

Original Research Article

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

ABSTRACT

Aims: *Microtrichia perotitii* DC belongs to the family Asteraceae and is widely distributed in West African countries. 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 and is locally used for treating pain related diseases such as toothaches, cuts and burns and rashes in children including skin diseases, rheumatism, diarrhea and jaundice. It is on the basis of its significance in tradi-tional medicinal usage that an attempt was made to isolate the active principles in the herb although previous phytochemical screening of the leaves of the herb has indicated the presence of carbohydrates, tannins, cardiac glycosides, triterpenes, proteins, flavonoids, alkaloids, and steroids.

Methods: The leaves of the herb were extracted using soxhlet apparatus that hot-extraction method with methanol. Fractionation of the crude extract (MPME) gave a white milky solid from the n-butanol fraction A(C₁) whose 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 upon comparison with literature indicate it to be an alpha-amyrin acetate.

Conclusion: This is the first time an alpha-amyrin acetate was isolated from the leaf of *Microtrichia perotitii*. 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, ethnomedicinal

INTRODUCTION

For centuries people have been living closely to their environment and solely rely on its available flora and fauna as a source of food and medicine and has therefore led to the societies' developing their plant pharmacopeias. This practice is most common in developing countries of Asia and Africa where 80 % of the population depend on herbal therapies because of their effectiveness, low cost and rare side effects when compared to synthetic drugs (Kim, 2005; WHO, 2008; Solomon, 2009; Vipin *et al*, 2015).

Traditionally, medicinal plants are used in ethnomedicine and the plants contain or produce bioactive compounds of known therapeutic properties in addition to their uses as food additives and perfumes (Mukherjee and Wahile, 2006; Morales *et al.*, 2008; Sunita *et al.*, 2010). The medicinal plants provide the most needed natural products either as plants extract, pure compounds or as standardized extracts and thus provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity (Cosa *et al.*, 2006).

Secondary metabolites otherwise known as natural products are seldom extracted out of the plants. Therefore, extraction of bioactive compounds from the plants and their quantitative and qualitative estimation is important for exploration of new biomolecules to be used by pharmaceutical and agrochemical industry directly or can be used as a lead molecule to synthesize more potent molecules (Krishnananda *et al.*, 2017).

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 (Hutchinson and Dalziel, 1963). In Nigeria it is found in northern part of the country where it is known as Maijankai or Sawun keke in Hausa, Osete in Igbo and Shaware pepe in Yoruba natives. The herb is an annual plant and is a diffuse much branched pubescent varying from 1ft in height. The leaves are obovate, obtuse, cuneately narrowed into petiole, coarsely toothed above $\frac{1}{2}$ - $1\frac{1}{2}$ inch long, $\frac{1}{3}$ - 1 inch broad. The petiole of lower leaves is 1 inch or more at the upper. Other features are in accordance to some members of the family (Daniel, 1877; Andrews, 1954, Watson and Dallwitz, 1992). Traditionally, *Microtrichia perotitii* is used for treating pain related diseases such as toothaches, cuts and 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 (Abdullahi *et al.*, 2011^a; Abdullahi *et al.*, 2013^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 has already been identified and authenticated by Mal. Musa M. of herbarium unit of the Department of Biological sciences, Ahmadu Bello University, Zaria and was given specimen number 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 (Vogel,1989; Brain and Turner, 1975; Ciulei, 1994; Wang and Weiler, 2006; Anees *et al.*,2009).

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 Woo *et al.*, 1980 (Figure 3.1).

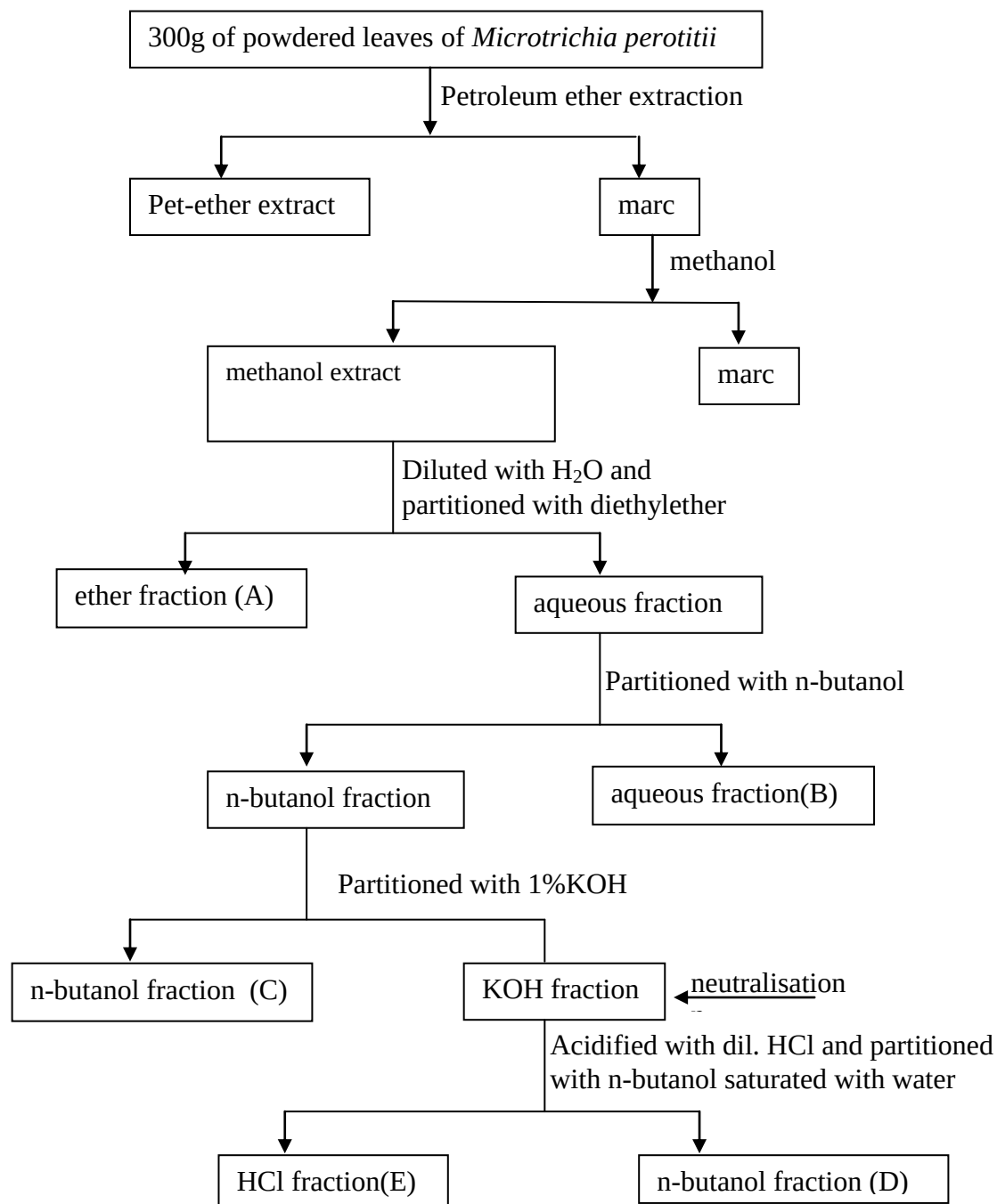


Figure 3.1: Schematic chart for the extraction (Woo *et al.*, 1980)

Each of the extract was evaporated gently over hot water bath for about 30min with intermittent removal from the bath. Thereafter each was transferred into sterilized bottle, labeled and kept in desiccator until use. Accordingly, 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. However, soluble fraction C was found to be most active in the studies (Abdullahi *et al.*, 2013).

Chromatographic analysis of soluble fraction C

The soluble fraction (0.2 g) was spotted on a pre-coated TLC plates and developed in butanol:acetic acid:water(8:1:1)and hexane:ethylacetate(7:3) solvent mixtures. R_f values were calculated for each spot after being spread with p-anisaldehyde (Sharma and Sarina, 2012; Rajauria and Abu-Ghannam, 2013). 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 %) (Zygmunt and Namiesnik, 2003; Franz *et al.*, 2013). Seventy six 40 ml fractions were collected and similar fractions were pooled together as A(C₁), B(C₂) and C(C₃). However, fraction A(C₁) produced a single profile and was therefore subjected to spectrophotometric analyses with FTIR (8400S HIMADZU), ¹H-NMR spectrophotometer (Bruker400Mhz), and ¹³C-NMR spectrophotometer(Bruker400Mhz).

RESULT AND DISCUSSION

Soluble fraction A(C₁) of the crude methanol extract of the leaves of *Microtrichia perotitii* gave a milky white powder weighing 13.0 gm with a melting point range of 224 – 226 °C and R_f value of 0.31. From the results of spectroscopic analyses, the IR, ¹H-NMR and ¹³C-NMR data of C₁ were consistent with those of published data for isolated α -amyrin acetate (Ali 2003; Vincent *et al.*, 2015).

The IR spectroscopic data of component A(C₁) showed observed absorptions signal at 2924.18 cm⁻¹for CH asymmetric for aromatic system and 2868.24 cm⁻¹ for C-H stretching in CH₃ and CH₂, 1726.35 cm⁻¹and 1654.98 cm⁻¹ (C=O stretching), 1458.23cm⁻¹ (C-H in CH₃) and 1374.33 cm⁻¹ (CH₃-/CH₃CO, 1112.96 cm⁻¹ C-H scissor for CH₂, 1025.20 cm⁻¹ C-H rocking for CH₂ and

152 726.22 cm⁻¹wagging for CH₂as shown in table1(Sultana and Saleem,2010; Krishnaveni and
153 Thaakur,2012; Niaz,2013; Arun *et al.*,2014; Punnam *et al.*,2014: Gopukumar *et al.*, 2016).

154 **Table 1: FTIR spectrum of compound A(C₁) of soluble fraction C of crude methanol**
155 **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

156
157
158 The ¹H-NMR of component A (C₁) showed signals for methyls, methylenes and methine
159 protons. In the up-field region of the spectrum singlets each of three protons integration
160 resonating at different wave numbers were assigned to eight methyl singlets thus; δ 0.83s, 3H-
161 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-
162 25;1.04,s,3H-27 while at the downfield region of the spectrum,a triplet proton signal at δ4.83
163 ppm was for the olefinic proton H-12, a multiplet at δ4.47 ppm was observed for oxymethine
164 proton (H-3)which suggested the attachment of an acetate moiety at position3.One of the methyl
165 singlets atδ1.57,s, was assigned to methyl of the acetate group attached at C-3 (Table2)(Segovlet
166 *al.*, 2011; Chander *et al.*, 2014; Goyal and Sharama, 2014).

167
168 The ¹³C-NMR spectrum of component A(C₁) showed thirty two prominent carbons resonance of
169 32 carbon atoms in the compound. The up-field resonance at δ29.7, 16.7, 16.6, 16.1, 18.1, 27.9,
170 27.5, andδ 25.3 ppm were due to the tertiary methyls while the signal at δ21.2 ppm was for the
171 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 with the carbonyl of the acetate resonating 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 gives 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 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	$\delta H(ppm)$ (Exptal)	$\delta H(ppm)$ (literature) ^a	$\delta C(ppm)$ (Exptal)	$\delta C(ppm)$ (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=Ali, 2003; b= Vincent *et al*, 2015, exptl = experimental

A confirmation of the structure of compound A(C₁) was further accomplished through 2D NMR experiments thus, ¹H-¹H COSY, NOESY, HMBC and HSQC spectra analyses (Niaz, 2013; Lea, *et al.*, 2014; Ragasa, *et al.*, 2014). On the basis of above observations along with some reported literature data compound A1(C₁) was identified as α -amyrin acetate (Feleke and Brehani, 2005; Razbor *et al.*, 2007; Catharina *et al.*, 2013; Lea *et al.*, 2014). The positive test of the soluble fraction C to libermann-Burchard test was an indication of the presence of a triterpenoid in the plant. However, this is the first time isolation of an α -amyrin acetate is being reported for the leaves of *Microtrichia perotitii*. α -amyrin acetate have been reported to possess analgesic and anti-inflammatory properties thus supporting some of the ethnomedicinal application of the plant. Other important activities of α -amyrin acetate include, anti-oxidant, sedative, anti-pyretic, anticonvulsant and anti-bacterial properties (Aragao *et al.*, 2009; Manguro *et al.*, 2009; Sob *et al.*, 2010; Dias *et al.*, 2011; Joyce *et al.*, 2011; Ali, 2013; Catharina, *et al.*, 2013; Lea, *et al.*, 2014; Singh *et al.*, 2014; Virgilio *et al.*, 2015), 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, a molecular docking study found alpha amyryin 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 indicates that α -amyryin probably displays its effects through interaction with the cannabinoid pathway; another study has also indicated evidence for participation in protein kinase C and protein kinase A pathways (Nnamonu *et al.*, 2016). α -amyryin acetate was isolated from the aerial roots of *Ficus benghalensis* L (Asteraceae) for its antidiabetic activity, *Hoya multiflora* from its sedative,

209 anxiolytic and anticonvulsant properties as well as significant anti-inflammatory activity in egg
210 albumen-induced paw edema(Aragão *et al.*, 2009; Jahan, *et al.*, 2014; Ebajo *et al.*, 2015).

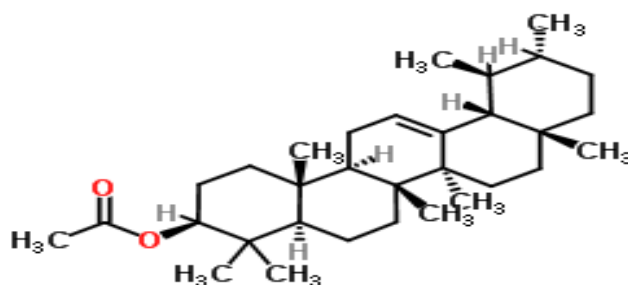


Figure 2: structure of α -amyrin acetate

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.

COMPETING INTERESTS

The authors have declared that no competing interests exist.

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