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Phytotoxic and Chemical Investigations of a Nigerian Medicinal Plant

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Abstract: *Pyrenacantha staudtii* is a medicinal plant endemic in Nigeria and used ethnomedicinally for the treatment of various diseases by the populace. In continuation of the studies on chemical constituents of *Pyrenacantha staudtii*, the study was aimed at validating the phytotoxic activity of the leaf extract and through a systematic chemical procedure, isolation and identification of the chemical principles of *Pyrenacantha staudtii* leaf was established. The investigation of the chemical constituents of *Pyrenacantha staudtii* leaves has led to the isolation and characterization of two known compounds oleanilic acid and β-amyrin. The compounds were isolated first time from *P. staudtii*. The structures of the compounds were established by spectral (MS and NMR) and chemical methods. The methanolic extract of *P. staudtii* exhibited significant phytotoxic activity against *Lemna minor* L. at 10 μg mL^{-1}. The result of the study has justified the phytotoxic potential of the plant.

Key words: Phytotoxic, activity, *Pyrenacantha staudtii*, leaf, Icacinaceae

INTRODUCTION

The use of plants for the treatment of diseases and infections is as old as mankind. In Africa the use of medicinal plants is currently attracting attention by scientists' in order to validate the ethnomedicinal applications as remedy against some diseases. The World Health Organization (WHO) supports the use of herbal medicines provided they are proven to be efficacious and safe. This scientific justification of herbal plants led to the systematic and chemical investigation of a local medicinal plants *Pyrenacantha staudtii*.

Pyrenacantha staudtii (Hutch and Dalz) Icacinaceae is one of the oldest plant medicines that have been used by human beings. The plant is a woody climber indigenous to Nigeria (West Africa). Many biological activities such as anticancer (Gill, 1992), anti-ulcer (Aguwa *et al*., 1986), antimalarial (Mesia *et al*., 2003), antiabortificient (Falodun *et al*., 2005; Falodun and Usifoh, 2006), abolition of painful uterine contractions, intestinal colic and dysmenorrheal effects have reported. The root bark and stem are also used in folk medicine for the treatment of cancer (Gill, 1992).

These activities are reported due to three kinds of main constituents, saponins, flavonoids and alkaloids. One of the alkaloids 3-carbomethoxylpyridine was isolated from the leaf and is used to prevent threatened abortion in gravid uterus (Falodun and Usifoh, 2006).

Until now, scanty chemical constituents have been isolated from the plant *Pyrenacantha staudtii*. Therefore, in our studies we reported for the first time the isolation, structure elucidation of Amyrin and oleanolic acid from the plant and its phytotoxic evaluation via activity guided isolation technique.

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MATERIALS AND METHODS

The plant samples were collected from Benin City, Nigeria in April, 2008 and identified by Dr. Aigbokhai (Taxonomist) at the Department of Botany, Faculty of Life Sciences, University of Benin, Nigeria. Botanical authentification was done at the Forest Research Institute of Nigeria (FRIN, Ibadan), where a herbarium specimen No FHT 107624 was deposited.

Extraction and Purification

The dried powdered leaf (2 kg) of *P. staudtii* was exhaustively extracted with fractionated methanol (4x10 L x 48 h) at room temperature. The greenish yellow extract was evaporated to dryness with the aid of a rotary evaporator at reduced pressure to yield a residue (230 g).

Isolation of Chemical Constituents

The methanolic extract was subjected to flash silica gel chromatography using gradient solvents of n-hexane, chloroform, ethylacetate and methanol upto 100% methanol to obtain 23 fractions (1-23). The various fractions were in turn subjected to repeated column chromatography to afford semi pure compounds. The precipitates from the chloroform and ethyacetate fractions were separately subjected to further column chromatography using isocratic solvent system. The compounds were analyzed with NMR, MS, IR, UV and 2D NMR experiments.

Phytotoxicity Assay

Phytotoxicity of the methanolic extract of *Pyrenacantha staudtii* was determined against *Lemna minor* L. according to the modified protocol of McLaughlin and co-workers (Meyer *et al*., 1982). The test extract was incorporated with E-medium at different concentrations at 10, 100 and 1000 μg mL^{-1} in MeOH. Conical flasks were inoculated with extract at the desired concentration prepared from the stock solution and allowed to evaporate overnight. Each flask was inoculated with 2 mL of E-medium and ten *Lemna minor* L. each containing a rosette of three fronds. Other flasks were supplemented with MeOH serving as negative control and reference inhibitor, i.e., paraquat serving as positive control. Treatments were replicated three times and the flasks were incubated at 37°C for 7 days. Growth of *Lemna minor* L., in a compound containing flask was determined by counting the number of fronds per dose and growth inhibition was calculated with reference to negative control.

RESULTS AND DISCUSSION

The concentrations at 1000, 100 and 10 μg mL^{-1} produced a phytotoxic activity of 50, 30 and 15%, respectively. The extract exhibited a significant phytotoxic effect against *Lemma minor* at the tested concentrations (Table 1).

The pytochemical studies led to the isolation and characterization of known compounds but reported first from *P. staudtii*.

Table 1: Phytotoxicity against *Lemna minor* L. of *P. staudtii* leaf extract

	No. of fronds		
Concentration (μg mL^{-1})	Extract	Control	Growth regulation (%)
1000	10	20	50
100	14	20	30
10	17	20	15

Compound 1

Colorless needles from methanol

HR-EI-MS m/z: 456.3610 (calcd. for $C_{30}H_{48}O_3$, 456.3603)

$[\alpha]$ D^{25}: + 78.9°C (c = 0.07, $CHCl_3$)

IR (KBr) υ_{max} cm^{-1}; 3400 - 2640, 1700, 1660 and 820.

% Yield : 2 mg

^1H NMR ($CDCl_3$, 300 MHZ): 5.24 (1H, t, J = 3.45 Hz, H-12), 3.60 (1H, dd, J = 4.1, 9.9 Hz, H -3), 1.12, 1.03, 0.98, 0.97, 0.91, 0.90 and 0.98 (3H, each, s, Me).

^{13}C NMR ($CDCl_3$, 300 MHZ): 183.4 (s, C-28), 143.4 (s, C-13), 122.7 (d, C-12), 79.4 (s, C-3), 55.2 (d, C-5), 47.6 (d, C-9), 46.5 (s, C-17), 4.9 (t, C-19), 41.6 (s, C-14), 41.0 (d, C-18), 39.1 (s, C-8), 38.7 (s, C-4), 38.4 (t, C-1), 37.1 (s, C-10), 33.8 (t, C-21), 33.0 (q, C-29), 32.6 (t, C-7), 32.4 (t, C-22), 30.6 (s, C-20), 28.1 (q, C-23), 27.7 (t, C-15), 27.2 (t, C-2), 25.9 (q, C-27), 23.5 (q, C-30), 23.4 (t, C-11), 23.4 (t, C-16), 18.3 (t, -6), 17.1 (q, C-26), 15.6 (q, C-24) and 15.3 (q, C-25).

EIMS m/z (re.int.): 456 $[M]^+$ (4) , 248 (98), 203 (60), 133 (53).

Compound 1 was obtained as colorless needles from the chloroform soluble fraction of the methanolic extract of this plant. It gave a positive reaction to triterpene. The HREIMS showed the molecular ion peak at m/z 456.3610, corresponding to molecular formula $C_{30}H_{48}O_3$ (calcd. for $C_{30}H_{48}O_3$, 456.3603). Apart from the molecular ion peak, the EIMS showed other prominent fragment ions at m/z 248, 203, 133 which were characteristic of Δ^{12}-amyrin skeleton. The fragmentation pattern is clearly shown in Fig. 1.

The IR showed absorption bands for hydroxyl groups (3400-2640 cm^{-1}), carbonyl of the carboxyl group (1700 cm^{-1}) and trisubstituted double bond (1600 and 820 cm^{-1}). The ^1H NMR spectrum of the compound showed signals for seven methyls singlets at 0.89, 0.90, 0.91, 0.97, 0.98 and 112. The tertiary nature of these methyls was evident from their sharp singlets in the ^1H NMR. The ^1H NMR spectrum also showed the presence of an olifinic proton resonating at δ 5.24 (1H, t, J = 3.4 Hz) corresponding to H-12. while the proton germinal to the hydroxyl group was observed at δ 3.60 (dd, J = 4.1, 9.9 Hz). The ^{13}C NMR assignments of various carbon atoms were substantiated by DEPT experiments, which revealed the presence of seven methyl, ten methylene, five methane and seven quaternary carbon atoms. The physical and spectral data of the compound was in complete agreement to the reported data in literature. The compound was identified as 3β-hydroxyolean-12-28-oic acid known as oleanolic acid.

Compound 2 was obtained as colorless needles from EtOAc.

HR-EI-MS m/z: 426.3825 (calcd. for $C_{30}H_{50}O$, 426.3861)

$[\alpha]$ D^{25}: + 100°C (c = 0.21, $CHCl_3$)

IR (KBr) υ_{max} cm^{-1}; 3510 - 3055, 1635, 820

% Yield : 1.43 mg,

^{1}HNMR ($CDCl_3$, 300 MHZ): 5.11 (1H, m, H-12), 3.19 (1H, dd, J = 4.5, 10.0 Hz, H -3), 1.02, 1.01, 1.08, 0.96, 0.93, 0.88, 0.85 and 0.80 (3H, each, s, Me).

^{13}C NMR ($CDCl_3$, 300 MHZ): 144.3 (s, C-13), 124.4 (s, C-12), 78.8 (d, C-3), 54.4 (s, C-5), 47.7 (d, C-9), 47.3 (d, C-18), 46.9 (t, C-19), 42.3 (s, C-14), 41.6 (t, C-22), 40.9 (s, C-8), 39.1 (s, C-4), 39.0 (t, C-1), 37.40 (s, C-10), 34.0 (s, C-17), 33.3 (q, C-29), 33.2 (t, C-7), 32.9 (t, C-21), 31.7 (s, C-20), 28.2 (q, C-23), 28.0 (q, C-28), 27.4 (t, C-2), 26.5 (t, C-16), 26.3 (t, C-15), 26.0 (q, C-27), 23.6 (t, C-11), 23.3 (q, C-30), 18.5 (t, -6), 16.9 (q, C-26), 15.6 (q, C-25) and 15.5 (q, C-24).

EIMS m/z (re.int.): 456 $[M]^+$ (4) , 248 (98), 203 (60), 133 (53).

Compound 2 isolated from ethylacetate fraction gave a molecular formula $C_{30}H_{50}O$ established through HREIMS showing molecular ion peak at m/z 426.3825 (calcd. 426.3861). The EIMS spectrum of compound 2 showed diagnostic peaks at m/z 257, 218, 207, 203 and 189 characteristic for β-amyrin skeleton with Δ^{12} unsaturation.

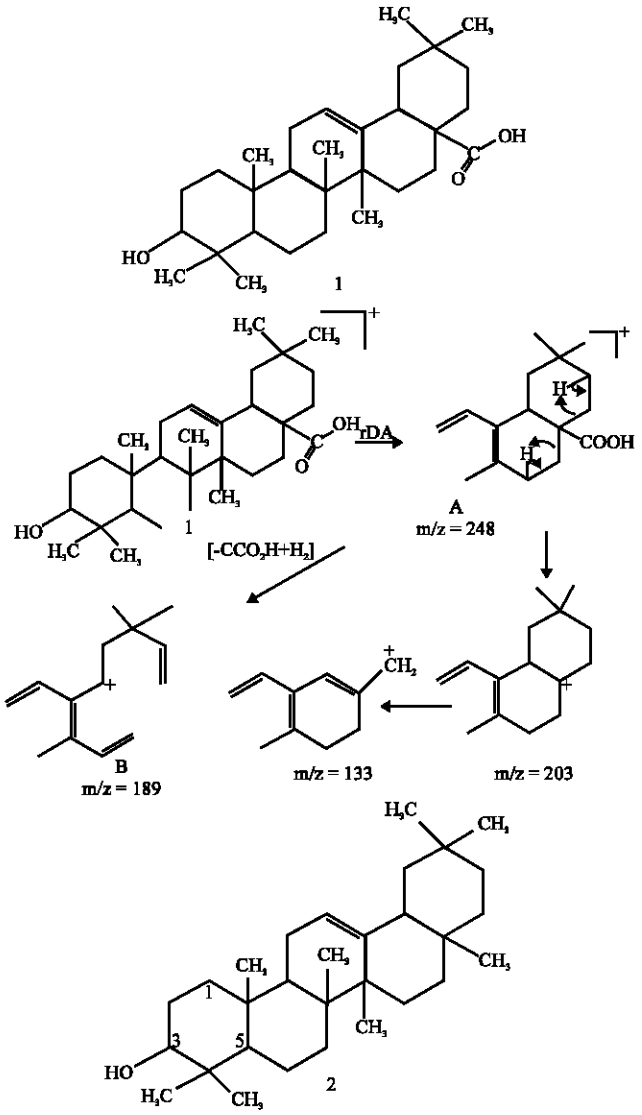

Fig. 1: Mass fragmentation pattern of 1

The ^{1}HNMR spectrum of 2 displayed eight tertiary methyl resonances at 1.08, 1.02, 1.01, 0.96, 0.93, 0.88, 0.85, 0.80 (all singlets). The carbinylic proton resonated at 3.19 in α and axial configuration as confirmed by a double doublet (J = 10.0 Hz, 4.50 Hz) and a multiplet at 5.11 was indicative of the olifinic proton. The ^{13}CNMR spectrum (BB and DEPT) of 2 displayed 30 carbon signals including eight methyl, ten methylene, five methane and seven quartinary carbon atoms. Comparison of these data with reported ones in literature, showed that compound 2 is β-amyrin.

CONCLUSION

The methanolic extract of *P. staudtii* at the various concentrations tested exhibited marked phytotoxic activity against *Lemna minor* L. Two compounds (2 and 1.43 mg each) were isolated from the plant, for the first time.

The result of the study established the phytotoxicity of the plant and further pharmacological investigation of the chemical constituent isolated will be carried out.

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