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Isolation of ursolic acid (3β-hydroxyurs-12-en-28-oic acid) from the leaves of *Eucalyptus grandis W. Hill ex Maiden*

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ABSTRACT

Ancient people used medicinal plant extract as ingredients in tradomedical portions and poisons. Over time, these traditional treatments have had the chance to become refined based on efficacy and safety. It was therefore hypothesised that plants that were used in the past and are still used today to treat symptoms associated with a particular disease condition are more likely to contain pharmacologically active metabolites than plants that have not been used continuously. The use of E. grandis in traditional medicines has been document. Ursolic acid (3β-hydroxyurs-12-en-28-oic acid) was isolated from the leaves of Eucarlyptus grandis. Column chromatography (normal phase), IR, LC-MS and NMR techniques were employed for the isolation and characterization of the compound. Presence of ursolic acid, a pentacyclic triterpene carboxylic acid in the leaves of E. grandis gives credence to the ethnomedical use of the plant leaves in the treatment of diseases.

Keywords: Ethnomedicine, Drug development, Tuberculosis, Natural products, Ursolic acid

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INTRODUCTION

Natural products continue to play a most significant role in the drug discovery and development process[1], and plants are recognized as a valuable and inexhaustible source of bioactive metabolites [2]. It is established that drugs derived from plants are known to have lesser side effects than the synthetic drugs.

Eucalyptus grandis is a tree belonging to the family Myrtaceae. It is native to the east coast of Australia; It is commonly known as rose gum or flooded gum. The glossy dark green leaves are stalked, lanceolate, and paler on their undersides, 10 to 16 cm (4-6.4 in) long and 2-3 cm (0.8-1.2 in) wide. They are arranged alternately along the branches. The white flowers appear from mid-autumn to late winter from April to August, and are arranged in groups of seven to eleven flower heads.

The flowers are followed by small pear-or cone-shaped gumnuts which measure 5-8 mm in length and 4-7 mm across [3-5]. Essential oil extracted from the leaves of *E. grandis* is reported to have antiseptic and disinfectant properties, decoction of the leaves are used in folklorik treatment of flu, bronchitis, pneumonia and respiratory infections [6].The plant has been reported of high performance index ***(I_P)*** in two independent ethnobotanical survey reports of medicinal plants for the treatment of tuberculosis and other respiratory diseases of Nupe tribe of Niger State, Nigeria, and Zulu tribe of Maputuland, KwaZulu-Nata province, Republic of South Africa [7,8]. This paper reports on the occurrence and characterization of ursolic acid in *E. grandis.*

MATERIALS AND METHODS

General chemical and analytical methods. Gravity column chromatography was done with glass columns packed with Merck silica gel 60 (0.040-0.063mu), 230-400 mesh. Column fractionations were monitored by thin layer chromatography (TLC) on aluminium- backed Merck silica gel 60 F_{254} plates using ascending technique. The plates were visualized by spraying with 10% sulphuric acid in methanol or 1:1 solution of 5% p-anisaldehyde in ethanol baking at 130^0C. Melting point was determined using Stuart Scientific SMP1 apparatus. The ^{1}H and ^{13}C NMR as well as DEPT **(Appendixes 1, 2 &3)** were recorded on a Bruker – Avance 400MHZ FT-NMR Spectrometer operating at 400MHZ using the residuall solvent ($CDCl_3$) peak as internal standard. The Electron-Impact Mass spectrometer (EI-MS) was carried out on Agilent Technologies 1200 series Binary SL and the infra-red spectra were run on Perkin Elmer Spectrum 100 FTIR Spectrometer. Standard phytochemical screening methods for alcoholic hydroxyl group and steroids were carried out as described by Harborne, 1998 [9].

Collection, Preparation, Extraction and Isolation of Ursolic acid

The leaves of *Eucarlyptus grandis* were collected in June 2012, at a paper Mill plantation around Empangeni area of Kwazulu – Natal, Republic of South Africa. The plant was identified by Mrs. N.R. Ntuli, of Botany Department, University of Zululand and voucher specimen was prepared and deposited.

In this work 500g of air-dried powdered leaves of *E. grandis* were subjected to cold maceration in an alkaline methanol. The concentrated extract yielded a solid residue (21.0 g). 10.0g of the solid was subjected to column chromatography using gradient elution of hexane-ethyl acetate (5% stepwise increase in polarity). A total of 220 fractions were collected and monitored with TLC; similar fractions were pooled together (GA-GK). Fraction GJ showed single spots with traces of impurity on TLC plate.

Further purification of the fraction led to the isolation of a creaming-yellow powder **(IBF03),** which was recrystallised from methanol (1.80 g).

The structure was confirmed by comparing the physical and NMR spectral data (^{1}H, ^{13}C &DEPT) with that of ursolic acid **(Fig. 1)** reported in literature **(Appendixes 1, 2 &3).**

RESULTS AND DISCUSSION

Ursolic acid is ubiquitous; occurring in many plants; sometimes as white, light green or yellow powder. It has been reported in *Rosmarinus officinalis, Glechoma hederaceae, Ilex paraguariensis, Ichnocarpus frutescens, Phoradendron juniperinum, Syzygium claviflorum, Hyptis capitata* [10,11].

Extraction of *E. grandis* leaves followed by repeated normal phase (NP) column chromatographic separation led to isolation of creamy-yellow powder, with melting point of 278-282^{o}C.

The compound tested positive for alcoholic hydroxyl group and gave a positive reaction to Liebermann-Salkoswski reagent (blue coloration). The UV spectrum displayed strong absorptions at 474, 442, and 422 nm and the IR (ATR, cm^{-1}) spectrum showed strong absorptions at 3562 (OH alcohol), 2937 (OH acid), 2865 (C=C) and 1698 (C=O). Others are 1460, 1385, 1385, 1306, 1030. The compound has a molecular formula $C_{30}H_{47}O_3$ as deduced from the mass spectrum (observed m/z 455.3522$[M+H]^+$).

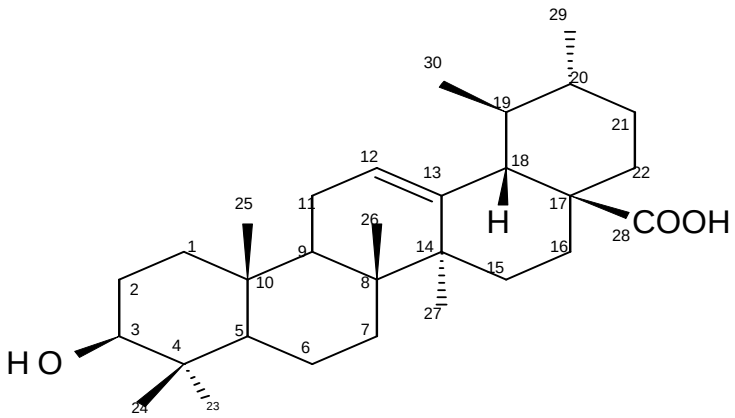

Figure1. Structure of Compound IBF03

The ^{1}H NMR spectrum of compound **IBF03** displayed seven proton signals (two secondary of the α –type triterpene) at δ 0.75 (3H, s, H-26) , δ 0.79 (3H, s, H-24), δ 0.83 (3H, d, J = 5.1Hz H-29), δ 0.8 4 (3H, s, H-26), δ 0.88 (3H, s, H-23), δ1.00, δ0.93 (3H, d, J =8.1Hz, H-30) and δ 1.03 (3H, s, H-27). This is further supported by a doublet at δ2.15 (1H, *J* = 11.25 Hz, H-19) indicating that H-18 and H-19 are trans to one another.[18] The signal at δ 3.21 (1H, *dd*, *J* = 9.0, 6.0Hz) was assigned to H-3 of the 3β – equatorial hydrogen and the olefinic signal at δ 5.24 (1H, dd, J = 13.5, 3.5 Hz) of H-12 which coupled to H-11 **(Appendix 1).**

The ^{13}C NMR spectrum of compound **IBF03** displayed 30 signals, **(Appendix 2),** of which seven quaternary carbons, seven methines, nine methylenes and seven methyls deduced from the DEPT experiments **(Appendix 3).** The most downfield signals resonated at δ180.9 was attributed to the carboxylic acid (C-28). The appearance of signals at δ125.7 and 138.0 indicated the presence of a double bond in urs-12-ene triterpenoid **(Table 1).**

Based on the physical, chemical and NMR spectra data in comparison with literature values, compound **IBF03** was identified as 3β-hydroxyurs-12-en-28-oic acid [12, 13].

Table1. ^{13}C NMR data of compound D (chemical shifts, δ, in ppm)

C	DEPT	δ_C^*	δ_C^* (Lit.)	C atom	DEPT	δ_C	δ_C (Lit.)
1	CH_2	38.5.	39.2t	16	CH_2	23.8	24.5t
2	CH_2	27.4	28.2t	17	C	46.9	48.1s
3	CH	78.9	78.2d	18	CH	52.3	53.6d
4	C	39.1	39.6s	19	CH	39.1	39.5d
5	CH	54.7	55.9d	20	CH	39.8	39.4d
6	CH_2	17.9	18.8t	21	CH_2	30.1	31.1t
7	CH_2	32.6	33.7t	22	CH_2	36.5	37.4t
8	C	38.9	40.1s	23	CH_3	28.2	28.8q
9	CH	46.9	47.9d	24	CH_3	16.8	16.5q
10	C	38.2	37.5s	25	CH_3	15.1	15.7q
11	CH_2	23.8	23.7t	26	CH_3	16.1	17.5
12	CH	125.5	125.7d	27	CH_3	23.2	24.0q
13	C	138.1	139.3s	28	C	180.7	179.7s
14	C	41.6	42.5s	29	CH_3	17.0	17.5q
15	CH_2	28.9	28.8t	30	CH_3	21.0	21.4q

Data obtained in Cdcl3+3 drops cd3od

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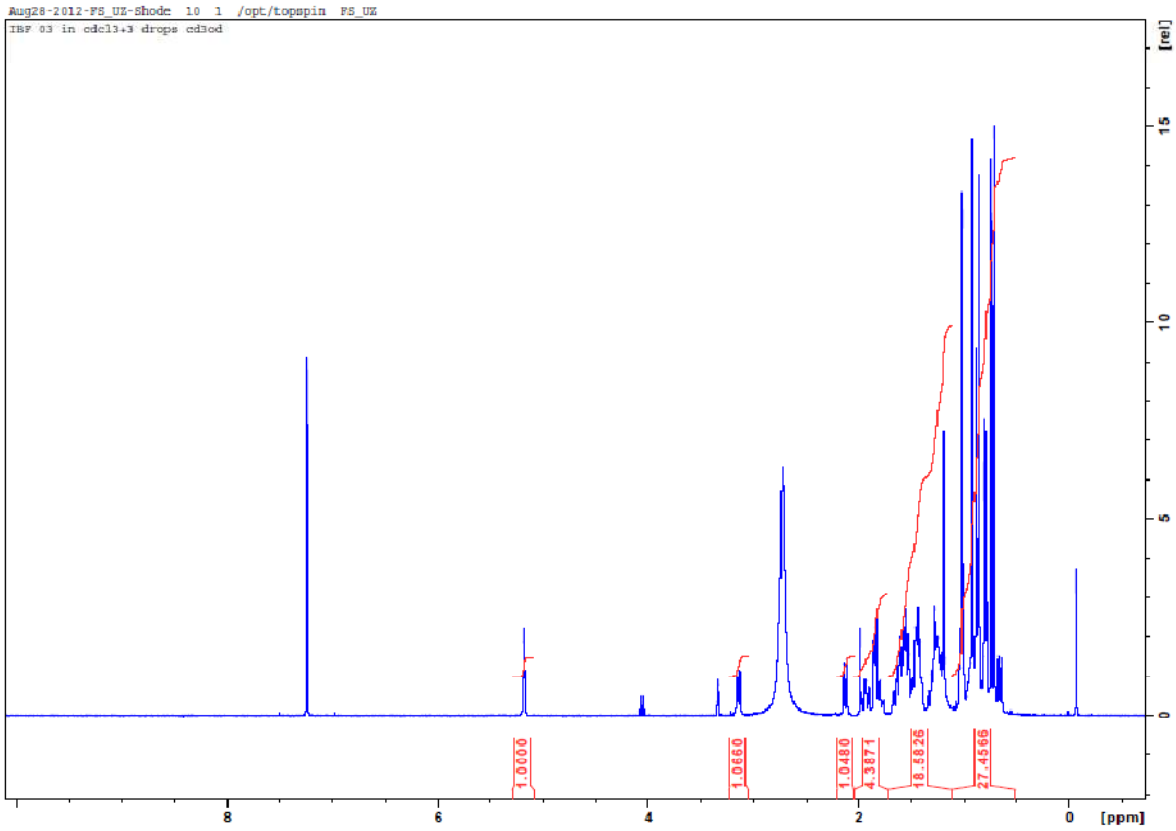
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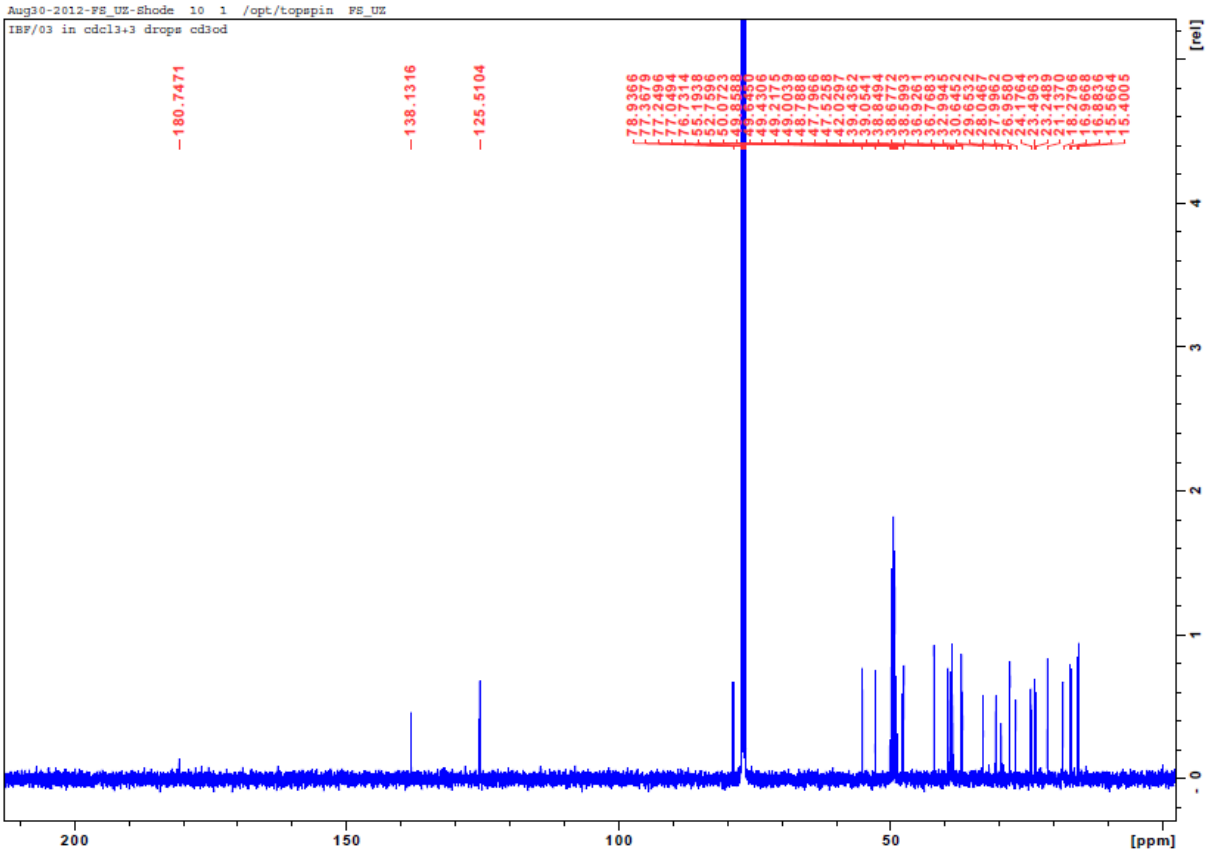
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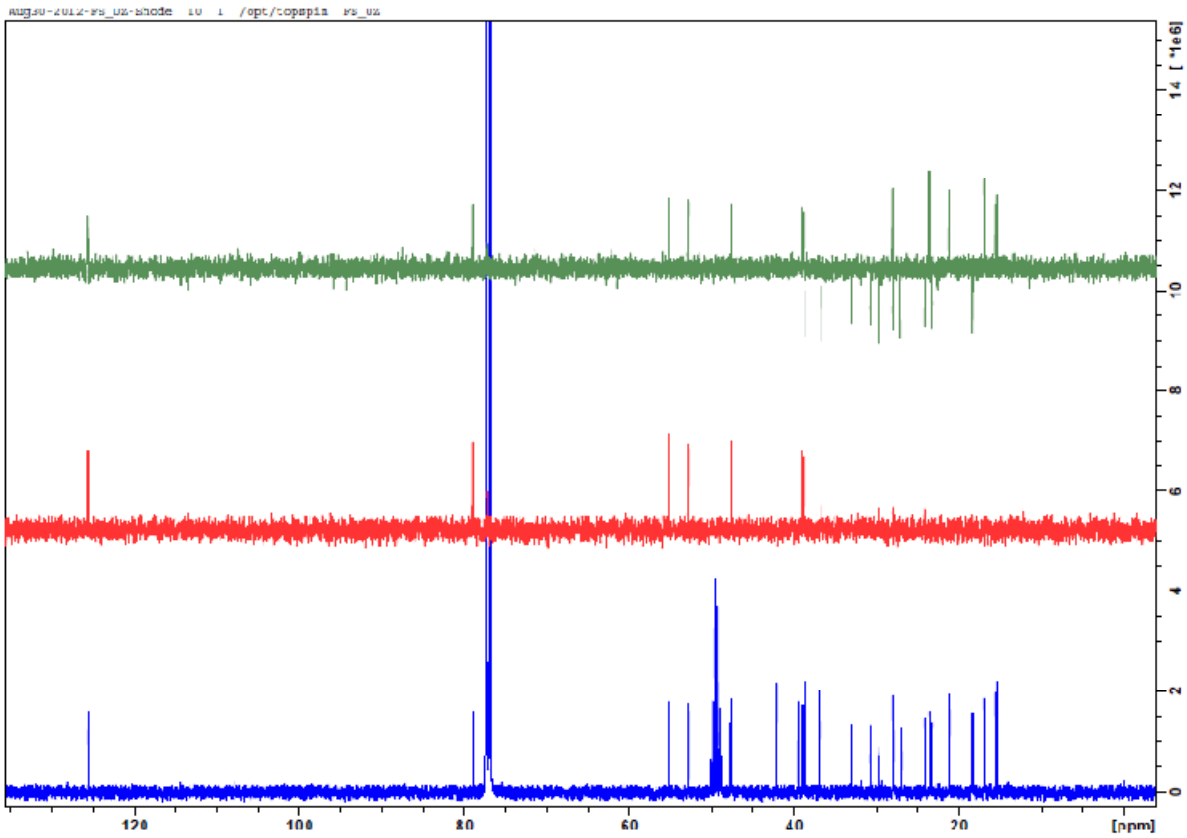
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