

# Phytochemical Screening and Chromatographic Separation of Methanolic Extract of Vernonia Lasioopus Stem Bark

CHOJI JOSEPH DANJUMA<sup>1</sup>, PROFESSOR JOHN BARNABAS NVAU<sup>2</sup>

<sup>1</sup>Plateau State University Bokkos

<sup>2</sup>Chemistry, Plateau State University, Bokkos

## I. INTRODUCTION

### 1.1 Background of Study

Medicinal plants, also called medicinal Herbs, have been discovered and used in traditional medicine practices since prehistoric time. Plants synthesize hundreds of chemical compounds for various functions, including defense and protection against insects, fungi, disease, and herbivorous mammals. (Gershenzon J, Ullah C 2022)

Medicinal plants have been helpful in treating both infectious and life style diseases (Gurib Fakim A 2006). These medicinal plants are considered as rich resources of ingredients which can be used in drug development and synthesis. Besides that, these plants play a critical role in the development of human cultures around the world (Rasool, 2012). Knowledge on their use has been preserved by passing it through oral communication and cultural practices by communities around the world. They are vital sources of easily accessible remedy used in the countryside healthcare system. (Rasool, 2012).

The earliest historical records of herbs are found from the Sumerian civilization, where hundreds of medicinal plants including opium are listed on clay tablets, c. 3000 BC. The Ebers Papyrus from ancient Egypt, c. 1550 BC, describes over 850 plant medicines. The Greek physician Dioscorides, who worked in the Roman army, documented over 1000 recipes for medicines using over 600 medicinal plants in *De materia medica*, c. 60 AD; this formed the basis of pharmacopoeias for some 1500 years. Drug research sometimes makes use of ethnobotany to search for pharmacologically active substances, and this approach has yielded hundreds of useful

compounds. Some include the common drugs aspirin, digoxin, quinine, and opium. The compounds found in plants are of many kinds, but most are in four major biochemical classes: alkaloids, glycosides, polyphenols, and terpenes. ).(Ahn, K. 2017)

The beginning of the development of herbal medicines was concurrent in the development of chemistry in isolation, purification and determination of plants compounds (Shakya et al; 2012).

Medicinal plants are widely used in non-industrialized societies, mainly because they are readily available and cheaper than modern medicines (Popovic Z et al 2013). The annual global export value of the thousands of types of plants with medicinal properties was estimated to be US\$ 60 billion per year and it is growing at the rate of 6% per annum. In many countries, there is little regulation of traditional medicine, but the World Health Organization coordinates a network to encourage safe and rational usage. Most botanical herbal market has been criticized for being poorly regulated and containing placebo pseudoscience products with no scientific research to support their medical claims. Medicinal plants face both general threats, such as climate change and habitat destruction, and the specific threat of over-collection to meet market demand.

#### 1.1.1 Scientific and Cultural Uses

Medicinal plants have been a vital source of both curative and preventive medical therapy preparations for human beings, which also has been used for the extraction of important bioactive compounds. (Thirumalai T, David E. Mar. 2020). It is estimated that a number of medicinal plant species has been

shown to be biologically active against various illnesses (Abena et al, 2007, Ajaiyeoba fadare, 2006,). Medicinal plants play a golden role as a traditional medicine and as a medical resource in almost all culture (Jamshidi-Kia et al 2018). Medicinal plants frequently used as raw material for extraction of active ingredients which is used in the synthesis of different drugs. Like in case of Laxatives, blood thinners, antibiotics and anti-malaria medication, contain ingredients from plants. Ogundare et al., (2002). Many plants are traditional sources for food and pharma product. Since the difference of a therapeutic and toxic effect depends on the dose, there are fluent transitions between consumption, therapy and toxicity of nutraceuticals in food, medicine, or plant (Jenzer and sadeghi, 2016).

#### 1.1.2 Future of medicinal plants

Medicinal plants have a promising future because there are about half million plants around the world, and most of them their medicinal activities have not been investigated yet, and their medicinal activities could be decisive in the treatment of present or future studies. (Jamshidi-Kai et al 2018).

#### 1.2. Aim of Study

To separate and Isolate the secondary metabolites in the bark of the plant *Vernonia lasiopus* stem.

#### 1.3. Objectives

- Plant Collection and Identification.
- Extraction and concentration of extract
- Isolation (Column and Thin Layer Chromatography)

#### 1.4. Significance of the Study

To verify and justify the claims made by traditional herbalist on the plant (*Vernonia lasiopus*) and also help with the possible preparation of alternative drugs to synthetic drugs that can cure bacterial disease without imposing side effect.

#### 1.5. Statement of the problem

Most products made from medicinal plants and products marketed by traditional healers had not been tested for their safety, efficacy is sometimes uneven quality, sometime containing dangerous metabolites. It has been noted that over the last few decades in the

evolution of drug-resistant disease pathogens which have caused treats to public health and even resulted to death of many lives. As a result, the study plant (*Vernonia lasiopus*) will be investigated for its ability to prevent the activities of certain disease pathogens from having effect in the host body.

#### 1.6. Scope/Delimitation

This work is focused and limited to extraction and chromatographic separation of the stem extract of *Vernonia lasiopus* only.

## II. LITERATURE REVIEW

### 2.1. VERNONIA LASIOPUS

*Vernonia lasiopus* is a plant that grows to a maximum height of about 3m and has smooth grayish brown bark. It has oval shaped and densely hairy leaves with pale mauve or white flowers in color. (Dharani N et al 2010) *V. lasiopus* belongs to the tribe of Vernonieae and the family Asteraceae with about 1500 described species. (Da-Silva JB, 2013). Most of the *Vernonia* species are found in temperate, tropical and sub-tropical areas, especially in South America, Asia, Africa and North America (Redonda-Martínez R, et al. 2012). *V. lasiopus* is widely distributed in bush land, grassland, woodland and forest. It grows in an altitude between 1000 to 2500m. In Ethiopia, *Vernonia* species are used to treat eye infections, wounds and bone related problems such as fracture. (Kalayou S, Haileselassie M. et al. 2012) The name *Vernonia lasiopus* is a synonym of *Baccharoides lasiopus*. (Pflanzenw. Ost-Afrikas, C: 403(1895)). The genus *Vernonia* is one of the major sources of bioactive sesquiterpenoids which are an important class of compounds due to their diverse biological activities (Magadula and Erasto, 2009). *Vernonia* is a genus of about 1000 species in the family Asteraceae. It is a coarse shrub 1.5m tall; stems tomentose. Leaves lanceolate, 4.5-9cm wide, cuneate to unwinged exauriculate petioloid base, margins serrate, apex acuminate, thinly scabridulous pubescent above, pale-tomentose beneath. Capitula in corymbiform cymes; involucre 11-15mm long; phyllaries 2-4 seriate.

The common name for *Vernonia lasiopus* is Ironweed  
Chemical constituents of *Vernonia lasiopus*

Several researches carried out on *Vernonia lasiopus* suggest that it contains different bioactive compounds, such as steroidal glycosides, triterpenoids, alkaloids, tannins, flavonoids, saponins, phenolics, terpenes, and several types of sesquiterpene lactones (Kimani 2021).

## 2.2. Plant of study: *Vernonia lasiopus*



Plate 1: Picture of *Vernonia lasiopus* (plant)

### 2.2.1. Uses of *Vernonia lasiopus*

*Vernonia Lasiopus* is reputed to have several health benefits traditionally where an infusion of powder leaves is used to cure indigestion, stomach-ache, malaria, and also as a purgative. A root decoction is said to be a very effective treatment for stomach-ache (Dharani et al, 2010)

*Vernonia Lasiopus* is used for treatment of children constipation and wound healing. (Toxicological survey of Africa medicinal plants, 2014)

*Vernonia Lasiopus* (O. Hoffman) pounded leaves and paste has been traditionally used to treat worms in infected wounds, hepatitis and kids constipation. Stem decoction has been used in *Vernonia* treatment of malaria, worms and non-bacterial infections, epilepsy, indigestion and during childbirth. (Kareru PG, Gachanja AN, Keriko JM, Kenji GM et al, 2008) *lasiopus* has also been in use traditionally in treating fever, abdominal pain, diarrhea, sores, venereal diseases, scabies and ascariasis among other ailments. (Galiwando, Julius 2009)

*lasiopus* are used traditionally as galactagogue, purgative and anthelmintic, and traditional medicine

for headache, liver diseases, skin diseases, respiratory infections, malaria, augment labor, convulsions, epilepsy, fainting, female reproductive problems, and gastro-intestinal problems.

## 2.3 Extraction

Extraction is the first step to separate the desired natural product from the raw materials.

Extraction methods include solvent extraction, distillation method, pressing and sublimation according to the extraction principle. Solvent extraction is the most widely used method. The extraction of natural products progresses through the following stages:

- The solvent penetrates through into the solid matrix
- The solute dissolves in the solvent
- The solute is diffused out of the solid matrix
- The extracted solute are collected

The extraction efficiency increases with the increase in extraction duration in a certain time range. Increasing time will not affect the extraction after the equilibrium of the solute is reached inside and outside the solid material. (CAS PubMed Google scholar)

Methods of Extracting Natural Products include:

1. Maceration
2. Percolation
3. Decoction
4. Reflux extraction
5. Soxhlet extraction
6. Pressurized liquid extraction (PLE)
7. Supercritical fluid extraction (SPE)
8. Ultrasound assisted extraction (UAE)
9. Microwave assisted extraction (MAE)
10. Hydro distillation and steam distillation
11. Enzyme assisted extraction
12. Pulsed electric field (PEF) extraction

**Thin Layer Chromatography:** It is an analytical technique that is used to separate and analyze complex biological or non-biological samples into their constituent. It is most popular for monitoring the progress of a chemical reaction or estimation of a

substance in a mixture. It is also one of the popular techniques for testing the purity of a sample in this method. The silica or alumina as a stationary phase is coated onto a glass or aluminum foil as thin Layer and then a sample is allowed to run in the presence of mobile phase (Trivedi and Chaudhary, 2014).

**2.3.2 Column Chromatography:** It is a chromatographic method that is used to isolate a single chemical compound from a mixture. This method is able to separate substance based on differential adsorption of compounds to the adsorbent, compounds move through the column at different rate allowing them to be separated in fractions (Kore et al 2018).

**2.3.3 Stationary Phase:** It refers to a solid, a gel or a liquid. It may be distributed on a solid. This solid may or may not contribute to the separation processes. The liquid may also be chemically bonded to the solid (bonded phase) or immobilized onto it (immobilized phase) (IUPAC Gold book)

**2.3.4 Mobile Phase:** It is the liquid or gas that flows through a chromatography system, moving the material to be separated at different rate over the stationary phase (Wiktionary).

### III. MATERIALS AND METHOD

#### 3.0.1 Apparatus/Equipment

Electric Weighing balance, Measuring Cylinder, Beaker, Funnel, Conical Flask, Filter Papers, Capillary Tubes, Masking Tapes, Thin Layer Chromatography (TLC) Plates, Stirring Rod, Distiller, Spray Bottle, Heating Mantle, Spatula, Scissors, Pencil And Meter Rule.

#### 3.0.2 List of Solvents

N-Hexane, Ethyl Acetate, Methanol, Acetone, Ethanol, Iodine Crystal, Sulphuric Acid (H<sub>2</sub>SO<sub>4</sub>), Chloroform, Ferric Chloride

#### 3.1.0 COLLECTION AND IDENTIFICATION OF PLANT MATERIAL

The stem bark of *Vernonia lasiopus* were collected from Mushere community in Bokkos Local Government by my supervisor Prof. John B. Nvau.

The plant was identified by Mr Azila an Ethnobotanist in college of Forestry Jos and transported to the organic Chemistry laboratory in Plateau State University, Bokkos for analysis.

#### 3.2.0 EXTRACTION OF PLANT MATERIAL

The stem bark of the plant *V. lasiopus* was air dried for 56 days (8 weeks). The air dried stem barks were grinded into coarse using Mortar and piston. 100g of the grinded plant were then extracted using 500ml of methanol as a solvent, after which another 500ml of methanol was used to re-extract the residue. The extract was concentrated using a rotary evaporator. The concentrated extract was used for TLC and Phytochemical screening of alkaloids, saponins, Phenols, tannins, steroids, terpenoids and Phytosterols

#### 3.2.1 METHOD USED FOR EXTRACTION

Maceration:

#### 3.3.0 THIN LAYER CHROMATOGRAPHY (TLC)

##### 3.3.1 SPOTTING

A concentrated portion of this plant was dissolved with methanol in a valve, and a silica gel TLC plate was cut using an ordinary house hold scissors.

The TLC plate was marked with a pencil 1cm from the bottom, during spotting; a capillary tube was dipped into a solution of acetone for cleaning up, after which it was dipped into the sample and spotted on the TLC plate marked with pencil. Then the spotted TLC plate was allowed to dry, after which it was dipped into a mixture of hexane and ethyl acetate in some few minutes to determine the movement of the compounds as the solvent travel up on the TLC plate, but not allowing it to reach the ending.

##### 3.3.2 VISUALIZATION

In order to visualize the movement of the solute travel on the TLC plate, 10ml of concentrated sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) was added to 90ml of ethanol and was used as a spraying agent.

The mixture of this solutions was poured into a spraying bottle and was sprayed on the TLC plate spot developed, after which the TLC plate was placed

on a hot plate for few minutes through which the movement of the solute travel was visualized clearly and the retention factor ( $R_f$ ) was been determine using this formula

$$R_f = \frac{\text{Distance travelled by the solute (cm)}}{\text{Distance travelled by the solvent (cm)}}$$

In this case, if the fraction has the same  $R_f$  value, it shows similar characteristic and are joined together in one value, but if after spotting, only one spot is visualized after the TLC plate development, it indicate a pure compound, the TLC will continue until all similar compounds are pulled together and pure compounds will be collected, isolated and ready for analysis.

#### 3.4.0 QUALITATIVE ANALYSIS OF PHYTOCHEMICALS IN VERNONIA LOSIOPUS

##### 3.5.1 TESTS FOR COMPOUNDS

The following compounds were tested and confirmed to be present.

##### SAPONINS

20ml of distilled water was added to 2g of plant material in a test tube and heated to boiling in a water bath. The extract was allowed to cool, then it was filtered and shaken for 5minutes, it was observed a fronting formation in the test tube which lasted for 30minutes, that confirmed the presence of saponins

##### PHENOLS

20ml of distilled water was added to 2g of plant material in a test tube and heated to boiling in a water bath. The extract was allowed to cool, then it was filtered. 5drops of 2% ferric chloride was added to the extract, a formation of bluish green and a sharp black colour was observed which confirmed the presence of phenol (Roopashee et'al 2008).

##### TANINS

0.5g of the sample was boiled in 10ml of distill water in a test tube, 5drops of 1% Ferric Chloride was added in the test tube, a blue brown colour was observed which confirmed the presence of tanins

##### TERPENOIDS

(Salkowski Test) About 0.5g concentrated crude methanolic extract of the plant sample was poured in a beaker, 2ml of chloroform was added in the beaker, and about 3ml of sulphuric acid ( $H_2SO_4$ ) was added carefully, it was observed to be a formation of surface layer that gives a brownish colouration at the interphase confirming the presence of terpenoids (Ugochukwu. et'al 2013).

##### STEROIDS

10ml of chloroform was added to 0.5g crude extract of the sample and 10ml of sulphuric acid was added side by side. The formation of a yellowish green layer indicates the presence of Steroids (Alhadin et'al 2015).

##### ALKALOIDS

Wagner's reagent was added to 0.5g of the sample extract. The formation of a brown/redish ppt indicates the presence of alkaloids. (sadoon et'al 2014).

##### PHYTOSTEROLS

10ml of chloroform was added to 0.5g of crude extract of the sample, and 5 drops of Sulphuric acid was added and shaken. The appearance of a golden colour confirms the presence of Phytosterols.

#### 3.6.0 PREPARATION OF SOME REGENTS

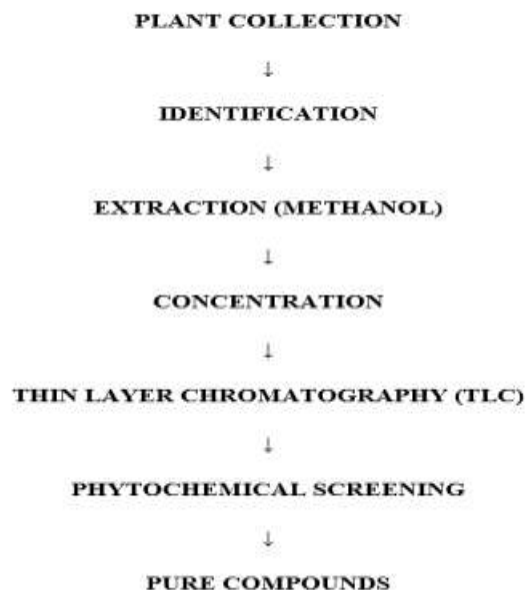
3.6.1 Preparation of 2% ferric chloride: 2g of Ferric Chloride was weighed and dissolved in 98g of distilled water.

3.6.2 Preparation of 1% ferric chloride: 2ml of the 2% ferric Chloride prepared above was measured and dilute in 2ml of distilled water.

3.6.3 Preparation of Wagner's reagent: 2g of iodine and 6g of potassium iodide were weighed, and dissolved in 100ml of distilled water.

3.6.4 Preparation of Spraying agent: 10ml of concentrated sulphuric acid ( $SO_4$ ) was measured and diluted with 90ml ethanol in an ice medium..

## STAPES IN METHODOLOGY



## IV. RESULTS AND DISCUSSION

### 4.1.0 RESULTS

The plant metabolites were extracted with methanol.  
The percentage yield of the extracts as shown in table 4.1 is based on the weight of the dried, crushed plant materials used.

TABLE 1: Table for percentage (%) yield of Vernonia Lasiopus plant

| Colour of the Extract | Solvent used for Extraction | Weight of sample used (g) | Weight of Extract(g) | Percentage yield of plant (%) |
|-----------------------|-----------------------------|---------------------------|----------------------|-------------------------------|
| Green                 | Methanol                    | 100                       | 3                    | 2                             |

Table showing Percentage (%) yield

TABLE 2: T.L.C Result for Methanolic Crude Extract in the ratio 3:1(Hexane/Ethylacetate)

| S/N | Spot Number | Retention Factor (Rf) Value |
|-----|-------------|-----------------------------|
| 1   | 1           | 0.94                        |
| 2   | 2           | 0.87                        |
| 3   | 3           | 0.61                        |
| 4   | 4           | 0.57                        |
| 5   | 5           | 0.52                        |
| 6   | 6           | 0.46                        |
| 7   | 7           | 0.27                        |

|   |   |      |
|---|---|------|
| 8 | 8 | 0.08 |
|---|---|------|

TABLE 3: T.L.C Result for Methanolic Crude Extract in the ratio 1:1(Hexane/Ethylacetate)

| S/N | Spot Number | Retention Factor (Rf) |
|-----|-------------|-----------------------|
| 1   | 1           | 0.95                  |
| 2   | 2           | 0.88                  |
| 3   | 3           | 0.83                  |
| 4   | 4           | 0.71                  |
| 5   | 5           | 0.66                  |
| 6   | 6           | 0.53                  |

|   |   |      |
|---|---|------|
| 7 | 7 | 0.35 |
| 8 | 8 | 0.08 |

TABLE 4: T.L.C Result for Methanolic Crude Extract in the ratio 1:2 (Hexane/Ethylacetate)

| S/N | Spot Number | Retention Factor (Rf) |
|-----|-------------|-----------------------|
| 1   | 1           | 0.96                  |
| 2   | 2           | 0.90                  |

|   |   |       |
|---|---|-------|
| 3 | 3 | 0.77  |
| 4 | 4 | 0.69  |
| 5 | 5 | 0.58. |
| 6 | 6 | 0.47  |
| 7 | 7 | 0.32  |
| 8 | 8 | 0.22  |
| 9 | 9 | 0.13  |

TABLE 5: Results for phytochemical screening test of *V. lasiopus*

| Test    | Saponin | Phenol | Tannin | Terpenoids | Steroids | Alkaloids | Phytosterols |
|---------|---------|--------|--------|------------|----------|-----------|--------------|
| Results | +++     | ++     | +      | +          | ++       | ++        | +            |

#### 4.2.0 DISCUSSION

Solvent used for the extraction of the 100g stem bark of *V. Lasiopus* gave a good yield result of 2%. The spot on the T.L.C plate using Hexane and Ethylacetate (1:2) solvent system also confirmed that it was an effective method for the extraction Table 4.4. The best solvent for T.L.C separation of the crude Methanolic extract is Hexane: Ethylacetate (1:2) that gave Nine (9) spots with the following Rf Values (0.13, 0.22, 0.32, 0.47, 0.58, 0.69, 0.77, 0.90, 0.96) with a better separation, while ratio 3:1 using the sample Hexane and ethylacetate solvent system and ratio 1:1 using the same solvent systems gave Eight(8) spots each as shown in table 4.2 and 4.3 with the following Rf Values (0.08, 0.27, 0.46, 0.52, 0.57, 0.61, 0.87, 0.94 and 0.08, 0.35, 0.53, 0.66, 0.71, 0.82,0.88, 0.95) respectively.

The result from the phytochemical screening of *V. Lasiopus* shows that the plant contains the following secondary metabolite. ( Saponins, Phenols, Tannins, Terpenoids, Steroids, Phytosterols and Alkaloid), as shown in Table 4.5. The Presence of these metabolites in the plant extract indicates the usefulness of the plant by the local to manage some diseases symptoms.

#### V. CONCLUSION AND RECOMMENDATION

##### 5.1 CONCLUSION

The result of this research project shows that the stem bark of the plant *V. Lasiopus* possess a variety of interesting active compounds, which upon further research could be used as starting material for drug discovery or manufacturing to remedy various diseases.

##### 5.2 RECOMMENDATION

It is recommended that further work could also be possible to isolate the structural or the active pure compounds and to identify what specific phytochemical is primary for any biological activity to improve human life, animals lives as well as to improve the environment.

#### REFERENCES

- [1] 2014, Toxicological survey of Africa medicinal plants.
- [2] Ahn, K.( 2017). The worldwide trend of using botanical drugs and strategies for developing global drugs. ( BMB Reports. 50).
- [3] Azwanida NN. 2015;4:196. A review on the extraction methods use in medicinal plants, principle, strength, and limitation. Med Aromat Plants. [Google Scholar]
- [4] Banu KS, Cathrine L. 2015;2:25–32 General techniques involved in phytochemical analysis. Int J Adv Res Chem Sci. . [Google Scholar]
- [5] Beena P, Rajesh KJ, Arul B. 2016;3:153–9. Preliminary phytochemical screening of *Cicer arietinum* in folklore medicine for hepatoprotection. J Innov Pharm Biol Sci. [Google Scholar]
- [6] Da-Silva JB, Temponi VDS, Gasparetto CM, Fabri RL, Aragão DMD, et al. (2013) *Vernonia condensata* Baker (Asteraceae): A promising source of antioxidants. Oxidative Medicine and Cellular Longevity 2013: 1-9.

- [7] Dharani N, Rukunga G, Yenesew A, Mbora A, Mwaura L, et al. (2010) Common antimalarial trees and shrubs of East Africa. A Description of Species and a Guide to Cultivation and Conservation Through Use. The World Agroforestry Centre (ICRAF), Nairobi, Kenya, p:
- [8] Dhawan D, Gupta J. 2017; 11:17–22. Comparison of different solvents for phytochemical extraction potential from *Datura metel* plant leaves. *Int J Biol Chem.*[Google Scholar]
- [9] Gershenzon J, Ullah C 2022. [https:// en.m. Wikipedia.org/ wiki/Medicinal-plants](https://en.m.wikipedia.org/wiki/Medicinal-plants)
- [10] Gurib Fakim A (2006) Medicinal plant: tradition of yesterday and drugs of tomorrow. *Mol Aspects Med* 27:1-93
- [11] Ingle KP, Deshmukh AG, Padole DA, Dudhare MS, Moharil MP, Khelurkar VC. 2017; 6:32–6. Phytochemicals: Extraction methods, identification, and detection of bioactive compounds from plant extracts. *J Pharmacogn Phytochem.* [Google Scholar]
- [12] Jamshidi-Kia, F., Lorigooini, Z., & Amini-Khoei, H. (2018). Medicinal Plants: Past History and Future Perspective. *Journal of Herbmed Pharmacology*, 1, 1-7.
- [13] Kalayou S, Haileselassie M, Gebre-egziabher G, Tiku'e T, Sahle S, et al. (2012) In-vitro antimicrobial activity screening of some ethnoveterinary Citation: Michael N Musila, Beatrice G Muthoni, Samson C Koech, Mathew P Ngugi, Wilton M Mbinda (2017) Evaluation of In vivo Toxicity of Methanolic Leaf Extract of *Vernonia lasiopus* (O. Hoffman). *J Pharmacogn Nat Prod* 3: 133. doi:10.4172/2472-0992.1000133 Page 5 of 6 *J Pharmacogn Nat Prod*, an open access journal 2472-0992 Volume 3 • Issue 1 • 1000133 medicinal plants traditionally used against mastitis, wound and gastrointestinal tract complication in Tigray Region, Ethiopia. *Asian Pac J Trop Biomed* 2: 516-522.
- [14] Kareru PG, Gachanja AN, Keriko JM, Kenji GM (2008) Antimicrobial activity of some medicinal plants used by herbalists in eastern province, Kenya. *The African Journal of Traditional, Complementary and Alternative medicines* 5: 51-55.
- [15] Kokwaro JO (1976) *Medicinal Plants of East Africa*, Kenya Literature Bureau, Kenya.
- [16] Kore, S. C., Majumdar, S. H., Suryawanshi, J. S., & Pimpodkar, N. V. (2018). Investigation on Chemical Changes Occur During Purification (Shodhana) of *Bhallataka* by HPTLC. *International Research journal of Pharmacy and Medical Sciences*, 3(1), 5-10.
- [17] Majekodunmi SO. 2015; 3:521–7. Review of extraction of medicinal plants for pharmaceutical research. *MRJMMS.* [Google Scholar]
- [18] MVS MK, Talluri VP, Rajagopal SV. 2015; 6:348–58. Purification and characterization of bioactive compound from the methanolic leaf extract of *Millingtonia hortensis* linn. *Int J Pharm Bio Sci.* [Google Scholar]
- [19] Ogundare, A. O., Adetuyi, F. C., & Akinyosoye, F. A. (2006). Antimicrobial activities of *Vernonia tenoreana*. *African Journal of Biotechnology*, 5(18).1663-1668. +
- [20] Pandey A, Tripathi S. 2014;2:115–9. Concept of standardization, extraction, and pre-phytochemical screening strategies for herbal drug. *J Pharmacogn Phytochem.* [Google Scholar]
- [21] Popovic Z., Matic R., Bojovic S. Stefanovic M., Vidakovic V. 2013. Ethnobotany and herbal medicine complementary and alternative medicine: An overview of publications in the field of I&C medicine 2001-2013. *J.*
- [22] Rasool H.B Medicinal plants. Importance and uses. *Pharmaceut. Anal. Acta.* 2012;3:e139. doi: 10.4172/2153-2435.1000e139.
- [23] Redonda-Martínez R, Villaseñor JL, Terrazas T (2012) Trichome diversity in the *Vernonieae* (Asteraceae) of Mexico: *Vernonanthura* and *Vernonia* (Vernoniinae). *J Torrey Bot Soc* 139: 235-247.



- [24] Shakya AK, Sharma N, Saxena M, Shrivastava S, Shukla S.(2012). Evaluation of the Antioxidant and Hepatoprotective Effect of Majoon-e-Dabeed-ul-ward against carbon Tetrachloride Induced Liver Injury. Exp Toxicol Pathol. 64(7-8),767- 73.