

## **Isolation and Characterisation of an Alpha-amyrin Acetate Isolated from the Leaves of *Microtrichia perotitii* DC (Asteraceae)**

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### **Authors' contributions**

This work was carried out in collaboration between all authors. Author MNA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors NI and IH managed the analyses of the study. Author YMK managed the literature searches. All authors read and approved the final manuscript.

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### **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(C<sub>1</sub>) 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.

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

**Conclusion:** An alpha-amyryn acetate was isolated from the leaf of *Microtrichia perotitii* for the very first time. Alpha-amyryn 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*.

**Keywords:** *Microtrichia perotitii*; asteraceae; soxhlet apparatus;  $\alpha$ -amyryn acetate; ethanomedicinal.

## 1. 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 ethanomedicine 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 Igbira 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, ½ - 1½ inch long and ⅓ – 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 [14,15].

## 2. MATERIALS AND METHODS

### 2.1 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.

### 2.2 Solvent Extraction

300 g of the powdered leaves of *Microtrichia perotitii* were refluxed with 3 L of petroleum ether (60-80°C) for 8 hours in a soxhlet apparatus. The extract was decanted off and fresh quantity of petroleum ether (3 L) was added again and then refluxed for another 5 hr. The defatted leaves

(marc) were completely dried and later extracted with 3 L 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].

### 2.3 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] (Fig. 1).

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].

### 2.4 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].

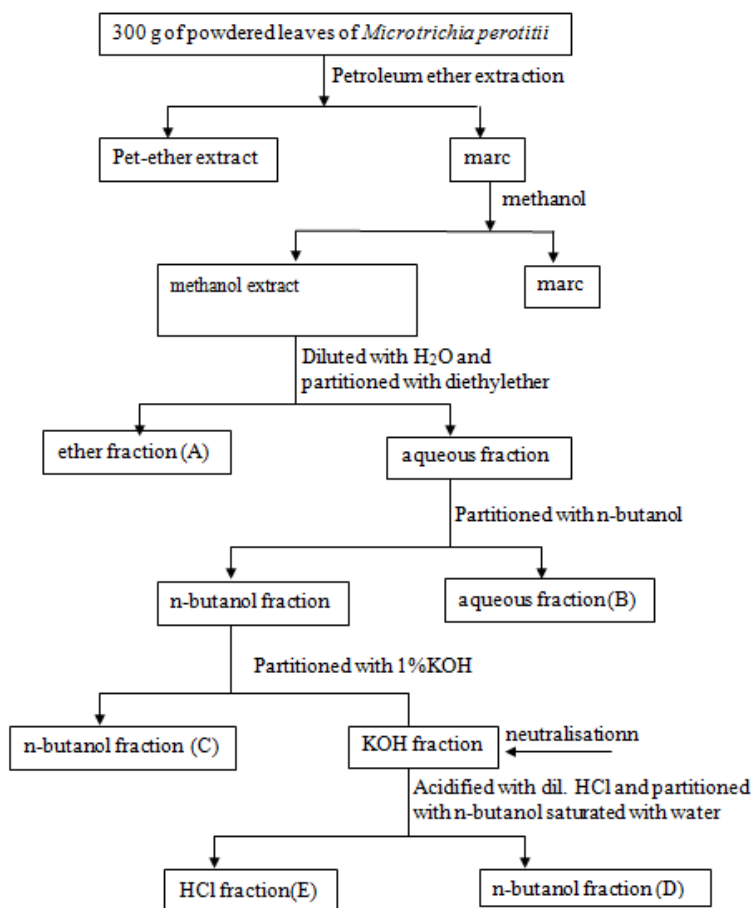


Fig. 1. Schematic chart for the extraction [21]

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<sub>1</sub>), B (C<sub>2</sub>) and C (C<sub>3</sub>). However, fraction A (C<sub>1</sub>) produced a single profile and was therefore subjected to spectrophotometric analyses with FTIR (8400S HIMADZU), <sup>1</sup>H-NMR spectrophotometer (Bruker 400 Mhz), and <sup>13</sup>C-NMR spectrophotometer (Bruker400Mhz).

### 3. RESULTS AND DISCUSSION

Soluble fraction A (C<sub>1</sub>) 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°C and R<sub>f</sub> value of 0.31. The data obtained from the spectroscopic analyses of C<sub>1</sub> 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<sub>1</sub>) showed observed absorptions signal at 2924.18 cm<sup>-1</sup> for CH asymmetric for aromatic system and 2868.24 cm<sup>-1</sup> for C-H stretching in CH<sub>3</sub> and CH<sub>2</sub>, 1726.35 cm<sup>-1</sup> and 1654.98 cm<sup>-1</sup> (C=O stretching); 1458.23cm<sup>-1</sup> (C-H in CH<sub>3</sub>) and 1374.33 cm<sup>-1</sup> (CH<sub>3</sub>-/CH<sub>3</sub>CO, 1112.96 cm<sup>-1</sup> C-H scissor for CH<sub>2</sub>, 1025.20 cm<sup>-1</sup> C-H rocking for CH<sub>2</sub> and 726.22 cm<sup>-1</sup>wagging for CH<sub>2</sub>as shown in Table1 [28,29,30,31,32,33].

The <sup>1</sup>H-NMR of compound A (C<sub>1</sub>) 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].

**Table 1. FTIR spectrum of compound A (C<sub>1</sub>) 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 <sub>3</sub>
1253.77	CH <sub>3</sub> -/CH <sub>3</sub> CO
1182.40	-CH <sub>2</sub> -
1112.96	CH
1025.20	C-O
726.22	CH

The <sup>13</sup>C-NMR spectrum of compound A (C<sub>1</sub>) 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.

A confirmation of the structure of compound A(C<sub>1</sub>) was further accomplished through 2D NMR experiments that is, <sup>1</sup>H-<sup>1</sup>H COSY, NOESY, HMBC and HSQC spectra analyses[30,37,38]. On the basis of above observations along with some reported literature data compound A (C<sub>1</sub>) was identified as an α-amyrin acetate [39,40,41,38]. 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].

**Table 2. NMR spectroscopic data of compound A (C<sub>1</sub>) 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) <sup>a</sup>	$\delta C(\text{ppm})$ (Exptal)	$\delta C(\text{ppm})$ (literature) <sup>b</sup>	Multiplicity (DEPT)	HMBC (H→C)
1	--	--	38.6	38.5	CH <sub>2</sub>	-
2	1.60	1.66	23.9	23.4	CH <sub>2</sub>	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 <sub>2</sub>	-
7	--	--	33.3	32.9	CH <sub>2</sub>	-
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 <sub>2</sub>	-
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 <sub>2</sub>	-
16	--	--	26.2	26.7	CH <sub>2</sub>	-
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 <sub>2</sub>	-
22	--	--	42.8	41.6	CH <sub>2</sub>	-
23	0.90s	0.85	29.7	28.1	CH <sub>3</sub>	3,4,5,24
24	0.84s	0.84	16.7	15.8	CH <sub>3</sub>	3,4,5,23
25	0.91s	0.96	16.6	14.2	CH <sub>3</sub>	1,5,9,10
26	0.98s	0.98	16.1	16.8	CH <sub>3</sub>	7,8,9,14
27	1.04s	1.04	18.1	17.6	CH <sub>3</sub>	8,13,14,15
28	0.83s	0.78	27.9	28.8	CH <sub>3</sub>	16,17,18,22
29	0.86s	0.77	27.5	23.3	CH <sub>3</sub>	18,19,20
30	0.87s	0.83	25.3	24.4	CH <sub>3</sub>	19,20,21
-	1.57bs	2.02	21.2	21.5	CH <sub>3</sub>	1'
COCH <sub>3</sub>	--	--	173.7	170.8	C	-
-C=O	--	--	--	--	--	--

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

This is the first time isolation of an  $\alpha$ -amyirin acetate is being reported for the leaf of *Microtrichia perotitii*.  $\alpha$ -amyirin 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  $\alpha$ -amyirin acetate include; antioxidant, sedative, anti-pyretic, anticonvulsant and anti-bacterial properties [26,41,38,42,43,44,45,46,47,48], 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  $\beta$  (ER $\beta$ ) and hence unlikely to bind to the human estrogen receptor and exhibit selective estrogen receptor modulation. Recent evidence indicated that  $\alpha$ -amyirin 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].  $\alpha$ -amyirin acetate was isolated from other families of plants based on its important biological activities [42,50,51].

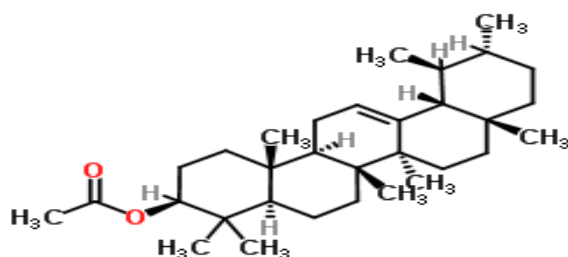


Fig. 2. structure of  $\alpha$ -amyrin acetate

#### 4. CONCLUSION

Although, *Microtrichia perotitii* has not been fully exploited for various studies however, the study of its leaves has led to the isolation of  $\alpha$ -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.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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