, but it originated from South America [4]. *Bidens pilosa* is sometimes known as Spanish needles, cobblers-pegs, pitchforks, blackjack, ghost needle weed etc. [5]. *Bidens pilosa* is locally known as abéręę

olóko in Yoruba. *Bidens pilosa* is a common weed that spreads widely. It has been used as spices and tea ingredient [5]. It has been reported to treat inflammation [6], malaria [7]; [8], microbial infections[9], as well as tumors, [10]; essential oils from BP has antioxidant properties [11]. The medicinal properties of *Bidens pilosa* have been recorded to be effective in treating more than 40 conditions such as digestive problems, immunological disorders, infectious diseases, and metabolic syndromes, whether used as a complete plant or in isolated components [12]. Some bioactive compounds belonging to flavonoids, terpenes, phytosterols, phenolic acids and unsaturated fats are associated with the plant pharmacological impacts [13];[14]). This study was directed to investigate the anti-mycobacterial activity of various fractions and components from the roots of the plant *Bidens pilosa* (BP) at various stages of purification through bio-assay guided techniques, based on the extensive potential applications of the plant.

## 2. Objectives

The study was targeted at evaluating the antimicrobial properties of the root parts of *Bidens pilosa* plant, against *Mycobacterium tuberculosis.* The phytochemical screening were carried out to identify the metabolites present. Extracts of the root part of *B. pilosa* were subjected to bio-assay activity. The bioactive components were further purified using column chromatography.

Spectroscopic analysis of isolates were carried out using $^{1}H$ NMR, $^{13}C$, HMBC and mass spectrometry for the structural elucidation of the compound.

## 3. Methods

### Preparation of Plant Samples

Root samples were gathered from Iyana-Iyesi village in Ado-Odo Ota local government, positioned at 6 degrees 41 minutes north and 3 degrees 41 minutes east. The samples were subjected to a full washing with tap water for dirt removal. Following this, the root samples were left to air-dry for a period of 14 days. Thereafter, the root parts were subjected to cold macerated in 95% methanol for 7 days. The extracts were filtered with glass funnel through a bed of cotton wool.

### Concentration of Extracts

The collected filtrates were concentrated with the rotary evaporator. The fractions derived from the root of *B. pilosa* were concentrated, weighed, and stored in the refrigerator for future utilization.

### Fractionation of plant extracts

The dried crude methanol extracts (10 g) of root of BP were dissolved in 1:1 chloroform and water mixture. A separating funnel was used for this process to obtain the chloroform and aqueous fractions, respectively. The aqueous fraction was kept in the fridge, while the chloroform fraction was allowed to air-dry. To the dried solid chloroform fraction, 100 mL of 95% methanol was added. The solution was placed in a 250 mL separating funnel. Hexane (100 mL) was added to the mixture in the separating funnel and allowed to mix. The methanol and hexane fractions were collected separately. A total of four separate fractions: chloroform, aqueous, hexane and methanol fractions were obtained. After drying, the different fractions were stored in a refrigerator until they were needed for subsequent use.

### Phytochemical analysis of extracts of *Bidens pilosa*

Analysis of the crude extracts and fractions from the root of the *B. pilosa* plant involved a phytochemical screening to determine the presence of compounds such as phenolics, flavonoids, glycosides, alkaloids, saponins, terpenoids, anthraquinones, cardiac glycosides, phlobatannins, and tannins, following established protocols. [15]; [16].

### Antimicrobial activity of root fractions of *B. pilosa*

In order to assess the effectiveness of the extracts against specific disease-causing microorganisms, the antimicrobial assay of the *B. pilosa* root fractions was conducted through the Agar diffusion method targeting the listed pathogens*: Staphylococcus aureus, Salmonella Typhi, Candida albicans, Trichophyton rubrum* according to the procedure described by [17]. Sterile petri

dishes were prepared using nutrient agar, which act as feed for the test organisms. Swab sticks inoculated with the test organisms or isolates at a 0.5% McFarland standard were used to seed sterile nutrient agar plates. A sterile cork borer was then employed to create wells approximately 9 mm in diameter in the plates. The concentrations of the extracts were adjusted to 100, 50, 25, 12.5, 6.25, 3.125, and 1.562 mg/mL using a serial dilution technique. The bored wells on the nutrient agar plates, which had been inoculated, received 0.2 mL of each extract at varying concentrations through the utilization of sterile 1 mL pipettes. The plant extract diffuses to the inoculated agar plates to either inhibit or kill the test organisms.

Following a 24-hour incubation period at 37°C, the plates were inspected for the growth and inhibition of test organisms. Observation of a clear zone around the disc shows that the growth of the test organism has been inhibited by the extract. The extent of antimicrobial activity displayed by the plant extract is commonly linked to the diameter of the inhibition zone This demonstrates that the plant extract's potency increases with the larger size of the inhibition zone. To ascertain the zone of inhibition, the measurement in millimeters from one end of the disk to the other end was taken. The minimum inhibitory concentration (MIC) is the lowest concentration of the diluted plant extracts that can inhibit the growth of specific test isolates on extracts of *B. pilosa.* A standard antibiotic, Gentamycin, was utilized at a concentration of 10 µg/mL.

**Preliminary assessment of bioactivity in plant samples**

Bioactivity screening was carried out on the crude methanol extracts and fractions of the BP plant as part of the preliminary analysis. Testing for antimicrobial effectiveness was carried out on standard MTB (H37R-v) and multiple clinical MTB strains, including those resistant to rifampicin, as per the guidelines provided by FMOH (2010)[18]. Some of the pathogens include *Staphylococcus aureus, Salmonella typhi, Candida albicans, Trichophyton rubrum* and *Mycobacterium tuberculosis* ($H_{37}Rv$). Agar dilution proportion method was employed for susceptibility testing of Mycobacterium tuberculosis, following the methodology outlined in an earlier study [19].

**Antimycobacterial activity of *B. pilosa* fractions**

The effectiveness of various fractions obtained from the root of *B. pilosa* against drug-susceptible Mycobacterium tuberculosis DS-MTB and drug-resistant Mycobacterium tuberculosis DR-MTB was determined in terms of antimycobacterial activity in Table 4 [20].

**Isolation of fractions**

There were four partitioned fractions from the crude methanol extracts of the plant. For each part of the plant, there is the aqueous, chloroform, methanol, and hexane fractions. Bioassay-guided fractionation was carried out on the partitioned fractions against the *Mycobacterium tuberculosis*, at every stage of the purification process of the plant parts. Further purification was carried over a silica gel (70-230 mesh) column (120.0 cm) diameter, 2.4 cm and successively eluted with solvents of increasing polarities, starting with *n*-hexane to 100% methanol, in order to obtain pure isolates.

**NMR analysis**

For a precise spectrum recording, a 20 mg sample was dissolved in 0.5 ml of deuterated chloroform (CDCl3), and subsequent 1H and 13C NMR spectra were acquired using a Bruker DRX500 NMR instrument set at 500 MHz. Trimethyl silane (TMS) served as the internal standard, with chemical shifts (δ) presented in parts per million (ppm) and coupling constants (j) specified in hertz (Hz).

## 4. Results

**Phytochemical Screening of root of *B. pilosa.***

Some of the secondary metabolites identified from the root of BP, when subjected to qualitative phytochemical screening showed the presence of the following compounds: terpenoids, quinones, alkaloids, quinones, tannins, cardiac glycosides and flavonoids.

**Table1: Phytochemical assessment of *B. pilosa* root fractions**

| Constituents/ Fractions | Methanol | Hexane | Chloro-form | Aqueous |
|---|---|---|---|---|
| Tannins | + | - | + | + |
| Saponins | - | - | - | - |

| | | | | |
|---|---|---|---|---|
| Flavonoids | + | + | - | + |
| Alkaloids | + | + | + | + |
| Quinones | + | + | + | + |
| Glycosides | + | - | - | + |
| Cardiac Glycoside | + | + | + | + |
| Terpenoids | + | + | + | + |
| Phenols | - | - | + | - |
| Steroids | - | + | + | - |

Key: + = trace - = not detected

**Table 2: Antimicrobial activities of *B. pilosa* root fraction by agar well diffusion Test**

| **Plant fractions** | ***Staphylococcus aureus*** | ***Salmonella* Typhi** | ***Candida albicans*** | ***Trichophyton rubrum*** |
|---|---|---|---|---|
| Chloro-form | 7.33[c] | 7.33[b] | 8.00[b] | 0.00[b] |
| Metha-nol | 14.33[a] | 15.33[a] | 15.33[a] | 8.33[a] |
| Hexa n-e | 8.00[c] | 8.00[b] | 9.67[b] | 0.00[b] |
| Crude | 10.67[b] | 8.33[b] | 8.33[b] | 8.00[a] |
| *P. Value* | <0.0001* | <0.0001* | <0.0001* | <0.0001* |

Diverse letters in a single column suggest notable distinctions among the plant extracts that were tested. *Significant at P≤0.05.

**Table 3: Minimum Inhibitory Concentration (mg/mL) of root fractions of *B. pilosa* by micro-tube dilution method using clinical isolates**

| **Plant Extract/fractions** | ***Staphylococcus aureus*** | ***Salmonella typhi*** | ***Candida albicans*** | ***Trichophyton rubrum*** |
|---|---|---|---|---|
| Chloroform | 100 | 100 | 50 | 100 |
| Methanol | 100 | 100 | 100 | 100 |
| Hexane | 12.5 | 6.25 | 6.25 | 100 |
| Crude | 25 | 100 | 100 | 100 |

**Table 4: Antimycobacterial activity of *B. pilosa* root fractions by proportion method**

| | DS-MTB (1-5) | | | | | DR-MTB | | H37Rv | P value |
|---|---|---|---|---|---|---|---|---|---|
| Plant Extract/Fraction | 1 | 2 | 3 | 4 | 5 | 1 | 2 | | |
| Chloroform fraction of BP root | R | R | S | R | R | R | R | S | |
| Hexane fraction BP root | S | S | S | S | S | S | S | S | |
| Methanol fraction of BP root | R | R | R | R | R | R | R | R | |
| Crude extract of BP root | R | R | R | R | R | R | R | R | |
| Control | S | S | S | S | S | S | S | S | 0.002 |

Keys: DS-MTB – Drug susceptible *Mycobacterium tuberculosis*; DR-MTB – Drug resistant *Mycobacterium tuberculosis*; H37Rv = *Mycobacterium tuberculosis* strain; S = Sensitive; R = Resistant. Control drugs: susceptible – Rifampicin (40 mg); resistant - Levofloxacin (200 mg), P value<0.05 is significant.

**Table 5: $^{1}H$ and $^{13}C$ NMR chemical shifts for C7**

| **Carbon #** | **$\delta^{13}C$ NMR (ppm)** | **$\delta^{1}H$ NMR (ppm)** | **Multiplicity** | **Coupling constant** |
|---|---|---|---|---|
| 1 | 173.3 | - | | |
| 2 | 33.43 | 2.1 | dt | 7.09, 7.09, 14.78 |
| 3 | 24.91 | 1.43 | m | |
| 4 | 26.89 | 1.09 | m | |
| 5 | 28.89 | 1.09 | m | |
| 6 | 28.89 | 1.09 | m | |
| 7 | 28.89 | 1.09 | m | |
| 8 | 33.43 | 1.86 | dd | 8.14, 15.36 |
| 9 | 129.41 | 5.16 | d | 7.46 |
| 10 | 127.7 | 5.15 | d | 7.46 |
| 11 | 37.15 | 2.6 | s | |
| 12 | 127.7 | 5.16 | d | 7.46 |
| 13 | 129.4 | 5.16 | d | 7.46 |
| 14 | 33.43 | 1.86 | dd | 8.14, 15.36 |
| 15 | 28.89 | 1.09 | m | |

| | | | |
|---|---|---|---|
| 16 | 31.69 | 1.09 | m |
| 17 | 21.97 | 1.09 | m |
| 18 | 13.7 | 0.85 | m |
| | 55.26 | 3.95 | m |
| | 13.8 | 1.09 | m |

This shows the $^{1}$HNMR, chemical shift for isolated fraction, 9, 12- octadecadienoic ethyl ester (C7).

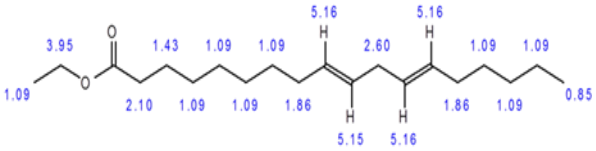


**Fig. 1: Structure of compound C7:**
(9, 12- octadecadienoic ethyl ester) or Ethyl Linoleate

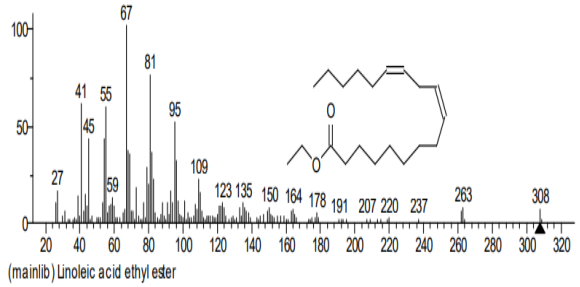


**Fig. 2: Mass Spectrum and structure of (9, 12- octadecadienoic ethyl ester) or Ethyl Linoleate**

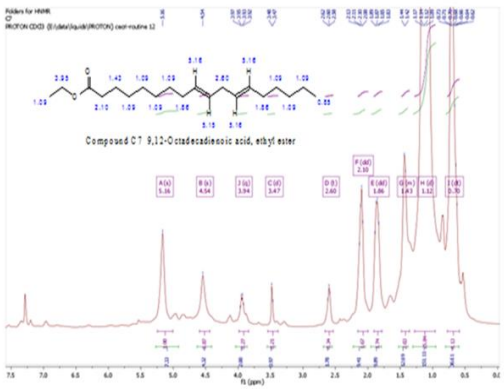


**Fig.3:1H NMR spectrum (500 MHz, CDCl2) of 9,12- octadecadienoic ethyl ester**

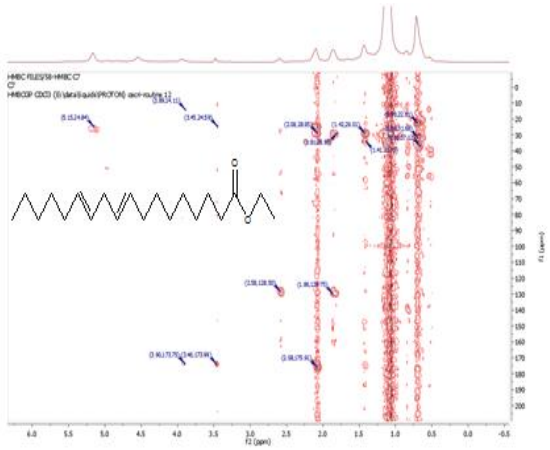

**Fig.4: HMBC spectra of compound C7**
(9,12-Octadecadienoic acid, ethyl ester or ethyl linoleate).

## 5. Discussion

The root fraction of *B. pilosa* contains phytochemicals such as alkaloids, terpenoids, and cardiac glycosides that are present in all fractions (Table 1). Other metabolites identified include: tannins, flavonoids, alkaloids, quinones, steroids and glycosides. Flavonoids are a group of compounds made of polyphenols. They possess several pharmacological benefits such as: antibacterial, anti-oxidant, boost the immune system, lower blood sugar and the blood pressure. Tannins were present in all fractions with the exception of the hexane fraction.

A previous study [21] demonstrated the presence of tannins in the root portion of *B. pilosa*. Earlier studies [22] identified similar metabolites from *Bidens pilosa* plant.

Antimicrobial activity of root extracts from *B. pilosa* was assessed using the agar well diffusion test to determine the zone of inhibition, with the experiment being conducted in triplicate. Statistical analysis using ANOVA was employed as shown in Table 2. The determination of minimum inhibitory concentration was carried out for various extracts against the chosen clinical isolates, as shown in Table 3. The hexane fraction showed minimum inhibitory concentrations of 6.25 mg/mL against *Salmonella typhi* and *Candida albicans,* and 12.5 mg/mL against *Staphylococcus aureus.* The bio-assay conducted on different extracts from the root sections of *B. pilosa* (Table 4) revealed the sensitivity of the hexane fraction to Mycobacterium tuberculosis. Preliminary open column chromatography was carried out on the

7.50 g of BP root extract (hexane fraction). Analyte sample for this extract was prepared by separately dissolving in 20 mL of hexane. An initial column chromatography analysis was performed on the extracts obtained from the roots of *B. pilosa*. The eluates collected from the preliminary column chromatography were further screened for bio-activity. Results obtained from this test was used as a guide to further purification. Silica gel 60 $F_{254}$ glass coated TLC aluminium coated plates was employed to monitor the purification process, until a single spot was observed. Eluted fractions showing similar $R_f$ values on TLC were pooled, then concentrated using rotary evaporator and allowed to dry. The compounds on the plate were visualized using ultraviolet light at wavelengths of 254 nm and 366 nm, as well as iodine and vanillin. The isolates were kept in the refrigerator for further spectroscopic analysis. The purification of BP root extract via open column chromatography, gave a total of seven eluates._The bioassay activity of these eluates identified $BPR_4$, as the most active fraction, from a solvent system composed of hexane, ethyl acetate, and methanol in a 1:4:5 ratio._The last combination obtained from BP root extract was collected at 10% hexane. Identified bioactive eluate, *Bidens pilosa* root forth extract ($BPR_4$), was a brown waxy substance, which was labelled **C7** and kept for further spectroscopic analysis. Pure compounds were analyzed using thin layer chromatography on TLC plates measuring 10 cm x 5 cm x 0.25 mm and were developed with different ratios of hexane to chloroform (8:2, 7:3, 5:5, 3:7). The compound was identified from the interpretation of $^1$H NMR, $^{13}$C, and HMBC. (Bruker DRX500, USA). Topsin software was used to process spectra obtained. The second purification of the hexane fractions of *B. pilosa* root by open column chromatography gave eight sub-fractions. Bioassay-guided fractionation of the hexane fractions of the root parts of BP, identified a bioactive fraction, BPR4, to be sensitive to the mycobacterial species. Further purification led to the isolation of a compound - 9, 12-octadecadienoic ethyl ester, (C7). A compound was extracted from the root of *B. pilosa* using open column chromatography from fraction BPR4, which was derived from the hexane fraction of the root extract of *B. pilosa.* The compound was characterized as 9,12,-Octadecadienoic acid, ethyl ester, $C_{20}H_{36}O_2$. The compound, C7 was found in the region, 0.85 - 5.16ppm, as shown in Fig. 3. At a chemical shift of 1.86 ppm, a doublet of doublet was identified with a coupling constant of 8.14 Hz and 15.36 Hz, as seen in Table 5. The methylenic protons at a chemical shift of 5.15ppm; 5.16ppm gave doublets with a coupling constant of 7.46 Hz. At a chemical shift of 1.09 ppm, a lot of protons were observed as multiplet.

$^{13}$CNMR ($CDC1_3$), the chemical shift for the carbon was from 13.7-173.3 ppm. A total of 20 carbons with different signals were observed. The presence of carbonyl compound was identified at 55.26ppm while the carbonyl carbon was observed at 173.3 ppm. The chemical shift values for the carbon atoms identified are in agreement with literature [23, 24]. Fig.1, shows the structure of the compound obtained from the hexane fraction of *B. pilosa* root, while fig.2 identified the mass spectrum of the compound isolated having a mass of 308.g/mol. The two dimensional analysis of the identified compound is shown in Fig.4. (HMBC). Earlier studies and GC-MS analysis identified this compound as a major component of some selected locally grown plant seeds from the oil samples, [25]; [26] ;[27]. Ethyl linoleate has several benefits which includes: antioxidant, antimicrobial, hepaprotective and hypocholesterolemic properties, which were identified by previous articles [28].

**Conclusion**

This study involved conducting extraction, purification, and analysis of the antibacterial and antimycobacterial properties of the root parts of *B. pilosa,* as well as screening for phytochemical compounds.

Phytochemical analysis revealed the presence of alkaloids, terpenes, flavonoids, and cardiac glycosides as secondary metabolites in the root of *Bidens pilosa.* Analysis of antimicrobial activity showed that the hexane fraction extracted from the root of *Bidens pilosa* is effective against certain clinical isolates. Further bio-assay activities against *Mycobacterium tuberculosis* species identified the hexane fraction more

sensitive than other fractions. This study investigated the extraction of bioactive compounds from the root portions of *B. pilosa* using bioassay-guided methods, as well as assessing their biological effects.

Through phytochemical analysis, a compound was isolated from the root portion of *B. pilosa* using multiple rounds of column chromatography. This compound was identified as: 9, 12-octadecadienoic ethyl ester (ethyl linoleate). This compound is a derivative of unsaturated fatty acid, which constitute the essential fatty acids recommended by professional societies as part of the dietary intakes. It can also be found in some plant oils such as: sunflower, canola, soybean, flaxseed and corn. The unsaturated fatty acid has exhibited antibacterial activity against infectious diseases, contribute to the reduction of the low-density lipoprotein (LDL), thereby reducing the risk of stroke or heart attack, as well as a diet supplement towards prevention of different types of cancer.

**References**

[1] World Health Organization (WHO). Global tuberculosis report 2022. Geneva, WHO, 2022. www.who.int/publications/i/item/9789240061729.

[2] Jagatap, V. R., Ahmad, I., & Patel, H. M. (2022). Recent updates in natural terpenoids as potential anti-mycobacterial agents. *Indian Journal of Tuberculosis*, *69*(3), 282-304.

[3] Bhalla, E. (2020). Traditional Uses of Medicinal Plants for Treatment of Widespread Diseases: A Review. *Epidemiology and Transmission of Infectious Diseases*, 150.

[4] Ruiz-Reyes, E., Mendoza-Cevallos, M. A., Polanco-Moreira, A. P., Segovia-Cedeño, D. G., Alcivar-Cedeño, U. E., & Dueñas-Rivadeneira, A. (2022). Phytochemical study of the plant species *Bidens pilosa* L.(Asteraceae) and Croton floccosus (Euphorbiaceae). *F1000 Research*, *11*(702), 702.

[5] Bartolome, A.P., Villasenor, I.M. and Yang, W. (2013). *B. pilosa* L. (Asteraceae): Botanical Properties, Traditional Uses, Phytochemistry, and Pharmacology. *Evidence-Based Complementary and Alternative Medicine*, Volume 2013, Article ID 340215, 51 pages.

[6] Hong M, Fan X, Liang S, Xiang W, Chen L, Yang Y, Deng Y, Yang M (2020) Total flavonoids of *Bidens pilosa* ameliorates Bone Destruction in Collagen-Induced Arthritis. Planta Med. https://doi.org/10.1055/a-1352-5124.

[7] Andrade-Neto VF, Brandão MGL, Oliveira FQ, Casali VWD, Njaine B, Zalis MG, Oliveira LA, Krettli AU (2004) Antimalarial activity of *Bidens pilosa* L. (Asteraceae) ethanol extracts from wild plants collected in various localities or plants cultivated in Humus Soil. Phytother Res 18:634–639. https://doi.org/10.1002/ptr.1510

[8] Evbuomwan, I. O., Stephen Adeyemi, O., & Oluba, O. M. (2023). Indigenous medicinal plants used in folk medicine for malaria treatment in Kwara State, Nigeria: an ethnobotanical study. *BMC Complementary Medicine and Therapies*, *23*(1), 324.

[9] Khan MR, Kihara M, Omoloso AD (2001) Anti-microbial activity of *Bidens pilosa*, Bischofia Javanica, Elmerillia Papuana and Sigesbekia Orientalis. Fitoterapia 72:662–665. https://doi.org/10.1016/S0367-326X(01)00261-1.

[10] Kviecinski MR, Felipe KB, Schoenfelder T, de Lemos Wiese LP, Rossi MH, Gonçalez E, Felicio JD arc;, Filho DW, Pedrosa RC (2008) Study of the Antitumor Potential of *Bidens pilosa* (Asteraceae) Used in Brazilian Folk Medicine. *J Ethnopharmacol 117*, 69–75, doi:https://doi.org/10.1016/j.jep.2008.01.017.

[11] Falowo AB, Mukumbo FE, Muchenje V (2019) Phytochemical constituents and antioxidant activity of Artemisia Afra and *Bidens pilosa* essential oil in ground pork. J Essent Oil-Bearing Plants 22:176–186. https://doi.org/10.1080/0972060X.2019.1574212

[12] Kuo, T. F., Yang, G., Chen, T. Y., Wu, Y. C., Tran Nguyen Minh, H., Chen, L. S., ... & Yang, W. C. (2021). *Bidens pilosa*: Nutritional value and benefits for metabolic syndrome. *Food Frontiers*, *2*(1), 32-45.

[13] Abdulkader, O. M., Sharaf, A. E. A., Fouda, H. M., & Elhaw, M. H. (2022). Phyto-Biochemical

Constituents and GC-MS Analysis of *Bidens pilosa* Leaves and Stems. *Egyptian Academic Journal of Biological Sciences, H. Botany*, *13*(2), 1-9.

[14] Mtenga, D. V., & Ripanda, A. S. (2022). A review on the potential of underutilized Blackjack (*Bidens pilosa*) naturally occurring in sub-Saharan Africa. *Heliyon*, e09586.

[15] Trease, G.E. and Evans, W.C. (1978) Pharmacology. 11th Edition, Bailliere Tindall Ltd., London, 60-75.

[16] Sofowora A., Ogunbodede E. and Onayade A. (2013). The Role and Place of Medicinal Plants In The Strategies For Disease Prevention. *African Journal of Traditional Complementary Alternative to Medicine,* **2(3):** 365-372.

[17] Mahesh, B. and S. Satish, (2008). Antimicrobial activity of some important medicinal plant against plant and human pathogens. World Journal of Agricultural Science, 4(S): 839-843.

[18] Federal Ministry of Health Nigeria (FMOH, 2010). National Tuberculosis and Leprosy Control Programme Workers' Manual. 5th ed. Abuja: FMOH.

[19] Ajanaku, C.O., Echeme, J.O., Mordi, R.C., Bolade, O., Okoye, S., Jonathan, H., Ejilude, O., (2018). In-Vitro Antibacterial, Phytochemical, Antimycobacterial Activities and GC-MS Analyses of *Bidens pilosa* Leaf Extract. *Journal of Microbiology, Biotechnology and Food Sciences,* 8(1): 721-725.

[20] Ajanaku, C.O., Echeme, J.O., Mordi, R.C., Ajani O.O., Okere, D.U., Kayode, A.A. (2019). Antimicrobial and GC-MS analysis of stem and root extracts of *Bidens pilosa* Lin. *Oriental Journal of Chemistry*, 35(2): 839-848.

[21] Silva, F.L, Fischer, D.C.H, Tavares, J.F, Silva, M.S, De-Athayde-Filho, P.F, Barbosa-Filho, J.M. (2011). Compilation of secondary metabolites from *B. pilosa* L. *Molecules*, **16**:1070–1102. https://doi.org/10.3390/molecules16021070

[22] Ruiz-Reyes E, Mendoza-Cevallos MA, Polanco-Moreira AP, Segovia-Cedeño DG, Alcivar-Cedeño UE, Dueñas-Rivadeneira A. Phytochemical study of the plant species *Bidens pilosa* L. (Asteraceae) and *Croton floccosus* (Euphorbiaceae). F1000Res. 2022 Oct 17;11:702. doi: 10.12688/f1000research.112653.2. PMCID: PMC9623199.

[23] Chang M., Wang G., Kuo, Y.H., Lee, C.K. (2000). The low polar constituents from *B. pilosa* L. var. minor (Blume) Sheriff. *Journal of Chinese Chemical Society* 47:1131-1136.

[24] Runde, M., Ogoko, E., Aletan, U., Suleiman, H. M., Imojara, A., & Hitler, L. (2023). Gas chromatography/mass spectrometry analysis and uv absorptivity of bio-oils extracted from some locally grown plant seeds in nothern Nigeria. *Heliyon*, *9*(11).

[25] Oladeji, O. S., Oluyori, A. P., & Dada, A. O. (2024). Landolphia (P. Beauv.) genus: Ethnobotanical, phytochemical and pharmacological studies. *Saudi Journal of Biological Sciences*, 103988.

[26] Adetuyi, B. O., Aruwa, C. E., Oni, P. G., Ogundolie, F. A., Olajide, P. A., Toloyai, P. E. Y., ... & Inobeme, A. (2024). Extraction and processing of essential oils and their application in food industries. In *Applications of Essential Oils in the Food Industry* (pp. 9-23). Academic Press.

[27] Tyagi, T., & Agarwal, M. (2017). Phytochemical screening and GC-MS analysis of bioactive constituents in the ethanolic extract of Pistia stratiotes L. and Eichhornia crassipes (Mart.) solms. *Journal of Pharmacognosy and phytochemistry*, *6*(1), 195-206.

[28] Nivetha, K., & Prasanna, G. (2016). GC-MS and FT-IR analysis of Nigella sativa L. seeds. *Int. J. Adv. Res. Biol. Sci*, *3*(6), 45-54.