

ISOLATION AND CHARACTERISATION OF AN ALPHA-AMYRIN ACETATE ISOLATED FROM THE LEAVES OF *MICROTRICHIA PEROTITII* DC (ASTERACEAE)

*Abdullahi, M. N.; **N. Ilyas.;**Hajara, I.; ** Kabir, Y. M

* Department of Applied Science. College of Science and Technology. Kaduna Polytechnic. Kaduna. Nigeria

** Department of Pharmacognosy and Drug Development. Ahmadu Bello University. Zaria. Kaduna State. Nigeria.

*Corresponding author

ABSTRACT

Aims: *Microtrichia perotitii* DC belongs to the family Asteraceae and is widely distributed in West African countries of Nigeria, Senegal, Mali, Port of Guinea, Sieraleone, Ivory Coast, Ghana and Dahomey. In Nigeria it is found in northern part of the country where it is known as *Maijankai* or *Sawun keke* in Hausa, *Osete* in Igbira and *Shaware pepe* in Yoruba languages. The herb is locally used for treating pain related diseases which includes toothaches, cuts and burns, rashes in children, skin diseases, rheumatism, diarrhea and jaundice. It is on the basis of its significance in trado-medicinal usage that an attempt was being made to isolate the active compounds in the herb. Previous phytochemical screening of the leaves of the herb has confirmed the presence of carbohydrates, tannins, cardiac glycosides, triterpenes, proteins, flavonoids, alkaloids, and steroids.

Methods: The leaves of the herb were extracted in methanol in a soxhlet apparatus. The crude extract obtained that is *Microtrichia perotitii* methanol extract(MPME) was fractionated in methanol, ether, aqueous, and n-butanol. Each of these soluble fractions was tested for analgesic and anti-inflammatory activities against laboratory animals (mice and rats). The most active fraction was n-butanol which was obtained as a white milky solid A(C₁) and its melting point range was 224 – 226 °C. The compound after re-crystallisation` was subjected to spectroscopic analyses with Infrared, proton and carbon-13 Nuclear Magnetic Resonance spectroscopy .

Result: The data obtained for the compound when compared with literature value was found to be an alpha-amyrin acetate.

Conclusion: An alpha-amyrin acetate was isolated from the leaf of *Microtrichia perotitii* for the very first time. Alpha-amyrin acetate has been reported to have broad spectrum of biological activities against some diseases and ailments and could therefore be the leading principle behind many of the ethnomedicinal applications of *Microtrichia perotitii*.

Key words; *Microtrichia perotitii*, asteraceae, soxhlet apparatus, α -amyrin acetate, ethanomedicinal

INTRODUCTION

For centuries, people have been living closely to their environment and as such solely rely on its available flora and fauna for source of food and medicine. This has therefore led to the communities to develop their plant pharmacopeias. These practices are most common in Asia and African countries where at least 80 % of the population depended on herbal therapies because of their effectiveness, low cost and rare side effects when compared to synthetic drugs [1], [2], [3], [4].

Medicinal plants are used in ethnomedicine and they produce bioactive compounds that have therapeutic properties in addition to their uses as food additives and perfumes [5], [6], [7]. The bioactive compounds (natural products) occur as either plant extracts, pure compounds or as standardized extracts and therefore provide unlimited opportunities for new drug discoveries [8]. The bioactive compounds are usually extracted from the plants and therefore, their quantitative and qualitative estimation is important for exploration as new biomolecules for use either by pharmaceutical or agrochemical industries directly or can be used as a lead molecule to synthesize more potent molecules [9].

Microtrichia perotitii DC belongs to the family Asteraceae and is widely distributed in West African countries of Nigeria, Senegal, Mali, Port of Guinea, Sieraleone, Ivory Coast, Ghana and Dahomey [10]. In Nigeria it is found in northern part of the country where it is known as *Maijankai* or *Sawun keke* in Hausa, *Osete* in Igbera and *Shaware pepe* in Yoruba natives. The herb is an annual plant and is a diffuse much branched, pubescent and varying from 1ft in height. The leaves are obovate, obtuse, cuneately narrowed into petiole, coarsely toothed, $\frac{1}{2}$ - $1\frac{1}{2}$ inch long and $\frac{1}{3}$ - 1 inch broad. The petiole of lower leaves is 1inch or more at the upper part. Other features are in accordance to some members of the family [11], [12],[13]. Traditionally, *Microtrichia perotitii* is used for treating pain related diseases such as toothaches, cuts, burns and rashes in children. Others include, skin disease, rheumatism, diarrhea and jaundice. Phytochemical investigation of the extracts from this herb showed that it is rich in many active compounds including carbohydrates, tannins, cardiac glycosides, triterpenes, proteins, flavonoids, alkaloids, and steroids [14^a],[15^b].

MATERIALS AND METHODS

Plant collection and identification

The herb *Microtrichia perotitii* was collected from Rigasa village, Kaduna State, Nigeria in the month of April 2016. The fresh herb was compared with authenticated sample earlier identified by Malam Musa M. of herbarium unit of the Department of Biological sciences, Ahmadu Bello University, Zaria. The voucher or specimen number given was 998 for future references. The fresh herb was allowed to dry under the shade for three weeks until all the leaves have fallen- off the stalk. They were reduced to coarse powder using traditional pestle and mortar.

Solvent extraction

300 g of the powdered leaves of *Microtrichia perotitii* were refluxed with 3L of petroleum ether (60-80 °C) for 8 hours in a soxhlet apparatus. The extract was decanted off and fresh quantity of petroleum ether (3L) was added again and then refluxed for another 5 hr. The defatted leaves (marc) were completely dried and later extracted with 3L of methanol in a soxhlet apparatus for 8 hours. Thereafter, the round bottom flask was removed and distilled gradually and the crude evaporated to dryness on boiling hot water bath. The extractable was allowed to cool and placed in a clean container and kept sealed in a desiccator until use and was marked MPME [16] - [20].

Fractionation of crude extract of the leaves of *Microtrichia perotitii* (MPME)

The methanol crude extracts of the leaves of *Microtrichia perotitii* (MPME) was fractionated using the scheme proposed by [21] (Figure1).

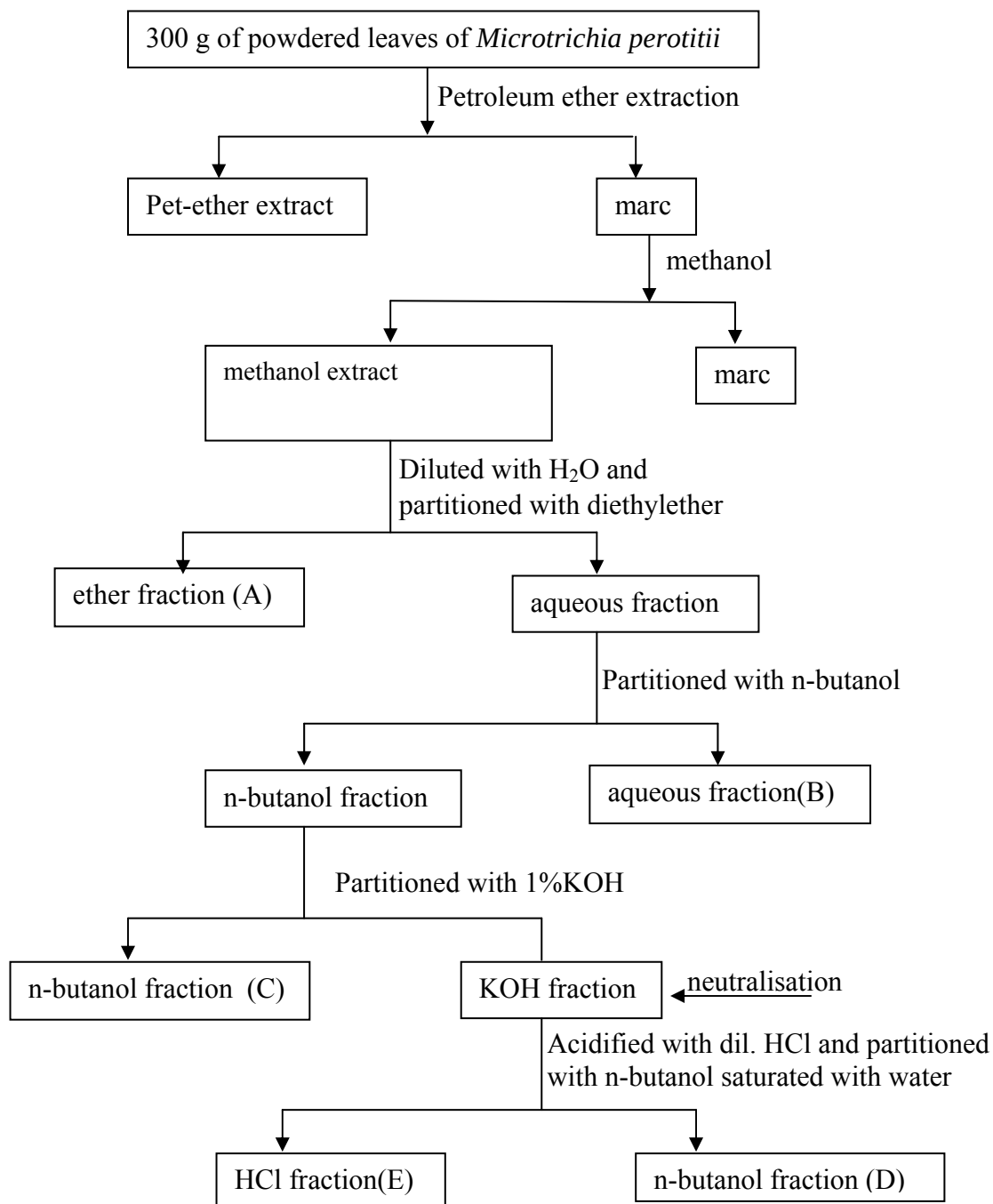


Figure 3.1: Schematic chart for the extraction [21].

Each of the extract was evaporated gently over hot water bath for about 30 min with intermittent removal. Thereafter, each was transferred into sterilized bottle, labeled and kept in desiccator until use. The residues were identified as; A (ether soluble Fraction), B (aqueous soluble fraction), C (n-butanol soluble fraction), D (n-butanol soluble fraction) and E (aqueous hydrochloric soluble fraction). All these soluble fractions were subjected to analgesic and anti-inflammatory studies with laboratory animals (Swiss albino mice and Wistar rats). However, the n-butanol soluble fraction C was found to be most active in the studies [15].

Chromatographic analysis of soluble fraction C

The n-butanol soluble fraction C (0.2 g) was spotted on a pre-coated TLC plates and developed in butanol: acetic acid: water (8:1:1) and hexane: ethyl acetate (7:3) solvent mixtures. R_f values were calculated for each spot after being spread with *p*-anisaldehyde [22], [23]. 4 gm. of the crude sample was dissolved in methanol and poured over a column (3 x 60 cm) packed with silica gel and hexane. The column was eluted with: Hexane (100 %), Hexane-Ethylacetate (2:1), Hexane-Ethylacetate (1:1), Hexane-Ethylacetate (1:2), Ethylacetate (100%), Ethylacetate-Methanol (2:1), Ethylacetate-Methanol (1:1), Ethylacetate-Methanol (1:2) and Methanol (100 %)[24],[25]. Seventy six 40 ml fractions were collected and similar fractions were pooled together as A (C_1), B (C_2) and C (C_3). However, fraction A (C_1) produced a single profile and was therefore subjected to spectrophotometric analyses with FTIR (8400S HIMADZU), ^1H -NMR spectrophotometer (Bruker400Mhz), and ^{13}C -NMR spectrophotometer (Bruker400Mhz).

RESULT AND DISCUSSION

Soluble fraction A (C_1) of the crude methanol extract of the leaves of *Microtrichia perotitii* produced a milky white powder weighing 13.0 gm with a melting point range of 224 – 226 $^{\circ}\text{C}$ and R_f value of 0.31. The data obtained from the spectroscopic analyses of C_1 were compared with those of published data for isolated α -amyrin acetate [26], [27]. The observed data are thus;

The FTIR spectroscopic data of compound A(C_1) showed observed absorptions signal at 2924.18 cm^{-1} for CH asymmetric for aromatic system and 2868.24 cm^{-1} for C-H stretching in CH_3 and CH_2 , 1726.35 cm^{-1} and 1654.98 cm^{-1} (C=O stretching), 1458.23 cm^{-1} (C-H in CH_3) and 1374.33 cm^{-1} (CH_3 -/ CH_3CO), 1112.96 cm^{-1} C-H scissor for CH_2 , 1025.20 cm^{-1} C-H rocking for CH_2 and 726.22 cm^{-1} wagging for CH_2 as shown in table1[28],[29],[30],[31],[32],[33].

Table 1: FTIR spectrum of compound A (C₁) of soluble fraction C of crude methanol crude extract of the powdered leaves of *Microtrichia perotitii* (MPME)

Vmax(cm-1)	Functional group
2924.18	CH
2868.24	CH
1726.36	C=O
1654.98	C=O
1458.23	C=C
1374.33	CH ₃
1253.77	CH ₃ -/CH ₃ CO
1182.40	-CH ₂ -
1112.96	CH
1025.20	C-O
726.22	CH

The ¹H-NMR of compound A (C₁) showed signals for methyls, methylenes and methine protons. In the up-field region of the spectrum singlets each of three protons integration resonating at different wave numbers were assigned to eight methyl singlets thus; δ 0.83s, 3H-28; 0.84,s,3H-24;0.86,s,3H-29; 0.87,s,3H-30; 0.90,s,3H-23; 0.98s, 3H-26, &δ0.91,s, 3H-25;1.04,s,3H-27 while at the downfield region of the spectrum, a triplet proton signal at δ4.83 ppm was for the olefinic proton H-12, a multiplet at δ4.47 ppm was observed for oxymethine proton (H-3) which suggested the attachment of an acetate moiety at position 3. One of the methyl singlets at δ1.57 ppm,s, was assigned to methyl of the acetate group attached at C-3 (Table 2)[34],[35],[36].

The ¹³C-NMR spectrum of compound A (C₁) showed prominent carbons resonance of 32 carbon atoms in the compound. The up-field resonance at δ29.7, 16.7, 16.6, 16.1, 18.1, 27.9, 27.5, and δ 25.3 ppm were due to the tertiary methyls while the signal at δ21.2 ppm was for the carbon of methyl acetate group. In the downfield region, the peak at δC 81.1 was assigned to the acetate-containing carbon at position 3 while the carbon of the acetate resonated at δC 173.7 and its

terminal methyl at δC 21.2. The presence of the acetate at C-3 was observed by the chemical shift of C-2 that resonated at δC 23.9. Two single protonated carbons had distinct signals at δC 51.5 and δC 55.6 for the carbons C-5 and C-18, respectively. The signal at δC 129.8 was ascribed to C-11 and that at δC 142.7 was attributed to C-12. The chemical shifts of C-12 and C-13 gave evidence to the position and orientation of the methyl C-29 and C-30. This is provided by the resonance of the 19 *p*-methyl at δC 40.8 in the equatorial position, which is in close proximity to the double bond. The DEPT analysis showed signals for the nine methyls, nine methylenes, seven methines and seven quaternary carbons.

Table 2: NMR spectroscopic data of compound A (C₁) of solvent fraction C of crude methanol extract of the leaves of *Microtrichia perotitii* (MPME)(chemical shifts and multiplicity)

Carbon No	$\delta H(\text{ppm})$ (Exptal)	$\delta H(\text{ppm})$ (literature) ^a	$\delta C(\text{ppm})$ (Exptal)	$\delta C(\text{ppm})$ (literature) ^b	Multiplicity (DEPT)	HMBC (H→C)
1	-	-	38.6	38.5	CH ₂	-
2	1.60	1.66	23.9	23.4	CH ₂	2,3
3	4.47	4.48	80.6	80.9	CH	1,2,4,23,24,1'
4	-	-	38.6	37.7	C	-
5	0.81	0.81	51.5	55.3	CH	4,6,10,23,24
6	1.57	1.51,1.34	18.2	18.3	CH ₂	-
7	-	-	33.3	32.9	CH ₂	-
8	-	-	37.8	39.7	C	-
9	1.58	1.54	43.3	47.7	CH	-
10	-	-	36.8	36.8	C	-
11	2.25	1.89	22.7	22.8	CH ₂	-
12	4.83	5.10	129.8	124.2	CH	9,11,14,18
13	-	-	142.7	139.5	C	-
14	-	-	42.8	42.1	C	-
15	-	-	27.9	28.2	CH ₂	-
16	-	-	26.2	26.7	CH ₂	-
17	-	-	32.5	33.8	C	-
18	2.23	1.29	55.6	59.0	CH	-
19	2.24	1.38	40.8	39.5	CH	18,20
20	2.27	1.98	41.7	38.9	CH	-
21	-	-	31.3	33.6	CH ₂	-
22	-	-	42.8	41.6	CH ₂	-
23	0.90s	0.85	29.7	28.1	CH ₃	3,4,5,24
24	0.84s	0.84	16.7	15.8	CH ₃	3,4,5,23
25	0.91s	0.96	16.6	14.2	CH ₃	1,5,9,10
26	0.98s	0.98	16.1	16.8	CH ₃	7,8,9,14
27	1.04s	1.04	18.1	17.6	CH ₃	8,13,14,15
28	0.83s	0.78	27.9	28.8	CH ₃	16,17,18,22
29	0.86s	0.77	27.5	23.3	CH ₃	18,19,20
30	0.87s	0.83	25.3	24.4	CH ₃	19,20,21
-COCH ₃	1.57bs	2.02	21.2	21.5	CH ₃	1'
-C=O	-	-	173.7	170.8	C	-

Key; a= [26]; b= [27], exptl = experimental

A confirmation of the structure of compound A(C₁) was further accomplished through 2D NMR experiments that is, ¹H-¹H COSY, NOESY, HMBC and HSQC spectra analyses[30],[37],[41].

On the basis of above observations along with some reported literature data compound A (C₁) was identified as an α -amyrin acetate [38],[39],[40],[41]. In the reported phytochemical screening of the leaf, n-butanol soluble fraction C gave positive test to Libermann-Burchard test thus indicating the presence of a triterpenoid in the plant [14], [15].

This is the first time isolation of an α -amyrin acetate is being reported for the leaf of *Microtrichia perotitii*. α -amyrin acetate have been reported to possess analgesic and anti-inflammatory properties and could therefore be responsible for the analgesic and anti-inflammatory activities of the herb as well being responsible for some of the ethnomedicinal applications of the herb. Other significant activities of α -amyrin acetate include; anti-oxidant, sedative, anti-pyretic, anticonvulsant and anti-bacterial properties [26], [40], [41], [42] ,[43], [44], [45], [46], [47], [48], anti-erectile dysfunction effects, growth inhibitory effect on Streptococcus , suppressive effects on male albino rat fertility , antihyperglycaemic , larvicidal , cytotoxic and antispasmodic , apoptosis of leukemia cells , antihepatotoxic and antioxidant properties.

A molecular docking study found alpha amyirin to be weakly docking with human estrogen receptors β (ER β) and hence unlikely to bind to the human estrogen receptor and exhibit selective estrogen receptor modulation. Recent evidence indicated that α -amyrin probably displays its effects through interaction with the cannabinoid pathway while in another study it indicated evidence of participation in protein kinase C and protein kinase A pathways [49]. α -amyrin acetate was isolated from other families of plants based on its important biological activities [42],[50],[51].

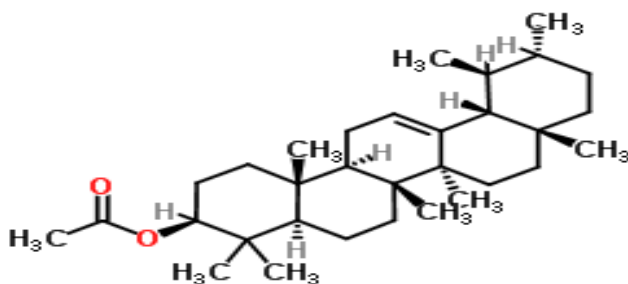


Figure 2: structure of α -amyrin acetate

CONCLUSION

Although, *Microtrichia perotitii* has not been fully exploited for various studies however, the study of its leaves has led to the isolation of α -amyrin acetate for the first time which is a compound with wide ranging biological activities. The isolation has also confirmed some of the ethnomedicinal application of the herb.

ACKNOWLEDGEMENT

The authors wish to acknowledge the contributions and support of our various institutions for providing the enabling environment to carry out this research. Need to be mentioned are all the technical staff of our research laboratories.

COMPETING INTERESTS

The authors have declared that no competing interests exist.

REFERENCES

1. Kim H.S., (2005). Do not put too much value on conventional medicines. *J.Ethnopharmacol.*100: 37-39
2. World Health Organization (2008). Fact sheet no134: Traditional Medicine. Accessed 14th and 17th July, 2009. Available <http://www.who.int/mediacentral/fs134/enl>
3. Solomon, M.A. (2009). Ethiopian Herbal Medicine Practice and the Recognition with Modern Medicine. *Pharmacog. Rev.* 3(5). Pp 37-40
4. Vipin, K. U.; Neeta, S. and Prakash, O. M. (2015). Characterisation and Screening of Bioactive Compounds in the Extract prepared from Aerial Roots of *Fiscus benghalensis*. *Int. J. Pharm. Sci. Res.*6 (12):5056 – 5069
5. Mulharerjee, P. K. and Wahile,A. (2006). Integrated Approaches towards Drug Development from Ayurveda and Other Indian system of Medicines. *J. Ethanopharmacol.*, 103:25 -35
6. Morales, G.; Paredes, S.P. and Loyola, L. A. (2008). Antimicrobial Activity of three *Baccaharis* Species used in the Traditional Medicine of Northern Chile *J.Moles*, 13: 790-794
7. Sunita, D.; Sudhir, K. K.; Satry, K. V and Rana, S. V. S.(2010) Phytochemical Screening of Methanolic Extract and Antibacterial Activity of Active Principles of Hepatoprotective Herb, *Eclipta alba*. *J. Ethanobot. Leaflets*, 14: 248 – 258
8. Cosa P, A J, Berghe, D V, Maes, L.(2006) Anti-infective potential of natural products: How to develop a stronger invitro ‘proof-of-concept’,*J.Ethnopharmacol.*,106:290–302
9. Krishnananda, P. I.; Amit, G. D.; Dipika, A.P.; Mahendra, S. D.;Mangesh, P. M.; andVaibhav, C. K.(2017). Phytochemicals: Extraction methods, identification and detection of bioactive compounds from plant extracts. *J. Pharmacog. Phytochem.* , 6(1):32-36
10. Hutchinson, J. and Dalzie, J.M. (1963). *Flora of West Tropical Africa*, 2nd edn Vol 12. Crown Agent for overseas Government and Administration Millbank, London SW 1.pp 325-397
11. Daniel, O., (1877). *Flora of Tropical Africa*. Vol III Umbelliferae to Ebenaceae. Published under the authority of the first commissioner of Her Majesty’s works. By L.Reeve and Co., 5 Henrietta Street, Covent. Garden London. Pp302-303
12. Andrews, F.W. (1954). *The Flowering Plants of the Sudan*, Vol.3 Compositae-Graminae. Published for the Sudan Govt. by T. Bande and Co Ltd. Arbroath. Scotland. Pp 1-63
13. Watson, L. and Dallwitz, M. (1992). *The Families of Flowering Plants: Description, Illustration, Identification and Information*. Accessed 18th August, 2008. Available <http://www.biodiversity.uno.edu/delta/>
14. Abdullahi, M. N.; Ibrahim, H. and N.Ilyas (2011). Phytochemical Screening and Biological Studies of the Leaves of *Microtrichia perotitii* DC (Asteraceae). *Brit. J. Med. Plants*,1(3): 88 – 97.

15. Abdullahi, M. N.; Ibrahim, H. and N.Ilyas (2013). Evaluation of Phytochemical Screening and Analgesic Activity of Aqueous Extract of The Leaves of *Microtrichia perotitii* Dc (Asteraceae) in Mice using Hotplate Method. *Med. Plant Res.*, 3:537-543
16. Vogel, A.I.; Brain, S.F.; Anthony, J.H.; Peter, W.G.S.; Austin, R.T. (1989). *Textbook of Practical Organic Chemistry*. 5th edn. Longman group UK Ltd. pp197-235, 254-394
17. Brain, K.R. and Turner, T.D. (1975). The Practical Evaluation of Phytopharmaceuticals. Wright-scientechnica Bristol. Pp4-9, 60, 76-79, 81-85,153.
18. Ciulei,I. (1994). *Methodology for the Analysis of vegetable drugs*. Chemical industries Branch. Division of Industrial operations, UNIDO. Romania. Pp21-97.
19. Wang, L. and Weiler, C.L. (2006). Recent Advances in Extraction of Nutraceuticals from Plants. *Trend in food Science and Technology*.Elsevier, 17: 300-312.
20. Anees, A.; Abbas, F.M.A.; Sufia, H.; Lim, H.K.(2009). Extraction, Separation and Identification of Chemical ingredients of *Elephantopus scaber* L. using factorial design of experiment. *Int. J. Chem.*, 1(1):36-48
21. Woo,S.W.; Shin, H.Y. and Kang, K.S.(1980). Chemistry and Pharmacology of Flavone-C-Glycoside from *Zizyphus* seeds. *The Kor. J. Pharmacog.*, 11(3&4):141-148.
22. Sharma,P. and Sarin,R.(2012). Isolation and Characterisation of Quercetin and Kaempferol *in Vivo* and *in vitro* from *Pedaliu murex*. *Int. Res. J. Pharm.*, 3(4):184-187.
23. Rajauria,G. and Abu-Ghannam, N.(2013). Isolation and Partial Characterization of Bioactive Fucoxanthin from *Himanthalia elongata* Brown Seaweed: A TLC-Based Approach. *Int. J. Anal. Chem.*, 13:1-8
24. Zygmunt, J. and Namiesnik, J. (2003). Preparation of samples of Plant material for Chromatographic Analysis. *J. Chroma. Sci.*, 41:109-116.
25. Franz,B.; Abraham,W. and Martin,S. (2013).Natural Product Isolation: How to get from biological material to pure compounds.*Nat. Prod., Report*, 30:525-545
26. Ali,N(2013). Brine shrimp Cytotoxicity of Crude Methanol Extract and Antispasmodic Activity of α -amyrin acetate from *Tylophora hirsuta* Wall. *BMC Compl. Alternat. Med.*, 13:135-141
27. Vincent.A. S. N.; Esperanza, M.G. A.; Chien-Chang, S.; Consolacion, Y. R.(2015). Metabolites from the Male Cone of *Cycas vespertilio*. *Int. J. Pharmacog. Phytochem. Res.*, 7(4); 727-731
28. Sultana,N.and Saleem,M.(2010). Phytochemical Studies On *Astonia scholaris*. *Zeitschrift für Naturforsch.*65b:203-210

29. Krishnaveni,A. and Thaakur,S.R. (2012). Phytochemical Investigations of *Sapindus emarginatus*. Int. J. Res. Pharmacog. Pharmacot.1 (2):93-96
30. Niaz,A. (2013). Brine shrimp Cytotoxicity of Crude Methanol Extract and Antispasmodic activity of α -amyrin acetate from *Tylophora hirsuta* Wall. BMC Compl. Alternat. Med., 13:135
31. Arun,K.; Shrabani,P.; Pingalkumari, S.;Thirugnanasambandan, S. and Kathiresan, K.(2014). Antibacterial and Phytochemical Assessment on various extracts of *Ipomoea pes-caprae* (L.) R.Brthrough FTIR and GC-MS Spectroscopic Analysis. Asian. J. Pharmaceut. Clin. Res., 17(3):134-138
32. Punnam, C.M.; Srivan,P.; Suresh, B.K. and Vijayachari,P.(2014). Anti-microbial Constituents from Leaves of *Glyptopetalum calocarpum* (Kurz.) Prain. Int. J. Pharmaceut. Chem. Sci., 3(4):871-875
33. Gopukumar S T, Princy Alexander, Jainambo M, Praseetha P K. (2016). Phytochemical Screening and FT-IR Analysis of *Ficus benghalensis* Fruits. Int. J. Pharmacog. Phytochem. Res., 8(9); 1529-1534
34. Segovl,J.F.O;Oliveira,V.I;Gonçavel,M.C.A.;Resck,I.I.;Silv,C.A.M.;Silveira,D. et al.(2011). Botanical Characterisation, Geographical Distribution and Phytochemistry Analysis of *Manilkara huberi*(Ducke) Stanhl Autochtonous in Amapa state, Brazil.UDC.58:550(73):34-39
35. Chander,M.P.; Srivani, P.; Suresh, K.B. and Vijayachari, P. (2014).Anti-microbial Constituents From Leaves of *Glyptopetalum calocarpum* (Kurz.) Prain. Int.J.Pharm.Chem. Sci., 3(4):871-875
36. Goyal,N. and Sharma, S.K. (2014).Bioactive Phytoconstituents and Plant Extracts from genus *Heliotropium*. Int. J. Green Pharm., 8(4):217-225
37. Ragasa,C.Y.; Ofelia, C.; Emelina,H.M.; Leonisa,O.B.and Chien-Chang,S.(2014). Chemical Constituents of *Ficus ampelas*. Res.J. Pharmaceut., Biol. Chem. Sci., 5(2): 355-359.
38. Feleke,S. and Brehane,A.(2005). Triterpene Compounds from the Latex of *Ficus sur*.L. Bul. Chem. Soc. Ethiopia, 19(2):307-310
39. Razbor, M.I.; Voncina, D.D.; Dolecek,V.; Ernest,V.(2007).Determination of Major Phenolic Acids, Phenolic Diterpenes and Triterpenes in Rosemary (*Rosmarinus officinalis* L.) by Gas chromatography and Mass Spectroscopy. Acta. Chim. Slov., 54:60-67
40. Catharina,E.F.; Thabat, deS.S.; Marcelo,D.M.V.F and Maria,A.C.K. (2013).Triterpene esters: Natural Products from *Dorstenia arifolia*(Moraceae). Moles, 18:4247-4256
41. Lea,H.R.;Anne,W.;Tianarilalaina,T.A.;Solofoniaina,G.A.;Ernest.J.B.R.;Amelie,R.;Fanantena nirainy,R.(2014). Triterpenes and Steroids from the stem bark of *Gambeya boiviniana* Pierre. J. Pharmcog. Phytochem. 3(1):68-72

42. Aragão, G.F.; Carneiro, L.M.V.; Junior, A.P.F.; Bandeira, P.N.; Lemos, I.L.G.; Viana, G.S.B (2009) Evidence for excitatory and Inhibitory Amino acids participation in the Neuropharmacological activity of alpha- and beta- amyrin acetates. *The Open Pharmacol. J.*; 3:9-16.
43. Manguro, L.O.A.; Opiyo, S.A.; Herdtweck, E.; Lemmen, P.(2009). Triterpenes of *Commiphora holtziana* oleo-gum resin. *Can. J. Chem.*, 87:1173-1179
44. Sob, S.V.T.K.; Wabbo, H. K.; Tchinda, A. T.; Tane, P.; Ngadju, B.T. and Ye, Y. (2010) Anthraquinones, sterols, triterpenoids and xanthenes from *Cassia obtusifolia*. *Bio. Sys. Ecol.*, 38:342-345
45. Dias, M.M.; Hamerski, L.; Pinto, A.(2011). Semi-preparative separation of α and β -amyrin by High Performance Liquid Chromatography. *QUÍMICA. NOVA*, 34(4):1-9.
46. Joyce, J.K.; Neil, A.K. and Hafizah, C. (2011). Triterpenoids from *Vernonia auriculifera* Hiern Exhibit Antimicrobial activity. *Afr. J. Pharm. Pharmacol.*, 5(8): 1150-1156
47. Singh, M.; Kaur, M.; Dangi, C.B.S.; Singh, H. (2014). Phytochemical and TLC Profiling of *Lawsonia intermis* (Heena). *Int. J. Pharmaceut. Res.*, 3(1):1-7
48. Virgilio, D.E.; Chien-Chang, S.; Consolacion, Y.R.(2015). Terpenoids and Sterols from *Hoya multiflora* Blume. *J. Appld Pharmaceut. Sci.*:5(04):33-39
49. Nnamonu, L.A.; Tor-Anyiin, T. A.; Ugbenyo, N. O.; and Anyam, J.V.(2016). Isolation and Characterization of α -Amyrin from Stem Bark of *Ficus exasperata* (Vahl). *Biotech. Int.*, 16(4): 1-7
50. Jahan, R.; Abdullah, Al-N.; Snehali, M. and Mohammed, R.(2014). Ethnopharmacological Significance of *Eclipta alba* (L.) Hassk. (Asteraceae). *Int. Schol. Res. Notice*, 2014:1-22
51. Ebajo, V. D.; Sherr, C-C.; Ragasa, C.Y. (2015). Terpenoids and Sterols from *Hoya multiflora* Blume. *J. Appld. Pharmaceut. Sci.*, 5(04):033-039