

Phytochemical investigations of leaf, fruit and root bark of *Olox manni* Oliv. Olacaceae

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ABSTRACT

Phytochemical examination of the petroleum ether and methanol crude extracts of the leaf, fruit and root bark of the *Olox manni* showed the presence of coumarins, steroid/triterpenes, saponins, fatty acids and tannins and absence of alkaloids in the plant. Volatile oils and flavonoids are present only in the fruit and leaf part of the plant. The petroleum extract of the leaf *Olox manni* Oliv. (Olacaceae) afforded a colourless crystals (*E*)-3-methyl-5-phenyl-2-pentenoic acid (C₁₂H₁₄O₂, m.p. 57-58 °C), which was identified by C H and spectral analysis (UV, IR, NMR, EIMS).

Keywords: *Olox manni*, phytochemical, extract

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INTRODUCTION

The plant *Olox manni* is plant specie in the family Olacaceae that are widely distributed in the tropics especially Nigeria, Sierra Leone, and Ghana. It is a climbing shrub up to 2 metres high leaves are lanceolate to ovate or elliptic up to 6 x 3 inches with 5 – 6 pairs at lateral looped nerves. Flowers are greenish white in axillary racemes. Fruits are orange when ripe about ½ x ¾ inches. Natural habitat is closed forest (Daziell, 1963) Decoction of the leaves and roots of the plant is used for yellow fever (Irvine, 1961).

Olacaceae families have been reported to contain fatty acids (Galliard and Mercer, 1975). The synthesis of (*E*) and (*Z*) -3-methyl-5-phenyl-2-pentenoic acid have also been reported (Stoermer and Pinhey, 1998).

EXPERIMENTAL

Plant Material

The whole plant (aerial and underground parts) material bearing fruits and leaves growing wild were collected around Zaria, Nigeria in July 1999. The plant was authenticated and deposited (Voucher specimen number 1697) in the Herbarium, Department of Biological Sciences, Ahmadu Bello University, Zaria.

Leaves were removed from the twigs, epicarp and mesocarp of the fruits and root bark were also removed with help of scrapper. All the plant parts were separately air dried, powdered, sieved, weighed and stored in airtight containers and subsequently referred to as powdered leaves, fruit and root bark.

Extraction

Powdered leaves of *Olox manni* (1.0kg) were successively extracted with soxhlet extractor petroleum ether (60 - 80°C) and methanol respectively. The same procedure was repeated with 1.0 kg each of powdered root bark and fruit.

Phytochemical screening

The petroleum and methanolic extracts of the leaves, fruit and root bark were separately subjected to preliminary phytochemical tests using standard techniques (Trease and Evans, 1984; Ciulei, 1981; Brain and Turner, 1975).

For saponins the Lieberman – Buchard, haemolytic and frothing tests were carried out and observations recorded. The Mayer's, Wagner and Hagner's tests were carried out for alkaloids, Lieberman – Buchard for steroids/triterpenoids, ferric chloride test was carried out for tannins, Schinoda test for flavonoids, Sudan IV test for volatile oils, bicarbonate test for free fatty acid, Borntrager's test for anthraquinone, ammonia- UV test for coumarins, Molisch test for carbohydrate and Keller- killani for cardiac glycosides.

Column Chromatographic Separation

Petroleum ether extract of the leaf was gradiently eluted on silica gel column chromatography with gradients of light petroleum ether, chloroform and methanol. This afforded colourless crystal (32mg) in the methanol region, which was characterised by elemental and spectral analysis.

RESULTS

Extraction

The various extracts obtained were weighed and percentage yields calculated as shown in Table 1.

Table 1: Percentage Yield of Extracts from *Olox manni*

	Percentage yield of Extracts	
	Petroleum ether Extract (%)	Methanol Extract (%)
Leaves	3.5	4.2
Fruits	5.6	8.4
Root Bark	5.3	9.2

Table 2: Summary of Phytochemical Constituents of the Extracts of the leaf, fruit and root bark. of *Olox manni*

Compound/group	Leaves		Fruit		Root bark	
	PE	ME	PE	ME	PE	ME
Steroid/triterpene	++	+	+	+	++	+
Saponin	Lieberman	NT	++	NT	+	NT
	-Buchard					
	Haemolitic	NT	+	NT	+	NT
	Frothing	NT	+++	NT	+	NT
Tannins	NT	++	NT	+	NT	++
Flavones	++	-	+++	++	-	-
Alkaloids	-	-	-	-	-	-
Volatile oils	+	-	++	-	-	-
Coumarins	+	-	-	+	+	-
Free carboxylic acid	-	++	-	+	-	+
Free anthraquinone	-	-	-	-	+	-
Combined anthraquinone	-	+	-	-	-	+
Carbohydrate	NT	++	NT	+	NT	++
Cardiac glycoside	NT	-	NT	-	NT	++

PE = Petroleum extract; ME = Methanolic extract; +++ = Copiously present; ++ = Moderately present
+ = Slightly present; - = Absent; NT = Not Tested

Phytochemical screening

The results of phytochemical test carried out on the various extracts are recorded as shown in Table 2

Isolation

Compound 1 on analysis was identified as (*E*)-3-methyl-5-phenyl-2-pentenoic by comparison of its IR, NMR and EI-MS spectra with that reported in literature (Stoermer and Pinhey 1998)

C H analysis : C 76.2 H 7.3 (Calculated C 75.8 H 7.4 %) UV (ethanol) 208,305 nm.

IR (CDCl₃) 3500-2800(br,s OH), 3104, 2950, 1694 (s, C=O), 1641, 1260, 968 cm⁻¹.

¹H-NMR (90 MHz, CDCl₃) 2.16 (3H, d, *J* 1.1 Hz, CH₃), 2.43 (2H, t, =C(CH₂), 2.73 (2H, t, Ph-CH₂), 5.61 (1H, m, =CH), 6.92-7.40 (5H, t, ArH), 9.98 (1H, b,s COOH).
¹³C-NMR (15 MHz, CDCl₃) 19.22 (CH₃), 34.02, 42.85 (CH₂), 115.7 (=CH); 126.2, 128.2 128.5 (ArCH), 140.9 (quat, C1'), 161.7 (quat, C3), 171.5 (quat, C1).
EI-MS 190(M⁺, 3%), 144(10), 91(100).

DISCUSSION

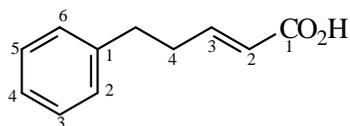
The phytochemical constituents in the various parts of the plant vary. This includes volatile oils, flavones/flavone glycosides that were found in leaf and fruit, alkaloids were found to be absent in all parts while saponins, triterpenoid/steroidal aglycone, and fatty acids were found in all parts of the plant. This finding is in line with the report by Mabry *et al.* (1968) that

Olacaceae family contains lot of fatty acid. The presence of saponins, cardiac glycosides and tannins in the fruits might be responsible for the toxic effects observed when the fruits of *Olox manni* (which are predominantly taken by monkeys) are consumed by humans and also when leaves of *Olox manni* are taken by animals. Presence of volatile oils and flavonoids has also been reported in fruits of other plants in the same family (Harborne, 1973). The flavonoids might be responsible for the yellow to orange colour of the fruits. Flavonoids detected in the petroleum ether extract could either be methylated or that they occur as free aglycone while those that appear in the methanol extracts are likely the glycosides.

From the petroleum extract of the leaf, (*E*)-3-methyl-5-phenyl-2-pentenoic was isolated. Its EIMS displayed a molecular ion peak at m/z 190 corresponding to a molecular formula $C_{12}H_{14}O_2$, a base peak at m/z 91 of the $C_7H_7^+$ benzylic (tropylium) cation and an alkyl benzyl radical $C_{11}H_{12}^+$ m/z 144 formed due to elimination of CO_2 . The IR spectrum showed broad singlet at $3500-2800cm^{-1}$ for OH, a singlet at $1694cm^{-1}$ for the C=O. The 1H -NMR of the OH on C_1 displayed at δ 9.98, aromatic protons at δ 6.92-7.40, and methyl protons at δ 2.16, protons at C_4 and C_5 at δ 2.43, at 2.73

respectively.

^{13}C -NMR showed 12 carbon signals. The methyl C atom at δ 19.22 C_4 , C_5 atoms at δ 34.02, δ 42.85 respectively, aryl C atom δ 126.2, 128.2, 128.5 and 3 quaternary C atom at δ 140.9 (C_1') 161.7(C_3), 171.5(C_1). Identification was confirmed by comparison of the UV, IR, 1H NMR, ^{13}C NMR with that reported in literature (



Stoermer and Pinhey, 1998).

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