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Isolation and NMR Characterization of Ursane-Type Triterpenoid from the Leaves of *Peperomia pellucida*

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ABSTRACT: *Peperomia pellucida* is a member of the *Piperaceae* family. Extraction of plant material was carried out by Soxhlet extraction method using hexane and ethylacetate as solvents respectively. The extract was concentrated using a rotary evaporator, followed by isolation and purification using column and thin layer chromatographic techniques. Fraction C20 showed a clearly defined single spot with R_f value of 0.51. Using ^{1}H-NMR, ^{13}C-DEPT, COSY, HSQC and HMBC and by comparison with literature values, the structure of the compound was established as an Ursane-type triterpenoid. The use of *P. pellucida* in ethnomedicine for the treatment of various ailments could be attributed to the Ursane-type triterpenoid and other bioactive chemical compounds present in the plant.

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Plant derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resources of drugs of traditional medicines, modern medicines, pharmaceutical intermediates and chemical entities for drug synthesis. The use of medicinal plants in the treatment and management of various ailments has increased significantly such that a greater number of people in Nigeria now rely on plant based medicines as their source of primary healthcare especially, in rural communities, (Karunamoorthi *et al*., 2013). *Peperomia pellucida* is a member of the *Piperaceae* family. The common names include pepper elder, shining bush plant, and English cow-foot. It is an edible plant native to South America and has been widely grown in many countries in the world, including West African countries. The plant grows in shaded and damp hard surfaces and is very common during rainy season.

It is characterized by succulent stems, freshly and heart-shaped leaves and tiny dots like seeds attached to the fruiting spikes. When crushed, it has a mustard-like odour. *P. pellucida* is one of the known medicinal plants used in various parts of the world for the treatment of various diseases and infections such as rheumatism, diarrhea, dysentery, convulsion, epilepsy, paralysis, tumour, joint and abdominal pain, cough, cold, fever, asthma, boils, acne, renal disorders, fatique, headache, wound, vaginal and kidney infections (Coe and Anderson, 1999; Khan and Omoloso, (2002). In Nigeria, the whole plant is used in the treatment of measles, convulsion, hypertension and bone fracture (Chukwuma *et al*., 2015). In India, it is used for pimples, white spots, wounds and stomach problems (Das *et al*., 2014; Kalita *et al*., 2015). It is used in Brazil for the treatment of hemorrhoid pain and kidney infections, while the aerial parts are used in Indonesia for stomachache, dizziness and headache (Santos *et al*., 2014; Waty *et al*., 2017). In Africa, it is used as a condiment, and eaten as a spicy leafy vegetable. The pharmacological activities of *P. pellucida* leaves such as hypotensive, anti-inflammatory, antioxidant, antipyretic, gastroprotective, antidiabetic and antibacterial activities have been reported (Khan and Omoloso, 2002; Nwokocha *et al*., 2012; Humzah *et al*., 2012).

Previous researchers isolated a good number of compounds from *P. pellucida*. Manalo *et al.,* (1983) isolated 2, 4, 5-trimethoxy styrene, campesterol, stigmasterol, and β-sitosterol. Xu *et al.,* (2006) isolated thirteen compounds, with five being novel. Khan *et al*., (2010) isolated patuloside A (3-β-Dglucopyranosyloxy- 1, 5, 6-trihydroxy-9H-xanthene-9-one). In the present research, we report the isolation and NMR characterization of an ursane-type triterpenoid from the leaves of *P. pellucida*. This is the first report of the isolation of an ursane-type triterpenoid from the leaves of *P. pellucida*.

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MATERIALS AND METHODS

Sample Collection and Preparation: P. pellucida leaves were harvested from Umunomo Ihitteafokwu in Ahiazu Mbaise Local Government Area of Imo State, Nigeria. The plant material was identified and authenticated at the Taxonomy Unit, Forestry Department, Micheal Okpara University of Agriculture Umudike, Nigeria. The leaves were handpicked and air dried for 30 days at room temperature. The dried leaves were milled to fine powder using a laboratory mill.

Extraction of Plant Chemicals and Compound Isolation: Extraction of plant material was carried out by Soxhlet extraction method using hexane and ethylacetate as solvents respectively. The extract was concentrated using a rotary evaporator at room temperature and left on the laboratory bench for 2 days. The column was washed with acetone and rinsed with n-hexane. The column was prepared by packing a glass column (2.5 cm by 80 cm) with slurry of silica gel (60.2 g) in 200 ml of n-hexane. The slurry was introduced in one smooth flow and the solvent drained off to the top of the column bed. A dry free flowing mixture of plant extract was introduced onto the silica bed. 100 ml of n-hexane was used to wash down sides of the column and also to fill it up. Solvent mixture of n-hexane and ethylacetate (90:10 ml) was introduced and collection of fractions in well labeled vials began just before the plant material travelled to the column neck. This continued for the following solvent mixtures - 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, 0:100. Thereafter, a more polar solvent, methanol (100 ml) was used to elute the more polar components from the column. A total of 57 vials were collected. Each fraction was spotted using a capillary tube on a precoated TLC plate and developed in a solvent mixture of 3:7 ml (ethylacetate: hexane). Fraction C20 gave a single spot on TLC with R_f value of 0.51. It was packaged in a vial and sent to University of Strathclyde, Glasgow, Scotland, for spectral analysis.

RESULTS AND DISCUSSION

The ^1H – NMR for fraction C20 (Tables 1 and 2) showed the presence of four olefinic protons at 5.70 ppm (H-12, d) J (1.36), 5.18 ppm (H-15, dd) J (15.20 Hz, 8.58 Hz), 5.04 ppm (H-16, dd) J (15.14 Hz, 8.68 Hz) and 4.52 ppm (H-30, d) J (1.64, 1.72). The signals at 3.55 ppm (H-3, tt) J (11.09, 4.63) are features of a sterol moiety. The signals at 1.03 ppm (H-1) and 1.87 ppm (H-22 ddt) J (13.47, 6.09, 3.41) are characteristic of methylene protons. The other signal at 0.83 ppm (H-24) is a methyl proton. The ^1H-^1H- COSY showed correlations at 5.04 ppm (H-16, dd), 5.18 ppm (H-15, dd), 5.07 ppm (H-12, d) and 4.52ppm (H-30 s) characteristics of vinylic protons. ^1H-^1H coupling signals were also observed at 3.55 ppm (H-3.td, J=11.09, 4.63 Hz) confirming the presence of a sterol moiety. The signals at 1.03 ppm (H-1 m) and 1.87 ppm (H-22 ddt) J (13.47, 6.09, 3.41) are characteristics of a methylene proton.

Table 1: ^1H-NMR chemical shift for fraction C20

Position of hydrogen	Chemical shift (ppm)	Assignment (type of proton)
1	1.03	$-CH_2$
2	1.67	$-CH_2$
3	3.55	-CH-OH
4	-	-
5	0.86	-CH
6	5.38	$-CH_2$
7	1.84	$-CH_2$
8	-	-
9	1.55	-CH
10	-	-
11	1.85	$-CH_2$
12	5.70	=CH
13	-	-
14	-	-
15	5.18	=CH
16	5.04	=CH
17	-	-
18	1.67	-CH
19	1.01	-CH
20	1.41	-CH
21	1.52	$-CH_2$
22	1.87	$-CH_2$
23	0.82	$-CH_2$
24	0.83	$-CH_3$
25	0.84	$-CH_3$
26	0.85	$-CH_3$
27	1.01	$-CH_3$
28	1.25	$-CH_3$
29	0.76	$-CH_3$
30	4.52	$-CH_3$

The DEPT-135 spectrum showed the presence 7 $-CH_3$, 8 $-CH_2$ and 9-CH carbons. The signals at 138.33 ppm (C-16), 129.30 ppm (C-15), 114.33 ppm (C-12) and 106.37ppm (C-30) are characteristics of the four vinylic carbons of the sterol moiety. Also, the signals at 71.84 ppm correspond to the methine carbon of the sterol moiety (C-3). Moreso, signals were also observed at 37.27ppm (C-22) and 40.50ppm (C-1) characteristics of methylene carbons.

The ^1H - ^{13}C HSQC spectra showed correlations between the carbon atom at (C-12) at 114.33 ppm and the proton (H-12) at 5.70 ppm, C-15 at 129.30 ppm and (H-15) at 5.18 ppm and the carbon atom (C-16) at 138.33 ppm and the proton (H-16) at 5.04 ppm as well as between the carbon atom (C-3) at 71.84 ppm and the proton (H-3) at 3.55 ppm. The signals at 40.50 (C-1) and 37.27 (C-22) were assigned to the methylene carbons at protons 1 and 22 respectively.

Also, ^{1}H - ^{13}C HSQC showed single bond coupling between carbon atoms (C-24) at 29.18 ppm and the proton (H-24) at 0.83 ppm as well as the carbon atom (C-25) at 28.26 ppm and the proton (H-25) at 0.84 ppm. The analysis of the ^{1}H, ^{1}H-^{1}H COSY, DEPT and HSQC were in agreement with reported literatures for an ursane-type triterpenoid (Pranab *et al*., 2012; Hayat *et al.,* 2005; Yan-Li *et al*., 2013).

Table 2: ^{13}C (DEPT) chemical shift of fraction C20

Position of carbon atom	Chemical shift (ppm)	Assignment
1	40.50	$-CH_2$
2	29.71	$-CH_2$
3	71.84	-CH-OH
4	-	-
5	56.79	-CH
6	18.80	$-CH_2$
7	31.90	$-CH_2$
8	-	-
9	50.18	-CH
10	-	-
11	24.32	$-CH_2$
12	114.33	=CH
13	-	-
14	-	-
15	129.30	=CH
16	138.33	=CH
17	-	-
18	55.52	-CH
19	56.08	-CH
20	56.79	-CH
21	31.68	$-CH_2$
22	37.27	$-CH_2$
23	21.10	$-CH_2$
24	29.18	$-CH_3$
25	28.26	$-CH_3$
26	19.41	$-CH_3$
27	26.11	$-CH_3$
28	19.41	$-CH_3$
29	19.05	$-CH_3$
30	106.37	$=CH_2$

Thus confirming that C20 isolated from *P. pellucida* leaves is an ursane-type triterpenoid.

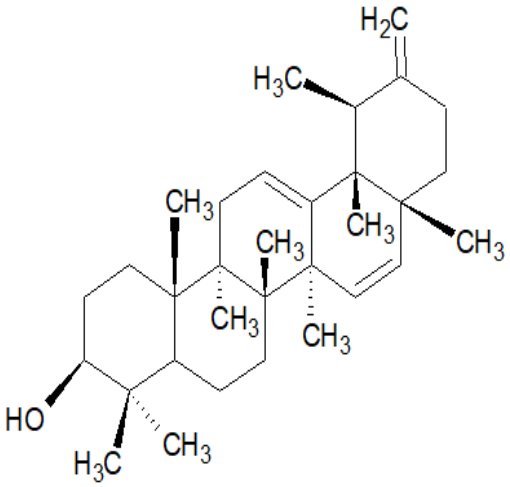

Fig 3. ursane - type triterpenoid

Conclusion: Peperomia pellucida leaves appears to be suitable for developing drugs that can be used to treat several diseases or disorders, this could be attributed to the ursane-type triterpenoid and other isolated bioactive compounds present in the leave. Utilization of this plant in suitable form can be beneficial in terms of promotion on health and disease therapy.

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