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Isolation and Characterization of Triterpenoid Saponins from *Ficus natalensis* subsp. *leprieurii* Leaves

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Abstract: *Ficus natalensis* subsp. *leprieurii* also known as the natal fig is a fruit-producing tree belonging to the family Moraceae and widely distributed in African countries and cultivated in Egypt. *F. natalensis* is common with a myriad of traditional and medicinal importance. Owing to the increased demand for natural products with new structural compositions, the current study aimed to separate and elucidate the structure of triterpenoid saponins from *F. natalensis* leaves. Our previous biological investigation of *F. natalensis* leaves revealed its potent antioxidant and anti-inflammatory characteristics, and its ability to alleviate Cd-induced reproductive toxicity. Phytochemical investigation of *F. natalensis* leaves revealed the separation and structure elucidation of seven (**1–7**) compounds belonging to triterpenoid saponins using NMR and MS data and identified for the first time in *F. natalensis*. The isolated compounds were identified as 3-*O*-β-D-glucopyranosyl (1 → 4) β-D-glucopyranosyl (1 → 4)–α-L-rhamnopyranosyl-quinovic acid-28-*O*-β-D-glucopyranosyl (1 → 4)–α-L-rhamnopyranosyl (1 → 2)–α-L-arabinopyranoside ester (**1**), 3-*O*-β-D-glucopyranosyl (1 → 4) α-L rhamnopyranosyl-quinovic acid-28-*O*-β-D-glucopyranosyl (1 → 4)–α-L-rhamnopyranosyl (1 → 2) α-L-arabinopyranoside (**2**), 3-*O*-β-D-glucopyranosyl–quinovic acid-28-*O*-β-D-glucopyranosyl ester (**3**), as 3-*O*-α-L-rhamnopyranosyl-quinovic acid-28-*O*-β-D-glucopyranoside ester (**4**), 3-*O*-β-D-glucopyranosyl oleanolic acid (**5**), 3-methoxy-oleanolic acid-28-*O*-α-L-rhamnopyranoside (**6**), and 3-*O*-α-L-rhamnopyranosyl-oleanolic acid-28-*O*-β-D-glucopyranoside ester (**7**). Among the identified compounds, compounds **1** and **2** were identified for the first time in nature according to Reaxys and Web of Science database.

Keywords: *Ficus natalensis*; natal fig; Moraceae; phytochemical investigation; triterpenoid saponins

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1. Introduction

Recently, natural product specialists have made great efforts towards the identification of novel natural products with potential safety and biological activities. Plants are the main source of secondary metabolites including alkaloids, flavonoids, saponins, steroids, and terpenoids with myriad of medicinal and health value that can protect humans from several diseases [1]. Triterpenoid saponins are an important metabolite class abundant in several plant species with a myriad of biological importance [2]. Moraceae (the fig family) is a family of flowering plants comprising about 38 genera and over 1100 species and widely distributed in tropical and subtropical regions [3]. The *Ficus* genus including about 850 species is considered as one of the most important members of Moraceae and is generally known as fig trees or figs [4]. Several *Ficus* species have been reported for their traditional importance as widely used in the treatment of constipation, rheumatic disease,

dyspepsia, and dysentery, besides their hypoglycemic, anthelmintic, and antihypertensive effects [4]. *Ficus natalensis* subsp. *Leprieurii* is an evergreen tree widely distributed in African countries and used traditionally alone or mixed with other medicinal plants to treat several ailments such as colds, cough, sore throat, and dysentery [5]. Several phytochemicals were reported from *F. natalensis* belonging to different classes including phenolics, triterpenoids, steroids, and volatiles [6,7]. Triterpenoid saponins were previously reported in the *Ficus* genus providing several biological importance for *Ficus* species [7]. Moreover, biological studies on *F. natalensis* leaves revealed an anti-microbial effect [8], and antibacterial activity [9]. Our previous biologically guided study on *F. natalensis* leaves revealed its potent antioxidant, anti-inflammatory, and effect to alleviate cadmium chloride testicular disruptions [5,10]. Moreover, three triterpenoid saponins with oleanolic acid aglycone were previously isolated from *F. natalensis* leaves [10]. Triterpenoid saponin with quinovic acid aglycone was previously isolated from *Ficus elastica* aerial root bark [11]. Hence, the main goal of this study was to separate, purify, and elucidate the structure of triterpenoid saponins from *F. natalensis* leaves.

2. Materials and Methods

2.1. Plant Material

Ficus natalensis subsp. *leprieurii* (Miq.) C.C. Berg leaves were gathered in September 2020 from the Horticultural Research Institute, Giza, Egypt. The botanical identification of the plant was supplied by Prof. Dr. Rim Hamdy, Taxonomy Department, Faculty of Science, Cairo University, Egypt. A voucher specimen was deposited at the herbarium of Pharmacognosy Department, Faculty of Pharmacy, Egyptian Russian University, Cairo, Egypt number (01 Fnal/2019).

2.2. General Experimental Procedures

NMR analysis was carried out using Bruker High-Performance Digital FT-NMR Spectrometer Advance III 400 MHZ for 1H & C 13. All samples were analyzed in DMSO-*d6* and TMS was used as an internal reference, with the chemical shifts expressed in ppm and coupling constants (*J*) in Hertz, (The analysis was done in the NMR unit at the Faculty of Pharmacy, Cairo University, Egypt). The mass analysis was carried out on a Direct Inlet part to the mass analyzer in Thermo Scientific GCMS model ISQ at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Nasr City, Cairo. The mass spectroscopy system was used to explore the characteristic fragmentation using EI mode and the expected molecular weight at 70 ev. Glass columns packed with silica gel are used for chromatographic separation. A glass column (60 × 2.5 mm) packed with Sephadex LH-20 was used for compound purification. Silica gel 60 F254 precoated aluminum sheets (20 × 20, 0.2 mm, thickness) and (E. Merck, Darmstadt, Germany) for thin layer chromatography. Spots were visualized by ethanol/H_2SO_4 and vanillin/H_2SO_4 spray reagents. Solvent system S1 (chloroform–methanol–water (7:2.5:0.5 *v*/*v*/*v*)) was used as a solvent system for TLC. $CHCl_3$ and then a $CHCl_3$/MeOH mixture were used as solvent systems for compound elution on column chromatography.

2.3. Extraction and Fractionation

2.3.1. Extraction and Fractionation of *F. natalensis* Leaves

The air-dried ground powdered leaves of *F. natalensis* (2.5 Kg) were macerated in 100% methanol with occasional stirring at room temperature till exhaustion. The methanol extract was concentrated and dried under vacuum (45 °C) to yield a dry total extract (340 g). The dried total extract (250 g) was suspended in water (350 mL) and subjected to defatting through fractionation with petroleum ether (PE) to remove the lipophilic compounds and chlorophyll to yield petroleum ether fraction (44 g) and the defatted aqueous phase. The defatted aqueous phase was further fractionated using solvents with different polarities including chloroform and *n*-butanol to yield the chloroform fraction (38.5 g) and *n*-butanol fraction (50 g). The remaining aqueous phase was dried and labeled as the aqueous

residual fraction (Aq R) (70 g). To precipitate the crude saponins, the *n*-butanol fraction (50 g) was suspended in 100 mL methanol and then poured onto 500 mL pure acetone with continuous stirring. The precipitate was filtrated and dried to yield (25 g) named as butanol precipitate. The aqueous residual fraction (70 g) was treated similarly to yield the second precipitate (15 g) named aqueous precipitate expected to encompass saponins and triterpenoid constituents in both precipitates (Figure 1).

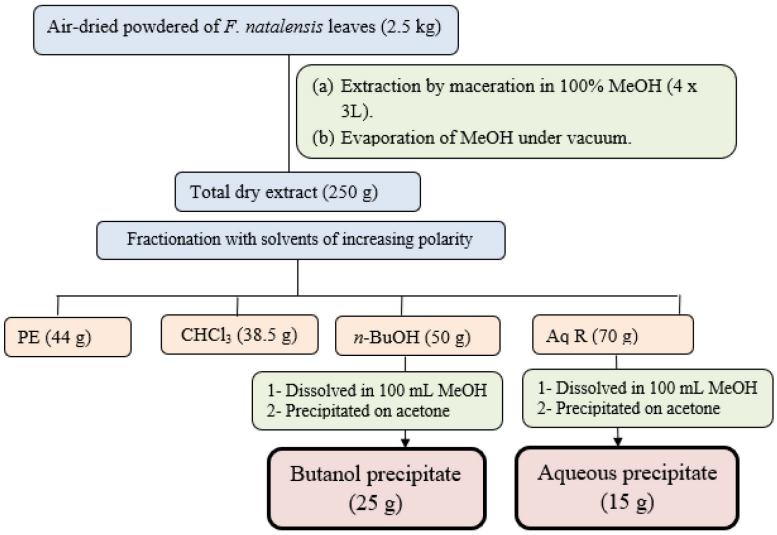


Figure 1. Extraction and fractionation scheme of *F. natalensis* leaves.

2.3.2. Purification of the Butanol Precipitate Fraction

The butanol precipitate (25 g) was purified on a glass column packed with silica gel and elution with gradient $CHCl_3$/MeOH mixtures. Similar fractions were collected according to their similarity pattern on TLC silica plates using solvent S1 and ethanol/H_2SO_4 and vanillin/H_2SO_4 spray reagents to give 19 major collective fractions (Figure 2). Fraction-6 (2 g) eluted from 80% $CHCl_3$ and 20% MeOH, was subjected to further purification using a silica gel column and $CHCl_3$/MeOH mixture as an eluent to give 10 main subfractions. Subfraction-5 (200 mg) was subjected to further purification using silica gel columns followed by Sephadex LH20 column using MeOH as an eluent to compound 4 (6 mg). Fraction-12 (4 g) eluted with 70% $CHCl_3$/30% MeOH (from the main column) was subjected to further purification on a silica gel column and eluted with $CHCl_3$/MeOH/H_2O mixtures with increasing polarity, to yield 20 main subfractions. Subfraction-6 (350 mg), subfraction-10 (200 mg), and subfraction-18 (450 mg) were subjected separately to further purification using silica gel columns using $CHCl_3$/MeOH as an eluent to yield compound 3 (5 mg), compound 2 (11 mg), and compound 1 (10 mg), respectively.

2.3.3. Isolation and Purification of the Aqueous Precipitate Fraction

The aqueous precipitate (15 g) was purified on a silica gel column and elution using $CHCl_3$/MeOH mixtures with increasing polarity, to afford 51 individual fractions each (250 mL). Similar fractions were collected using TLC silica plates using the solvent S1 and ethanol/H_2SO_4 and Vanillin/H_2SO_4 as spray reagents to give 13 major collective fractions, (Figure 3). Fraction-9 (3 g) eluted with 70% $CHCl_3$ and 30%MeOH, was subjected to further purification on a silica gel subcolumn and eluted with $CHCl_3$/MeOH mixture to yield 9 main subfractions. Subfractions-6 (400 mg) and subfraction-8 (200 mg) were subjected separately to further purification using silica gel columns followed by Sephadex LH-20 column using MeOH as an eluent to give compound 5 (9 mg) and compound 6 (5 mg), respectively. Fraction-11 (2 g) eluted with 60% $CHCl_3$ and 40% MeOH, was further purified on a silica subcolumn and eluted with $CHCl_3$/MeOH mixture to yield 12 main subfractions.

Subfraction-5 (200 mg) was subjected to more purification on the silica column followed by Sephadex LH-20 column to yield compound 7 (6 mg).

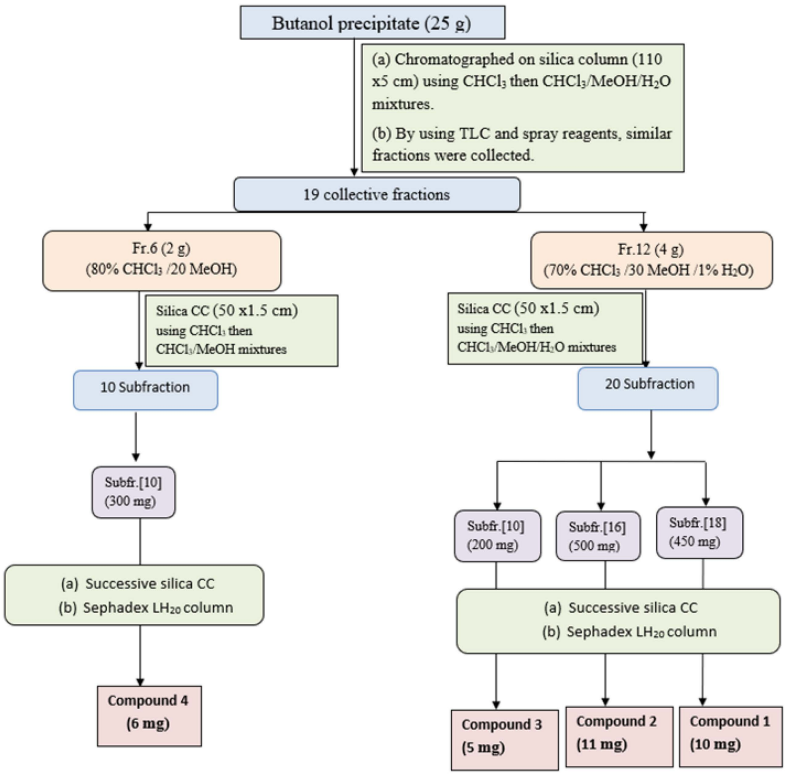


Figure 2. Isolation and purification scheme of compounds isolated from n-butanol precipitate.

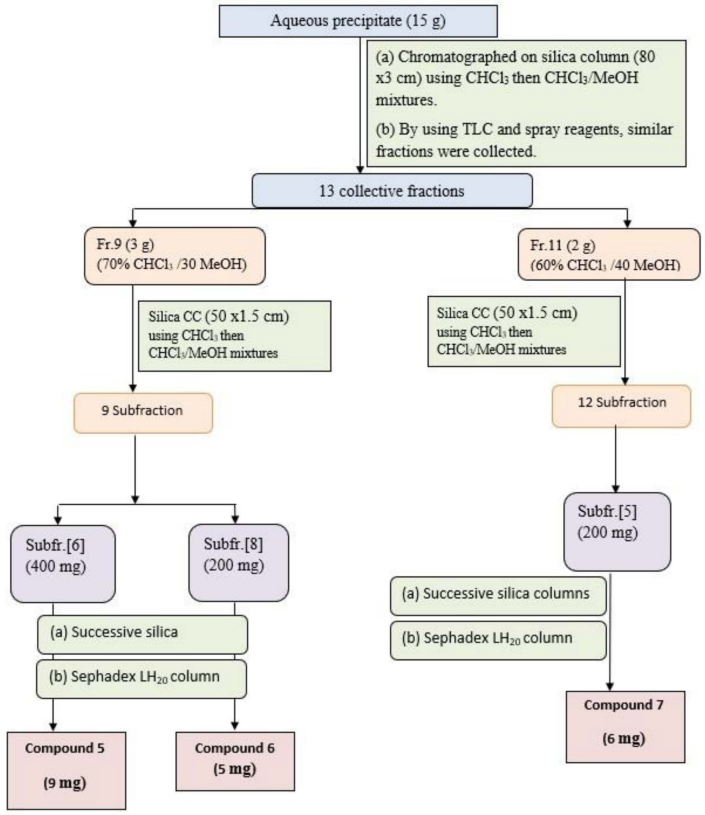


Figure 3. Isolation and purification scheme of compounds isolated from aqueous precipitate.

2.3.4. Acid Hydrolysis for Sugars

Acid hydrolysis for all compounds containing sugars was performed using 1 M HCL on water bath for 2 h at 80 °C, then removing solvent under reduced pressure. Sugars were extracted from the aqueous phase (10 mL) and washed with methylene chloride (15 mL). The combined methylene chloride extract was washed with water to yield the aglycone moiety after evaporation [2,12].

3. Results and Discussion

Phytochemical investigation of *F. natalensis* leaves led to the isolation and identification of 7 compounds belonging to triterpenoid saponins and the detailed identification of the pure compounds as mentioned below.

3.1. Compound **1**

Compound **1** was obtained as a creamy white amorphous powder (10 mg). Chromatographic properties: R_f = 0.33 (S1) on silica gel TLC plate and appeared as purple color with ethanol/H_2SO_4 and vanillin/H_2SO_4 spray reagents. ^{1}HNMR (400MHz, DMSO-d6) and ^{13}CNMR (100 MHz, DMSO-d6) spectroscopic data are summarized in Table S1. The ^{1}HNMR spectrum (Figures S1 and S2) of compound **1** revealed the presence of six overlapped methyl signals that appeared at δH 0.84, 0.90, 0.86, 0.87, 0.99, and 1.01 ppm assigned to methyl groups H-23, H-24, H-25, H-26, H-29, and H-30, respectively. Two proton resonances were detected corresponding to oxygenated methine (originally secondary alcoholic groups) at δH 3.80 (m) assigned for H-3 and olefinic proton at δH 5.31 (brt) assigned for H-12, together with the absence of a dd signal in the range of δH 2.2–3.0, suggestive that compound **1** belongs to the ursane series [13,14]. The ^{13}CNMR spectrum (Figure S3) showed signals for two carboxyl carbons assigned for C-27 and C-28 at δC 179.90 and δC 174.36, respectively. Moreover, an ethylenic carbon C-12 was detected at δC 130.25, a quaternary carbon C-13 at δC 136.97 and C-19 at δC 38.82, as well as C-20 at δC 39.02. ^{1}HNMR and ^{13}CNMR revealed the characteristic signals corresponding to an ursane-type triterpene skeleton for the aglycone having an olefinic bond at C-12 and two carboxyl groups at C-27 and C-28, which falls in accordance with the structure of quinovic acid (3β-ol-urs-12-en-27,28-dioic acid) [14].

^{1}H-NMR spectrum showed six anomeric proton signals at δH 5.61 (d, J = 1.5), 4.36 (d, J = 7.8), 4.64 (d, J = 7.8), 4.55 (d, J = 3.0), 5.15 (d, J = 1.5), and 4.78 (d, J = 7.8) assigned for H-1′, H-1′′, H-1′′′, H-1′′′′, H-1′′′′′, and H-1′′′′′′, respectively, two methyl signals were observed at δH 0.99 and 1.01 corresponding to C-6 of two rhamnose moieties. ^{13}CNMR spectrum exhibited six anomeric signals at δC 98.5, 100.25, 102.41, 92.65, 97.32, and 104.60 corresponding to C-1′, C-1′′, C-1′′′, C-1′′′′, C-1′′′′′, and C-1′′′′′′, respectively, indicating the presence of six saccharide moieties. The downfield shift of C-3 at δC 82.32 and the upfield shift due to esterification of C-28 at δC 174.36 showed that the sugar chains are located at these positions. The acid hydrolysis using 1 N HCl and the comparison of the hydrolysate with authentic sugars using PC co-chromatography led to the identification of sugars as rhamnose, glucose and arabinose. All 1D NMR signals assigned in NMR spectra were finally confirmed using homo- and hetero-nuclear long-range coupling 2D HSQC, and HMBC spectra. A direct correlation was observed in HSQC and HMBC spectra of compound **1**, different J^2 and J^3 sets of correlations between hydrogens and carbons were assigned. HSQC (Figure S4) revealed the major correlation of olefinic proton H-12 (5.31) with the corresponding carbon at δC 130.25 and of H-3 at δH 3.80 with the corresponding carbon C-3 at δC 82.32 confirmed the down field shift of C-3, which confirmed the substitution of C-3 position. The absence of any downfield shift of any of the carbons of two glucose units (glc 2 and glc 3) suggested that they are terminal sugars. The small coupling constant of the anomeric protons of rhamnose moieties (J = 1.5) led to configuration assignment as α sugar, while the large coupling of the glucose units' anomeric protons (J = 7.8) led to the identification of their configuration as β sugars [14]. HMBC spectrum (Figures S5 and S6) revealed that the attachment of one of the sugar chains is at

C-3 from the correlation of H-1′ (rham 1) at δH 5.61 with C-3 at δC 82.32. The downfield shift of C-4 of the rhamnose moiety together with the downfield shift of C-4 of the glucose unit (glc 1) led to the assumption of the sequence of the first saccharide chain to be D-glucopyranosyl (1 → 4)-D-glucopyranosyl (1 → 4)-L-rhamnopyranosyl. The attachment of the second sugar chain at C-28 was recognized from the upfield shift of the carbon of the carboxyl group at δC 174.36 (as compared to non-esterified quinovic acid), indicating its esterification by the sugar chain. The chemical shift of C-1 arabinose assigned it as the first sugar attached to the carboxylic group. Moreover, the downfield shift of its C-2 at δC 75.27 suggested that it is the point of attachment of the second rhamnose moiety (rahm. 2). The downfield shift of C-4 of the second rhamnose allowed for the identification of the point of attachment of the terminal glucose (glc 3), with the second saccharide chain proposed to be D-glucopyranosyl (1 → 4)-L-rhamnopyranosyl (1 → 2)arabinopyranosyl. The EI-MS had a molecular ion peak $[M]^+$ at *m/z* 1365.51 (calcd. 1365.60), corresponding to the molecular formula $C_{63}H_{96}O_{32}$ (calcd. $C_{63}H_{97}O_{32}$). Consequently, from the aforementioned NMR data and comparing with previously reported literature of structurally related compounds [13,14], compound **1** was assigned as 3-O-β-D-glucopyranosyl (1 → 4) β-D-glucopyranosyl (1 → 4)–α-L-rhamnopyranosyl-quinovic acid-28-O-β-D-glucopyranosyl (1 → 4)–α-L-rhamnopyranosyl (1 → 2)–α-L arabinopyranoside ester (Figure 4) which is reported for the first time from *F. natalensis* leaves. By searching for the compound novelty in nature on Reaxys and Web of Science databases, compound **1** was identified for the first time from nature.

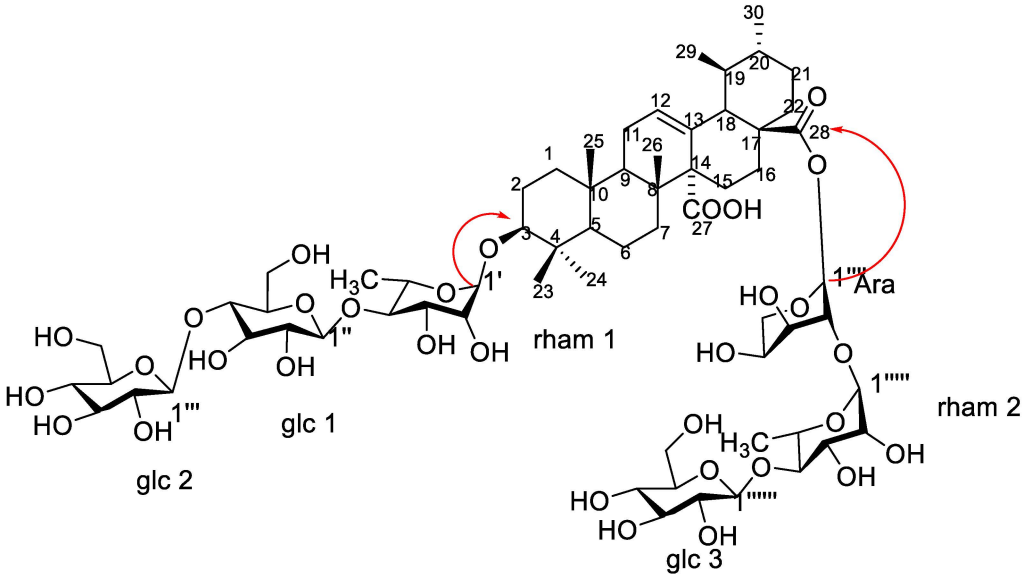


Figure 4. Structure and major correlations of compound **1**.

3.2. Compound **2**

Compound **2** was isolated as a yellowish-white amorphous powder (11 mg). Chromatographic properties: R_f = 0.35 (S1) on the silica gel TLC plate gave a pink color with ethanol/H_2SO_4 and vanillin/H_2SO_4 spray reagents. ^{1}HNMR (400 MHz, DMSO-d6) and ^{13}CNMR (125 MHz, DMSO-d6): Spectroscopic data are summarized in Table S1. ^{1}H-NMR spectrum (Figure S7) of compound **2** revealed the presence of six angular methyl overlapped signals that appeared at δH 0.84, 0.86, 0.99, 0.87, 0.86, and 1.01 ppm assigned for methyl groups H-23, H-24, H-25, H-26, H-29, and H-30, respectively. Another two proton resonances were noticed at δH 3.18 (m) and δH 5.32 (brt) assigned to H-3 (oxygenated methine) and H-12 (olefinic proton) characteristic for the triterpenoid skeleton.

^{13}CNMR spectrum (Figures S8 and S9) showed two signals at δC 179.73 and δC 177.82, characteristic of carboxyl carbons C-27 and C-28, respectively. Moreover, an ethylenic carbon C-12 was detected at δC 130.15 as well as a quaternary carbon C-13 at δC 138.36.

The comparison of ^{1}HNMR and ^{13}CNMR spectral data of **2** with that of compound **1** proved their similarity as ursane-type triterpene aglycones, with an olefinic bond at C-12 and two carboxyl groups at C-27 and C-28, which is identified as quinovic acid (3β-ol-urs-12-en-27,28-dioic acid). ^{1}HNMR spectrum showed five anomeric proton signals at δH 4.84 brs, 4.26 (d, J = 8), 5.15 (d, J = 3.0), 4.69 brs, and 4.90 (d, J = 7.8) assigned for H-1′, H-1′′, and H-1′′′, and H-1′′′′ and H-1′′′′′, respectively. Moreover, ^{13}CNMR spectra exhibited five anomeric signals at δC 98.51, 104.63, 92.66, 97.35, and 102.44, corresponding to C-1′, C-1′′, C-1′′′, C-1′′′′, and C-1′′′′′, respectively, indicating the presence of five sugar moieties attached to the aglycone. ^{13}CNMR data of C-3 at δC 82.35 and of C-28 at δC 177.82 confirmed sugar attachments at these positions. Acid hydrolysis using 1 N HCl and the comparison of the hydrolysate with authentic sugars using PC co-chromatography led to the identification of sugars to be rhamnose, glucose, and arabinose. The spectral data of the sugar moieties of compound **2** showed a large similarity with compound **1**, though without one of the glucose units. This led us to the assumption that the sequence of the first saccharide chain is D-glucopyranosyl (1 → 4)-L-rhamnopyranosyl which is located at C-3, and the second saccharide chain is D-glucopyranosyl (1 → 4)-L-rhamnopyranosyl (1 → 2)-L arabinopyranosyl esterifying C-28. The EI-MS with molecular ion peak $[M]^{+}$ at *m/z* 1234.41 (calcd. 1234.61) corresponds to the molecular formula to be $C_{59}H_{94}O_{27}$ (calcd. $C_{59}H_{94}O_{27}$). Consequently, from the aforementioned 1D NMR data and comparison with previously reported literature of related compounds [13,14], compound **2** is proposed to be 3-O-β-D-glucopyranosyl (1 → 4)–α-L rhamnopyranosyl-quinovic acid-28-O-β-D-glucopyranosyl (1 → 4)–α-L rhamnopyranosyl (1 → 2)–α-L arabinopyranoside (Figure 5) which is isolated for the first time from *F. natalensis* leaves. By searching for the compound novelty in nature on Reaxys and Web of Science databases, compound **1** was identified for the first time from nature.

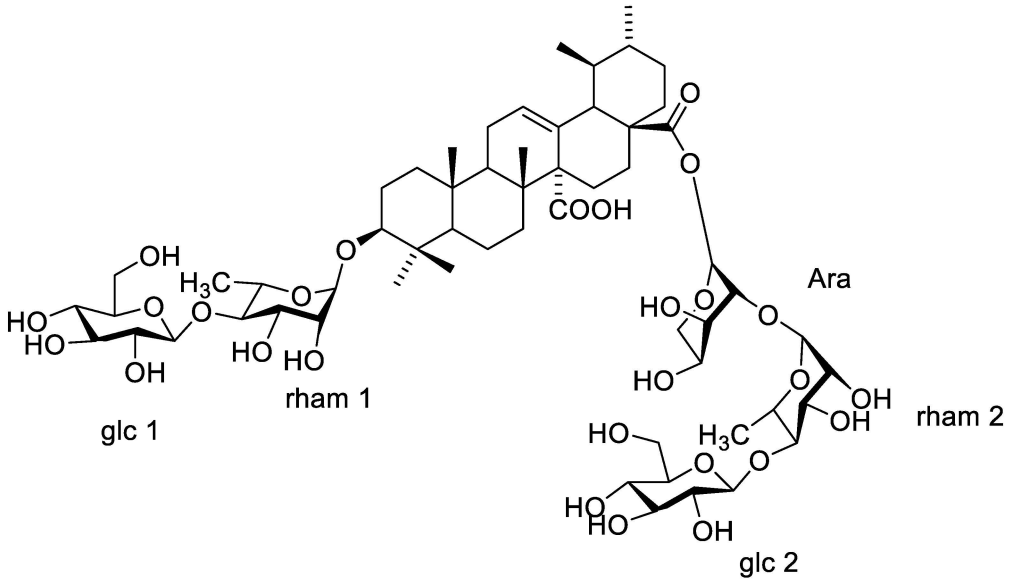


Figure 5. Structure of compound **2**.

3.3. Compound **3**

Compound **3** was isolated as a yellowish-white amorphous powder (5 mg). Chromatographic properties: R_f = 0.38 (S1) on silica gel TLC plate gave a rose-pink color upon spraying with ethanol/H_2SO_4 and vanillin/H_2SO_4 spray reagents. ^{1}HNMR spectrum (400 MHz, DMSO-d6) and ^{13}CNMR (125 MHz, DMSO-d6): Spectroscopic data are summarized in Table S2. ^{1}H-NMR and ^{13}CNMR revealed the characteristic peaks of quinovic acid (3β-ol-urs-12-en-27,28-dioic acid). From ^{1}H-NMR, it was possible to identify the six methyl signals at δH 0.84, 0.88, 0.88, 0.87, 0.86, 0.90 ppm that were assigned for methyl groups H-23, H-24, H-25, H-26, H-29, and H-30, respectively. Oxygenated methine of H-3

and H-12 olefinic proton resonances were detected at δH 3.52 and at δH 5.32 (brt). ^{13}CNMR spectrum showed two signals at δC 178.86 and δC 175.11 characteristic for carboxyl carbons C-27 and C-28, respectively. Moreover, an ethylenic carbon C-12 was detected at δC 130.11 as well as a quaternary carbon C-13 at δC 138.58. Moreover, ^{1}HNMR spectrum showed two anomeric proton signals at δH 5.18 (d, J = 7.8) and 4.81 (d, J = 7.8) assigned for H-1′ and H-1″, respectively, indicating the presence of two β sugar moieties. ^{13}CNMR spectrum also exhibited two anomeric signals at δC 107.71 and 104.47 corresponding to C-1′ and C-1″, respectively, proving the presence of two sugar moieties. The rest of the sugar carbon shifts led to the identification of the sugar units to be two glucose units (Table S1). The data of C-3 and C-28 at δC 82.99 and δC 175.11, respectively, verified the location of the glucose moieties. The EI-MS with molecular ion peak $[M]^+$ at *m/z* 810.98 (calcd. 810.44), corresponding to the molecular formula to be $C_{42}H_{66}O_{15}$ (calcd. $C_{42}H_{66}O_{15}$). All the aforementioned 1D NMR data are in accordance with that published for glycoside B isolated from *Anthocephalus cadamba* bark [15,16]. Consequently, compound **3** was assigned as 3-O-β-D-glucopyranosyl-quinovic acid-28-O-β-D-glucopyranosyl ester (Figure 6) which is isolated for the first time from *F. natalensis* leaves.

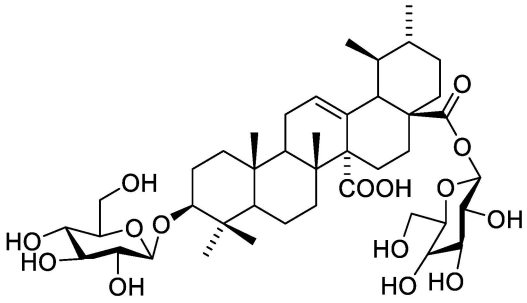


Figure 6. Structure of compound **3**.

3.4. Compound **4**

Compound **4** was isolated as a yellowish-white amorphous powder (6 mg). Chromatographic properties: R_f = 0.37 (S2) on silica gel TLC plate yielded a rose-pink color upon spraying with ethanol/H_2SO_4 and vanillin/H_2SO_4 spray reagents. ^{1}HNMR spectrum (400 MHz, DMSO-d6) and ^{13}CNMR (125 MHz, DMSO-d6); Spectroscopic data are summarized in Table S2. ^{1}HNMR and ^{13}CNMR data showed characteristic NMR signals of another quinovic acid (3β-ol-urs-12-en-27,28-dioic acid). ^{1}HNMR chemical shifts of compound **4** revealed the presence of six methyl signals at δH 0.84, 0.95, 0.90, 0.87, 0.97, 1.04 ppm assigned for methyl groups H-23, H-24, H-25, H-26, H-29, and H-30, respectively. H-3 oxygenated methine appeared at δH 3.52, while olefinic H-12 was assigned at δH 5.33 (brt). ^{13}CNMR spectrum showed two signals at δC 181.82 and δC 175.32, characteristic of carboxyl carbons C-27 and C-28, respectively. Moreover, carbon C-12 was detected at δC 129.39 as well as a quaternary carbon C-13 at δC 134.27 proving the presence of a double bond between C-12 and C-13. ^{1}HNMR spectrum showed two anomeric proton signals at δH 5.41 (d, J = 1.5, 1H′) and 4.90 (d, 1H″) together with a methyl doublet at δH 1.01 (6′-H). Additionally, the ^{13}CNMR spectrum exhibited two anomeric signals at δC 97.05 and 103.54, corresponding to C-1′ and C-1″, indicating the presence of one rhamnose moiety and one glucose moiety. Other sugar carbons were assigned at the range of δC 60–80. The attachment of rhamnose at C-3 was confirmed from the downfield shift of C-3 at δC 90.57 and the value of its anomeric proton while the location of glucose at C-28 was assigned by the upfield shift of C-28 at δC 175.32. The structure was confirmed by EI-MS which showed a molecular ion peak [M]+ at *m/z* 794.82 and prominent fragments at *m/z* 632.28 $[M\text{-}162]^+$, corresponding to the removal of one hexose unit, and at *m/z* 484.8, corresponding to $[M\text{-glc-rham-2H}]^+$, consistent with the molecular formula to be $C_{42}H_{66}O_{14}$. All the previous data are in accordance with that published for gongganoside B isolated from Bhesa paniculata bark [16,17]. Therefore, compound **4** was confirmed as 3-O-α-L-rhamnopyranosyl-quinovic

acid 28-O-β-D-glucopyranoside ester (Figure 7), which was previously reported from *Ficus elastica* aerial root bark [11] and identified for the first time from *F. natalensis* leaves.

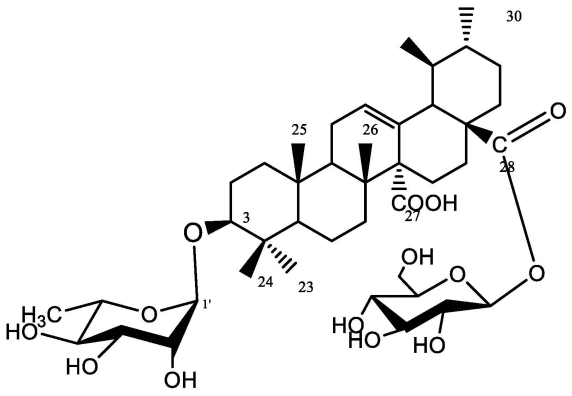


Figure 7. Structure of compound **4**.

3.5. Compound **5**

Compound **5** was obtained as a white amorphous powder (9 mg). Chromatographic properties: R_f = 0.46 (S1) on silica gel TLC plate. It gave a violet color with ethanol/H_2SO_4 and vanillin/H_2SO_4 spray reagents. ^{1}HNMR spectrum (400 MHz, DMSO-d6) and ^{13}CNMR (100 MHz, DMSO-d6): Spectroscopic data are summarized in Table S3. The ^{13}CNMR spectrum showed seven methyl carbon signals at δC 29.31, 17.62, 14.95, 18.03, 26.21, 33.84, and 23.49 assigned to C-23, C-24, C-25, C-26, C-27, C-29, and C-30, respectively. An ethylenic carbon was observed at δC 123.71 ppm assigned for C-12, together with a quaternary carbon signal C-13 at δC 139.30 ppm and one carboxylic carbon at δC 183.99 ppm corresponding to C-28. The signals of C-19 and C-20 were observed at δC 46.31 and 31.73, respectively, proving the location of C-29 and C-30 methyls at C-20.

The ^{1}HNMR spectrum showed the presence of 7 overlapped methyl signals at δH 0.84, 0.88, 0.88, 0.87, 1.34, 0.90, and 0.86 ppm corresponding to protons of seven angular methyls H-23, H-24, H-25, H-26, H-27, H-29, and H-30. An oxygenated methine proton of a terpene moiety appeared at δH 3.20 ppm attributed to H-3, and a trisubstituted olefinic proton appeared as a broad triplet at δH 5.32 ppm. ^{1}HNMR and ^{13}CNMR spectra led to the identification of 8 to be an oleanane-type triterpene skeleton, with an olefinic bond at C-12 and one carboxyl group at C-28, known as oleanolic acid [18]. Additionally, the ^{1}HNMR spectrum showed one anomeric proton signal at δH 5.18 (d, J = 7.8), indicating the presence of one β sugar unit. An anomeric carbon was assigned at δC 101.53 in ^{13}CNMR, the data of the sugar carbons led to the identification of the sugar unit as glucose [2]. The attachment of this glucose moiety at C-3 was confirmed by the downfield shift of C-3 carbon at δC 84.40. The EI-MS with molecular ion peak $[M]^+$ at *m/z* 618.42 (calcd. 618.41), corresponds to the molecular formula to be $C_{36}H_{58}O_8$ (calcd. $C_{36}H_{58}O_8$). Consequently, based on 1D NMR data and by comparing with reported literature of related compounds [19,20], compound **8** was assigned as 3-O-β-D-glucopyranosyl oleanolic acid (Figure 8), which was isolated from *Albizia gummifera* bark and the whole plant of *Patrinia saniculaefolia* [19,20], and is reported for the first time from *F. natalensis* leaves.

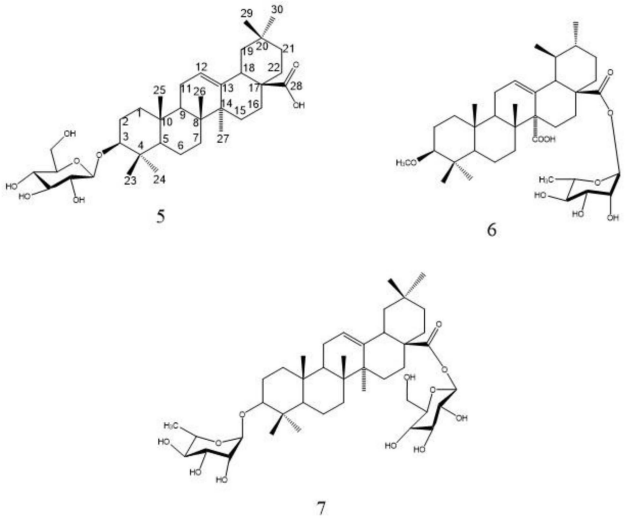


Figure 8. Structure of compounds **5**, **6**, and **7**.

3.6. Compound **6**

Compound **6** was isolated as a yellowish-white amorphous powder (5 mg). Chromatographic properties: R_f = 0.43 (S1) on silica gel TLC plate. It gave a pink color with ethanol/H_2SO_4 and vanillin/H_2SO_4 spray reagents. ^{1}HNMR spectrum (400 MHz, DMSO-d6) and ^{13}CNMR (100 MHz, DMSO-d6): Spectroscopic data are summarized in Table S3. Spectral data of compound **6** were superimposed with those of compound **5** which prove the presence of the same oleanane-type triterpene skeleton, with an olefinic bond at C-12 and one carboxyl group at C-28 (oleanolic acid) [13,21]. The point of difference between **6** and **5** lies in the presence of one anomeric proton signal at δH 4.87 (d, J = 1.5) at ^{1}HNMR spectrum assigned for H-1′ together with methyl signal at δC 18.35 (C-6′) indicating the presence of one α-L-rhamnose moiety. Additionally, the ^{1}HNMR spectrum revealed the presence of a singlet signal at δH 3.51 integrated for three protons of the methoxy group. ^{13}CNMR showed one anomeric signal at δC 98.34 corresponding to C-1′ and methoxy carbon at δC 51.67. The upfield shift of C-3 at δC79.49 as compared to the non-substituted one led to the identification of the site of attachment of the methoxy group, while the upfield shift of C-28 at δC 179.9 proved the location of the rhamnose moiety. The EI-MS showed a molecular ion peak $[M]^+$ at *m/z* 616.48, consistent with the molecular formula to be $C_{37}H_{60}O_7$. From the discussed 1D NMR, EI-MS data and by comparing with previously reported literature of structurally related compounds [13,21], compound **6** was assigned as 3-methoxy-oleanolic acid-28-O-α-L-rhamnopyranoside which is isolated for the first time from *F. natalensis* leaves.

3.7. Compound **7**

Compound **7** was isolated as a white amorphous powder (6 mg). Chromatographic properties: R_f = 0.41 (S2) on a silica gel TLC plate. It gave a violet color with ethanol/H_2SO_4 and vanillin/H_2SO_4 spray reagents. ^{1}HNMR spectrum (400 MHz, DMSO-d6) and ^{13}CNMR (100 MHz, DMSO-d6), spectroscopic data are summarized in Table S3. Comparison of the spectral data of 7 with those of compounds **5** and **6** led to the identification of its aglycone as oleanane-type triterpene, with an olefinic bond at C-12 and one carboxyl group at C-28 (oleanolic acid) [12,18]. The points of difference were the presence of two anomeric proton signals at δH 5.19 with a small coupling constant together with a doublet signal of CH3-6′ at δC 1.02 indicating the presence of rhamnose moiety and at δH 4.69 indicating the presence of β sugar in 1H NMR. Moreover, two anomeric carbons at δC 104.47 and 102.43 were identified in the 13C NMR spectrum. The EI-MS with molecular ion peak $[M]^+$ at *m/z* 764.41 (calcd. 764.99), corresponding to the molecular formula to be $C_{42}H_{68}O_{12}$ (calcd. $C_{42}H_{68}O_{12}$). A comparison of the sugar carbon values with the reported literature

confirmed the presence of one β-D-glucose unit and one α-L-rhamnose unit [13,21]. The attachment of the sugar units at C-3 and C-28 was confirmed by their chemical shift at δC 82.21 and 175.0, respectively. Consequently, compound **7** was identified as 3-O-α-L-rhamnopyranosyl-oleanolic acid-28-O-β-D-glucopyranoside ester which is isolated for the first time from *F. natalensis* leaves.

4. Conclusions

The detailed chromatographic separation of seven triterpenoid saponins was introduced herein. The isolated compounds were identified according to their spectroscopic analysis including 1D ^{1}HNMR, ^{13}CNMR, 2D HSQC, and HMBC along with MS to confirm the deduced structure. Seven compounds (1–7) were identified for the first time from *F. natalensis* belonging to bidesmoside triterpenoid saponins with quinovic acid and an oleanolic acid aglycone. Compounds **1** and **2** were reported for the first time from nature according to Reaxys and Web of Science database. Further screening of the biological activities of the isolated compounds is recommended in future work. In addition, the application of large-scale metabolomic approaches including UPLC-MS/MS is recommended for metabolites profiling of *F. natalensis* leaves to profile more saponins present at lower levels and compare it with fruits as well as plants from other sources to provide conclusive evidence on saponins profile in *F. natalensis*.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/separations10090478/s1, Table S1.1H- and 13C-NMR spectroscopic data of compounds (**1**–**2**) in DMSO-*d6*; Table S2: ^{1}H -and ^{13}C-NMR spectroscopic data of compounds (**3**–**4**) in DMSO-*d6*; Table S3: ^{1}H -and ^{13}C-NMR spectroscopic data of compounds (**5**–**7**) in DMSO-*d6*. Figure S1:^{1}HNMR spectrum of compound **1**; Figure S2: Magnified ^{1}HNMR spectrum of compound **1**; Figure S3:^{13}CNMR spectrum of compound **1**; Figure S4: HSQC spectrum of compound **1**; Figure S5: HMBC spectrum of compound **1**; Figure S6: Magnified HMBC spectrum of compound **1**; Figure S7:^{1}HNMR spectrum of compound **2**; Figure S8: ^{13}CNMR spectrum of compound **2**; Figure S9: Magnified ^{13}CNMR spectrum of compound **2**.

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