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Novel benzophenone and xanthones from leaves and root bak of Salacia nitida (Benth.) N.E. Br.

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ABSTRACT: Salacia species are widely used in traditional medicine for the treatment of several pathologies such as diabetes, liver disorders and skin infections. In Africa, Salacia nitida is used for its beneficial effects against typhoid fever and malaria. However, despite the many traditional uses of this plant, few chemical studies have been carried out on this species. Our study aims to extract, isolate and identify phytocompounds from the leaves and root bark of S. nitida and evaluate their biological potential. Extraction by successive maceration followed by flash chromatography allowed the isolation of five compounds whose structures were elucidated by spectroscopic techniques (NMR and HRMS) and by comparison with literature data. These are two benzophenones, 4'-hydroxy-2,4,6-trimethoxybenzophenone (1) and 4'-hydroxy-2,4,6-trimethoxyphenone-β-D-glucopyranose (2), from leaves, and three xanthones, salacin A (3), salacin B (4) and mangiferin 5 from the root bark of Salacia nitida. Compounds 1 and 5 are already known in the literature. All crude extracts and compounds 1 and 2 were evaluated for their antitrypanosomal activity. Some extracts showed a significant effect on Trypanosoma brucei gambiense.

KEYWORDS: Salacia nítida, phytocompounds, antiprotozoal, Trypanosoma brucei gambiense.

1 INTRODUCTION

The genus Salacia (Celastraceae) has approximately 200 species found in South America, Asia and Africa **[1].** In many countries, species of this genus are used in traditional medicine for the treatment of several pathologies such as diabetes, malaria and skin infections **[2], [3], [4].** Previous phytochemical works reported pentacyclic triterpenoids **[5], [6].** Flavonoids **[7], [8],** saponin **[9], [10],** xanthanones, benzophenones **[11],** and thiosugarsulfoniums **[12], [13]** from Salacia species. Their secondary metabolites showed variety of biological activities such as antidiabetic, antimicrobial, and antiplasmodial **[11], [14], [15].** The species *Salacia nitida* (Benth.) N.E. Br. commonly in Nigeria under the name Akorkon or Enyim ocha, is a medicinal plant used for the treatment of typhoid fever and malaria. Indeed, previous studies have shown that extracts of this plant possess antiprotozoal and antidiabetic activities **[16], [17], [18], [19].**

With a view to searching for new bioactive compounds, extracts of S. nitida were explored with a view to isolating new molecules and evaluating their biological activities. The investigations focused on the products from the leaves and bark of the roots of *Salacia*

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nitida. Five compounds were isolated and identified, two benzophenones (compounds **1** and **2**), three xanthones (compounds **3, 4** and **5**), including two compounds (**1** and **5**) already known. These compounds were characterized by spectroscopy methods. All extracts and isolated compounds (**1** and **2**) were evaluated for their antiprotozaol activity against Trypanosoma brucei gambiense. Here we report the isolation, structure elucidation and antitrypanosomal activity of these extracts, compounds **1** and **2**.

2 MATERIAL AND METHODS

2.1 GENERAL EXPERIMENTAL PROCEDURES

Optical rotations were obtained at 25°C on a Polar 32 polarimeter. UV spectra were recorded at 25°C on a Jasco J-810 spectropolarimeter. The NMR spectra were recorded on a Bruker AM-300 (300 MHz), AM-400 (400 MHz), and AM-600 (600 MHz) (Bruker, Karlsruhe, Germany) equipped with a microprobe TXI 1.7 mm. NMR spectrometers were calibrated using solvent residual signals as references. Analytical HPLC runs were carried out using an Agilent LC-MS system consisting of an Agilent 1260 Infinity HPLC hyphenated with an Agilent 6530 ESI-Q-TOF-MS operating in positive polarity. Silica 330 and 24 g Grace cartridges were used for flash chromatography using an Armen instrument spot liquid chromatography flash apparatus. Sunfire® preparative C18 columns (150 x 4.6 mm, i. d. 5 µm, Waters) were used for preparative HPLC separations using a Waters Delta Prep (Waters Co., Milford, MA, USA) consisting of a binary pump (Waters 2525) and a UV-visible diode array detector (190–600 nm, Waters 2996).

2.2 PLANT MATERIAL

Whole *S. nitida* plants were collected in the Azito area of Abidjan in July 2019. The botanical identification was carried out by Professor MALAN Djah François, from the Nangui ABROGOUA University, Abidjan, Côte d'Ivoire. During four weeks, the collected leaves and root bark were dried in the shade at 25 °C in the laboratory and powdered (250 µm) using an electric grinder.

2.3 EXTRACTION AND PURIFICATION OF COMPOUNDS

2.3.1 COMPOUNDS 1 AND 2

The dried leaves of *Salacia nitida* (650 g) were chopped and extracted successively by maceration with petroleum ether (3 × 2 L, 24 h each), dichloromethane-methanol (1: 1 v/v, 2 × 2 L, 24 h each) and methanol-water (1: 1 v/v, 2 × 2 L, 24 h each) at room temperature to give a 6.5 g, 46 g and 50 g extracts respectively after the removal of the solvent. The dichloromethane-methanol (46 g) extract was partitioned between biphasic system heptane/acetone/water (103: 85: 12, v / v / v) to give corresponding extracts. The upper extract was submitted to flash chromatography using a Silica 330 g Grace cartridge. The rate of flow was 100 mL/min, and the mobile phases were cyclohexane, AcOEt, MeOH and water. The steps of the gradient are as follows: 100 % cyclohexane to 100 % AcOEt at 30 min, 100 % AcOEt to 100 % mixture AcOEt-MeOH (9: 1) at 30 min, and to mixture AcOEt-MeOH (9: 1) to 100 % mixture AcOEt-MeOH-Water (5: 4: 1) at 30 min. Fraction F5 gave 1 g of 4'-hydroxy-2,4,6-trimethoxybenzophenone (compound **1**). The purification of fraction F6 (0.45 g) by repeated flash chromatography reverse-phase (C18, ACN-H2O + 0.1% AF, gradient), gave compound **2** (145 mg).

2.3.2 COMPOUNDS 3, 4 AND 5

The dried root bark of *Salacia nitida* was pulverized. The plant powder (1 kg) was macerated extracted at room temperature with a mixture of dichloromethane-methanol (1: 1 v/v) (2 x 3 L for 24 hours) and methanol-water (1: 1 v/v, 2 × 3 L, 24 h each). The extract obtained (10 g) was subjected to flash chromatography using a column of 120 g of silica with a gradient of ACN / H2O (10 to 100%) to 85mL / min to give 16 fractions (F1-16), according to their TLC profiles. Flash chromatography in normal phase was carried out on the F8 fraction (473 mg) to give five subfractions (F81-F85). The F85 fraction (62 mg) was selected and taken by preparative HPLC separation using a gradient of ACN-H2O with 0.1% formic acid (10 to 30%) to give the products compound **3** (1.8 mg), mangiferin **5** (2.5 mg) and compound **4** (1.6 mg).

2.4 ANTITRYPANOSOMIAL ACTIVITY

The extracts and products were evaluated in vitro on blood forms of Trypanosoma brucei gambiense (strain FéoITMAP/1893), responsible for Human African trypanosomiasis. The parasites were maintained in HMI9 medium whose composition consisted of Dulbecco's medium modified by Iscove (Gibco, BRL) supplemented with 36 mM $NaHCO_3$, 1 mM hypoxanthine, 0.08 mM bathocuproin, 0.16 mM thymidine, 0.2 mM 2-mercaptoethanol, 1.5 mM L-cysteine, 10% heat-inactivated fetal bovine serum, 100 IU penicillin and 100 µg/mL streptomycin **[20].**

Two-fold serial dilutions of the compounds were made in 100 µL of HMI9 medium in 96-well microplates. Parasites were then added to each well (200 µL of a suspension at 4104 cells/mL). After 72 h of incubation at 37°C in 5% CO_2, 20 µL of 450 µM resazurin was added

to each well and incubated further for 6 h at 37°C in 5% CO_2. In living cells, resazurin is reduced to resorufin. This conversion is monitored by measuring the absorbance at specific wavelengths of resorufin (570 nm) and resazurin (600 nm) using a Multiskan MS microplate reader (Labsystems, France). The activity of the compounds was expressed as IC_{50}. Pentamidine di-isethionate was used as a reference compound **[20].**

3 RESULTS AND DISCUSSION

From the leaves and the roots of *Salacia nitida,* two benzophenones (**1** and **2**) and three xanthone (**3, 4** and **5**), were isolated respectively and structurally characterized.

3.1.1 COMPOUNDS 1 AND 5

The analysis of the spectroscopic data of compounds **1** and **5** (**Table 1**), by comparison with those of the literature, allowed to identify these compounds, as being4'-hydroxy-2,4,6-trimethoxybenzophenone (**1**) and mangiferin (**5**) (Fig.1), structures already known **[11].**

Table 1. NMR spectroscopic data for compounds 1 and 5 in CD_3OD and DMSO-d_6 respectively (δ in ppm)

Atoms	*1*	
	δ_H *m* (*J* in Hz)	δ_C
C=O	-	194.2
1	-	111.3
2, 6	-	158.8
3, 5	6.16 *s*	91.0
4	-	162.5
1'	-	131.7
2', 6'	7.56 *d* (8.78)	132.8
3', 5'	6.82 *d* (8.78)	115.4
4'	-	160.6
2-*O*-CH_3	3.67 *s*	56.0
4-*O*-CH_3	3.85 *s*	55.6
6-*O*-CH_3	3.67 *s*	56.0

Atoms	*5*	
	δ_H *m* (*J* in Hz)	δ_C
1	-	161.7
2	-	107.4
3	-	163.5
4	6.35 *s*	93.2
4a	-	156.1
5	6.75 *s*	102.0
6	-	151.4
7	-	144.2
8	7.31 *s*	106.8
8a	-	110.4
9	-	178.7
9a	-	101.2
10a	-	151.4
Glycosyl group of 4		
1'	4.6 *d* (7.6)	73.1
2'	2.34 *m*	70.6
3'	3.19 *m*	78.8
4'	4.02 *t* (9.5)	70.2
5'	3.15 *m*	81.5
6'	3.4 *dd* (12.4, 2.2) 3.68 *dd* (12.4, 5.3)	61.4

The chemical structures of compounds **1** and **5** are shown below (Fig.1).

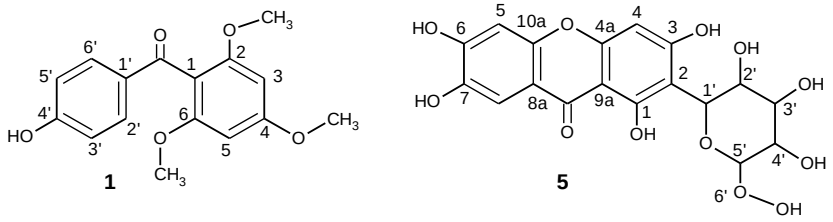


Fig. 1. Chemical structures of compounds 1 - 5 isolated from Salacia nitida.

3.1.2 COMPOUNDS 2

Compounds **2** was isolated as a pale-yellow amorphous powder with the molecular formula $C_{22}H_{26}O_{10}$. Its chemical structure was established based on HR-ESI-MS with ^{1}H NMR and ^{13}C NMR spectra.

A signal at *m/z* 451.1608 $[M + H]^+$(calcd. for $C_{22}H_{26}O_{10}$ 554.1250) and 289.1608 [M + hexose + H] $^+$corresponded to typical *O*-glucoside terminal to be $C_{22}H_{26}O_{10}$ from the protonated molecular ion $[M + H]^+$at *m/z* 451.1608 (calcd. for $C_{22}H_{26}O_{10}$ 554.1250) in the HR-ESI mass spectrum (Fig.2). The presence of the ketone functionality was supported by the ^{13}C NMR signal at δc 194.2 (Fig.3). In the ^{1}H NMR spectrum (Fig.4), the aromatic signals at δ_H 6.29 (2H, *s,* H-3 and H-5) and the signals at δ_H 7.72, (2H, *d, J* = 8.95 Hz, H-2' and H-6') and 7.12, (2H, *d, J* = 8.95 Hz, H-3' and H-5') were assigned to a 1,2,4,6-tetrasubstituted B ring and a 4'-monosubstituted A ring, respectively. A characteristic doublet signal of an anomeric proton with a large coupling constant (J = 7.62 Hz) at δ_H 5.04 and the remaining ^{1}H and ^{13}C NMR data of the sugar unit indicated the presence of a *β*-glucose moiety. Finally, two singlets' signals at δ_H 3.87 (3H, *s*) and 3.68 (6H, *s*) revealed the presence of three methoxyl substitutions. The position of the methoxyl groups and glucose was determined by the HMBC correlations. The ^{13}C and DEPT NMR spectroscopic data showed 6 carbon signals assignable to *β*-glucopyranose **[21], [22].** Of the remaining 16 signals; 12 carbons are most likely to constitute the trisubstituted and monosubstituted aromatic rings. One forms a carbonyl function due to its chemical shift (δc 194.2) and the one remaining indicates the presence of a methoxyl groups. The ^{13}C NMR and 2D NMR (COSY, HMBC, HSQC and NOESY) (Fig.5-8) signals indicated that the trisubstituted aromatic ring B (δc 158.8, 158.8 and 162.5) is substituted with three methoxyl, respectively and that the aromatic ring A is substituted with a glucosyl moiety (δc 160.6). The aromatic rings A and B are normally expected to be linked with a carbonyl group (δc 194.2). HMBC correlations of compound **2** indicated that the glucosyl group was linked from the $C_{4'}$-OH position.

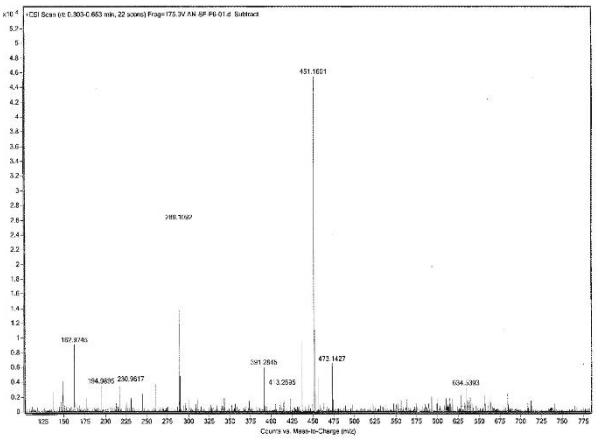


Fig. 2. HR-ESI-MS spectrum of compound 2

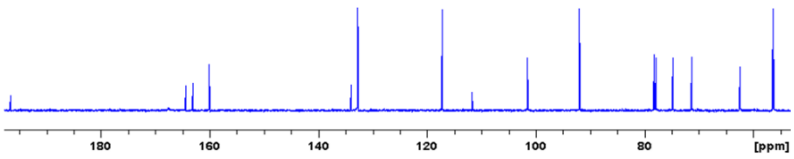


Fig. 3. ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound 2

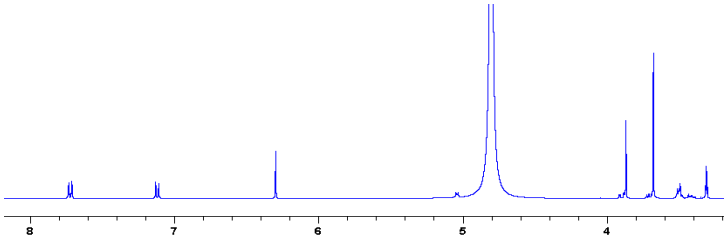


Fig. 4. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound 2

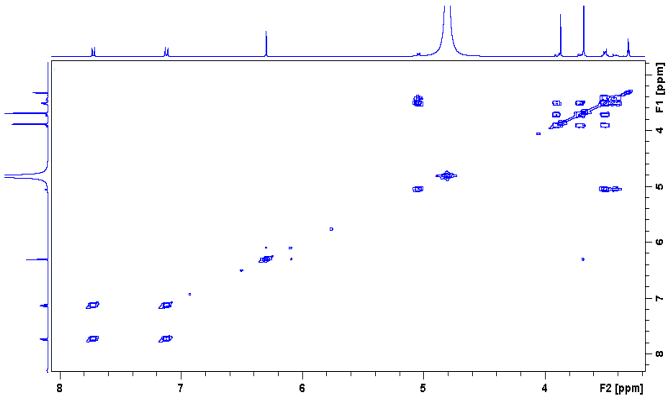


Fig. 5. COSY spectrum of compound 2

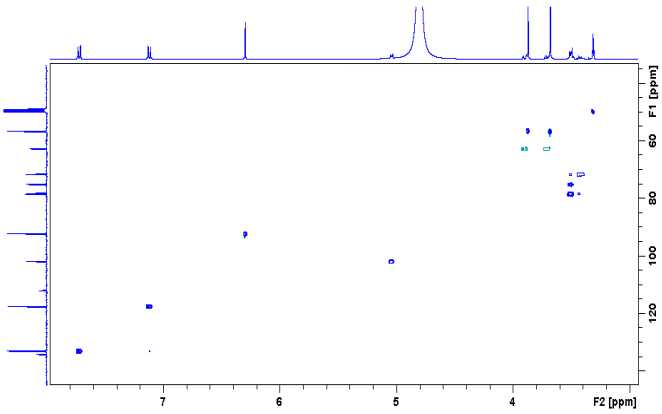


Fig. 6. HSQC spectrum of compound 2

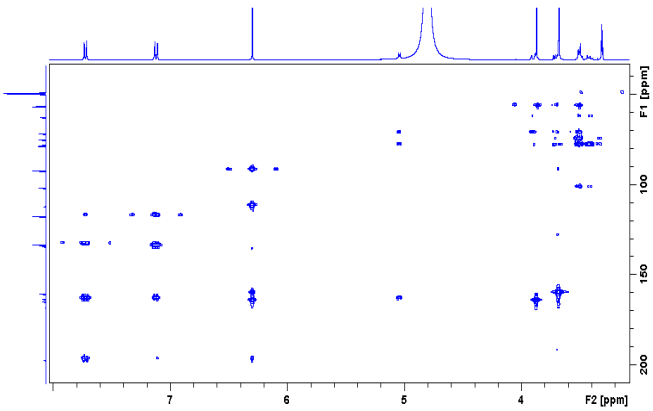


Fig. 7. HMBC spectrum of compound 2

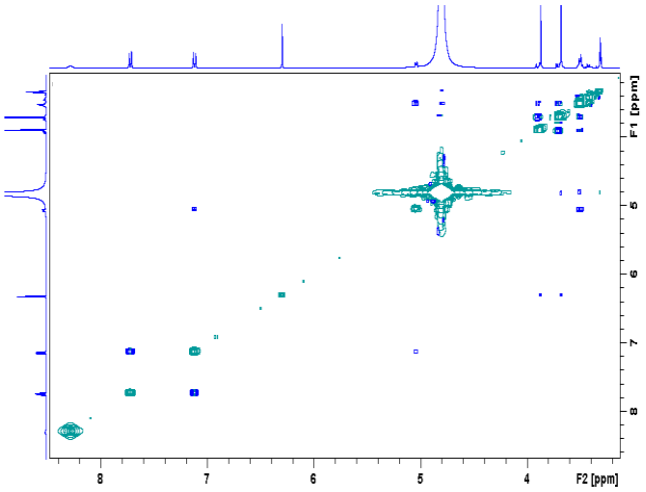


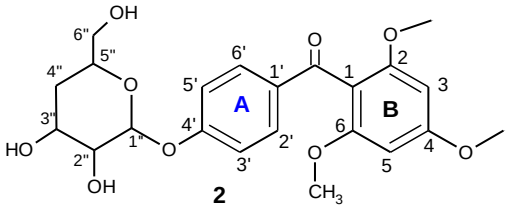
Fig. 8. NOESY spectrum of compound 2

The ^{1}H NMR and ^{13}C NMR spectral data are grouped in Table 2.

Table 2. NMR spectroscopic data for compound 2 in CDCl3 (δ in ppm)

Atoms	δ_H *m* (*J* in Hz)	δ_C
C=O	-	194.2
1	-	111.6
2, 6	-	158.8
3, 5	6.29 *s*	91.9
4	-	162.5
1'	-	133.9
2', 6'	7.72 *d* (8,95)	132.6
3', 5'	7.12 *d* (8,95)	117.1
4'	-	160.6
2-*O*-CH$_3$	3.68 *s*	56.3
4-*O*-CH$_3$	3.87 *s*	56.1
6-*O*-CH$_3$	3.68 *s*	56.3
1''	5.04 *d* (7,62)	101.5
2''	3.50 *m*	74.7
3''	3.52 *m*	77.8
4''	3.42 *m*	71.3
5''	3.48 *m*	78.1
6''	3.70 *dd* (12.6, 5.54) 3.89 *dd* (12.16, 2.05)	62.4

Based on the above evidences this compound was expected to be a glucopyranoside of a benzophenone derivative and elucidated as a new natural product, 4'-hydroxy-2,4,6-trimethoxyphenone-*β*-D-glucopyranose (Fig.9).



2

Fig. 9. Chemical structure of compound 2

3.1.3 COMPOUNDS 3 AND 4

Compound 3: Salacin A or 1,3,6,7-tetrahydroxy-xanthone-2-C-β-D-xylopyranosyl- (1→2) -β-D-glucopyranoside

Salacin A (1,3,6,7-tetrahydroxy-xanthone-2-C-β-D-xylopyranosyl- (1→2) -β-D-glucopyranoside (**3**) was obtained as a yellow powder, and Its chemical structure was established based on HR-ESI-MS with ^{1}H NMR and ^{13}C NMR spectra (Fig.10-16). Its molecular formular was deduced to be $C_{24}H_{27}O_{15}$ from HR-ESI-MS (m/z 555.1316 $[M+H]^+$) (Fig.10). The ^{1}H NMR spectrum of **3** (Fig.11) contained resonances for two anomeric protons [δ_H 4.95 (1H, d, *J* = 10.4 Hz) and 4.3 (1H, d, *J* = 7.17 Hz)] and three uncoupled aromatic protons (δ_H 6.34, 6.82, and 7.46) (Table 3). All these data suggested **3** to be a xanthone glycoside with à disubstituted **A** rang and a trisubstituted **B** ring. The NMR data of **3** were similar to those of mangiferin **[23]** except that the signals associated the presence of a xylose moiety **[21], [22].** In the HMBC spectrum of **3** (Fig.15), the xylose anomeric proton at δ_H 4.3 correlated with the resonance for C-2' (δ_C 82.1) of the glucosyl residue, and the glucosyl anomeric proton at δ_H 4.95 correlated with the resonances for C-2 (δ_C 107.6), C-1 (δ_C 164.7), and C-3 (δ_C 165.5) of the aglycone. Combining this information with the analysis of the chemical shift of the anomeric carbon of the glucosyl moiety (δ_C 73.6), **3** was deduced to be a xanthone C-glycoside, with the sugar chain located at C-2 of the aglycone. Thus, **3** was defined as **Salacin A**.

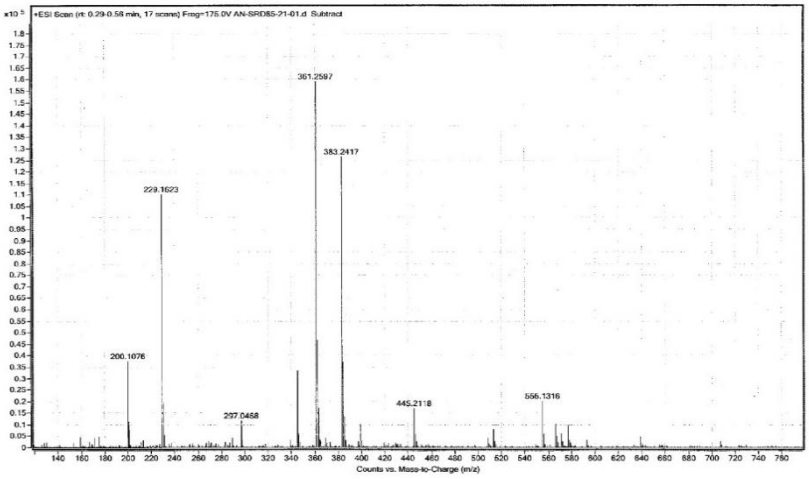


Fig. 10. HR-ESI-MS spectrum of compound 3

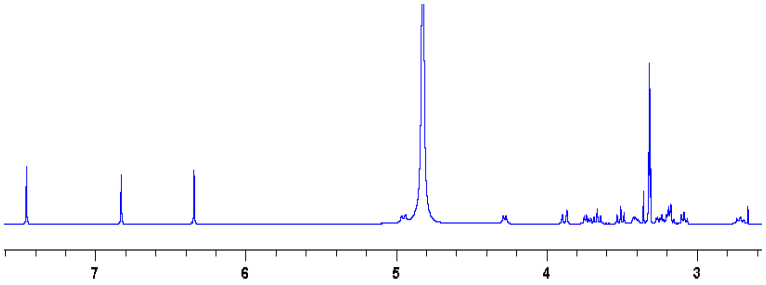


Fig. 11. ^{1}H NMR (400 MHz, CD_3OD) spectrum of compound 3

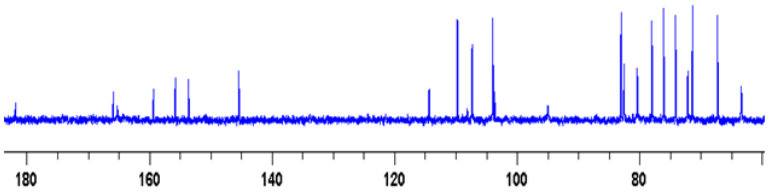


Fig. 12. ^{13}C NMR (100 MHz, CD_3OD) spectrum of compound 3

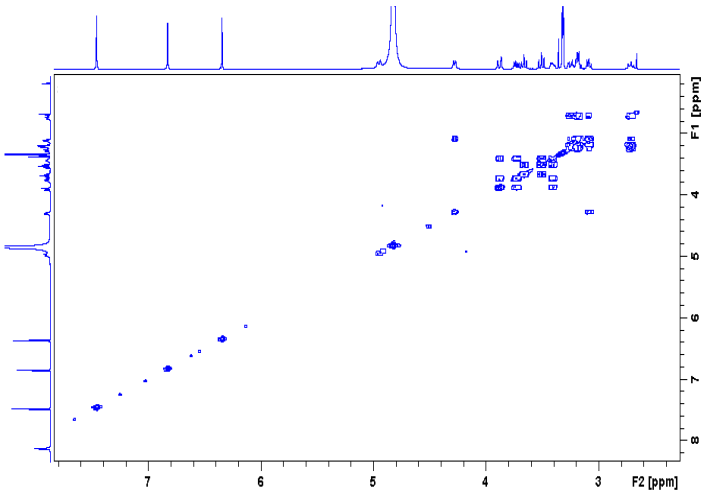


Fig. 13. COSY spectrum of compound 3

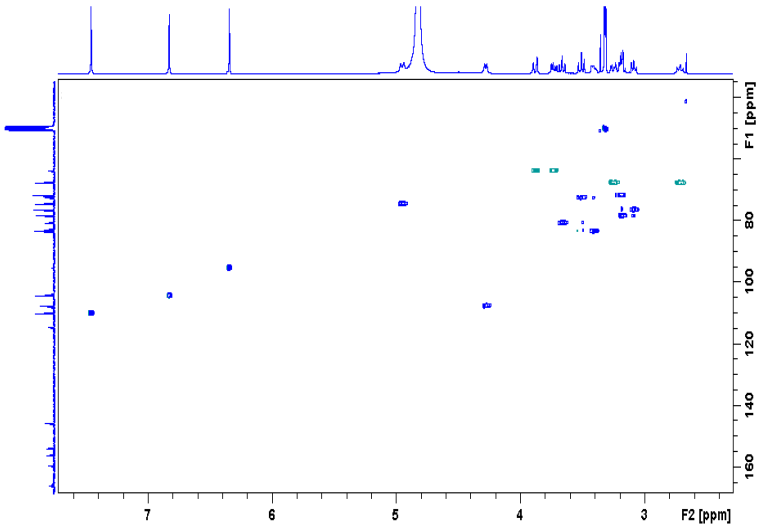


Fig. 14. HSQC spectrum of compound 3

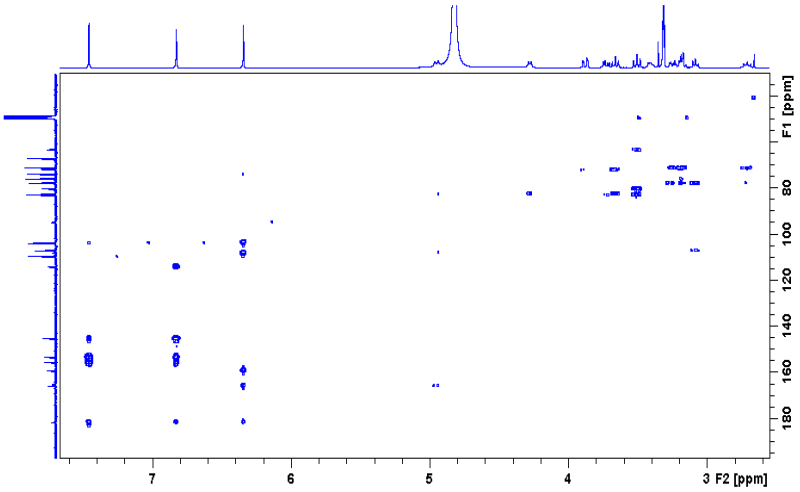


Fig. 15. HMBC spectrum of compound 3

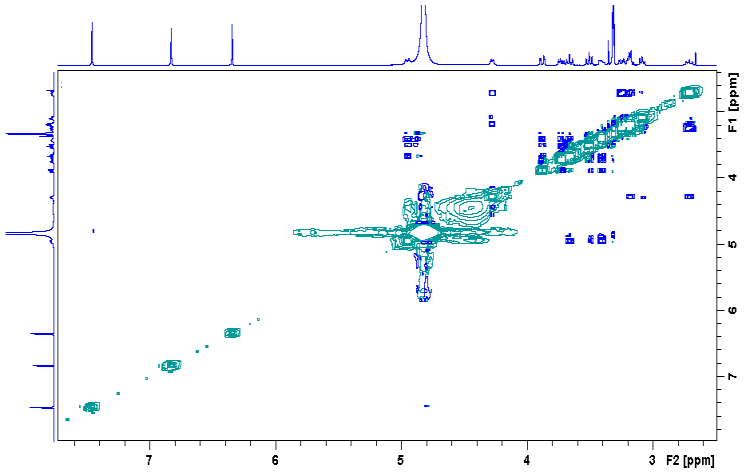


Fig. 16. NOESY spectrum of compound 3

Compound 4: Salacin B

Salacin B (**4**) was obtained as light-yellow powder, and its chemical structure was established based on HR-ESI-MS with ^{1}H NMR and ^{13}C NMR spectra (Fig.17-28). Its molecular formula was determined to be $C_{19}H_{17}O_{10}$ by HR-ESI-MS (m/z 405.0657, [M+H] $^{+,}$ calcd for $C_{19}H_{17}O_{10,}$ 405.0671), and ^{13}C NMR spectroscopic data (Table 2), indicated 12 degrees of unsaturation. The ^{1}H NMR spectrum of **4** demonstrated the resonances for three aromatic protons [δ_H 6.01, 6.32, 6.88 (each 1H, s)], four oxygenated methylene protons [δ_H 3.45 (1H, d, *J* = 11.74 Hz, H-5'b), δ_H 3.16 (1H, d, *J* = 11.74 Hz, H-5'a), δ_H 2.92 (1H, d, *J* = 16.22 Hz, H-6'b), δ_H 2.41 (1H, d, *J* = 16.22 Hz, H-6'a)], and three oxygenated methine protons [δ_H 3.32 (1H, m), δ_H 3.37 (1H, m), δ_H 3.29 (1H, m)]. The ^{13}C NMR spectrum of **4** exhibited 19 carbons resonances comprising one keto carbonyl carbon (δ_C 180.6), six sp^2 oxygenated tertiary carbons (δ_C 156.7, 165.0, 157.1, 151.7, 144.7, 150.8), three sp^2 quaternary carbons (δ_C 106.1, 110.1, 102.5), three sp^2 methine carbons (δ_C 89.1, 101.9, 106.6), one sp^3 oxygenated tertiary carbon (δ_C 115.3), three sp^3 oxygenated methine carbons (δ_C 70.1, 68.8, 69.7), one sp^3 oxygenated methylene carbon (δ_C 66.1) and one sp^3 methylene carbon (δ_C 34.7). The HMBC (Fig. 21) correlations from H-5 to C-6/C-7/C-8a, and H-8 to C-10a/C-7/C-9 indicated the linkage of two benzene rings via (4a, *O,* 10a) -ether bond forming a xanthone skeleton. The relative configuration of **4** was established on the basis for the NOESY spectrum, which showed NOE correlations of H-5'a/H-3', H-2'/H-4', and H-2'/H-6a'. Thus, the structure and the absolute configuration of **salacin B** was defined.

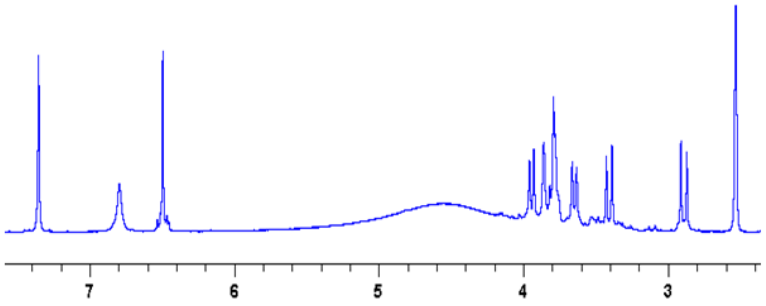


Fig. 17. ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 4

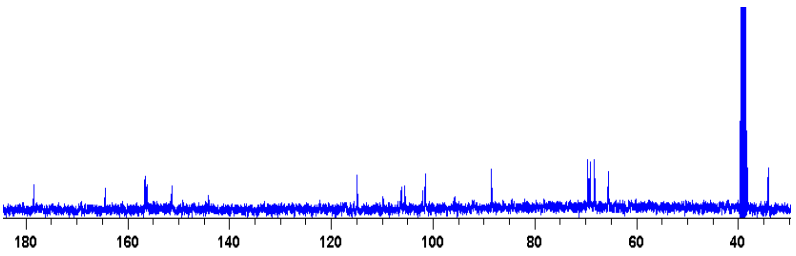


Fig. 18. ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound 4

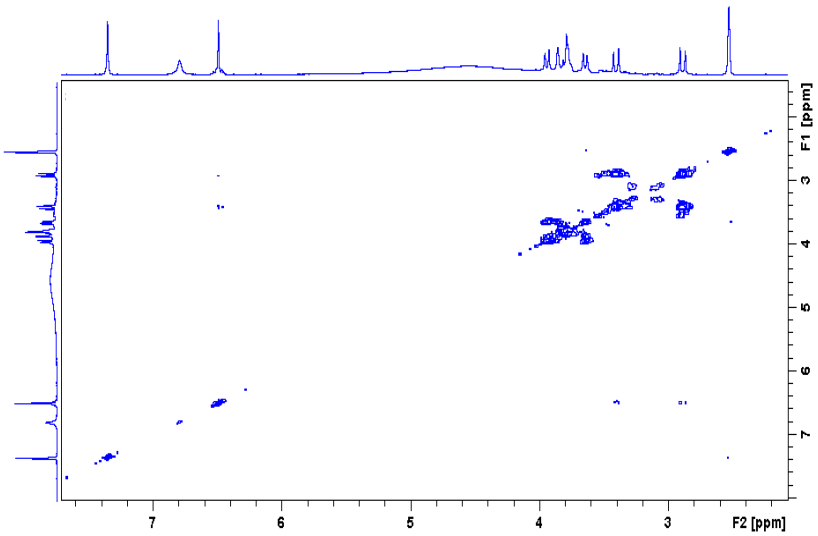


Fig. 19. COSY spectrum of compound 4

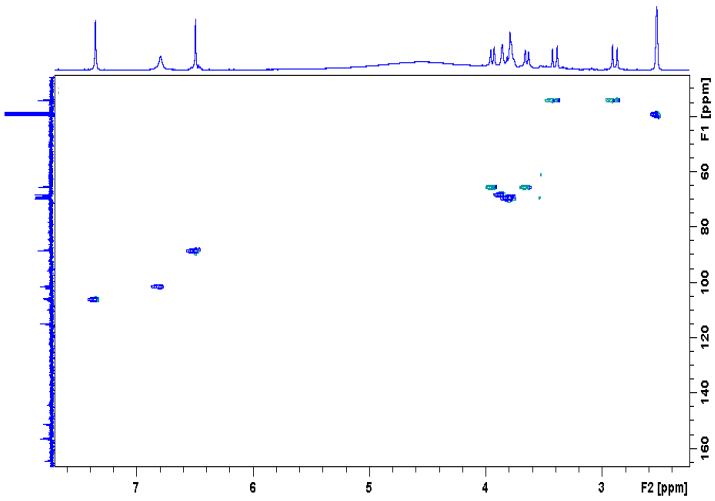


Fig. 20. HSQC spectrum of compound 4

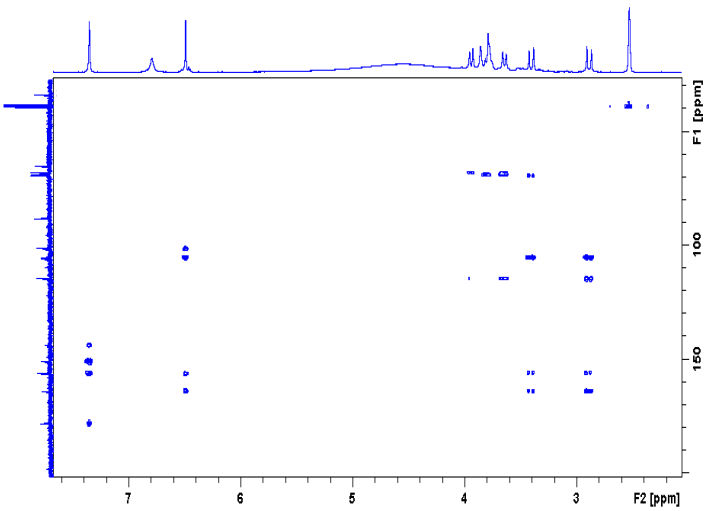


Fig. 21. HMBC spectrum of compound 4

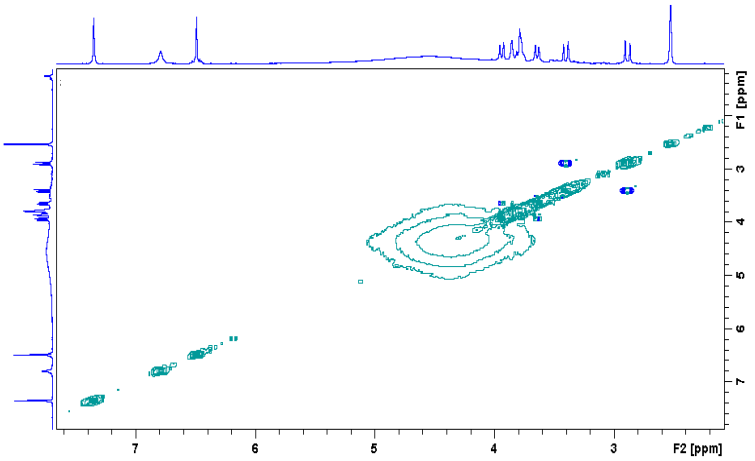


Fig. 22. NOESY spectrum of compound 4

Table 3. NMR spectroscopic data for compounds 3-4 in CD3OD and DMSO-d6 respectively (δ in ppm)

Atoms	*3*	
	δ_H *m* (*J* in Hz)	δ_C
1	-	164.7
2	-	107.6
3	-	165.5
4	6.34 *s*	94.5
4a	-	158.9
5	6.82 *s*	103.9
6	-	153.1
7	-	145.0
8	7.46 *s*	109.3
8a	-	114.0
9	-	181.1
9a	-	103.6
10a	-	155.3
1'	4.95 *d* (10.4)	74.0
2'	4.42 *m*	82.1
3'	3.65 *t* (8.95)	80.3
4'	3.5 *t* (9.4)	71.7
5'	3.39 *m*	82.6
6'	3.73 *dd* (11.3, 5.2) 3.87 *dd* (11.3, 2.24)	61.3
1''	4.3 *d* (7.17)	106.9
2''	3.08 *t* (7.22)	75.9
3''	3.18 *m*	77.9
4''	3.19 *m*	70.9
5''	2.70 *t* (11.1) 3.23 *dd* (11.1, 5.1)	66.8

Atoms	*4*	
	δ_H *m* (*J* in Hz)	δ_C
1	-	156.2
2	-	105.5
3	-	164.4
4	6.50 *s*	88.4
4a	-	156.6
5	6.79 *s*	102.0
6	-	144.2
7	-	156.2
8	7.35 *s*	106.2
8a	-	109.8
9	-	178.5
9a	-	101.5
10a	-	149.2
6'	2.89 *d* (16.05) 3.41 *d* (16.05)	34.1
1'	-	114.9
2'	3.79 *m*	69.6
3'	3.80 *m*	69.2
4'	3.86 *m*	68.3
5'	3.65 *d* (11.74) 3.95 *d* (11.74)	65.6

The chemical structures of salacin **A** and **B** were defined as shown in Figure 23.

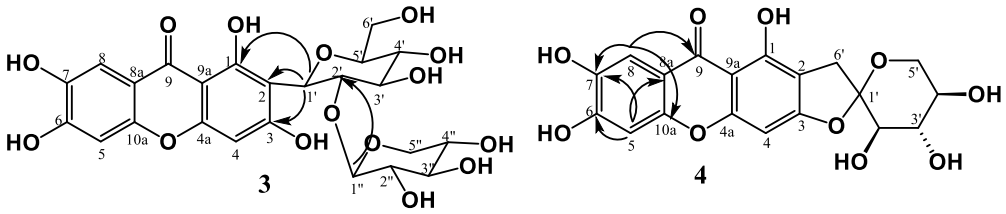


Fig. 23. Chemical structures of salacin A and B

4 ANTITRYPANOSOMIAL ACTIVITY

All extracts and compounds **1-2,** were evaluated for their antitrypanosomial activity on *Trypanosoma brucei* gambiense (Table 4).

Table 4. In vitro antitrypanosomal activity of all extracts and compounds 1-2

Extracts and compounds	*Trypanosoma brucei* gambiense IC_{50} ± SD, (µg/mL)
SFE	8.85 ± 0.31
SFD	25.46 ± 4.41
SFM	87.06 ± 3.67
SRD	91.21 ± 9.72
SRM	2.08 ± 0.12
1	17.49 ± 1.23
2	71.50 ± 6.12
Pentamidine	**0.0004±0.00007(0.0012±0.0002 µM)**

SFE: *Petroleum ether extract from S. nitida leaves;* ***SFD****: Dichloromethane-methanol (50: 50) extract from S. nitida leaves;* ***SFM****: Methanol-water (50: 50) extract from S. nitida leaves;* ***SRD****: Dichloromethane-methanol (50: 50) extract from the root bark of S. nitida;* ***SRM****: Methanol-water (50: 50) extract from the root bark of S. nitida*

Two extracts demonstrated antitrypanosomial with an $IC_{50} < 10$ µg/mL. SFE and SRM extracts were active on *Trypanosoma brucei* gambiense (8.85 ± 0.31 and 2.08 ± 0.12 µg/mL, respectively). Compounds **1-2** were, however, inactive on *Trypanosoma brucei* gambiense.

5 CONCLUSION

Three new phenolic compounds, 4'-hydroxy-2,4,6-trimethoxyphenone-β-D-glucopyranose and two salacins **A** and **B,** as well as two known polyphenols (**1** and **5**), were isolated from the leaves and the bark of the roots of *S. nitida*. Two were from benzophenone and three from xanthone. This is the second report of benzophenone and xanthone from S. nitida. Evaluation of the biological potential of pure crude extracts showed good antitrypanosomal activity. Additional studies will be necessary to isolate and identify the main active molecules of this plant.

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