SOME POLYHYDROXY COMPOUNDS FROM ETHYL ACETATE AND ALCOHOL EXTRACTS OF *FICUS ITTEOPHYLLA*

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ABSTRACT

The polyhydroxy compounds, glycerol, mannitol, glucose, quinic acid and lactic acid were identified from the ethyl acetate and alcoholic extracts of the plant Ficus itteophylla. These compounds were analysed by 3-part TLC with authentic samples, 1D and 2D-NMR. Their acetates were also investigated.

INTRODUCTION

The *Ficus* species belong to the family **Moracea** which consists of trees, shrubs and a few woody herbs found predominantly in the tropics. Some species of *Ficus* show a remarkable development of aerial roots, and they may start life as epiphytes. Epiphytic *Ficus* has an initial saprophytic mode of life.

Plants in the *Ficus* family have a lot of medicinal, food and other uses. Such include uses as fodder[1] , cure for diarrhoea[2], pile and haemorrhoids[3-4], treatment of diabetes[5-6], treatment of bacterial, microbial, fungal and viral infections[7-8]. Their use in the treatment of asthma and snake bites has also been reported[9]. The fig is an article of food in Southern Nigeria[10]

Many researchers have investigated and isolated various types of chemical compounds from different species of the genus *Ficus* . which may account for pharmacological actions, medicinal and other uses of these plants. Examples of such compounds are acids[11-12], terpenes[13], steroids[14-17], flavones and coumarins[18-19], sugars and their derivatives[20-23] and alkaloids[24-31]. The isolation and identification of hordenine and catechol from *F. itteophylla* have been reported[32]. In the search for more of the constituents of this plant, we identified the polyhydroxyl compounds glycerol, mannitol, glucose, quinic acid and lactic acid from the alcoholic extracts.

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EXPERIMENTAL

Equipment

All solvents used were redistilled on a vertical still and all NMR data were obtained from a 400 MHz Bruker NMR Spectrophotometer model ADVANCE DPX_{400}. IR spectra were obtained from Perkin-Elmer IR Spectrophotometer 1600 Series FT IR, with Hewlett Packard Color Pro Printer while GC-MS and UV spectra were respectively obtained from Hewlett Packard Gas Chromatographic Machine GC 5890 + TRIO-1 Mass Spectrometer by VG Lab Ltd., and CE660 Multimude Computing UV Spectrophotometer 6000 Series. TLC was carried out on 0.2 mm

layer of silica (Merck, Kieselgel $60F_{254}$) on aluminium sheets while column chromatography was carried out on silica gel (60-120 mesh size).

Extraction

Powdered bark of the plant *Ficus itteophylla* (600 g) was packed into a Soxhlet apparatus and extracted with water (3.5 L) for 7 days. The extract was concentrated on the rotary evaporator and dried under high vacuum to give a solid extract (yield 97.16 g, 16.2%). The extract (85.0 g) was packed into a thimble of a 500 cm^3 capacity Soxhlet extractor and extraction carried out using increasingly polar solvents; petroleum spirit, ethyl acetate, chloroform, acetone, ethanol and methanol (600 cm^3 each). Table 1 gives the results obtained.

Table 1:Solvents, percentage extraction and extract weight per 85 g of crude aqueous extract.

Solvent	*% Extraction*	*Extract weight (g 85 g)*
Petroleum spirit	0.05	0.04
Ethyl acetate	1.24	1.05
Chloroform	0.02	0.02
Acetone	1.62	1.38
Ethanol	14.15	12.03
Methanol	14.80	12.58

Analysis of ethyl acetate extract

Ethyl acetate extract (1.20 g) was purified by column chromatography on silica using petroleum spirit, ethyl acetate, chloroform, acetone and methanol as mobile phase thus increasing solvent polarity stepwisely. Five fractions were collected as follows: Fraction 1 (10 mg, R_f 0.90), Fraction 2 (150 mg, R_f 0.70), Fraction 3 (150 mg, R_f 0.65), Fraction 4 (150 mg, R_f 0.50) and Fraction 5 (440 mg, R_f 0.44, 0.30 and 0.20). Mobile phase for TLC was $CHCl_3$:acetone (80:20).

Fraction 5 was re-chromatographed and the main component on analysis gave δ_H (D_2O) 0.7-1.1 (m), 1.20 (d, *J* 6.9 Hz), 1.3-2.2 (m), 2.3 (m) 3.45 (dd *J* 11.8, 6.5 Hz), 3.5-3.95 (m), 4.08 (quartet *J* 6.9 Hz), 4.04 -4.5 (m), and 6.25-7.25 (m). 3-part TLC with authentic lactic acid, eluent $CHCl_3$:acetone (80:20), R_f 0.36 and ^{1}H-NMR spectra δ_H (D_2O) 1.21 (3H, d, *J* 6.9Hz), 4.01 (1H, quartet, *J* 6.9 Hz) δ_H (CD_3OD) 1.36 (3H, d, *J* 6.7 Hz), 4.18 (1H, quartet, *J* 6.6 Hz). Identical shifts and coupling constants were measured when fraction 5 was rerun in CD_3OD δ_H (CD_3OD) 0.8-1.1 (m), 1.28 (d, *J* 6.8 Hz), 1.7-3.0 (m), 3.2-4.0 (m), 4.05 (quartet, *J* 6.8 Hz), and 6.2-7.6 (m).

Analysis of EtOH extract

TLC of EtOH extract was carried out in a variety of solvent mixtures but there was no appreciable separation. The ^{1}H-NMR spectrum of the extract is δ_H (D_2O) 2.1 (d), 2.25 (d), 3-4 (m, α-hydroxyl protons), 7.25 (d, *J* 7.5Hz, *p*-substituted aromatic), and 7.55 (d, *J* 8.1 Hz, *p*-substituted aromatic). Action of Fehling's solution on an aqueous solution of this extract gave a brick-red precipitate of Cu_2O.

Acetylation

EtOH extract (5.0 g, 0.027 mol, based on hexose monosaccharide, 180 Daltons) was dissolved in pyridine (0.81 moles, 30 equiv., 64 g) and acetic anhydride (0.27 moles, 10 equiv., 27.5 g) was added dropwise into the stirred mixture over a period of 1hr. at 0 °C. The reaction was monitored by TLC until 3-part TLC with starting material indicated the completion of acetylation..

The reaction mixture was worked up and concentration gave a crude semi-solid (2.80 g, 56%). δ_H 2.08 (m, acetoxy protons), 2.22 (d), 2.40 (d), 3.1 (m), 3.75-4.8 (m),

4.9 (m), 5.1-5.3 (m), 5.4-5.6 (m), 5.7 (d) 7.33 (m), and 8.62 (s). Column chromatography of the sample was done on silica. Elution commenced with neat petroleum spirit with the polarity increased at a fast rate (25% increments) to chloroform, then at a slow rate (5% increments) to acetone and finally MeOH. Five fractions were collected as follows: Fraction 1 (10 mg, R_f = 0.80 and 0.76), Fraction 2 (20 mg, R_f 0.76 and 0.74), Fraction 3 (1530 mg, R_f 0.75 and 0.65). Fraction 4 {40 mg, R_f 0.56 (streaking)} and Fraction 5 (600 mg, R_f 0.45, 0.30, & 0.20). Mobile phase was $CHCl_3$:acetone (80:20). Two of these fractions are reported below.

Fraction 2: Spectra analysis gave δ_H 2.08 (m, acetoxy methyl groups), 2.20 (m), 3.85 (m, H-5 proton of β-glucose pentaacetate), 4.12 (m), 4.28 (m + d, *J* 2.7Hz), 5.12 (m), 5.24 (t), 5.5 (d, *J* 2.3 Hz), 5.72 (d, *J* 8.3Hz, H-1 proton of β-glucose pentaacetate), 5.84 (d), 6.35 (d, *J* 3.7 Hz, H-1 proton of α-glucose pentaacetate). 5-part TLC with β-glucose pentaacetate and α-glucose pentaacetate, R_f 0.22 in petroleum spirit:Et_2O (1:1). This integration ratio together with TLC result shows the presence of a 1:1 mixture of β- and α-glucose pentaacetate.

Fraction 3: Spectra analysis gave δ_H 2.00 (m, 9H, $3CH_3C{=}O$) 2.24 (s), 3.64 (m), 3.75 (m), 4.18 (2H, dd, *J* 12.0, 6.0Hz, H-1& H-3 triacetin), 4.32 (2H, dd, *J* 12.0, 4.3 Hz, H-1′& H-3′ triacetin), 5.18 (1H, tt, *J* 5.8, 4.4 Hz, H-2 triacetin), and 7.1-7.2 (m). The 2D-^{1}H-^{1}H *J* correlated NMR of this fraction indicates the correlation between the major peaks in the 1D-NMR plot which suggests the presence of propane-1, 2, 3-triacetate.(triacetin) **GC-MS DB** 17 [49 °C (4min.) x 15 °C /min, 325 °C (10 min)] RT 13.18 (propane-1, 2, 3-triacetate) 158 (3%), 145 (55%), 115(35%), 103 (65%), 43 (100%). 3-part TLC with an authentic sample of triacetin in 20% acetone in $CHCl_3$ gave a single spot at R_f 0.35.

Analysis of MeOH extract

TLC of this extract was done in a variety of solvent systems including n-BuOH: acetic acid: water (4:1:5) but no separation was achieved. The ^{1}H-NMR analysis of this fraction gave δ_H (D_2O)1.20 (dd, *J* 13.5, 10.9Hz), 1.70 (dd, *J* 13.5, 10.9 Hz,), 1.85 (dd, *J* 3.7, 2.9 Hz), 1.95 (m), 2.4 (s), 2.75 (s), 3.42 (dd, *J* 9.3,3.1 Hz), 3.3-3.8 (m), 4.0 (quartet, *J* 3.4 Hz), 4.5 (d, *J* 7.9 Hz), 5.12 (d, *J* 3.5 Hz), 6.75 (d, *J* 8.4 Hz), 6.85 (d, *J* 2.6Hz, d, *J* 8.0 Hz), 7.1 (d), and 7.3 (d). When a solution of the extract in water was treated with Fehling's solution and heated for 15 minutes in a water bath, a brick-red precipitate of Cu_2O was formed.

Acetylation

MeOH extract (2.0 g, 0.011 mol, based on hexose monosaccharide, 180 Daltons) was dissolved in pyridine (26.33 g, 0.33 mol, 30 equiv.) and acetic anhydride (12.2 g, 0.12 mol, 10 equiv.) were mixed at room temperature. The mixture was stirred at room temperature, followed by 3-part TLC which ascertained the completion of acetylation after 3 days. The reaction mixture was worked up to give the product (0.60 g, 30% based on extract weight). The ^{1}H-NMR gave δ_H 1.20 (m), 2.0 (acetoxy methyl protons), 2.20 (m), 2.48 (d), 2.6 (d), 3.0 (m), 3.35-4.35 (m), 4.8 (m), 5.1 (m), 5.4 (m), and 5.6 (d). 2D-NMR of the sample shows a lot of correlations between the groups. The sample was purified by flash column chromatography in a silica-gel column. Elution started with neat petrol and the polarity was increased stepwisely by addition of Et_2O, then $CHCl_3$ and finally acetone. Five main fractions were collected as follows: Fraction 1 (< 0.01 g, R_f 0.82), Fraction 2 (0.01 g, R_f 0.80 and 0.60),

Fraction 3 (< 0.01 g, R_f 0.42 and 0.30), Fraction 4 (0.01 g, R_f 0.30) and Fraction 5 (0.48 g, R_f 0.05). Mobile phase was petroleum spirit:$CHCl_3$ (1:1). Fractions 4 and 5 were analysed.

Fraction 4: δ_H 2.0 (m, acetoxy methyl protons), 2.02-2.28 (m), 2.30 (s), 3.6-4.0 (m), 4.08 (dd), 4.20 (dd), 5.02 (m), 5.12 (s), 5.6 (d), 6.57 (m), 6.85 (dd), 6.95 (dd), and 7.07 (m). 3-part TLC was carried out with authentic mannitol hexaacetate in EtOAc:petroleum spirit (6:4), a single spot was obtained at R_f 0.58.

Fraction 5: This is the main fraction from this column (480 mg). δ_H 1.2 (m), 2.05 (m for acetoxy groups), 2.27 (d), 2.55 (d), 2.78 (d), 3.1 (m), 3.7 (m), 4.02-4.37 (m), 4.88 (m), 5.12 (m), and 5.5 (m). TLC indicated that sample was a mixture, therefore it was run through a silica-gel column using n-hexane, acetone and MeOH as mobile phase increasing the polarity stepwisely from n-hexane to MeOH. Five fractions were obtained from this column. They were labelled as Fraction F5.1 (< 0.01 g, R_f 0.80), Fraction F5.2 (0.12 g, R_f 0.6), Fraction F5.3 (0.13 g, R_f 0.50), Fraction F5.4 (0.13 g, R_f 0.29, and 0.22) and Fraction F5.5 (0.08 g, R_f 0.01) in hexane:$CHCl_3$ (1:1) mobile phase.

Fraction 5.2 was analysed as follows: TLC of this fraction in a variety of solvents indicated the presence of one main component. δ_H 2.0 (15H, 5 x CH_3CO), 2.2 (2H, d, *J* 9.6Hz, **f**), 2.5 (1H, d, *J* 11.6Hz, **e**), 3.05 (1H, dd, *J* 11.5Hz, 5.6 Hz, **d**), 4.8 (1H, dd, *J* 6.2, 5.8 Hz, **c**), 5.12 (2H, complex separations, *J* 4.5-5.0 Hz, **2b**), and 5.43 (1H, t, *J* 4.7 Hz, **a**); a - f are explained under "Discussion". All assignments were for quinic acid. 2D-NMR of this sample was run and it gave the correlations between the hydrogen atoms. **GC-MS** DB-17 [49 °C (4 min.), x 15 °C/min, 325 °C (10 min)], RT (min) 18.68 m/z (EI+) 301 (43, M-59, M- CH_3COO ?), 242 (5), 199 (3), 110 (35, PhCOOH), 94 (100, PhOH).

RESULTS AND DISCUSSION

Aqueous extract of the bark of the plant *F. itteophylla* is used in Northern Nigeria in the treatment of pile, dysentery and heamorrhoids[1]. Five fractions were collected. Fraction 5 obtained from the column of the ethyl acetate portion on analysis was found to be lactic acid. The presence of lactic acid in this extract was confirmed by both 3-part TLC and from comparison of the ^{1}H-NMR of the unknown and standard lactic acid in D_2O and CD_3OD. A comparison of the chemical shifts is shown in Table 2. The $^3J_{1,2}$ coupling constant in D_2O was 6.9 Hz for both the standard and the unknown, whereas in CD_3OD, the value was 6.7 Hz for the standard and 6.8Hz for the unknown. The experimental uncertainty is ± 0.375 Hz. The ^{1}H-NMR in D_2O spectrum contains a doublet at δ 4.0 and quartet at δ 1.15.

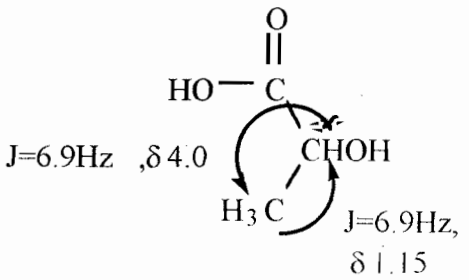
Table 2: Chemical shift comparisons for lactic acid standards and unknowns

	D_2O		CD_3OD	
	Standard	Unknown	Standard	Unknown
2-H	4.01	4.10	4.15	4.05
3-H	1.21	1.20	1.36	1.28
Δδ	2.80	2.80	2.79	2.77
ΔΔδ	0		0.02	

The doublet is due to coupling between the **-CH_3** and the H-atom of the α- H attached to the carboxyl group, while the quartet is due to the coupling of the α-H and the-**CH_3** group. The resonance positions are at low fields due to the electron- withdrawing effects of the oxygen atoms of the carboxyl groups.

$H_3C.CHOH\ COOH.$

Lactic acid.



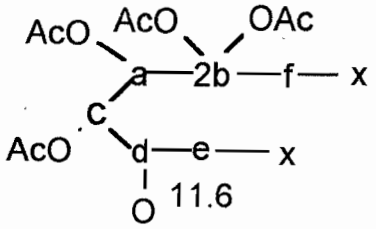
The ethanol extract in D_2O gave a ^{1}H-NMR spectrum with complex multiplets in the range δ 3-4 which could not be interpreted individually, but were assigned as protons adjacent to hydroxyl groups. The presence of anomeric protons was clearly shown by a pair of doublets (δ 5.22 Hz, *J* 4.1Hz; δ 4.43, *J* 8.0 Hz) on either side of the residual water peak. The large coupling in the upfield multiplet can only be due to a *trans*-diaxial relationship between the protons at C1 and C2. This suggested that these signals could be attributed to β-glucose and that the downfield peak was due to α-glucose. The mixture was acetylated and separated by column chromatography. ^{1}H-NMR of the third fraction revealed a mixture of compounds with signals at δ 6.35 (*J* 3.6 Hz) and δ 5.75 (*J* 8.3 Hz). These signals are shifted downfield relative to those in the underivatised sugar indicating that the anomeric positions were acetylated. Comparison with spectra[33] for authentic α-glucose peracetate and β-glucose peracetate confirmed the assignment.

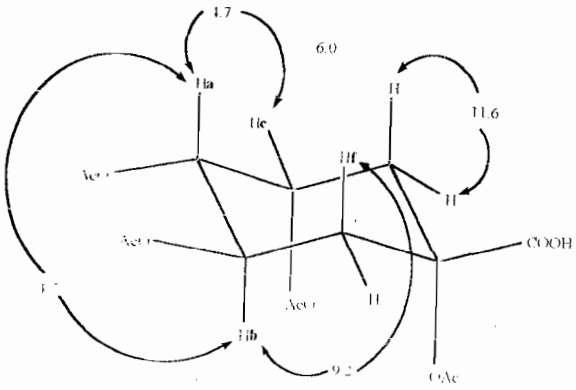
The next fraction from column chromatography of the acetylated extract gave a ^{1}H-NMR spectrum with signals at δ 4.18 and 4.3. These were both doublets of doublets (*J* 12.0, 6.0 Hz and *J* 12.0, 4.3 Hz respectively). The large coupling constant indicates that these protons must be geminal. There were two singlets at δ 2.07 and 2.08; ratio 2:1 respectively. The chemical shifts of the doublet of doublets indicated that they were adjacent to acetylated hydroxyl groups. There was also a complex signal at δ 5.18 (tt *J* 5.8, 4.4 Hz) which integrated to half a proton relative to each of the doublet of doublets. 2D - *J* resolved COSY -^{1}H-NMR showed correlation between peaks at δ 5.25, 4.22 and 4.18. This indicates a plane of symmetry in the molecule and the simplest interpretation consistent with the data was propane-1,2,3-triacetate (triacetin, the acetyl derivative of glycerol). The MS gave a base peak at m/z 158 which is consistent with the loss of CH_3COOH from the triac[illegible] molecular ion. The assignment was confirmed by comparison with authentic material[34]. Hence, ethanol extract contains both α– and β-D-glucose, and propane-1, 2, 3-triol.

The polyhydroxy compounds identified from the MeOH extract includes α- and β-D-glucose, quinic acid, and mannitol. Mannitol was identified by a comparison of position and shape of peaks in the ^{1}H-NMR spectra of the crude extract in D_2O with those of authentic sample in the same solvent and as an acetyl derivative from the fractions obtained on acetylation of crude MeOH extract. The ^{1}H-NMR of the fraction has features similar to that of authentic mannitol hexaacetate. Worthy of note are the doublets at δ 4.08 and 4.20 which on the standard are at 4.05 and 4.22 respectively. 3-part TLC between the authentic mannitol hexaacetate and this fraction in petroleum spirit:EtOAc (6:4) have the same R_f-value at 0.55 which also confirms the presence of mannitol hexaacetate.

The second fraction from the column fractions of the acetylated MeOH extract was found to be quinic acid tetraacetate. The ^{1}H-NMR chemical shifts and coupling constant values for identified protons (a - f) are illustrated as shown . 2D-NMR of this sample was run and it gave the correlations between the hydrogen atoms. Both the 1D

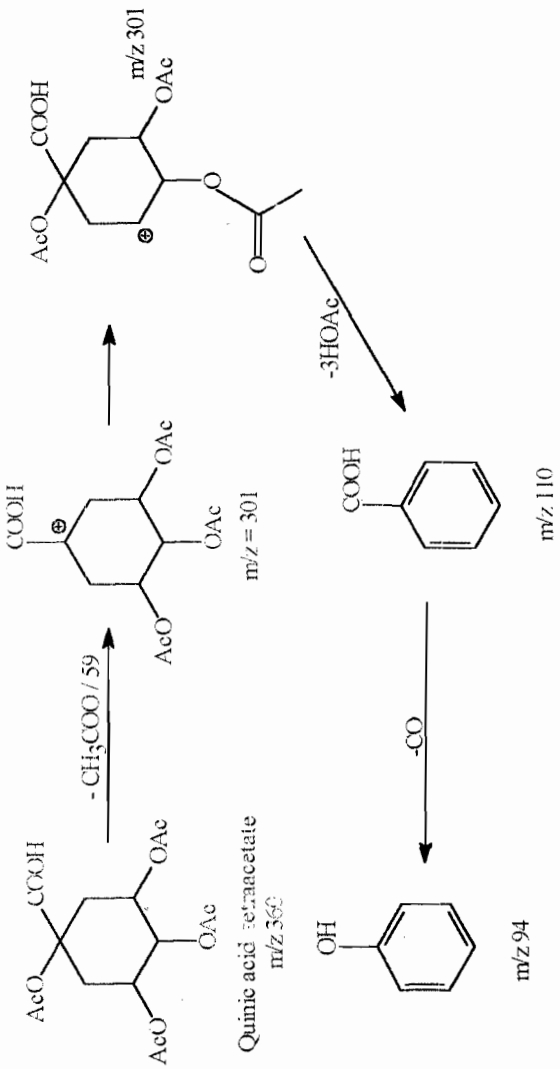
and 2D NMR spectra were run in C_6D_6 to improve the dispersion of the peaks. Both spectra show the correlations as on the $CDCl_3$ spectra.





To further analyse the structure, DEPT (i.e. Distortionless Enhancement by Polarisation Transfer) and ordinary carbon-13 spectra were run. These spectra indicate the presence of one 4^{o} carbon atom, three CH carbons, and two CH_2 carbons together with the acetate groups. Also, the carbon-hydrogen correlation spectrum was run and it gave results consistent with the presence of 6 carbon atoms in which three are CH, two are CH_2's and one quaternary carbon. On analysis of all these spectral data, it was concluded that the compound present in this fraction is quinic acid tetraacetate.

GC-MS DB-17 49 °C (4 min.), x 15°C/min, 325 °C (10 min) RT (min) 18.68 m/z (EI+) 301 (43, M-59, M-CH_3COO ?), 242 (5), 199 (3), 110 (35, PhCOOH), 94 (100, PhOH). This data is consistent with the following fragmentation, indicating a molecular ion peak less $CH_3CO_2^-$ fragment at **m/z** 301 and fragments at 110 (PhCOOH) and 94 (PhOH) which are characteristic of quinic acid tetraacetate fragmentation pattern.



Glycerol (propane-1, 2, 3-triol) is identified from the EtOH and MeOH extracts of *F. itteophylla*. Glycerol is used as a solvent, humectant, emollient, sweetener, in cosmetics, liquid soaps, in confectionery, as a shock absorber fluid, and as a fermentation nutrient in the production of antibiotics. Therapeutically, it is used as a

diagnostic aid in ophthalmology and pharmaceutical aid as humectant solvent

Mannitol is soluble in glycerol (1 g dissolves in 18 ml of glycerol) and it is not surprising that both of them are found together in the EtOH and MeOH extracts. In pharmacy, mannitol is used as an excipient and diluent for liquids and solids. In the food industry, it is used as anticaking and free-flow agent, flavouring agent, lubricant and release agent, stabilizer and thickener and nutritive sweetener. It is therapeutically classified as diuretic and diagnostic aid in renal function[36]. Hence, the presence of mannitol in the plant extract may help in lubrication, making the flow easier in the colon therapy for relieving haemorrhoids and dysentery.

The MeOH extract of the plant is found to contain quinic acid which was identified as the tetraacetate. Quinic acid is soluble in water, alcohol and glacial acetic acid. It is found in *Cinchona* bark, tobacco leaves, carrot leaves, apples, peaches, pears, plum, etc. It is a food acidulant with good taste characteristics but its use is limited by cost[37].

CONCLUSION

Mannitol, glycerol, α- and β-D-glucose, lactic acid and quinic acid were found in the ethyl acetate and alcoholic extracts of *Ficus itteophylla*.

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