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Original Article

Anti-caries activity of selected Sudanese medicinal plants with emphasis on *Terminalia laxiflora*

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ABSTRACT

In Sudan, some medicinal plants, such as *Acacia seyal*, *Calotropis procera* and *Balanites aegyptiaca* have been used to prevent or treat oral health problems. The stem and stem bark of *Terminalia laxiflora* Engl., Combretaceae, are used as antiseptics for mouthwash to prevent gingivitis and thrush in Africa. Methanol and 50% hydroethanolic extracts of 25 plants that are used in traditional Sudanese medicine for several diseases and cavity disorders were screened for anti-cavity activities. *T. laxiflora* methanolic wood extracts, which exhibited such activity, were investigated. The crude extracts were assayed for their antimicrobial activities against *Streptococcus sobrinus* in terms of minimum inhibitory concentration and glucosyltransferase inhibition. The active extract of *T. laxiflora* wood was subsequently fractionated by different chromatographic techniques. Isolated compounds were identified by spectroscopic methods and assessed for *S. sobrinus* and glucosyltransferase inhibitory effects. Methanolic extracts of *Terminalia brownii* (bark), *T. laxiflora* (wood), *A. seyal* (bark), *Persicaria glabra* (leaves) and *Tamarix nilotica* (stem) showed good activities against both *S. sobrinus* and glucosyltransferase (MIC $\leq$ 1 mg/ml, IC_{50} values <50 μg/ml). Over all plant extracts, *T. laxiflora* demonstrated the good combined activities (MIC 0.5 mg/ml, glucosyltransferase, IC_{50} 10.3 μg/ml); therefore, its methanolic wood extracts were selected for further phytochemical studies. Four constituents were isolated by chromatographic techniques and identified by spectroscopic techniques. Pharmacological evaluation of the obtained compounds showed that flavogallonic acid dilactone had comparatively good antibacterial activity. In the glucosyltransferase inhibitory test, terchebulin displayed potent activity with an IC_{50} of 7.5 μM. The screening presented in this study showed that methanol extracts of *T. laxiflora* wood possessed promising anti-cavity effects.

Introduction

Dental caries is defined as an irreversible microbial disease of the calcified tissues of the teeth, characterized by demineralization of the inorganic portion and destruction of the organic substance of the tooth (Rajendran et al., 2009).

Bacterial plaque composed of native oral flora accumulated on dental surfaces embedded in an extracellular polysaccharide (EPS) matrix and is the primary etiologic agent of dental caries (Kolenbrander et al., 2006; Koo et al., 2009). Out of the seven species of mutans streptococci group, *Streptococcus sobrinus* have been one of the most commonly implicated in the pathogenesis of dental cavity; moreover, it produces exoenzymes named glucosyltransferases (GTF), which play critical roles in the synthesis of glucan and EPS to providing sites on dental surfaces for microbial colonization, in addition to adherent glucan for bacterial coherence (Paes Leme et al., 2006; Bowen and Koo, 2011; Nishimura et al., 2012; Hashizume-Takizawa et al., 2014).

Medicinal plants have been a great source of novel drug compounds from long time. Plant derived products have made large contributions to the well being of human health. Scientists across the globe have reported antimicrobial properties of several medicinal plants but still a very few of this enormous potential drug has been scientifically screened (Siqueira and Rocas, 2005; Karuppiah and Rajaram, 2012; Gauniyal and Teotia, 2014). In Sudan, various medicinal plants have been used to prevent or treat oral health problems. This study investigated some plants that are used in traditional Sudanese medicine as mouth detergents such as *Acacia seyal*, *Calotropis procera* and *Balanites aegyptiaca* (El Ghazali et al., 2003; Khalid et al., 2012). Different parts of *Terminalia laxiflora* Engl., Combretaceae, are used to prevent gingivitis and thrush in Congo; the stems are used as chewing sticks and also macerated

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stem bark are used as antiseptic to wash mouth (Fasola et al., 2013).

In the present study, 25 plant species were selected and evaluated for their anti-cariogenic activity in terms of inhibition of *S. sobrinus* bacterial growth and GTF inhibitory effects by using their methanolic and 50% hydroethanolic extracts. Methanolic extracts of *T. laxiflora* wood demonstrated significant combined activities; thus, it was further fractionated in order to identify the active compounds responsible for the biological activities.

Materials and methods

Reagents

All materials were purchased from Wako, Japan except *p*-iodonitrotetrazolium (INT) violet, which was from Sigma-Aldrich Co. Ltd, Japan.

Plant materials and extraction

The plants were collected from Khartoum state (Khartoum, Omdurman and Shambat cities) and Elgadarif state of Sudan. Voucher specimens are deposited in the Horticultural Laboratory, Department of Horticulture, Faculty of Agriculture, University of Khartoum (Table 1).

Plant materials were shade dried and powdered before extraction; they were each extracted three times for 12 h, with methanol and 50% hydroethanol. The extracts were filtered and the solvent was removed under reduced pressure using rotary evaporator. The concentrated extracts were then dried with a freeze dryer.

Fractionation, purification and identification of pure compounds from Terminalia laxiflora

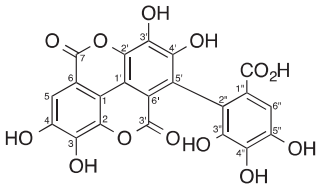
Fractionation and isolation of *Terminalia laxiflora* Engl., Combretaceae, wood were performed by the method described by Muddathir et al. (2013). Methanolic crude extracts (5 g) were chromatographed on medium pressure liquid chromatography (MPLC) using octadecyl-silica (ODS) column (YMC-DispoPackAT ODS-25, particle size 25 μm; column size 120 g, (40 mm × 188 mm), Japan), chromatography pump (Co. No. 540 Yamazen, Osaka, Japan) with pressure of 1.2 MPa, UV detector at 280 wavelength (UV-10V Yamazen, Osaka, Japan) and fraction collector (SF-2120, Advantec Tokyo Ltd, Japan).

The column was conditioned with the first eluent used for separation for 30 min with flow rate of 0.5 ml/min. Then water containing increasing proportions of methanol stepwise elution to obtain two fractions (F1 and F2). F1 (0.89 g) was passed through column chromatography (40 mm × 430 mm) on a Sephadex LH-20 (18–111 μm, GE Healthcare Bio-Sciences Corp, Tokyo, Japan) that revealed four subfractions. These four subfractions, F (1a, 1b, 1c and 1d) introduced through preparative high performance liquid chromatography (HPLC) with reversed phase Inertsil ODS-3 column (10 mm × 250 mm, GL Sciences Inc., Tokyo, Japan) and monitored at 280 nm. Solvent system used 10–100% gradient methanol in water with 0.05% TFA programmed for 60 min at a flow rate of 5 ml/min to give compound (**1**) (14 mg), compound (**2**) (8.5 mg) and compound (**3**) (5 mg). F2 (0.50 g) was also subjected to preparative HPLC under the same condition to isolate compound (**4**) (17.5 mg). The compounds purity was confirmed using analytical HPLC (Shimadzu SIL-20A) with reversed phase Inertsil ODS-3V column (5 μm (4.6 mm × 250 mm), GL Sciences Inc., Tokyo, Japan), flow rate: 1 ml/min, wavelength: 280 nm, gradient program: methanol:0.05% TFA aqueous solution for 60 min (Fig. 1).

The isolated compounds from *T. laxiflora* wood methanol extract, exactly compounds (**1**) and (**2**) were identified using the ^{1}H,^{13}C nuclear magnetic resonance (NMR). Spectra were recorded in methanol-d_4 with a JEOL EC600 MHz NMR (Tokyo, Japan). Additionally, ultra-performance liquid chromatography-time-of-flight mass spectrometry (UPLC-TOFMS, Waters Xevo^{TM} QTof MS, Waters, Milford, MA, USA) was performed using a C_{18} column (2.1 mm × 100 mm, Waters) with MeOH/H_2O = 5/95 (30 min), 100/0 (10 min) with a linear gradient as eluent. The UPLC-TOFMS data were collected in negative ionization mode (Fig. 2).

*Flavogallonic acid dilactone (**1**)*

Tan powder. ^{1}H NMR (in CD_3OD): δ 7.26 (s), 7.50 (s). ^{13}C NMR (in CD_3OD): δ 108.1 (C-1, 1′), 110.1–114.4 (C-6, 6′), 112.8 (C-5), 113.3 (C-6″), 117.5–120.2 (C-5′, 2″), 124.9 (C-1″), 135.7 (C-2), 136.3 (C-3), 136.5 (C-4), 137.8 (C-2′), 139.2 (C-3′), 143.2 (C-4′), 144.1 (C-3″), 145.9 (C-4″), 147.8 (C-5″), 158.9–160.4 (C-7, 7′), 168.9 (C-7″); UPLC-TOFMS *m/z* 469 $[M-H]^-$ (calcd. for C_{21} H_{10} O_{13} 470.2963).



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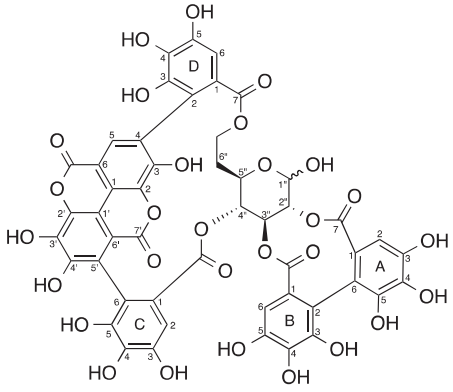
*Terchebulin (**2**)*

Tan powder. ^{1}H NMR (in CD_3OD): δ 3.04 (t, *J* = 11.6 Hz, one of the H-6″), 4.21 (t, *J* = 10.3 Hz, H-5″), 4.48 (t, *J* = 8.9 Hz, one of the H-6″), 4.78 (t, *J* = 11.0 Hz, H-4″), 4.98 (dd, *J* = 3.5, 9.7 Hz, H-2″), 5.23 (d, *J* = 2.8 Hz, H-1″), 5.64 (t, *J* = 9.6 Hz, H-3″), 6.37 (s, H-B6), 6.42 (s, H-D6), 6.56 (s, H-A2), 6.79 (s, H-C2), 7.48 (s, H-5). ^{13}C NMR (in CD_3OD): δ 63.4 (C-6″), 68.5 (C-4″), 69.0 (C-5″), 74.1 (C-3″), 74.2 (C-2″), 90.2 (C-1″), 106.4 (C-B6), 106.5 (C-D6), 106.8 (C-A2), 108.5 (C-C2), 112.0–114.0 (C-A6, B2, 5, 5′, 1, 1′, 2, 2′, 6, 6′), 116.0 (C-C6), 122.2 (C-D1), 123.5 (C-B1, C1), 125.1 (C-A1), 135.9 (C-B4), 136.1 (C-A4), 137.5 (C-C4), 137.6 (C-D4), 138.4 (C-3), 139.1 (C-D3), 140.7 (C-3′), 141.7 (C-D2), 143.4–143.6 (C-A5, B3, C5), 144.5–144.6 (C-A3, B5, C3, D5), 147.4 (C-4′), 150.3 (C-4), 158.3 (C-7′), 159.5 (C-7), 166.9 (C-D7), 167.0 (C-C7), 168.9 (C-A7), 169.5 (C-B7); UPLC-TOFMS *m/z* 1083.07 $[M-H]^-$ (calcd. for C_{48} H_{28} O_{30} 1084.7179).



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Compound (**3**) and compound (**4**) were identified by comparing the retention time with standard of the highest grade (purity >97.0%) gallic acid (Nacalai Tesque, Inc., Kyoto, Japan) and ellagic acid (Sigma, Japan) respectively.

Table 1
Minimum inhibitory concentration (MIC) and glucosyltransferase (GTF) inhibitory activities of selected Sudanese medicinal plant extracts.

No.	Botanical name	Family	Examined part	Voucher specimen	Collection place	Extracts	MIC (mg/ml)	GTF (%)
1	*Aristolochia bracteolata* Lam.	Aristolochiaceae	Whole plant	SD-SH-04	Shambat	M	–[a]	–[b]
2						E	1.0	08.0 ± 4.3h
3	*Calotropis procera* (Aiton) Dryand.	Apocynaceae	Leaves	SD-SH-11	Shambat	M	4.0	–
4						E	–	06.7 ± 8.9h
5	*Ambrosia maritima* L.	Asteraceae	Aerial part	SD-SH-03	Shambat	M	4.0	–
6						E	–	08.9 ± 7.5h
7	*Xanthium brasilicum* Vell.	Asteraceae	Leaves	SD-SH-12	Shambat	M	–	–
8						E	4.0	–
9	*Balanites aegyptiaca* (L.) Delile	Zygophyllaceae	Leaves	SD-KH-15	Khartoum	M	4.0	–
10						E	4.0	–
11			Bark			M	4.0	–
12						E	–	–
13	*Adansonia digitata* L.	Malvaceae	Fruit pulp	SD-OD-27	Omdurman	M	–	57.0 ± 1.0d
14						E	–	13.7 ± 4.8g,h
15	*Guiera senegalensis* J.F. Gmel.	Combretaceae	Leaves	SD-OD-40	Omdurman	M	2.0	70.4 ± 1.0c,d
16						E	–	69.6 ± 3.0c,d
17	*Terminalia brownii* Fresen.	Combretaceae	Bark	SD-GF-02	Elgadarif	M	0.5	88.5 ± 2.1a,b
18						E	4.0	90.1 ± 2.1a,b
19	*Terminalia laxiflora* Engl.	Combretaceae	Wood	SD-KH-03	Khartoum	M	0.5	87.2 ± 2.6a,b
20						E	4.0	80.5 ± 1.7a,b,c
21	*Vernonia amygdalina* Delile	Asteraceae	Leaves	SD-KH-19	Khartoum	M	4.0	–
22						E	4.0	–
23	*Euphorbia hirta* L.	Euphorbiaceae	Aerial part	SD-SH-37	Shambat	M	–	–
24						E	–	–
25	*Ricinus communis* L.	Euphorbiaceae	Leaves	SD-SH-36	Shambat	M	4.0	–
26						E	4.0	–
27	*Acacia tortilis* (Forssk.) Hayne	Fabaceae	Bark	SD-KH-07	Khartoum	M	2.0	–
28						E	4.0	–
29			Wood			M	–	–
30						E	4.0	11.2 ± 9.0h
31	*Acacia seyal* var. *fistula* (Schweinf.) Oliv.	Fabaceae	Bark	SD-KH-06	Khartoum	M	–	82.9 ± 3.0a,b,c
32						E	–	89.4 ± 0.1 a,b
33			Wood			M	–	–
34						E	–	50.3 ± 1.0e
35	*Acacia seyal* Delile	Fabaceae	Bark	SD-GF-05	Elgadarif	M	1.0	91.5 ± 5.1a,b
36						E	1.0	85.2 ± 3.1a,b,c
37			Wood			M	–	–
38						E	4.0	34.8 ± 4.3f
39	*Cassia acutifolia* Delile	Fabaceae	Leaves	SD-SH-24	Shambat	M	2.0	–
40						E	2.0	–
41	*Parkinsonia aculeata* L.	Fabaceae	Leaves	SD-SH-02	Shambat	M	4.0	–
42						E	4.0	07.1 ± 5.0h
43	*Khaya senegalensis* (Desr.) A. Juss.	Meliaceae	Bark	SD-SH-14	Shambat	M	4.0	88.7 ± 3.0a,b
44						E	–	77.8 ± 3.8b,c,d
45	*Peganum harmala* L.	Nitrariaceae	Seed	SD-OD-20	Omdurman	M	2.0	–
46						E	–	–
47	*Argemone mexicana* L.	Papaveraceae	Leaves	SD-KH-39	Khartoum	M	4.0	–
48						E	–	–
49			Seed			M	1.0	–
50						E	–	–
51	*Persicaria glabra* (Willd.) M. Gómez	Polygonaceae	Leaves	SD-SH-A-03	Shambat	M	1.0	87.2 ± 9.0a,b
52						E	–	78.2 ± 0.6b,c,d
53	*Ziziphus spina–christi* (L.) Desf.	Rhamnaceae	Bark	SD-SH-06	Shambat	M	4.0	94.5 ± 2.3a
54						E	4.0	27.0 ± 5.0f,g
55			Leaves			M	2.0	–
56						E	–	–
57	*Solanum dubium* Dunal	Solanaceae	Fruits	SD-SH-34	Shambat	M	–	–
58						E	–	–
59	*Tamarix nilotica* (Ehrenb.) Bunge	Tamaricaceae	Stems	SD-OD-10	Omdurman	M	1.0	85.4 ± 0.3a,b
60						E	–	91.4 ± 2.2a,b
61	*Tribulus terrestris* L.	Zygophyllaceae	Aerial part	SD-SH-33	Shambat	M	–	–
62						E	–	–

M, methanol; E, 50% hydroethanol. Values were expressed as mean ± SD; $n = 3$. Means with different letters in the same column were significantly different at the level ($p < 0.05$).

[a] No inhibitory activity at concentration of 4 mg/ml.

[b] No inhibition at 100 μg/ml.

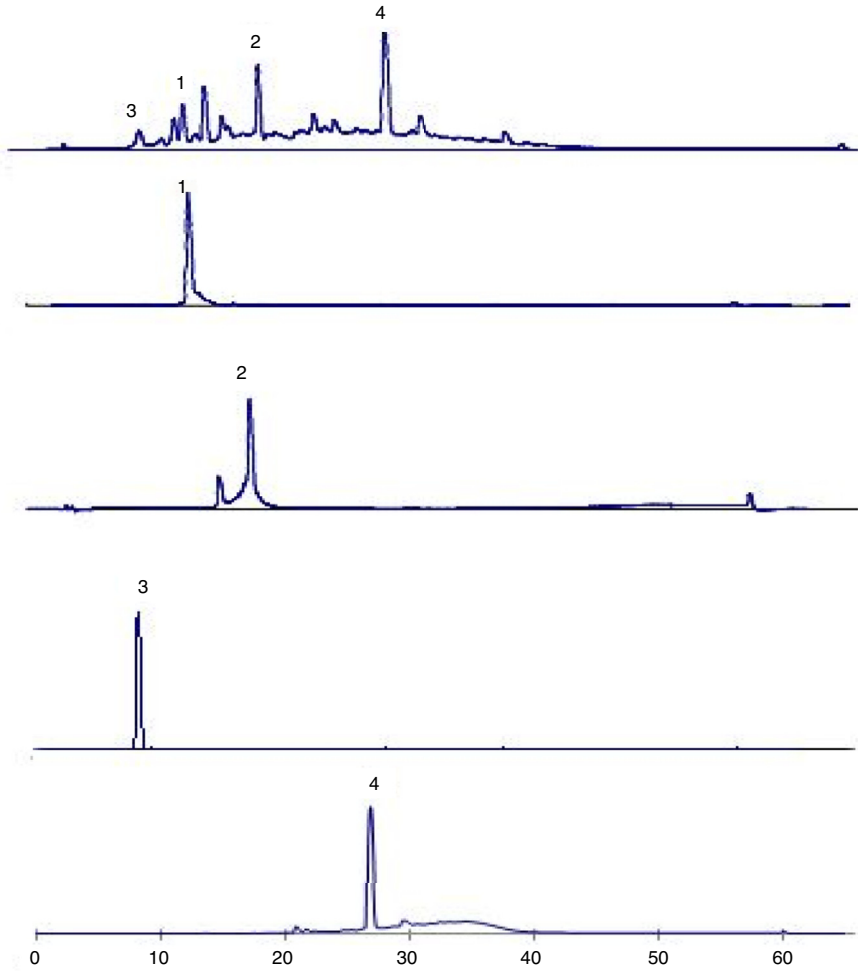

Fig. 1. HPLC fingerprint of methanol extract of *Terminalia laxiflora* and their isolated compounds. (**1**) Flavogallonic acid dilactone [retention time: 13.6 min], (**2**) terchebulin [retention time: 17.3 min], (**3**) gallic acid [retention time: 8.8 min] and (**4**) ellagic acid [retention time: 26.7 min].

Biological activities of the crude extracts and isolated compounds

Determination of the minimum inhibitory concentration (MIC)

MIC was determined by the broth dilution method according to Iwaki et al. (2006). *S. sobrinus* 6715 was cultured in a Brain-Heart Infusion Broth. The crude extracts, fractions or pure compounds were tested for antibacterial activity in sterile 96-well plates. The inoculums were prepared by diluting the broth culture to approximately 10^6 cells/ml. To each well containing sample, 100 μl of microbial inoculums were added, followed by addition of medium to achieve a final volume of 200 μl. The tested sample was prepared in a concentration range of 4000–31 μg/ml using a two-fold dilution method. Solvent and medium controls were included in each test plate. In order to dissolve the sample extracts, 20% dimethyl sulfoxide (DMSO) was used in this study. The final concentration of DMSO alone in the well showed no inhibitory effect on *S. sobrinus* growth. The experiments were performed in triplicate. Chlorhexidine was included in the assays as positive control. The cultures were incubated for 24 h at 37 °C under anaerobic conditions. Microbial growth was indicated by the addition of 50 μl of (0.2 mg/ml) INT to the culture and incubated at 37 °C for 2 h. The MIC was defined as the lowest concentration that inhibited the color change of INT (Eloff, 2001).

Assay for GTF inhibitory activity

Streptococcus sobrinus 6715 was cultured for 20 h at 37 °C in 4 l of Todd Hewitt broth. After centrifugation of the culture at 1300 × *g* for 10 min at 4 °C, the cells were collected and then extracted with 8 M urea for 1 h while stirring. The crude enzyme solution was dialyzed against 10 mM sodium phosphate buffer (pH 6.0). The crude enzyme solution was stored in a freezer at −80 °C.

GTF were incubated in 300 μl of 0.1 M phosphate buffer (pH 6.0) containing 1% sucrose, 0.5% dextran T-10, in the presence or absence of a sample at 37 °C for 3 h. The volume of the crude GTF solution used in the assay was determined by measured turbidity around 1.0 absorbance at 590 nm (Mitsunaga et al., 1997).

$$\text{Inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Statistical analysis

The inhibitory percentage and IC_{50} values of GTF were expressed as the mean (mean ± standard deviation). The significant differences between samples were assessed by one-way analysis of variance (ANOVA) followed by pairwise comparison of the mean using Tukey's multiple comparison test. Values were determined to be significant when p was less than 0.05 ($p < 0.05$).

Results

Twenty-five plant species that are used in traditional Sudanese medicine were investigated. Sixty-two methanolic and 50%

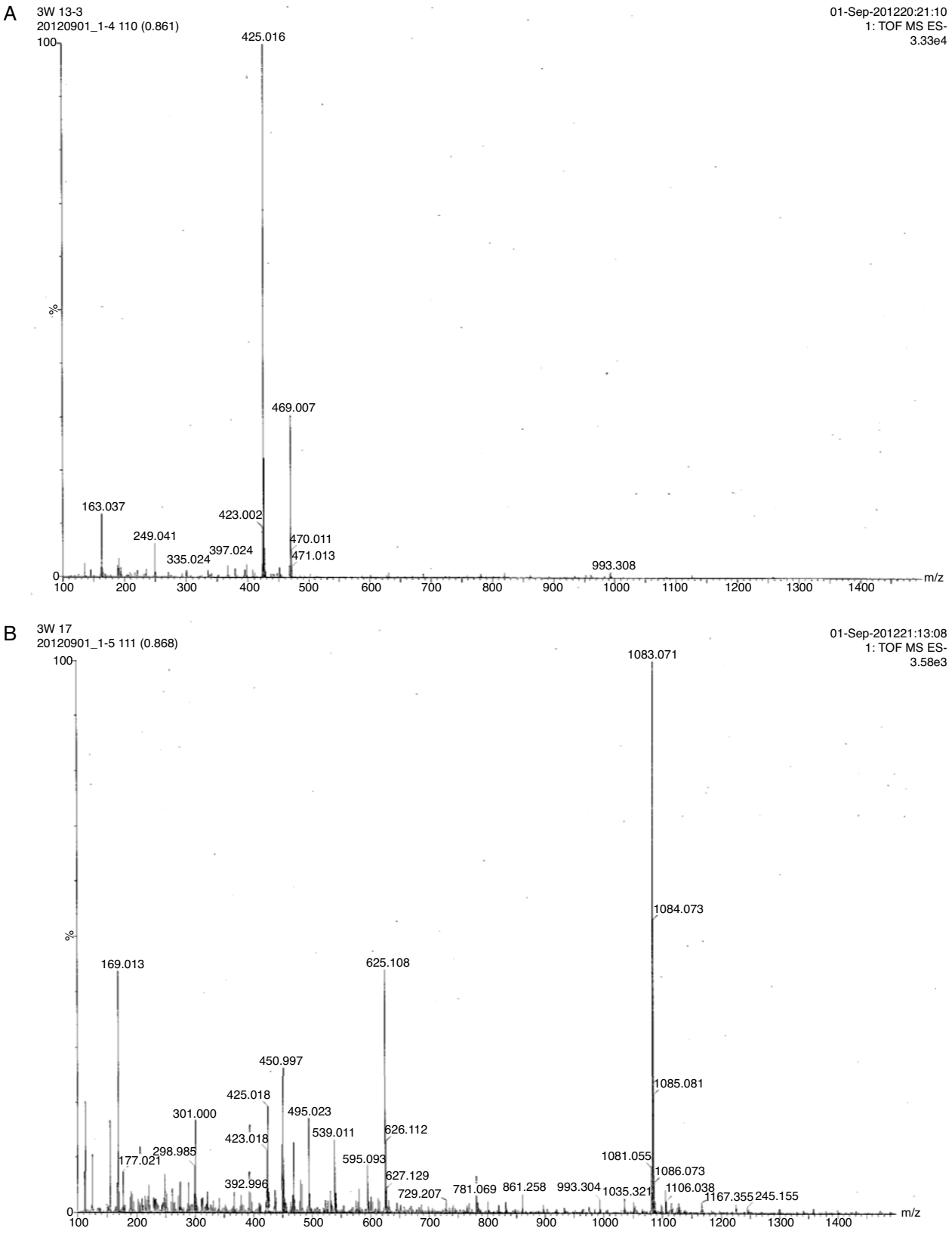

Fig. 2. Mass spectra of flavogallonic acid dilactone (A) [*m*/*z* 469] and terchebulin (B) [*m*/*z* 1083] by UPLC-TOFMS in negative ionization mode.

Table 2
IC_{50} values (μg/ml) obtained by the potent methanolic extracts against glucosyltransferase (GTF) enzyme.

Botanical name	Part used	GTF IC_{50} (μg/ml)
A. seyal	Bark	03.8 ± 1.7a
T. brownii	Bark	09.2 ± 3.4a
T. laxiflora	Wood	10.3 ± 2.2a
K. senegalensis	Bark	23.6 ± 2.6 b
P. glabra	Leaves	27.1 ± 4.1b,c,d
A. digitata L.	Fruit pulp	35.9 ± 3.2c,d,e
Z. spina–christi	Bark	38.8 ± 3.5d,e,f
A. seyal var. *fistula*	Bark	47.2 ± 3.6e,f
T. nilotica	Stems	49.8 ± 4.9f

Means with different letters in the same column were significantly different at the level ($p < 0.05$); $n = 3$.

Table 3
Anti-*S. sobrinus* and glucosyltransferase (GTF) inhibitory activity of compounds isolated from *T. laxiflora* wood.

Compound	MIC (mg/ml)	GTF IC_{50} (μM)
Terchebulin	1	7.5 ± 2.7a
Gallic acid	–[a]	NC
Ellagic acid	1	1017.5 ± 4.1c
Flavogallonic acid dilactone	0.5	149 ± 1.6b
Chlorhexidine[b]	0.0004	5.8 ± 3.3a

NC, the IC_{50} could not be calculated because the activity was less than 50% at highest concentration. Means with different letters in the same column were significantly different at the level ($p < 0.05$); $n = 3$.

[a] No inhibitory activity at concentration of 4 mg/ml.

[b] Positive control.

hydroethanolic extracts were prepared; and their antibacterial activities against *S. sobrinus* and GTF enzyme inhibitory activities were investigated.

Antibacterial assay

To evaluate the antibacterial activity of selected Sudanese medicinal plant extracts against *S. sobrinus* using dilution methods. The MIC were determined in Table 1. Among 62 plant extracts 35 extracts showed antibacterial activity. Also noteworthy the methanolic extracts of Combretaceae family; *Terminalia brownii* (bark) and *T. laxiflora* (wood) demonstrated highest antibacterial activity (MIC of 0.5 mg/ml) among them.

GTF enzyme inhibitory activity

Inhibitory effects over 55% on GTF enzyme activity were demonstrated by 18 of the 62 plant extracts at 100 μg/ml (Table 1); of these thirteen extracts exhibited inhibitory activity more than 80%. Table 2 shows the IC_{50} values of GTF inhibitory activity of nine methanolic extracts ranged between 3.8 and 49.8 μg/ml. *A. seyal* (bark), *T. brownii* (bark) and *T. laxiflora* (wood) display a significant inhibitory activity.

Combined activities of the crude extracts and isolated compounds

Methanolic extracts of *T. brownii* (bark), *T. laxiflora*, *A. seyal* (bark), *Persicaria glabra* (leaves) and *Tamarix nilotica* (stems) demonstrated MIC ≤ 1 mg/ml against *S. sobrinus* and IC_{50} less than 50 μg/ml against GTF enzyme.

Methanolic wood extracts of *T. laxiflora* which showed good combined activities (MIC 0.5 mg/ml, GTF, IC_{50} 10.3 μg/ml), were selected for further purification, crude methanolic extracts after subjected to MPLC resulted in two fractions F1 (MIC 0.5 mg/ml, GTF 96.4%) and F2 (MIC 1 mg/ml, GTF 93.1%); moreover purification revealing the presence of flavogallonic acid dilactone (**1**), terchebulin (**2**), gallic acid (**3**), and ellagic acid (**4**). Antibacterial and GTF inhibitory activities for isolated compounds are shown in Table 3. Flavogallonic acid dilactone (**1**) demonstrated relatively good antibacterial activity, with MIC of 0.5 mg/ml. Terchebulin (**2**) and ellagic acid (**4**) showed moderate antibacterial activity, however, terchebulin (**2**) displayed potent activity against GTF enzyme.

Discussion

Antibacterial and GTF enzyme inhibitory activities of extracts

In this study solvent selection relied on previous studies mentioning that methanol is classified as a polar solvent due to the presence of hydroxyl group. Nevertheless, there is also methyl group presence in methanol, which is sort of non-polar, so it has ability to extract various types of compounds and increase extract's yield. Furthermore, it can give higher concentrations of bioactive molecules from plants such as different classes of phenolic compounds. 50% hydroethanol yield high content of phenolic and flavonoid compounds (Ahmad et al., 2009; Caunii et al., 2012; Thanh et al., 2016). Some studies mentioned that polyphenols, such as flavonoids, phenolic acids and tannins showed anti-enzyme, antibacterial and/or anti-biofilm activities (Gulati et al., 2012; Livia et al., 2016).

The MIC values of the methanol extracts were relatively lower than those of the 50% hydroethanolic extracts, implying more active antibacterial composition in methanol than in 50% hydroethanolic extracts. These findings were similar to those reported by Samuelsen (2000). Ncube et al. (2012) believed that the crude extract will be active when having MIC values less than 8 mg/ml, whilst Gibbons (2005) suggested that isolated phytochemicals should demonstrate at least MIC < 1 mg/ml. In this study, MIC values of 0.5 mg/ml were considered an indication of good antibacterial activity.

Zhi et al. (2016) stated that inhibition of GTF activity and the consequential polysaccharide synthesis may diminish the virulence of cariogenic biofilms, which could be an alternative strategy to eradicate dental caries. Methanolic extracts of *A. seyal* (bark), *T. brownii* (bark) and *T. laxiflora* (wood) showed significant inhibitory activities on GTF. A previous study mentioned that methanolic extracts of these plants contain condensed and hydrolysable tannins (Muddathir and Mitsunaga, 2013). Yamauchi et al. (2016) reported that *T. brownii* (bark) contained gallic acid, punicalagin, terchebulin, ellagic acid 4-*O*-α-L-rhamnopyranoside, ellagic acid and 3,4,3′-tri-*O*-methylellagic acid. Plant polyphenols shared with catechin-based oligomeric forms (condensed tannins) and/or gallate ester form compounds (hydrolysable tannins) display strong anti-GTF activities (Yanagida et al., 2000).

Biological activities of compounds isolated from Terminalia laxiflora *wood*

Our chemical profiling of the methanol extracts of the *T. laxiflora* wood disclosed the presences of four compounds as we described in experimental part. Flavogallonic acid dilactone (**1**) showed ^{1}H NMR spectral pattern with two ^{1}H singlets (δ H 7.26 and 7.50). ^{13}C NMR spectrum displayed signals of nine non-oxygenated aromatic carbons (δ C 108.1, 108.1, 110.1, 112.8, 113.3, 114.4, 117.5, 120.2, 124.9) and nine oxygenated carbons (δ C 135.7, 136.3, 136.5, 137.8, 139.2, 143.2, 144.1, 145.9, 147.8) of three aromatic rings. The spectrum showed also signals of two lactonized carbonyl carbons (δ C 158.9 and 160.4) and a carboxylic carbon (δ C 168.9). Data confirmed by UPLC-TOFMS (m/z 469 $[M-H]^-$). These spectroscopic data were the same to literature values reported by Orabi et al. (2015) and Tanaka et al. (1986). The chemical structure has been

excellently reported by several authors (Grimshaw and Haworth, 1956; Tanaka et al., 1986, 1996; Kinjo et al., 2001; Hirano et al., 2003; Shuaibu et al., 2008; Ibrahim et al., 2014; Orabi et al., 2015). Flavogallonic acid dilactone (**1**) was isolated earlier from *Terminalia catappa* in free form and as a single-bonded acyl unit on their tannin pyranose cores, such as terflavins A–D (Tanaka et al., 1986).

The ^{1}H and ^{13}C NMR data of terchebulin (**2**) were similar to those reported by Lin et al. (1990). The UPLC-TOFMS (m/z 1083 $[M-H]^-$) data was in agreement with Silva et al. (2000). Previously Silva et al. (2000) reported that terchebulin was the main compound present in *Terminalia macroptera* root. Gallic acid (**3**) and ellagic acid (**4**) were also detected in *T. laxiflora* wood extract; these two compounds were isolated from different plants of the genus *Terminalia* (Silva et al., 2000; Shuaibu et al., 2008).

Data in Table 3 showed that flavogallonic acid dilactone (**1**) exhibited good antibacterial activity (0.5 mg/ml). From previous study, the activity of *Propionibacterium acnes* was inhibited at concentration 0.25 mg/ml (Muddathir and Mitsunaga, 2013). The MIC for gallic acid (**3**) has no activity toward *S. sobrinus* up to 4 mg/ml. Kang et al. (2008) stated that the MIC of gallic acid against *S. sobrinus* was 8 mg/ml. The MIC of ellagic acid (**4**) was 1 mg/ml. Sarabhai et al. (2013) mentioned that pure ellagic acid did not exert much biofilm inhibiting effect.

According to our findings, ellagic acid (**4**) showed weak GTF inhibitory activity (IC_{50} 1017.5 μM). Even though Sawamura et al. (1992) suggested that the use of ellagic acid will not affect the ecological balance of oral bacterial flora, but it could be a possible anti-carries agent through its GTF inhibitory action.

Terchebulin (**2**) exhibited significance activity against GTF (IC_{50} 7.5 μM), when compared to the positive control of chlorhexidine (IC_{50} 5.8 μM). Oolong tea fraction rich in high molecular weight polyphenols inhibited the synthesis of glucan non-competitively (Matsumoto et al., 2003).

Chlorhexidine demonstrated the strongest activity against *S. sobrinus* and in addition, it showed good activity against GTF (Table 3). Nonetheless, it has been reported that chlorhexidine was found to be a cytotoxic agent to murine fibroblast cell lines, human alveolar bone cells and human osteoblastic cell line. These results approve that chlorhexidine is not cell type specific (Cabral and Fernandes, 2007; Giannelli et al., 2008; Faria et al., 2009; Li et al., 2014). Toxicity assay with mouse fibroblasts showed that ellagic acid, terchebulin and flavogallonic acid isolated from *Terminalia avicennoides* stem bark, had $IC_{50} \geq 1500$ μg/ml as well as they did not affect the integrity of human erythrocyte membrane of the human (Shuaibu et al., 2008).

Conclusion

In the present study, *T. laxiflora* wood demonstrated significant anti-cavity activity. These results justify the use of this plant for oral care in traditional African medicine. The promising results of antimicrobial and GTF inhibitory activity shown here, suggests terchebulin (**2**) and flavogallonic acid dilactone (**1**) could be considered for further pharmacological studies, evaluating the toxicity and development of a natural anticariogenic agent for dental caries.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Authors' contributions

EAMM, AMM, KY and TM all contributed to the writing of this article. EAMM and AMM obtained samples. TM designed the study and supervised the laboratory work. EAMM and AMM performed the different assays and statistical analysis. KM contributed to compound identification.

Conflicts of interest

The authors declare no conflicts of interest.

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