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Extraction Isolation and Phytochemical Screening of Leaves and Stems Of Bidens Pilosa and Evaluation of Antifungal Potential of Extracts

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Abstract: *Bidens pilosa is a representative perennial herb, globally distributed across temperate and tropical regions. B. pilosa has been traditionally used in foods and medicines without obvious adverse effects. Despite significant progress in phytochemical and biological analyses of B. pilosa over the past few years, comprehensive and critical reviews of this plant are anachronistic or relatively limited in scope. The present review aims to summarize up-to-date information on the phytochemistry, pharmacology, and toxicology of B. pilosa from the literature. B. pilosa is a major crop weed, threat to native fauna, and a physical nuisance. It is considered one of the most noxious annual weeds in East Africa. B. pilosa had strong allelopathic effects which is beneficial in enhancing its capacity in interspecific competition and to promote its invasion. It is used as a folkloric medicine for the treatment of various diseases and used extensively by indigenous people, especially in Africa, for the treatment of a variety of ailments. Various compounds with biological activity, mainly, polyacetylenes and flavonoids have been isolated and identified in all parts of the plant. Pharmacognostic studies and phytochemical screenings of B. pilosa had also shown the presences of other compounds with biological activities which include terpenes, essential oils, tannins, polysaccharides, phenols, amino acids, ascorbic acid and organic acids.*

Key words: *Biden Pilosa, antifungal activity, essential oil, flavonoids, terpenes, treatment.*

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I. Introduction

Natural products, such as plants extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity. According to the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. The use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases.**[1]**

Plants have formed the foundation of complicated traditional medicine systems for thousands of years. Medicinal herbs are applied to treat a wide range of disease categories. The first written documentation of the use of medicinal herbs dates from the 26th century BCE in Mesopotamia, and the first record of the use of medicinal herbs by the Egyptians and Greeks dates from 18th century BCE and the 5th century BCE, respectively. Starting around the 11th century BCE, the Chinese and Indians started to develop herbal medicine systems, Chinese herbal medicine, and Ayurvedic medicine, respectively, that continue to be widely practiced today. Therefore, since antiquity, medicinal herbs have played a prominent role in human health. **[2]**

B. pilosa is an easy-to-grow herb that is widely distributed all over the world. It is considered to be a rich source of food and medicine for humans and animals. There is increasing global interest in the use of B. pilosa as shown by the many studies conducted on the plant in recent years. The folkloric use of B. pilosa has been recorded in America, Africa, Asia, and Oceania. To explore the potential clinical application of B. pilosa, it is important to link its traditional use with rigorous evidence-based scientific study. The present review focuses on recent studies on the botany, traditional usage, phytochemistry, pharmacology, and toxicology of B. pilosa. The information provided here highlights the possible usefulness of B. pilosa and its isolated compounds and offers insights into possible future research directions. **[2,3]**

PLANT PROFILE

B. pilosa are divided into three groups: (1) the botany, ethnomedical uses, plant chemistry, pharmacology, and biosafety of B. pilosa; (2) scientific studies that validate the ethnomedical uses of B. pilosa; and (3) the therapeutic and future research potential of B. pilosa.

Table 1: Taxonomy of B. pilosa [3]

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Asterales
Family	Asteraceae
Genus	Bidens
Species	Bidens Pilosa L.

Figure 1: *B. pilosa* (a) and its flowers (b)

***Bidens pilosa* L.**

B. pilosa is an annual, erect and ruderal herb originating from South America and now found in almost all tropical and subtropical region countries. It grows to a height of up to 1.5 m, branching from the base and its yellow flowers have 5-15 mm diameter.

Figure 1. *Bidens pilosa* L.

Morphology

Erect annual herbs 60-90 cm. high. Stem quadrangular, grooved, branches apposite. Leaves pinnately compound, usually 2.5-13.5 cm long including petiole, leaflets 3-5. Heads 21-42 in compound cymes terminating main stem and lateral branches, and 0.7-1 cm in diameter including ray florets, peduncles 1-9 cm long; outer involucral bracts spatulate-tipped, 2.5-5 mm long; ray florets absent or 4-7 per head, rays white or yellowish, 2-8 mm long; disk florets 35-75 per head, perfect, corollas yellow; pappus of 2-3 barbed awns 1-2 mm long. Achenes dark brown or black, straight, wingless, 8-16 mm long, setose. [4]

Extraction

Extraction is the crucial first step in the analysis of medicinal plants, because it is necessary to extract the desired chemical components from the plant materials for further separation and characterization. The basic

operation included steps, such as pre-washing, drying of plant materials or freeze drying, grinding to obtain a homogenous sample and often improving the kinetics of analytic extraction and also increasing the contact of sample surface with the solvent system. Proper actions must be taken to assure that potential active constituents are not lost, distorted or destroyed during the preparation of the extract from plant samples. [3] If the plant was selected, then it is needed to prepare the extract as described by the traditional healer in order to mimic as closely as possible the traditional 'herbal' drug. The selection of solvent system largely depends on the specific nature of the bioactive compound being targeted. Different solvent systems are available to extract the bioactive compound from natural products. The extraction of hydrophilic compounds uses polar solvents such as methanol, ethanol or ethyl-acetate. For extraction of more lipophilic compounds, dichloromethane or a mixture of dichloromethane/methanol in ratio of 1:1 are used. In some instances, extraction with hexane is used to remove chlorophyll. [4]

The other modern extraction techniques include solid-phase micro-extraction, supercritical-fluid extraction, pressurized-liquid extraction, microwave-assisted extraction, solid-phase extraction, and surfactant-mediated techniques, which possess certain advantages. These are the reduction in organic solvent consumption and in sample degradation, elimination of additional sample clean-up and concentration steps before chromatographic analysis, improvement in extraction efficiency, selectivity, and/ kinetics of extraction. The ease of automation for these techniques also favors their usage for the extraction of plants materials.[3]

A brief summary of the experimental conditions for various methods of extraction for plants material

	Soxhlet extraction	**Sonification**	**Maceration**
Common Solvents used	Methanol, ethanol, or mixture of alcohol and water	Methanol, ethanol, or mixture of alcohol and water	Methanol, ethanol, or mixture of alcohol and water
Temperature (^{o}C)	Depending on solvent used	Can be heated	Room temperature
Pressure applied	Not applicable	Not applicable	Not applicable
Time required	3–18 hr	1 hr	3-4 days
Volume of solvent required (ml)	150–200	50–100	Depending on the sample size

Phytochemicals [4,5]

Interest in basic research and application of B. pilosa has increased since its first identification in 1753. This is mainly due to its wide application in medicines, foods, and drinks. Around 116 publications have documented the exploitation and medical use of B. pilosa. To date, 201 compounds comprising 70 aliphatics, 60 flavonoids, 25 terpenoids, 19 phenylpropanoids, 13 aromatics, 8 porphyrins, and 6 other compounds, have been identified from this plant as compiled previously. The structures of these compounds are pre-sented in Tables 4, 5, 6, 7, 8, 9, and 10, respectively. However, the association between B. pilosa phytochemicals and their bioactivities is not yet fully established and should become a future research focus. In the present review, we explore possible associations, describe the importance of the known compounds in relation to their biological activity and discuss their likely mechanisms of action. Compelling evidence suggests that the various diverse bioac-tivities reported for B. pilosa reflect its phytochemical com-plexity. [5]

Phytochemical screening assay

Phytochemicals are chemicals derived from plants and the term is often used to describe the large number of secondary metabolic compounds found in plants. Phytochemical screening assay is a simple, quick, and inexpensive procedure that gives the researcher a quick answer to the various types of phytochemicals in a mixture and an important tool in bioactive compound analyses. A brief summary of the experimental procedures for the various phytochemical screening methods for the secondary metabolites.[5] After obtaining the crude extract or active fraction from plant material, phytochemical screening can be performed with the appropriate tests as to get an idea regarding the type of phytochemicals existing in the extract mixture or fraction.

Extracts tested and the standard bactericidal agent [6]

Crude extracts (ethanolic, water and petroleum ether) and fractions of stem bark and seed of *Z. chalybeum* were tested. Most reports suggested that extracts of the seed and stem were most often used. There was no report of use of leaves at all. Extracts of only stem bark of *W. ugandensis* were tested. No other part of the plant was reported to be medicinally useful. Broad spectrum tetracyline antibiotic capsules was used to produce a standard bacterial killing per-centage for comparison with that of the medicinal plant extracts. [6]

Bactericidal and antifungal tests Agar well diffusion assay

Wells were made on Muller Hinton agar plates using asterile borer. Two to five hour broth cultures of *S. aureus* and *E. coli* were centrifuged at 1956.5g for 15 mins (3x) and reconstituted in 1% gelatin in normal

saline. The turbidity of the reconstituted organisms was adjusted to that of turbidity standard (prepared by adding 0.5ml of 1% $Bacl_2$ to 99.5ml of 0.36N H_2S0_4). Both the standard and bacte-rial suspensions were agitated a vortex mixer immediately prior to use. A sterile cotton swab (on a wooden applica-tor stick) was dipped into the standardized bacterial sus-pension. Broth was expressed from the swabs by press-ing and rotating the swabs firmly against the inside of the tube above the fluid level. The swab was then evenly streaked in three directions over the entire surface of the agar plate to obtain uniform inoculums; a final sweep of the agar rim was made with the cotton swabs. The plates were allowed to dry for 3-5 minutes after which 20ml of the test samples and the controls were dispensed into each well. The concentration of the test samples were 50 mg/ ml of 1% GNS. The plates were incubated at 37^oC for 24hrs during which activity was evidenced by the pres-ence of a zone of inhibition surrounding the well. Zone sizes were measured in millimeters compared to standard tetracycline. **[7]**

Filter paper disc assay

The procedure for inoculation of the plates was essen-tially as given above (agar well diffusion assay). However, instead of drilling wells on the agar, filter paper discs (Whatman No. I) impregnated with 100mg of extracts were applied on the surface of the agar and the plate incubated overnight. Plates on which *Candida albicans* had been applied were also tested. **[7]**

Tube incubation and colony plate assays

Staphylococcus aureus and *E. coli* were cultured on Mueller Hinton Agar. Four colonies of the organisms were taken and grown in 10ml of peptone water for 4 hrs in a shaker to obtain logarithmic phase organisms. The organisms were centrifuged for 15 minutes at 1956.5g and washed twice in 1% GNS. The organisms were finally centri-fuged to obtain a pellet. The pellet was reconstituted with 1% GNS and the turbidity adjusted in order to obtain a turbidity visually comparable to that of the standard (pre-pared by adding 0.5ml of 1% $Bacl_2$ to 99.5ml of 0.36N H_2S0_4). This was crosschecked spectrophotometrically (470nM filter). Serial dilutions were made from this point to 10^{-4}. 10ml of the organisms were plated in duplicate unto Mueller Hinton plates and incubated overnight. A colony count was done on a colony counter after 18hrs. This gave a value of 150 colony forming units (CFU's). 100ml of these organisms at this dilution (equivalent to$1.5x10^3$ CFU's) were incubated with 200ml of plant ex-tract. Preliminary studies indicated that 20ml of this mix-ture when plated at zero time gave a growth of about 100 CFU's. The mixture was incubated at 37^oC on a shaker and 20 ul samples removed and plated at varying periods of time of 0 mins, 30 mins, 60 mins and 90mins onto Mueller Hinton plates in duplicate. The plates were then incubated at 37^0c and the colonies counted after 18hrs. Tetracycline capsules (BP) obtained from Kampala phar-maceuticals Ltd were used as positive controls. Follow-ing standard methods of colony counting of the Ameri-can Public Health Association[4]. mainly colonies between 30-300 were considered. A final concentration of bacte-ria killed or which grew were calculated using the for-mula: **[8,9]**

$$\text{Percentage of organisms killed} = \left(1 - \frac{\text{CFU per ml of test}}{\text{CFU per ml in GNS at Omins}}\right) \times 100.$$

The analysis of all the differences between means was performed using a paired t-test[5]. The confidence in-terval was set at 95% ($p<0.05$). In all cases means are shown with ± standard error.

Determining antifungal activity[8]

Microdilution assay

The plant extracts (100 ml) were serially diluted 50% with water in 96 well microtitre plates (Eloff,1998), and 100 ml of fungal culture was added to each well. Amphotericin B was used as the reference antibiotic and 100% acetone as the negative control. It was previously shown (Eloff et al., 2007) that the final concentration of acetone in the microplate well that the fungi are subjected to has no influence on the growth of fungi. As an indicator of growth, 40 ml of 0.2 mg/ml p-iodonitrotetrazolium violet (INT) dissolved in water was added to the microplate wells. The covered microplates were incubated for three to five days at 35°C at 100% relative humidity after sealing in a plastic bag to minimize fungal contamination in the laboratory. The MIC was recorded as the lowest concentration of the extract that inhibited antifungal growth. The colourless tetrazolium salt acts as an electron acceptor and is reduced to a red-coloured formazan product by biologically active organisms (Eloff, 1998). Where fungal growth is inhibited, the solution in the well remains clear or shows a marked reduction in intensity of colour after incubation with INT.

In order to determine which plants can be used for further testing, not only the MIC value is important, but also the total activity. Since the MIC value is inversely related to the quantity of antifungal compounds present, the quantity of antifungal compounds present was calculated by dividing the quantity extracted in milligrams from 1g leaves by the MIC value in mg/ml. The total activity is used to determine to what volume an extract from 1 g of plant material can be diluted and still inhibit the growth of the test organism (Eloff, 1999). Furthermore, it can also be used to evaluate losses during isolation of active compounds and the presence of synergism (Eloff, 2004).

The total activity can be calculated as:

$$\text{Total activity} = \frac{\text{Quantity of material in mg extracted from 1 g of plant material}}{\text{Minimum inhibitory concentration (mg/ml)}}$$

In the previous study, the total activity of the six plant species (*Bucida buceras*, *Breonadia salicina*, *Harpephyllum caffrum*, *Olinia ventosa*, *Vangueria infausta* and *Xylotheca kraussiana*), were calculated (Mahlo et al., 2010). Total activity for crude extract in this case provided an indication of the volume to which the crude extract or fraction can be diluted and still kill the microorganism.

Assay for free radical scavenging (DPPH) [8,9]

The antioxidant activities of each plant extracts were determined using the qualitative assay 2, 2-diphenyl-1-picrylhydrazyl (DPPH). This assay is preferred because it is used to provide stable free radicals (Fatimi et al., 1993).

A solution of 0.2% DPPH in methanol was prepared and then sprayed on the plates (until they became wet) and allowed to dry in a fume cupboard. The presence of antioxidant compounds was indicated by yellow bands against a purple background on TLC plates.

***Acetylene compound* [9]**

The acetylenes are one class of aliphatic hydrocarbons that has a taxonomically interesting distribution pattern in higher plant families; they occur regularly in only five families, namely the Campanulaceae, Asteraceae, Araliaceae, Pittosporaceae and Umbelliferae . Within the Asteraceae family, these compounds are widely distributed in the Heliantheae tribe . The genus *Bidens* is known to produce compounds of this class. They occur in all parts of the plant, often accumulating in roots.

The principal representative of the C_{13}-polyacetylenes is 1-phenylhepta-1,3,5-triyne. This C_{13}-phenylacetylene is abundant in *B. pilosa* and is present in leaves, stems and roots of the species. The compound is biologically active and several studies have reported that it strongly absorbs long-wave UV radiation, and the activity is altered upon exposure to light (photo activation).

The occurrence of C_{17}-acetylenes is rare in the genus, being limited to the Hawaiian species of *Bidens*, while one compound was related to *B. pilosa* grown in China. Also, three C_{14}-acetylenes, with one being common in species of genus *Coreopsis*, and another, a new compound, were reported first in *B. pilosa*.

Another group of polyacetylenes isolated from *B. pilosa* are the polyacetylene glucosides (PAGs), which are glycosides of polyacetylenes in which a sugar moiety (glycose or rhamnose) is joined to a polyacetylene through an *-O-* glucosidic linkage. Of even more restricted distribution, these have been reported for only two families, Asteraceae and Campanulaceae.

Phenylthiophenes, classified as C_{13}-acetylene and related compounds, are related to only occur in *Coreopsis* and in Hawaiian *Bidens*, however a phenylthiophene and its glycosylate were reported for *B. pilosa* growing in China.

***Flavonoids* [9,10]**

Flavonoids are the class of compound of higher occurrence in the species and are described as chemotaxonomic markers at lower hierarchical levels of the Asteraceae. According to the *Bidens* genus, the flavonoid profile of *B. pilosa* is a complex one that includes aurones, chalcones, flavanones, flavones and flavonols with a wide variety of *O*-methylation patterns and glycosylations , totaling 58 different compounds isolated to date.

Anthochlors (aurones and chalcones) are found in a number of plant families, including the Asteraceae. However research indicates that, despite some variations, anthochlors are good markers for the taxonomic subtribe Coreopsidinae (Heliantheae tribe), thus representing the only case in the family Asteraceae in which a certain type of flavonoid is taxonomically diagnostic at the sub tribal level.

Species of *Bidens* typically contain the chalcones butein (3,4,3’,4’-tetrahydroxychalcone,), okanin (3,4,2’,3’,4’-pentahydroxychalcone) and their 4’-glycosides. Of the aurones, maritimetin (6,7,3',4'-

tetrahydroxyaurone) and sulfuretin (6,3',4'-tetrahydroxyaurone) and their glycosides are commonly found in the genus. These compounds have been reported for *B. pilosa.*

Flavones and flavonols identified from members of *Bidens* are for the most part commonly encountered compounds, *i.e*., glycosides of apigenin, luteolin, kaempferol and quercetin, *B. pilosa* maintains that standard, however some flavonols present methoxy substitutent groups at their positions 3, 6, 7, 3' and/or 4', as in jacein centaureidin and its glycoside centaurein.Among the flavones 5-*O*-methylhoslundin was reported, a compound previously isolated only from *Hoslundia opposite* (Lamiaceae). This unusual compound presents methoxy substituted groups in C5 and C7 and a pyranone derivative at C6.

Other compound classes **[11,12]**

Several other compound classes have been isolated from different parts of *B. pilosa* and are listed in Table 1. Among these, aliphatic hydrocarbon derivatives and simple aromatic hydrocarbons have been reported, although these constituents are rather ubiquitous in plants. Long chain saturated unbranched hydrocarbons between C_{21} and C_{33} have been isolated of *B. pilosa*. Of the saturated unbranched alcohols, the compound 2-butoxyethanol is the only ether-ethanol, while for the unbranched aliphatic carboxylic acid and ester group, three compounds have ether-ester functions . The simple aromatic hydrocarbons and simple phenylpropanoid compounds form two small groups of natural products in *B. pilosa*. In the first, vanillic, salicylic and protocatechuic acids and their derivatives are predominant, while the phenylpropanoids are represented by coumaric, ferulic and caffeic acid. In this group, one new disubstituted acetylacetone was described for *B. pilosa* growing in India.

Also in the phenylpropanoids group, caffeoyl ester derivatives are fairly reported for the specie, and some esters formed by the combination of two caffeic acids to one quinic acid or one caffeic acid to one erythronic acid. The only coumarin described for *B. pilosa* is usually found in other species of the family.

Of the mevalonate pathway, several sesquiterpenes, sterols and triterpenes have been isolated of leaves from *B. pilosa*. The sesquiterpenes reported were characterized by GC-MS . These are divided into mono- and bicyclic, commonly found in leaf extracts from Asteraceae. In the diterpenes, acyclic phytane diterpenoids have been reported; among them phytyl heptanoate is an unusual compound that has an aliphatic chain of seven carbon atoms linked to the terminal acid portion. **[13]**

The most abundant sterols from *B. pilosa* are stigmasterol and sitosterol, which are ubiquitous compounds of plant cell membranes. Stigmasterol derivates, sitosterol glucoside and phytosterin B, a phytosterin first isolated in *B. pilosa* has also been reported. Among the triterpenes, only squalene is an acyclic one. The friedelanes and lupeol derivatives are the more common triterpenes reported for *B. pilosa*. Among the tetraterpenes *β*-carotene is reported to be present in high concentration in young leaves of *B. pilosa*.

Chlorin (=2,3-dihydroporphyrin) and its derivatives – including chlorophyll, pheophytin, chlorophyllin, pheophobide, and many other closely related analogues – are found in most higher plants, algae, and even bacteria. For *B. pilosa* two new pheophytins, with peroxide functionalities in ring *E* were reported, besides another six pheophytins. **[13]**

Only two representatives of the class of nitrogen-containing natural products, one being the nucleoside thymidine are reported. One thyophene was reported from *B. pilosa*. One disaccharide was isolated from an entire *B. pilosa.* Also, two miscellaneous representatives were reported, a quinone linked to an aliphatic chain and one compound of unidentified structure.

The content of essential oil from flowers, leaves and stems of *B. pilosa* has been analyzed by GC-MS in China, Japan, USA, Cameroon, Nigeria and Iran. In this review, the series of components identified as being commonly found in plants containing essential oils and present mostly in very small quantities are not listed. It is then just a brief comment about the main and unusual constituents. In the species a series of mono- and sesquiterpenes have been detected. The major constituents are the sesquiterpenes germacrene-D and *β*-caryophyllene. Polyacetylenes, including 1-phenylhepta-1,3,5-tryin have been identified in root oil and aerial parts. A chromone, known as precocene I, isolated from oil of the leaves from *B. pilosa* also was reported.

Table 17: Antibacterial activity of essential oils and flower extracts from B. pilosa[14]

	Mean zone of inhibition (mm)			
Strain	**Leaf essential oil**	**Flower essential oil**	**Leaf extract**	**Flower extract**
Micrococcus flavus	12.7 ±0.3	8.7 ±0.3	10.2 ±0.2	10.8 ±0.3
Bacillus subtilis	17.3±1.9	11.7 ±0.2	10.9 ±0.2	10.3 ±0.2
Bacillus cereus	19.0 ±1.4	11.2 ±0.3	11.8 ±0.4	18.5 ±1.0
Bacillus pumilus	12.3±0.7	10.8 ±0.2	10.5 ±0.4	7.7 ±0.2
Escherichia coli	13.7±0.4	20.3±0.7	10.2 ±1.1	14.0 ±1.3
Pseudomonas ovalis	12.5±0.8	13.7±1.5	10.2 ±0.6	12.5 ±0.6

Table : Antifungal activity of B. pilosa1[15]

Part/extract	Concentration (ppm)	Strain, % Inhibition		
		Cortiicum rolfsii	Fusarium solani	Fusarium oxysporum
Essential	100	85.7 ±0.9	68.2 ±0	74.5 ±1.7
oils	250	96.0 ±0.8	77.9 ±1.8	87.9 ±0.4
Leaves				
Aqueous Extracts	100	44.6 ±1.7	60.5 ±2.1	71.6 ±0.7
	250	94.2 ±0.3	68.9 ±0.7	82.4 ±1.9
Essential	100	60.4±0.9	89.2 ±0.4	86.9 ±0.5
oils	250	89.4 ±1.2	98.0 ±0.3	94.9 ±0.6
Flowers				
Aqueous Extracts	100	33.1±1.1	71.4 ±0.7	57.3 ±2.2
	250	66.1±1.4	91.2 ±0	90.0 ±0.7

Table: Antifungal activity of *B. pilosa* root extracts

Strain	**LC_{50} (mg/mL)**		
	Acetone extracts	**Methanol extracts**	**Water extracts**
Aspergillus niger	0.14	0.06	0.07
Aspergillus flavus	10.91	6.58	0
Penicillium notatum	0.05	0.05	0.05

TABLE . Preparation and frequency of use

Preparation	**N° informers using the preparation**
Infusion	02
Decoction	04
Tincture	03
Syrup	01
Medicinal wine	01
Juice	01
Poultice	02

Chromatographic techniques for identification of Biden Pilosa[14, 20]

Thin-layer chromatography (TLC) and Bio-autographic methods

TLC is a simple, quick, and inexpensive procedure that gives the researcher a quick answer as to how many components are in a mixture. TLC is also used to support the identity of a compound in a mixture when the R_f of a compound is compared with the R_f of a known compound. Additional tests involve the spraying of phytochemical screening reagents, which cause color changes according to the phytochemicals existing in a plants extract; or by viewing the plate under the UV light. This has also been used for confirmation of purity and identity of isolated compounds.

Bio-autography is a useful technique to determine bioactive compound with antimicrobial activity from plant extract. TLC bioautographic methods combine chromatographic separation and *in situ* activity determination facilitating the localization and target-directed isolation of active constituents in a mixture. Traditionally, bioautographic technique has used the growth inhibition of microorganisms to detect anti-microbial components of extracts chromatographed on a TLC layer. This methodology has been considered as the most efficacious assay for the detection of anti-microbial compounds. Bio-autography localizes antimicrobial activity on a chromatogram using three approaches: (i) direct bio-autography, where the micro-organism grows directly on the thin-layer chromatographic (TLC) plate, (ii) contact bio-autography, where the antimicrobial compounds are transferred from the TLC plate to an inoculated agar plate through direct contact and (iii) agar overlay bio-autography, where a seeded agar medium is applied directly onto the TLC plate. The inhibition zones produced on TLC plates by one of the above bioautographic technique will be use to visualize the position of the bioactive compound with antimicrobial activity in the TLC fingerprint with reference to R_f values. [7,8] Preparative TLC plates with a thickness of 1mm were prepared using the same stationary and mobile phases as above, with the objective of isolating the bioactive components that exhibited the antimicrobial activity against the test strain. These areas were scraped from the plates, and the substance eluted from the silica with ethanol or methanol. Eluted samples were further purified using the above preparative chromatography method. Finally, the components were identified by HPLC, LCMS and GCMS. Although it has high sensitivity, its applicability is limited to micro-organisms that easily grow on TLC plates. Other problems are the need for complete removal of residual low volatile solvents, such as *n*-BuOH, trifluoroacetic acid and ammonia and the transfer of the active compounds from the stationary phase into the agar layer by diffusion. Because bio-autography allows localizing antimicrobial activities of an extract on the chromatogram, it supports a quick

search for new antimicrobial agents through bioassay-guided isolation. The bioautography agar overlay method is advantageous in that, firstly it uses very little amount of sample when compared to the normal disc diffusion method and hence, it can be used for bioassay-guided isolation of compounds. Secondly, since the crude extract is resolved into its different components, this technique simplifies the process of identification and isolation of the bioactive compounds. [16]

High performance liquid chromatography

High performance liquid chromatography (HPLC) is a versatile, robust, and widely used technique for the isolation of natural products. Currently, this technique is gaining popularity among various analytical techniques as the main choice for fingerprinting study for the quality control of herbal plants. Natural products are frequently isolated following the evaluation of a relatively crude extract in a biological assay in order to fully characterize the active entity. The biologically active entity is often present only as minor component in the extract and the resolving power of HPLC is ideally suited to the rapid processing of such multicomponent samples on both an analytical and preparative scale. Many bench top HPLC instruments now are modular in design and comprise a solvent delivery pump, a sample introduction device such as an auto-sampler or manual injection valve, an analytical column, a guard column, detector and a recorder or a printer1. [16,17]

Chemical separations can be accomplished using HPLC by utilizing the fact that certain compounds have different migration rates given a particular column and mobile phase. The extent or degree of separation is mostly determined by the choice of stationary phase and mobile phase. Generally the identification and separation of phytochemicals can be accomplished using isocratic system (using single unchanging mobile phase system). Gradient elution in which the proportion of organic solvent to water is altered with time may be desirable if more than one sample component is being studied and differ from each other significantly in retention under the conditions employed. [17]

Purification of the compound of interest using HPLC is the process of separating or extracting the target compound from other (possibly structurally related) compounds or contaminants. Each compound should have a characteristic peak under certain chromatographic conditions. Depending on what needs to be separated and how closely related the samples are, the chromatographer may choose the conditions, such as the proper mobile phase, flow rate, suitable detectors and columns to get an optimum separation. [18]

Identification of compounds by HPLC is a crucial part of any HPLC assay. In order to identify any compound by HPLC, a detector must first be selected. Once the detector is selected and is set to optimal detection settings, a separation assay must be developed. The parameters of this assay should be such that a clean peak of the known sample is observed from the chromatograph. The identifying peak should have a reasonable retention time and should be well separated from extraneous peaks at the detection levels which the assay will be performed. UV detectors are popular among all the detectors because they offer high sensitivity and also because majority of naturally occurring compounds encountered have some UV absorbance at low wavelengths (190-210 nm). The high sensitivity of UV detection is bonus if a compound of interest is only present in small amounts within the sample. Besides UV, other detection methods are also being employed to detect phytochemicals among which is the diode array detector (DAD) coupled with mass spectrometer (MS). Liquid chromatography coupled with mass spectrometry (LC/MS) is also a powerful technique for the analysis of complex botanical extracts. It provides abundant information for structural elucidation of the compounds when tandem mass spectrometry (MS^n) is applied. Therefore, the combination of HPLC and MS facilitates rapid and accurate identification of chemical compounds in medicinal herbs, especially when a pure standard is unavailable. [16,18]

The processing of a crude source material to provide a sample suitable for HPLC analysis as well as the choice of solvent for sample reconstitution can have a significant bearing on the overall success of natural product isolation. The source material, e.g., dried powdered plant, will initially need to be treated in such a way as to ensure that the compound of interest is efficiently liberated into solution. In the case of dried plant material, an organic solvent (e.g., methanol, chloroform) may be used as the initial extractant and following a period of maceration, solid material is then removed by decanting off the extract by filteration. [19] The filtrate is then concentrated and injected into HPLC for separation. The usage of guard columns is necessary in the analysis of crude extract. Many natural product materials contain significant level of strongly binding components, such as chlorophyll and other endogenous materials that may in the long term compromise the performance of analytical columns. Therefore, the guard columns will significantly protect the lifespan of the analytical columns.

Non-chromatographic techniques[14,19]

Immunoassay

Immunoassays, which use monoclonal antibodies against drugs and low molecular weight natural bioactive compounds, are becoming important tools in bioactive compound analyses. They show high

specificity and sensitivity for receptor binding analyses, enzyme assays and qualitative as well as quantitative analytical techniques. Enzyme-linked immunosorbent essay (ELISA) based on MAbs are in many cases more sensitive than conventional HPLC methods. Monoclonal antibodies can be produced in specialized cells through a technique known as hybridoma technology. [16] The following steps are involved in the production of monoclonal antibodies via hybridoma technology against plant drugs:

(i) A rabbit is immunized through repeated injection of specific plant drugs for the production of specific antibody, facilitated due to proliferation of the desired B cells.
(ii) Tumors are produced in a mouse or a rabbit.
(iii) From the above two types of animals, spleen cell (these cells are rich in B cells and T cells) are cultured separately. The separately cultured spleen cells produce specific antibodies against the plants drug, and against myeloma cells that produce tumors.
(iv) The production of hybridoma by fusion of spleen cells to myeloma cells is induced using polyethylene glycol (PEG). The hybrid cells are grown in selective hypoxanthine aminopterin thymidine (HAT) medium.

Fourier-transform infrared spectroscopy (FTIR*)*

FTIR has proven to be a valuable tool for the characterization and identification of compounds or functional groups (chemical bonds) present in an unknown mixture of plants extract. In addition, FTIR spectra of pure compounds are usually so unique that they are like a molecular "fingerprint". For most common plant compounds, the spectrum of an unknown compound can be identified by comparison to a library of known compounds. Samples for FTIR can be prepared in a number of ways. For liquid samples, the easiest is to place one drop of sample between two plates of sodium chloride. The drop forms a thin film between the plates. Solid samples can be milled with potassium bromide (KBr) to and then compressed into a thin pellet which can be analyzed. Otherwise, solid samples can be dissolved in a solvent such as methylene chloride, and the solution then placed onto a single salt plate. The solvent is then evaporated off, leaving a thin film of the original material on the plate. [6,19]

TESTING OF PHRMACOLOGY ACTIVITY

Pharmacological Action

The whole plant (roots and arial part) of *Bidens pilosa* L. has been reported for various biological activities as following

Anti-mycobacterium, Antimicrobial, IFN-α promoter, Anti-Angiogenic, Anticancer, Antidiabetic, Antimalarial and antibacterial. Antiinflammatory and antiallergic, Immunomodulatory, Antioxidative, Gastric antisecretory and Antiulcer etc.

Antibacterial activity

Antibacterial activity of Methanolic Leaf extract of *Bidens pilosa* (with two-fold serial dilutions of plant extracts from 8.0- 0.25 mg/ml) was evaluated by agar dilution method against *S. aureus*, *S.* epidermis and *B. subtilis.* Minimum inhibiting concentrations (MIC) were determined for extracts with antibacterial activity > 0.60, Extract showed the MIC value as in *S. aureus* (2.0), *S. epidermis* (8.0), *and B. subtilis* (4.0). MIC values were taken as the lowest concentration of extract that completely inhibited bacterial growth after 18 h of incubation at 37°C. Neomycin was used as the reference and appropriate controls with no extract 'and solvent were used.

Anti-inflammatory and antiallergic activity[16]

Dried powder of *Bidens pilosa* used for *In vitro* experiments, the crude drug was extracted with water for 24 hr at 100℃. In the In vitro experiments the suspension of *Bidens pilosa* in 0.25% carboxy-methyl-cellulose sodium (CMC-Na) was used. The animals (male wistar rates (200 g), Male ddY mice (18-20 g), and male BALB/c mice (18-20 g), were administered pharmacologic dose of *Bidens pilosa* 100, 250, 500 mg/kg. Oral administration of *Bidens pilosa* suspension in CMC-Na solution on the production of IgE. The level of IgE in serum after 10 days of antigen immunization was higher than that at 5 or 15 days. *Bidens pilosa* dose dependently, in conjugation with cyclophosphamide as a positive control, inhibited the serum IgE level after 10 days of immunization. These results suggest that *Bidens pilosa* have the potential to regulate the host immune response to type I allergy. Therefore *Bidens pilosa* or flavonoids of *Bidens pilosa* may suppress the production of IgE by improving the helper T cell balance.

Determination of antifungal activity-

Antifungal activity of the above mentioned extracts was determined, using agar-well diffusion method. The extracts were dissolved in DMSO to obtain final concentration of 100 mg/ml. 100 µl of test compound was

introduced into the well and plates were incubated at 28°C ± 2°C for 48 hours. Dimethyl sulfoxide (DMSO) was used as a negative control. *clotrimazole* is used as standard fungicide at 50 ppm. [20,22]

Sr. No.	***Test organism***	***Zone of inhibition (mm)***				
		Leaf extract		***Latex extract***		***CLOTRIMAZOLE***
		Aqueous	***Ethanol***	***Aqueous***	***Ethanol***	***(50 ppm)***
1.	*Alternaria alternata*	*09*	*14*	*10*	*15*	*25*
2.	*Aspergillus flavus*	*07*	*09*	*10*	*13*	*28*
3.	*A. fumigatus*	*08*	*12*	*09*	*12*	*20*
4.	*A. niger*	*08*	*10*	*11*	*15*	*30*
5	*A.terrus*	*09*	*11*	*10*	*13*	*29*
6.	*Cuvularia lunata*	*08*	*11*	*12*	*16*	*32*
7.	*Fusarium solani*	*10*	*13*	*12*	*20*	*32*
8.	*Rhizoctonia solani*	*10*	*13*	*09*	*16*	*30*
9.	*Penicillium chrysogenum*	*12*	*15*	*13*	*18*	*35*
10.	*Rhizopus stolonifer.*	-	*0*	-	-	*22*

Antimalarial Activity[15, 23]

Dried plant roots were powdered (100 g each), extracted by percolation with 80% ethanol at room temperature, and the solvent evaporated to dryness at 45°C maximum. Dried extracts were submitted to *in vivo* and *in vitro* antimalarial assays or chemical analysis. Swiss albino adult mice, weighing 20–22 g, were used for the antimalar ial and toxicity tests. The *in vitro* antimalarial activity of *B. pilosa* tested against three *P. falciparum* isolates with various susceptibilities to chloroquine was similar, based on each inhibitory concentration dose (ICs). The IC50 of plants cultivated in standard soil ranged from 25 to 36 µg/mL. [17]

T helper cell modulator

Cytopiloyne (a polyacetylene) functions as a T cell modulator that may directly contribute to the ethnopharmacological effect of *Bidens pilosa* extract on preventing diabetes. Cytopiloyne was able to inhibit the differentiation of human Th0 cells into Th1 cells in a more effective way. CD4+ T cells were isolated from lymph nodes of BALB/c mice for Th cell differentiation study. It is found that cytopiloyne concentration-dependently (1-5 µg/ml) decreased the percentage of INF-γ-producing cells (i.e. Th1 cells) from 72.0% to 59.8%. Since Th1 and Th2 cell differentiation is cross-regulated and mutually antagonized next examined whether cytopiloyne could modulate Th2 cell differentiation. It is found that an addition of cytopiloyne to the differentiating Th cells increased the percentage of mouse IL-4-producing cells from 23.7% to 30.9% in a concentration-dependent manner. Cytopiloyne at these doses did not show any cytotoxicity toward the differentiating cells even after 24 h incubation. So it is concluded that cytopiloyne inhibited Th1 cell differentiation but increased Th2 cell differentiation in mouse T cells.

Activity on KCl- and norepinephrine-induced contractions of rat aorta[17]

Effect of leaf aqueous extract of *Bidens pilosa* L. on KCl- and norepinephrine-induced contractions of rat aortic strips was studied. In aortic strips with endothelium intact, contractions induced using 60 mM KCl and 10^{-5} M norepinephrine were dose-dependently relaxed by the extract, a more significant effect being seen with norepinephrine- induced contractions. Following mechanical damage to the aortic endothelium, inhibition of contractions was more prominent (105%) with the norepinephrine-induced contractions compared with KCl-induced contractions (15%) when the maximal dose (8 mg/ml) of the extract was used. The results suggest that the relaxation effect of the extract may be due to the blockade of the influx of extracellular Ca^{2+} into the cell. [17, 19]

Activity on various gastric ulcer models in rats

The methanol, cyclohexane and methylene chloride extracts of *Bidens pilosa* was studied for anti-ulcerogenic activity using the HCl: Ethanol gastric necrotizing solution. The methylene chloride extract, which showed the highest activity (100% inhibition) at a dose of 750 mg/kg compared with the methanol and cyclohexane extracts (41 and 46% inhibition, respectively), was further tested using the indomethacin-HCl:ethanol, absolute ethanol and pylorus ligation-induced ulcer methods. Pre-treatment with indomethacin significantly reduced the protective effect of the extract against HCl: ethanol solution to 31%. The extract had very little gastric mucosal protection against absolute ethanol (9.8% inhibition at 750 mg/kg) compared with the controls and neither reduced gastric acid secretion *in vivo* nor the acidity of gastric juice following i*n vitro* incubation. [14, 19]

Hypotensive effects in rats

The effects of the aqueous (150–350 mg/kg) and meth ylene chloride (150–300 mg/kg) extracts of *Bidens pilosa* was performed on fructose-induced hypertension in rats. The aqueous and methylene chloride extracts of *Bidens pilosa* reversed the high blood pressure and hypertriglyceridemia developed due to fructose feeding but did not have any effects on plasma levels of insulin and glucose. High doses of the extracts reduced plasma creatinine levels and tended to increase plasma cholesterol. These results suggest that the extracts of *Bidens pilosa* possess hypotensive effects whose mechanism of action is not related to insulin sensitivity.

Stimulate IFN-expression[18]

Hot water crude extracts from *Bidens pilosa* and its butanol subfraction increased IFN-γ promoter activity up to two- and six-fold, respectively. Finally, centaurein (EC50 =75µg/ml) and its aglycone, centaureidin (EC50 = 0.9µg/ml), isola ted from this butanol subfraction, augmented IFN-γ promoter activity by about four-fold. Consistent with the role of centaurein or its aglycone in IFN-γ regulation, it showed that centaurein induced the activity of NFAT and NFκB enhancers, located within the IFN-γ promoter, in Jurkat cells. Overall, the results showed that centaurein regulated IFN-γ transcription, probably via NFAT and NFκB in T cells.

Gastric antisecretory and antiulcer activities

The ethanolic extract (0.5-2 g/kg) decreased the gastric juice volume, acid secretion, as well as pepsin secretion in pylorus ligated rats. *Bidens pilosa* extract showed antiulcer activity against indomethacin-induced gastric lesions. The extract effectively inhibited gastric haemorragic lesions induced by ethanol, and with an effective dose of 2 g/kg being more potent than sucralfate (400 mg/kg). *Bidens pilosa* ethanolic extract exerts a cytoprotective effect in addition to its gastric antisecretory activity that could be due, partly at least, to the presence of flavonoids of which quercetin was identified by HPLC. **[20, 21]**

Immunosuppressive activity

The immunomodulatory effect of the methanolic extract obtained from dried leaves of *Bidens pilosa* L. and the polyacetylene 2-*O*-*β*-D-glucosyltrideca-11*E*-en-3,5,7,9-tetrayn 1,2-diol, isolated from it was investigated. The extract inhibited the proliferative response in two *in vitro* models: **human lymphocytes** stimulated by 5 µg/ml phytohemagglutinin or to 100 nM 12-*O*-tetradecanoyl phorbol-13-acetate plus 0.15 µM ionom ycin and **murine lymphocytes** stimulated by 5 µg/ml concanavalin A (Con A) or in the mixed l eukocyte reaction (IC_{50} =12.5 to 25 µg/ml) 2-*O*-*β*-D-glucosyltrideca-11*E*-en-3,5,7,9-tetrayn 1,2-diol was 10-fold more potent than the original extract in blocking both human and murine lymphocyte proliferation (IC_{50}= 1.25 to 2.5 µg/ml. In mice, the intraperitoneal administration of methanolic extract of *Bidens pilosa* significantly reduced the size of the popliteal lymph node after the inflammation induced by zymosan. One week after the injection of zymosan (150 µg) in the foot pad, PLN weighed 4.6 ±6 mg in comparison with 0.5 ±0.07 mg of the contralateral non-inflamed foot pad. The intraperitoneal treatment with 10 mg extract from day 2 to day 6 after zymosan injection reduced the PLN weight to 1.8 ±0.3 mg. **[20]**

Antioxidant activity

Aqueous infusion of *Bidens pilosa* is studying for antioxidant activity by its protective effect on the hemolysis induced by an initiator of radicals such as 2,2´-azobis (2-amidinopropane) dihydrochloride (AAPH). The amount of *Bidens pilosa* infusion that halved the hemolysis induced by AAPH was 6 µl, which coresponds to an I C_{50} of 1.19 mg of dry weight per milliliter of infusion. Thus, the oxidative hemolysis of erythrocytes induced by AAPH was suppressed by an aqueous infusion of *Bidens pilosa*, which is a very active antioxidant and exerts its protective effect at low amounts. **[18]**

Protection from oxidative damage in normal human erythrocytes

The ethanol (EtOH) and ethyl acetate/ethanol (EA/EtOH) extracts from the whole *Bidens pilosa* plant have the property to protect normal human erythrocytes against oxidative damage *in vitro*. It was determined that the oxidative hemolysis and lipid/protein peroxidation of erythrocytes induced by the aqueous peroxyl radical [2,2´-azobis(2-amidinopropane) dihydrochloride (AAPH)] were suppressed by both EtOH (50–150 µg/ml) and EA/EtOH (25–75 µg/ml) extracts of *Bidens pilosa* in concentration- and time-dependent manners. *Bidens pilosa* extracts also prevented the decline of superoxide dismutase (SOD) activity and the depletion of cytosolic glutathione (GSH) and ATP in erythrocytes. These results imply that *Bidens pilosa* may have protective antioxidant properties. **[18,19]**

Protective effects on animal liver injury and liver fibrosis

Total flavonoids of *Bidens pilosa* L. (TFB) (25, 50 and 100 mg/kg) were administered via gavages daily for 10 days to CCl_4 treated mice as well as TFB (30, 60 and 90 mg/kg) administered for 6 weeks to CCl_4 treated rats. The results showed that TFB (50 and 100 mg/kg) effectively reduced the CCl_4 induced elevated liver index, serum ALT, AST levels, hepatic MDA content, and restored hepatic SOD, GSH-Px activities in acute liver injury mice. TFB (60 and 90 mg/kg) treatment significantly inhibited NF-κB activation in liver fibrosis of rats. The histopathological analysis suggested that TFB reduced the degree of liver injury in mice and severity of liver fibrosis in rats. These results suggested that TFB had a protective and therapeutic effect on animal liver injury, which might be associated with its antioxidant properties and inhibition of NF-κB activation

Anticancer and antipyretic activity

The extract from whole plant *Bidens pilosa* L was extracted with *n*-hexane, chloroform and methanol extract (E1- E3). Screening of different extracts and fractions has been conducted using the *in- vitro* comet assay for anticancer and the antipyretic action, which was done with *in-vivo* models. *n*-hexane extract shows remarkable anticancer activity and methanolic extract bears maximum antipyretic activity. In the antipyretic activity, paracetamol was used as the standard test drug. The most promising material (LC50 < 1500 µg / ml) was F1 ethyl acetate fractions of methanolic extract and methanolic crude extract of whole plants. The extract obtained from the whole plant of *Bidens pilosa* L. showed a significant cytotoxic effect to methanolic extract against Hela cells by *in vitro* method and showed a comparable antipyretic activity effect to paracetamol in rabbit pyrogen test. **[8, 14]**

Toxicology

Despite its use as an ingredient in food for human con-sumption, studies on systemic toxicity (e.g., acute, subacute, chronic and subchronic toxicities) of B. pilosa in humans and animals are still inadequate and insufficient. So far, acute, and/or subchronic toxicities have been evaluated in rats and mice. **[16]** Oral acute and 28-day toxicities of water extract of B. pilosa leaves were evaluated in Wistar rats. An oral dose of water extract of B. pilosa leaves at 10 g/kg BW showed no obvious mortality or changes in the appearance in rats. The same extract at 0.8 g/kg BW/day, once a day, showed no obvious sub-chronic toxicity in rats over 28 days, as measured by survival rate, body weight, and gross examination of organs.[22] These data are consistent with our data indicating that oral delivery of the water extract of the B. pilosa whole plant at 1 g/kg BW/day, once a day, is safe in rats over 28 days (unpublished data). Taken together, these studies suggest that ingestion of B. pilosa aqueous extract at up to at 1 g/kg BW/day, once a day, is highly safe in rats. In addition, the acute toxicity of aqueous and ethanol extracts of B. pilosa in mice have been reported. Five- to six-week-old mice with weights between 28 and 35 g received a peritoneal injection of both extracts at the different doses. The LD50, the dose that causes 50% lethality, of the aqueous and ethanol extracts in mice was 12.30 g/kg BW and 6.15 g/kg BW, respectively. **[22]** A complete toxicological study has not been completed for humans. Furthermore, the drug interactions of B. pilosa with other drugs are unknown. Further safety verification and clinical trials should be performed before B. pilosa can be considered for medicinal use. **[22]**

Contraindications[20,22]

(i) *Bidens pilosa* has evidenced weak uterine stimulant activity in guinea pigs. As such, it should not be used during pregnancy.
(ii) This plant contains several coumarin derivatives. Coumarins are a group of chemicals that thin the blood. Those on blood thinning medications such as Warfarin should use *Bidens pilosa* with caution and monitor these possible effects.
(iii) The plant has been documented to lower blood sugar levels in several animal studies. Those with hypoglycemia or diabetes should only use *Bidens pilosa* under the supervision of a qualified health care professional and monitor their blood sugar levels accordingly.
(iv) *Bidens pilosa* has been documented with hypotensive activity in several animal studies. People with heart conditions and those taking antihypertensive drugs should consult their doctors prior to using this plant to monitor these possible effects.
(v) The desired hybridoma is selected for cloning and antibody production against a plant drug. This process is facilitated by preparing single cell colonies that will grow and can be used for screening of antibody producing hybridomas.
(vi) The selected hybridoma cells are cultured for the production of monoclonal antibodies in large quantity against the specific plants drugs.
(vii) The monoclonal antibodies are used to determine similar drugs in the plants extract mixture through enzyme-linked immunosorbent essay (ELISA). **[25]**

Herbal preparation[22]

An odd or unusual characteristic of this unique herbal drug is its wealth in multi-species formulas that have been used across the centuries. These formulations thus represent a social heritage, and their ethnobotanical information can add much to the understanding of local folk medical systems. While *Bidens pilosa* L. and *Cissus sicyoides* are principal in mixtures for respiratory problems, which form the major ethno-medical category in terms of number of preparations. Juice extract of *Bidens pilosa* L. (Arial part) and Solanum *torvum* (leaves) was used to treat Catarrh. Decoction of (aerial part), *Cassia fistula* (fruits), *Cissus sicyoides* (fruits), *Crescentia cujete* (fruits), *Phyla scaberrima* (aerial part), *Ruellia tuberosa* (root) and bee's honey was used to provide coolness at the uterus and treat menstrual irregularity. **[24, 26]**

II. Conclusion

The phytochemical investigation of leaves and stems of *Bidens pilosa* has shown the presence of alkaloids, glycosides, flavonoids, saponins,steroids, tannins. The extract , it has shown maximum anti-oxidant activity. Therefore, these extracts can be further used to cure many disease which can undergo free radical mechanism through its inhibitory action.

The presence of major phyto-constituent in leaves extract has shown the maximum antifungal activity then the stems of *Bidens pilosa*.

Conflict of interest: Nil

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