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Constituents of the stem bark of *Sacoglottis gabonensis* (Baill.) Urb. (Humiriaceae) show weak growth inhibitory effect against the mouse lymphoma cell line L5178Y

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Abstract

Three secondary metabolites, including methyl 3,5-dihydroxy-4-methoxybenzoate (1), eriodictyol (2) and bergenin (3) were isolated from the ethylacetate fraction of the methanol extract of the stem bark of *Sacoglottis gabonensis* (Baill.) Urb. (Humiriaceae). When evaluated for their cytotoxic activity against the mouse lymphoma cell line L5178Y (10 μgml^{-1}), only 2and 3showed weak activity with a growth inhibitory effect of 27.3 and 15.2%, respectively.

Keywords: Isolation, Cytotoxicity, *Sacoglottis Gabonensis*

1. Introduction

One of the foremost causal agents of morbidity and mortality globally is cancer [1, 2]. A projection of almost 12 million deaths arising from it by 2030 has been made [2]. Several studies have been carried out while a lot is still on-going on the chemoprotective action of medicinal plants; they include anticarcinogenic properties [3, 4]. A lot of these are indicating promising results. In previous studies, the stembark of *Sacoglottis gabonensis* (Baill.) Urb. (Humiriaceae) was reported for its ability to reduce sperm concentration in male rats [5]. Also, the sperm immobilisation potential of its constituents *vivo* has been reported [6]. The extract of the plant and the bergenin resulting from its purification has also been reported for their natural antioxidant defences, brain glucose protecting effect. They have also been reported to inhibit white blood cells proliferation both in differential and total counts [7]. This study aimed at reporting the cytotoxicity of the phytoconstituents against the mouse lymphoma cell line since by exploring its inhibitory potential on the WBC proliferation.

2. Materials and Methods

2.1 General procedures

^{1}H, ^{13}C and 2D NMR were run on AVANCE DMX 600 Nuclear magnetic resonance spectrometer. Liquid chromatography-Mass spectrometer; model Agilent 1100 series attached to a Thermoquest LCQ Deca XP was used for detection of the molecular weight while fractions were monitored with HPLC with the use of a Dionex UltiMate 3000 high performance liquid chromatography system (Thermo scientific) attached to a photodiode array detector (DAD-3000RS). The UV detection (235, 254, 280, and 340 nm) of the array of compounds was carried out. A C-18 prefilled column with a dimension of 125 high and 4 mm internal diameter (Knauer, Germany) was employed with the solvent systems: methanol: formic acid (0.1%) in water) at 0 h., followed by methanol (10%) at 0.08 h., methanol (10%); 0.58 h., methanol (100%) at 0.75 h., and a flow rate of 60 mL/h. HPLC purification was carried out using a semi-prep, high performance liquid chromatography system (Lachrom-Merck Hitachi (Pump L7100 and UV detector L7400). A prefilled separation column of 30 cm in height with an internal diameter of 0.8 cm (Europhere 100-C18 (Knauer, Germany) was used with a flow- rate of 300 mL/h. was used. Open column chromatography was executed with the use of a polar adsorbent (Silica gel 60 M (Merck MN)), Diaion HP20 (Mitsubishi Chemicals) was utilized as well as by monitoring in UV (254 and 366 nm).

2.2 Plant material

The stem bark of *S. gabonensis* was harvested at Oron, Akwa Ibom State, Nigeria after proper identification by Prof, A.T. Oladele of the Faculty of Agricultural Sciences, University of PortHarcourt, Rivers State of Nigeria and authentication at the Forestry Herbarium, Ibadan, Nigeria where a voucher specimen; FHI109851 was banked.

2.3 Extraction and isolation

About 0.5kg of dried coarsely powdered stem bark of *S. gabonensis* was subjected to cold marceration in 80% MeoH and concentrated *in vacuo* at 25ºC to afford 5%$^{w}/_{w}$ extract brown. Distilled water suspended extract was put through partitioning successively using a separating funnel with normal hexane, EtOAc and normal butanol (400 mL, thrice, each) and the resulting fractions were also concentrated *in vacuo* to yield hexane 1.3, 7.3, 8.5 & 5.1 g of the successive fractions (n-hexane (A), ethyl acetate (B), n-butanol (C) and aqueous (C), respectively). Based on HPLC characteristics, B was solubilised in DCM and adsorbed onto the stationary phase (silica gel for TLC) and run using a normal phase with silica gel under vacuum using n-hexane: ethylacetate (100:0) and gently increasing the polarity (by 20%) to 0:100, then with a subsequent addition of dichloromethane: methanol (100:0) and gradually increasing the polarity (by 20%) to 3:2 and the collected fractions (500 ml each) were concentrated *in vacuo*. A total of 10 fractions were pulled together based on TLC characteristics. Fraction 3 (80 mg) eluted with n-hex:EtoAc (60:40) of fraction 2 was solubilised in dichloromethane/methanol (1:1) subjected to chromatography with Sephadex LH-20 as the stationary phase with a column of 50 cm high and an internal diameter of 0.04 m) employing dichloromethane/methanol (1:1) as the eluting solvent. Fractions were collected using flow rate of 20 drops/2 seconds. Collection of 110 fractions (in 20 mL test tube each) was made and bulked to 7 sub-fractions coded SBE2A–G. Sub-fractions SBE2B, D and F were further purified due to yield. Sample SBE2B and D were ultimately cleaned on semi preparative HPLC to achieve offer compounds 1 & 2 while SBE2F was subjected to open column chromatography (50 cm x 0.04 m) employing HP-20 and MeOH: H_2O (1:0), and increasing water content gradually by 10% to grant the third compound.

2.4 Bioassay

The cytotoxicity assay was carried out by the method earlier described [8].

3. Results and Discussion

Isolated compound 1 (Fig 1), yellow in colour, existing physically as a solid and characterised as methyl-3,5-dihydroxy-4-methoxybenzoate, has absorption maximal (methanol) peaks at 260.5 and 298 nm, a retention time of 15.050 min (HPLC) and $C_9H_{10}O_5$ was deduced as the molecular formula as shown by the Electrospray ionization in mass spectroscopy spectral data. At Presence of pseudo-molecular ion peaks at *m/z* (% Intensity) At 199.0 $[M+H]^+$ and 197.1 $[M-H]^-$ (100) (base peak), +ve and *-ve* ESI-MS showed pseudo-molecular ion peaks; an indication of molecular weight of 198 $gmol^{-1}$. The latter was also justified by the molecular ion peak of 394.8 $[2M+H]^-$ (82) present on the -ve ESI-MS. The proton NMR data is as presented in Table 1. Comparing these data with already existing compound indicated that it is methyl,3,5-dihydroxy-4-methoxybenzoate [9, 10].

Table 1: ^{1}H -NMR spectra data of compound 1

Compound 1 (CH_3OD, 500 MHZ)			**Methyl,3,5-dihydroxy-4-methoxybenzoate (CH_3OD, 500 MHZ)**	
			[10]	[9]
Atom number	δH [ppm]	H/H COSY	δH [ppm]	δH [ppm]
	3.83 (s, $OCOCH_3$)		3.82 (s, $OCOCH_3$)	3.72 (s, $OCOCH_3$)
	3.86 (s, OCH_3)		3.85 (s, OCH_3)	3.76 (s, OCH_3)
2,6	7.02 (s, 2H)		7.01 (s, 2H)	6.92 (s, 2H)
UV maximum absorbance in MEOH	260.5 and 298 nm		Not Available	262 and 295 nm

Isolated compound 2 (Fig 1), a solid, which is characterised by its light yellowish white colour was characterised as eriodictyol. Its UV maximum absorbance (λ_{max} ^{MeOH}) was 288 nm while its molecular formula ($C_{15}H_{12}O_6$) was provided by the ESI-MS spectra. It was characterized respectively by both +ve & -ve ESI-MS having pseudo-molecular ion peaks at *m/z* (% Intensity) 287.2 $[M-H]^-$ (83) (base peak) and 289.2 $[M+H]^+$ (100) (base peak), depicting a molecular weight of 288 $gmol^{-1}$. Proton NMR data is as presented in Table 2. Similar data from literature [11] was used for its proper identification eriodictyol.

Table 2: ^{1}H -NMR spectra data of compound 2

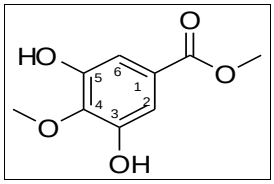
Atom No.	Compound 2 (CD_3OD)		Eriodictyol (DMSO-d_6) [11]
	^{1}H NMR (ppm)	H/H COSY	^{1}H NMR (ppm)
1			
2	5.27 (1H, s)		5.36 (1 H)
3a, 3b	2.69 (1H, dd), 3.06 (1H, dd)	3a/3b	2.66 (1H, dd), 3.15 (1H, dd)
4			
5			
6,8	5.87 (2H, m)	3b	5.85 (2H)
7			
9			
10			
1’			
2’	6.91 (1H, s)		6.87 (1H, s)
3’			
4’			
5’,6’	6.79 (2H, s)		6.74 (2H, s)

Isolated compound 3 (Fig 1), a solid and a white crystal by nature was characterised to be bergenin. It has a maximum (UV λ_{max} ^{MeOH}) peaks of 230, 288 & 328 nm and a retention time of 12.027 min (HPLC). Its molecular formula as deduced by ESI-MS spectral was $C_{14}H_{16}O_9$ in which both +ve & –ve modes indicated pseudo-molecular ion peaks at *m/z* (% Intensity) 329.0 $[M+H]^+$ (100) (base peak) and 327.2 $[M-H]^-$ (100) (base peak), respectively, which is calculated for 328 $gmol^{-1}$ molecular weight. The molecular formular

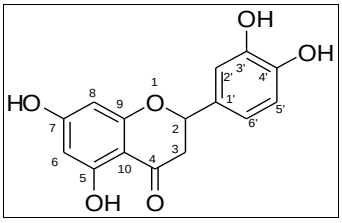
was also justified with the associated molecular ion peak at *m/z* (% Intensity) 654.9 $[2M+H]^-$ (20). The proton NMR data is a shown in Table 3. The latter data were in consonant those in the literatures for bergenin [12, 13].

Table 3: ^{1}H -NMR spectra data of compound SBE9 – bergenin (59)

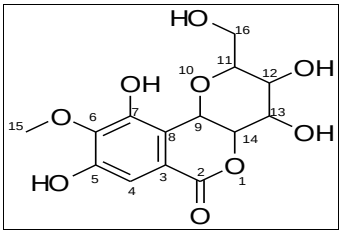
Compound 3 (CH_3OD, 500 MHZ)			**BERGENIN (Nasser *et al.*, 2009)**	**BERGENIN (Nasir *et al.*, 2011)**
Atom number	δH [ppm]	H/H COSY	δH [ppm]	δH [ppm]
1				
2	3.81 (t, 1H)	11	3.60 (1H,m)	3.81 (dd, 1H)
3	3.44 (t, 1H, oxygenated methine proton)	11	3.49 (1H, dd)	3.42 (1H)
4	4.05 (1H)	10b	3.99 (1H, dd)	4.02 (1H)
4a	4.05 (1H)	10b	4.99 (1H, dd)	4.10 (1H)
5				
6				
6a				
7	7.07 (1H, aromatic)		6.48 (m)	7.08 (1H)
8				
9				
10				
10b	4.95 (1H)		5.68 (1H,d)	4.95 (1H)
11	3.68 (ddd, 2H, oxygenated methylene protons)	4,4a	3.80 (2H, d)	3.68 (2H, m)
12	3.90 (t, 3H, oxygenated methyl protons, OCH_3)		3.76 (3H, s)	3.95 (OCH_3)



Methyl,3,5-dihydroxy-4-methoxybenzoate (1)



Eriodictyol (2)



Bergenin (3)

Fig 1: Isolated compounds from *Sacoglottis gabonensis* bark

4. Conclusion

When tested for their cytotoxicity against the mouse lymphoma cell line L5178Y (10 μg/ml), Eriodictyol and bergenin were weakly active and showed a growth inhibition of 27.3 and 15.2%, respectively while showed no activity.

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