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Structure Determination of Compounds from the Bark of Ficus ingens (Miq.) Miq. using 1D NMR Spectral Methods

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Abstract
This study was carried out to investigate chemical constituents on the bark of *Ficus ingens (Miq.) Miq*. Three compounds were isolated from methanol extract of the bark *Ficus ingens (Miq.) Miq*. and it were identified as compound-1 [tetrahydro-2-(hydroxymethyl)-6-(terahydro-3,4-dihydroxy-2,5-bis(hydroxymethyl)fura-2-yloxy)-2H-pyran-3,4,5 triol], compound-2 [dihydroxy-2-(3, 4, 5) trihydroxyphenyl) chromenylium-2-4) methoxy-6-methyloxane-3, 4, 5 triol] and compound-3 [6- methoxycyclohexane-1,2,3,4,5-pentaol]. The structures of the compounds were elucidated by means of ^{1}H-NMR, ^{13}C-NMR and DEPT-135 spectral data and comparison with literature reports.
Keywords: chemical constituents; *Ficus ingens (Miq).Miq.*; methanol;
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Introduction

Ficus ingens commonly called fig is a plant of high medicinally values possessing many of the biological activities like antibiotic and analgesic wide spread in northern and eastern sub-Saharan Africa. with a more or less contiguous range from Senegal in the west, eastwards to Eritrea and southwards to the Eastern Cape, South Africa [1]. It is found on rock faces and outcrops, rocky slopes, riparian and wade fringes, and in dense woodlands Substrates include lava flows, coral and limestone in drier, exposed areas and sandstone or dolomite in bushveld [2]. In northern Nigeria the figs, of and in Kenya the leaves and figs, of *F.ingens* have been recorded as famine food [3]. In South Africa a decoction of the bark mixed with cow feed is said to increase the flow of milk [18], though the leaves have been shown to be toxic to cattle, and sometimes to sheep [4]. When ripe, the figs are readily eaten by several species of bird [5]. *Ficus in genes*is have different specious and many active compounds were isolated from this different specious for instance *Benghalensis* bark; 20-tetratriaconthene-2-one, 6 heptatriacontene-10-one, pentatriacontan-5-one, β-sit sterol, β-d-glycoside and memo inositol In addition, the fruit extract of *F. benghalensis* exhibited antitumor activity , while the methanol extract of *F. benghalensis* possesses antioxidant. *F. sycomorus* extracts are used in Folk medicine in the treatment of infertility and sterility in human. *Ficus capensis* extract was used for treatment of a zoosperm. *Ficus asperifolia* extract has been reported to have an estrogenic effect in female rats [6]. The chemical constituents and hepatoprotective effect of whole plant of *F. ingens* (Miq.)Miq.(Moraceae) extract against carbon tetrachloride-induced acute liver damage in male Wistar albino rats. SC injection of CCl_4 to rats showed significant elevation of liver marker enzymes (ALT, AST, ALP and LDH) in their serum after 24 h of intoxication. The ethanol extract of *F. ingens* to take different doses 100,200&400 mg/kg. The results showed 400 mg/kg is significant [7].

Materials and Methods

Plant Collection and Identification

The bark of *Ficus ingens* (Miq.)Miq. was collected from *Amuru* village, *Horro Guduru, Wellega* Zone, Oromia Region Ethiopia, which is 383 km west of Addis Ababa. The plant was identified by prof. Legesse Negash and specimen was deposited at the National Herbarium (Voucher Diriba Borena 001/2015) in the department of biology, Addis Ababa University.

Experimental procedures

Extraction

The collected plant material was cut in to smaller pieces to facilitate drying and dried under room temperature. The dry plant material was taken separately and grinded to a uniform size using an electric grinder. The pulverized powder (250 g) was successively extracted by maceration with 1000 ml n- hexane, dichloromethane, ethyl acetate, and methanol for three consecutive days each at room temperature. The extracts were filtered using WhatmanNo.1 filter paper (150 mm) and evaporated under vacuum to obtain the respective crude extracts.

Isolation of compounds

The separation, isolation and purification of compounds was carried out by a gravity column chromatography using Merck silica gel 60 (0.040-0.063 mm) and monitored by thin layer chromatography (TLC; Merck 20 × 20 cm silica gel 60 F254 aluminum sheets). The extracts were crudely separated on a 4 cm diameter column using appropriate solvent systems which gave the best separation on TLC. Fraction sizes of 100 mL each were collected. The elution progress of each fraction and sub fractions was monitored by TLC using n-hexane, EtOAc, dichloromethane, chloroform, methanol and mixtures of different solvent system by increasing polarity of solvent depending on their TLC. Visualization of the chromatogram was achieved by spraying of appropriate reagents.

Spectroscopic analysis

Pure fractions from column chromatography were characterized by using IR, UV and nuclear magnetic resonance (^{1}H-NMR ^{13}C-NMR, DEPT and the spectra were recorded in $CDCl_3$ and DMSO-d_6 with Tetramethylsilane (TMS) as internal standard. Complete structure determination was achieved by comparing the IR and NMR data obtained with that in literature.

Isolation of compounds form methanol extract of Ficus ingens

4.5 g of MeOH extract was adsorbed on silica gel and charged on to column packed with silica gel using chloroform. The column was eluted using methanol: chloroform (as shown in Table 1)

Table 1: The fraction that collected using methanol: chloroform

Fractions	Solvent	Ratio	Yield of each fraction	Sample code
1-2	Chloroform	100%	10.12 mg	F-1 and F-2are discarded
3-10	MeOH:$CHCl_3$	1:9	30.10 mg	F-3
11	MeOH:$CHCl_3$	2:8	21.04 mg	F-11
12-13	MeOH:$CHCl_3$	3:7	14.16 mg	F -12
14	MeOH:$CHCl_3$	5:5	31.31 mg	F-14 Compound 2
15-17	MeOH:$CHCl_3$	6:4	0.25 mg	F-15
18-19	MeOH:$CHCl_3$	7:3	25.31 mg	Compound-3(F-18)
20	MeOH:$CHCl_3$	7:3	31.32 mg	Compound-2(F-20)
21—23	MeOH:$CHCl_3$	8:2	19,11 mg	F-23
24-30	MeOH:$CHCl_3$	8:2	27.13 mg	Compound-1 (F-24)
31-44	MeOH:$CHCl_3$	9:1	18.00 mg	F-31
45-60	MeOH:$CHCl_3$	100%	40.01 mg	F-60

Fractions 3-10 eluted with methanol: chloroform ratio (1:9, V/V) afforded compound 1 with a 1:1 (V/V) methanol: chloroform elution. Another fraction, F-14 was obtained which yielded compound 2 or F-14. Methanol: chloroform - (7:3) elution afforded compound 3, F-18.

Characterization of the compounds

Characterization of compound 1

Compound 1 was obtained as a white crystal from fractions F-20 and F-24. The ^{1}H-NMR (400 MHz, $CDCl_3$) of compound 1 depicts signals which appear at δ_H 5.24, 5.17, 5.09 , 3.77, 3.38 and 4.54 that were doublets, the signals which appeared at δ_H 4.86, 4.41, 3.51and 3.11 were multiples and the signals at δ_H 3.89 and 3.63 that were triplets (see Table 2).

Table 2: ^{1}H-NMR (400MHz, $CDCl_3$) spectral data of compound 1

Hydrogen atoms	^{1}H-NMR δ (ppm)
1	3.77 (d,2H)
2	-
3	3.38(d,2H)
4	4.54
5	5.09(d,2H)
6	3.89(t,3H)
7	5.09 (d,2H)
8	4.86 (m, 3H)
9	4.41 (m,3H)
10	3.51(m,3H)
11	5.24 (d,2H)
12	3.63(t,3H)

The ^{13}C-NMR spectrum of compound 1and its DEPT-135 showed a well resolved resonance of 12 carbon atoms,

indicated the presence of one di-oxygenated quaternary carbons(Cq) at δ_C 104.44 and three oxy-methylene carbons (CH_2-O) at δ_c 62.45, 62.57 and 60.89 . The spectrum showed presence of eight oxy-methine carbon signals at δ_c 77.40, 72.05, 82.97, 92.18, 74.68, 73.29, 70.24, and 77.40 are shows CH carbons. Table 3

Table 3: ^{1}HNMR^{13}C NMR and DEPT-135 for Compound 1

No	^{13}CNMR δ (ppm)	DEPT-135 δ (ppm)	Remark
1	62.45	Down	CH_2
2	104.44	-	Quaternary
3	77.40	Up	CH
4	72.05	Up	CH
5	82.97	Up	CH
6	62.57	Down	CH_2
7	92.18	Up	CH
8	74.68	Up	CH
9	73.29	Up	CH
10	70.24	Up	CH
11	77.40	Up	CH
12	60.89	Down	CH_2

Based on its ^{1}H-NMR, ^{13}C-NMR and DEPT-135 data, the compound 1 was identified as tetrahydro-2-(hydroxymethyl)-6-(terahydro-3,4-dihydroxy-2,5-bis(hydroxymethyl)fura-2-yloxy)-2H-pyran-3,4,5 triol. Figure 1

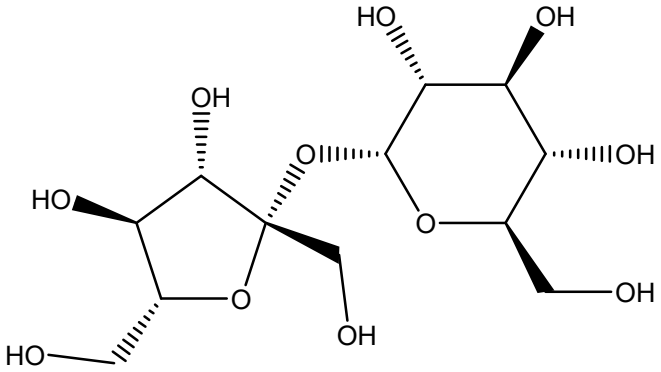

Figure 1: The proposed structure of compound 1

Characterization of compound 2

Compound 2 (F-14) 31.31 mg was isolated using column chromatography from polar fraction as a yellow crystal. TLC analysis by methanol: chloroform (5:5) showed a single spot with R_f = 0.7 staining yellow under Uv light. This compound was highly polar and ^{1}H- NMR, ^{13}C-NMR and DEPT-135 spectral data of the compound is listed in table 4.

Table 4: ^{13}C-NMR and DEPT-135 for Compound 2 (F-14)

No	^{13}C-NMR(ppm)	DEPT-135	Remark
1	20.3	Up	CH_3
2	29.20	Up	C-H
3	29.50	Up	CH
4	54.23	Up	CH
5	55.63	Up	CH
6	56.27	Up	CH
7	57.33	Up	CH
8	63.19	Up	CH
9	63.43	Up	CH
10	63.85	Up	CH
11	65.54	-	Quaternary
12	68.55	Down	Oxy methylene
13	71	Up	CH
14	72	Up	CH
15	73	Up	OH
16	74	Up	CH
17	85	Up	CH
18	92	Up	CH
19	96	Up	CH
20	103	Up	CH
21	104.95		Quaternary
22	109	Up	CH
23	112	Up	= CH
24	114	Up	
25	138.08	-	Quaternary
26	152.88	-	Quaternary
27	171	-	Quaternary

The ^{13}C-NMR and DEPT-135 indicates that compound 2 (F-14) has 27 carbon atoms. The spectra showed at C_δ 20.31 oxy methyl carbons, the spectra showed at δ_c 65.54, 104.95, 138.08, 152.88, and 171.89 are shows quaternary carbons. The spectra showed at δ_c 63 shows CH_2.

In the IR (KBr) spectrum, the absorption at 3408 cm^{-1} shows the presence of alcohol and the absorption at the 2924 cm^{-1} showed the of alkane CH_3. A strong absorption and at 1723 cm^{-1} indicated the presence of ester functional group. And a week band at 1621 cm^{-1} showed the presence of alkene C=C stretch. The presence of absorption bands 1078 cm^{-1} illustrated C-O stretches of ester functional group, a band at 625 cm^{-1} showed the presence of tri substituted double bond.

Based on the above NMR and IR spectrum data the structure was proposed for the compound 2 (F-14) was identified as dihydroxy-2-(3, 4, 5) trihydroxyphenyl) chromenylium-2-4) methoxy-6-methyloxane-3, 4, 5 triol. Figure 2

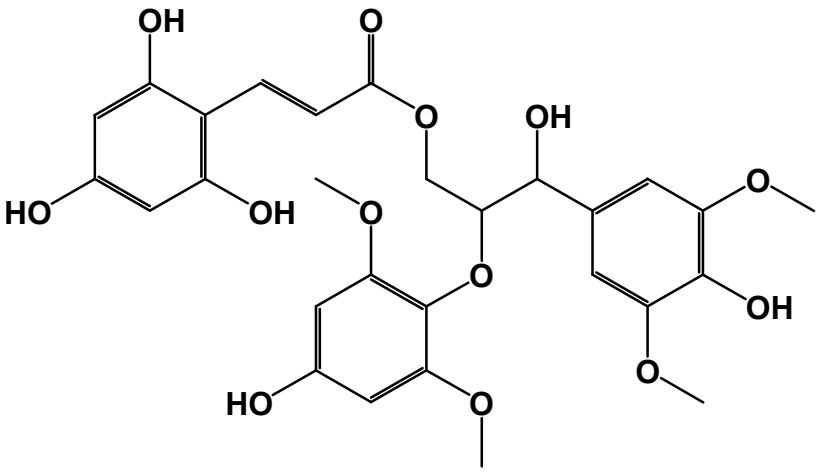

Figure 2: The proposed structure of compound 2

Characterization of compound 3

The ^{1}H-NMR, spectral data of compound 3 depicts the signals appearing at δ_H 4.61, 4.33, 3.77, 3.62, 3.41, 3 and 3.21 as multiples. The signal at δ_H 5.1 is appeared as a singlet. Table 5

Table 5: ^{1}H-NMR (400MHz, $CDCl_3$) spectral data of compound 3

Hydrogen atoms	^{1}H-NMR δ (ppm)
1	3.77 (m,1H)
2	3.4(m,1H)
3	3.21(m,1H)
4	3.6(m,1H)
5	4.3(m,1H)
6	5.12(s,3H)
7	4.6 (m,1H)

The singlet signal appears at δ_H 5.12 indicates the presence of methyl group bearing 3 proton attached to oxy-carbon. The multiple signals at δ_H 4.61, 4.33, 3.77, 3.62, 3.41, 3 and δ_H 3.21 are six methine each integrated to one proton in a ring.

^{13} C-NMR and DEPT-135 Table 8 indicate that Compound 3 has 7 carbon atoms. The spectra showed at δ_C 81.37 an oxy-methine signal CH-O and at δ 57.44 methyl carbon signal. Further, the spectra showed five oxy-methine signals at δ 68.25, 70.93, 72.58, 72.37, and 73.69.

Table 6:^{13}C-NMR and DEPT-135 for Compound 3

No	^{13}C-NMR δ (ppm)	DEPT-135 δ (ppm)	Remark
1	68.25	Up	CH-O
2	70.93	Up	CH-O
3	72.58	Up	CH-O
4	72.37	Up	CH-O
5	73.69	Up	CH-O
6	81.37	Up	CH-O
7	57.44	Up	-OCH_3

Based on the above ^{1}H-NMR and ^{13}C-NMR spectrum data the structure was proposed for compound 3, which is 6-methoxycyclohexane-1,2,3,4,5-pentaol. Figure 3

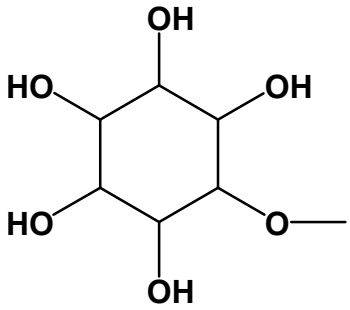

Figure 3: The proposed structure of compound 3

Conclusion

This work resulted in the isolation of three new compounds that is not isolated from the plant in previous study, the compound was coded as compound 1: tetrahydro-2-(hydroxymethyl)-6-(terahydro-3,4-dihydroxy-2,5-bis(hydroxymethyl)fura-2-yloxy)-2H-pyran-3,4,5 triol, compound 2: (F-14) was identified as dihydroxy-2-(3, 4, 5) trihydroxyphenyl) chromenylium-2-4) methoxy-6-methyloxane-3, 4, 5 triol and compound 3: 6-methoxycyclohexane-1,2,3,4,5-pentaol. The structure of the compound was characterized on the basis of spectral data (^{1}H-NMR, ^{13}C-NMR, DEPT-135 and IR) as well as comparison with the literature data.

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