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Identification of Some Chemical Constituents of the Leaves of *Alstonia boonei* and *Bridelia ferruginea*

Oluwatoyin Adenike Fabiyi[1*], Olubunmi Atolani[2], Gabriel A. Olatunji[3]

[1] Department of Crop Protection, University of Ilorin, P.M.B. 1515, Ilorin, Nigeria
[2] Department of Chemistry, University of Ilorin, P.M.B. 1515, Ilorin, Nigeria
[3] Department of Industrial Chemistry, University of Ilorin, P.M.B. 1515, Ilorin, Nigeria

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ABSRACT

This study aims to investigate the phytochemical composition of the leaves of Alstonia boonei and Bridelia ferruginea. The leaves of the two plants were extracted successively with n-hexane and ethyl acetate, concentrated and fractionated over gravity silica gel column chromatography. Some fractions obtained were analyzed using gas chromatography mass spectroscopy (GC-MS). GC-MS results indicated that the main constituents of the fractions from Alstonia boonei were fatty acid methyl esters, while the main constituents of the fractions from Bridelia ferruginea were fatty acid esters (47.46%) and triterpenoids (43.39%). Each of the fractions has at least one exclusive tetracyclic or pentacyclic triterpenoid present. Friedelan-3-one and clionasterol were present in the Alstonia boonei fractions, while beta-amyrin, lup-20(29)-en-3-one, lupeol, alpha-amyrin acetate and 4,4,6a,6b,8a,11,11,14b-octamethyl-1,4,4a,5,6,6a, 6b,7,8,8a,9,10,11, 12,12a,14,14a,-octadecahydro-2H-picen-3-one were present in the B. ferruginea fraction. Obviously, B. ferruginea is a reservoir to many pentacyclic triterpenoids. The pentacyclic triterpenoids may be used as a biomarker in the chemo taxonomical classification of the plants whilst acting as relevant contributors to the acclaimed biological benefits of B. ferruginea reported in traditional medical practices. The leaves of the two plants contain many bioactive compounds which may find application in pharmaceutical formulations as well as nutraceuticals and bio-pesticides preparations.

1. Introduction

Alstonia boonei de wild (Apocynaceae) is a medicinal plant that is widely used in folkloric medicine in Africa. Various therapeutic properties have been attributed to *A. boonei*. The stem bark extract of *A. boonei* has been investigated and reported to possess anti-inflammatory, analgesic and antipyretic activities (Olajide et al. 2000). An infusion of the bark is used as anti-venom for snake bites. The stem bark also finds application in the treatment of painful micturition, rheumatism and asthma (Ojewole 1984; Asuzu and Anaga 1991). There are also reports of application in the treatment of fever, malaria, dysentery, toothache, and inflammations (Danquah et al. 2012). The stem bark extract is applied topically to reduce oedema and to clear sore (Majekodunmi et al. 2008). In Ghana, it is given to assuage toothache and after child delivery, to aid in placenta expulsion (NNMDA 2006). The bark extract is known to contain some indole alkaloids which include alstonine, porphine and alstonidine as well as some triterpenoids (Phillipson et al. 1987; Nathaniel et al. 2010). *Bridelia ferruginea* benth (Euphorbiaceae) is an indigenous plant in Nigeria, used extensively for herbal preparations. Bright red extracts of the stem bark are commonly sold in Nigerian markets as mouth-wash. A decoction of the stem bark is used in African folklore for the treatment of dysentery, piles and gynaecological disorders (including sterility) while, the decoction of the leaves is used to treat diabetes. As a result of the coloured nature of the extracts, the extracts have been used as a source of dye among locals (Addae-mensah and Achenbach 1985). The plant is applied in the treatment of physical and mental ailments (Keay et al. 1989). Latex from the stem bark and leaves are used

* Corresponding author email: fabiyitoyinike@hotmail.com

to aid lactation in nursing mothers, while the bark extract is used as arrow poison and in the treatment of syphilis (Iwu 1993). In south-western Nigeria, the leave extract is employed in dressing of sores, wounds and burns. Wet mashed leaves and powdered dried leaves are used on inflammations, while the stem bark extract is used to expel worms in children (Oliver-bever 1986). All parts of the two plants contain important bioactive phytochemicals. Alkaloids such as vincamajine, echitamidine and echitamine have been detected in *Alstonia boonei* (Kweifio 1991; Raymond-Hammet 1999; Salina 2001), while flavonoids and flavonoid glycosides are reported in *Bridelia ferruginea* leaves (Addae-Mensah 1985). The leaf extracts of both *Alstonia boonei* and *Bridelia ferruginea* have been investigated for their nematicidal potential against *Meloidogyne incognita* Infecting *Corchorus olitorius* in both *in vitro* and field experiments (Fabiyi et al. 2012a, 2012b). From the foregoing, a lot of investigation has been carried out on stem bark of the two plants whereas the chemical constituent of *Alstonia boonei* and *Bridelia ferruginea* leaves has not been thoroughly investigated. Hence this study was carried out to determine some chemical constituents of the leaves of the plants responsible for some of the acclaimed therapeutic and nematocidal properties reported by traditional users.

2. Materials and Methods

Plant Materials: The leaves of *Alstonia boonei* were collected from Fiditi village in Oyo state area of Nigeria, while *Bridelia ferruginea* leaves were obtained from Bode saadu village in Kwara state Nigeria. The *Alstonia boonei* and *Bridelia ferruginea* plants were authenticated by a Taxonomist at the Department of Plant Biology at the University of Ilorin, Ilorin, Nigeria where voucher specimens UIH 001/1035 and UIH 002/313 respectively had been previously deposited.

Extraction: The plant materials (*Alstonia boonei* and *Bridelia ferruginea* leaves) were air dried at room temperature for two months after which they were pulverized using the laboratory mill (Christy and Norris Ltd Machine type 8). 1000g each of the pulverized materials were packed in a 10 litre aspirator and extracted successively with n-hexane (Hex) and ethyl acetate (EtOAc) for five days each. Solvents were re-distilled to ascertain purity before use. The extracts were decanted, filtered and concentrated to obtain the four crude extracts coded ABH (*Alstonia boonei* hexane extract), ABE (*Alstonia boonei* ethyl acetate extract), BFH (*Bridelia ferruginea* hexane extract) and BFE (*Bridelia ferruginea* ethyl-acetate extract).

Column Chromatography Fractionation

The crude extracts (30 g each) were subjected to different open column chromatography, cc on silica gel (100-120 mesh grade) using a glass column 10 by 50 cm. (Simon, 2006). In each case, the elution was initiated with petroleum ether at a steady flow rate of 1.5 ml per minute and fractions collected in volume of 50 ml. Further elution was carried by mixture of increasing volume of dichloromethane in petroleum ether until the last colourless fraction was obtained with dichloromethane. ABH afforded thirty-nine fractions which were pooled into three groups (fraction 1 to 8, fraction 9-27 and 28-39) based on the thin layer chromatography, (TLC) profile. ABE afforded forty-nine fractions which were pulled to three combined fractions (fractions 1-19, 20-34 and 35-49) based on the TLC profile. Similarly, the cc of BFH yielded forty-eight fractions pulled to three sub-groups (fractions 1-28, 29-38 and 39-48). The various combined fractions were concentrated using rotary evaporator under vacuum and three major samples namely ABH 28-39 (ABHB), ABE 20-34 (ABHB) and BFH 29-38 (BFHB) were subjected to gas chromatography mass spectroscopy (GC-MS) analysis on the basis of their TLC profile.

GC/GC-MS Analyses

The chemical compositions of the fractions were analysed using GC/MS-QP 2010 PLUS; Shimadzu interfaced with a finigan MAT ion trap detector. The column, RTX5MS column was packed with 100% dimethylpolysiloxane. The column temperature was initially held at 60^{o}C for 5 min with injection volume of 1µL and then programmed to rise at the rate of 5^{o}C/min to 250^{o}C. The injector temperature was set at 200^{o}C, whereas the detector (mass spectrophotometer) temperature was maintained at 250^{o}C.Carrier gas, helium was at a linear velocity of 46.3 cm/sec and pressure of 100.2 kPa. Ionization mode was electron impact at a voltage of 70 eV. The identification of the chemical components was carried out by matching their mass spectral with those of NIST library.

3. Results and Discussion

The chemical composition of the fraction ABHB obtained from *Alstonia boonei* using GC/GC-MS revealed that the major compounds are fatty acid esters (Table 1). Hexadecanoic acid methyl ester also known as methyl palmitate (22.96%) is the major fatty acid ester obtained in the ABHB fraction. Other abundant fatty acid esters include 10-octadecanoic acid methyl ester (13.74%), octadecanoic acid, methyl ester (9.08%), hexadecanoic acid ethyl ester (6.37%), 9-octadecenoic acid, ethyl ester (5.88%), heptacosanoic acid, methyl ester (5.53%) and octadecanoic acid ethyl ester (3.97%). Hydrocarbons such as heneicosane, tetradecane, hexadecane and tridecane were also detected in minute quantities. Friedelan-3-one (5.18%) also known as friedelin, a pentacyclic triterpenoid was detected in a significant yield. The GC-MS analysis of ABEB also revealed major constituent (Table 2) of the fraction to be fatty acid esters. Major fatty acid esters obtained in the fraction include hexadecanoic acid, methyl ester (17.87%), hexadecanoic

acid ethyl ester (14.04), 10-octadecenoic acid methyl ester (12.23%), octadecanoic acid methyl ester (10.19%), 9-octadecenoic acid, ethyl ester, (E)- (9.87%) and 9,12-octadecadienoic acid methyl ester (7. 03%).Gamma sit sterol (11.07%) is the major none fatty acid in the fraction. Other none fatty acid includes tetra tetracontane and 1-nonadecanal while one compound with relative abundance of (2.45%) was unidentifiable. The chemical composition of fraction BFHB (Table 3) indicated Methyl 5,9,23-nonacosatrienoate (45.63%) as the major compound. Other compounds in significant yield include alpha-amyrin acetate (19.05%), lupeol (10.39%), lup-20(29)-en-3-one (6.48%), beta-amyrin (5.31%), 17-pentatriacontene (4.27%), 1-Cyclohexene-1-butanol, 2, 6, 6-trimethyl (3.9%) and a pentacyclic triterpenoid, 4,4,6a,6b,8a,11,11,14b-octamethyl-1,4,4a,5,6, 6a, 6b,7, 8, 8a, 9,10,11,12,12a,14,14a-octadecahydro-2H-picen-3-one (2.16%). The fatty acid esters which include hexadecenoic acid methyl ester, 9-octadecanoicacid, methyl ester (E) and octadecanoic acid methyl ester were obtained in low amount in the fraction. The three fractions analysed indicated the presence of hexadecenoic acid methyl ester, palmitic acid and hexadecenoic acid ethyl ester. Apart from that, chemical composition of the ABHB and ABEB fractions indicated the presence of pentadecanoic acid methyl ester, 10-octadecenoic acid methyl ester, octadecanoic acid methyl ester, 9-octadecenoic acid ethyl ester (E), eicosanoid acid methyl ester, heneicosanoic acid methyl ester and octadecanoic acid ethyl ester.

Table 1
Chemical composition of fraction ABHB from *Alstonia boonei*

Peak no	RT	% RA	Component
1	18.55	1.15	Hexadecane
2	22.08	1.36	Tetradecane
3	26.64	0.76	Tridecane
4	28.27	1.32	Pentadecanoic acid, methyl ester
5	28.55	1.80	2-pentadecanone-6,10,14-trimethyl
6	29.55	22.96	Hexadecanoic acid, methyl ester
7	29.96	1.80	n-Hexadecanoic acid
8	30.31	6.37	Hexadecanoic acid, ethyl ester
9	30.66	1.42	Heptadecanoic acid, methyl ester
10	31.40	13.74	10-octadecanoic acid, methyl ester
11	31.67	9.08	Octadecanoic acid, methyl ester
12	32.04	5.88	9-octadecenoic acid, ethyl ester
13	32.31	3.97	Octadecanoic acid, ethyl ester
14	33.00	6.90	Pentadecanal
15	33.50	2.63	Eicosanoic acid, methyl ester
16	34.34	0.81	Heneicosanoic acid, methyl ester
17	35.14	5.53	Heptacosanoic acid, methyl ester
18	35.45	2.53	Octadecanoic acid, phenyl methyl ester
19	36.81	1.95	Tetracosanoic acid, methyl ester
20	38.80	2.85	Heneicosane
21	39.78	5.18	Friedelan-3-one

RT indicates retention time on the column in minutes. % A indicates percentage relative area (peak area relative to the total peak area).

Table 2
Chemical composition of fraction ABEB from *Alstonia boonei*

Peak no	RT	% Area	Component
1	26.78	0.32	Methyl tetradecanoate
2	28.27	1.22	Pentadecanoic acid, methyl ester
3	28.55	0.76	2-pentadecanone acid, methyl ester
4	29.55	17.87	Hexadecanoic acid, methyl ester
5	29.96	0.98	n-Hexadecanoic acid
6	30.32	14.04	Hexadecanoic acid, ethyl ester
7	30.65	0.99	Hexadecanoic acid,15-methyl-methyl ester
8	31.31	7.03	9,12-octadecadienoic acid, methyl ester
9	31.40	12.23	10-octadecenoic acid, methyl ester
10	31.67	10.19	Octadecanoic acid, methyl ester
11	32.05	9.87	9-octadecenoic acid,ethyl ester,(E)-
13	33.50	1.62	Eicosanoic acid, methyl ester
14	34.01	0.10	Eicosanoic acid, ethyl ester
15	34.34	0.51	Heneicosanoic acid, methyl ester
16	35.39	11.07	Clionasterol
17	35.65	1.48	Octadecanoic acid, ethyl ester
18	35.93	0.62	Tricosanoic acid, methyl ester
19	36.51	1.63	1-Nonadecanal
20	36.81	1.19	Tetracosanoic acid, methyl ester
21	37.45	0.83	Ethyl tetracosanoate
22	38.64	2.45	Unidentified
23	38.80	2.90	Tetratetracontane

RT indicates retention time on the column in minutes. %RA indicates percentage relative area (peak area relative to the total peak area).

Each of the fractions has at least one exclusive tetracyclic or pentacyclic triterpenoid; ABHB, friedelan-3-one (**1**), ABEB, clionasterol also known as gamma-sit sterol (**2**) and BFHB, beta-amyrin (**3**), lup-20(29)-en-3-one (**4**), lupeol (**5**), alpha-amyrin acetate (**6**) and 4,4,6a, 6b, 8a, 11, 11, 14b-octamethyl-1,4,4a, 5,6, 6a,6b, 7,8, 8a, 9,10, 11,12, 12a,14,14a,-octadecahydro-2H -picen -3-one (**7**) (Fig 1). It is obvious that *Bridelia ferruginea* is a repertoire of many functional pentacyclictriterpenes. Fatty acid esters accounted for 78.19% in ABHB, 80.87% in ABEB and only 1.83% in BFHB (Fig 2, 3 and 4). The most significant part of BFHB is fatty acid esters (47.46%) and terpenoids (43.39%). Aliphatic alcohols were absent in ABHB and ABEB but are present in BFHB in minute quantity. Several aliphatic acid esters dominated the fractions from *Alstonia boonei* many of which are being reported for the first time in the plant. Lupeol palmitate, lupeol linoleate, triterpenoids, pentacyclic triterpenoids, steroids, palmitic and linoleic acid esters have been reported to be present in the leaves of *Alstonia boonei* (Faparusi and Bassir 1982; Kweifo-okai and Carol 1992). Fatty acids are important bioactive compounds that have been reported to be present in plant' parts with variety of functions (Atolani et al. 2009 and 2011). Fatty acid esters from the leave of *Kigelia pinnata* have been reported to possess significant degree of cytotoxicity (Atolani et al. 2013). Friedlan-3-one and some fatty acid esters were found in the ABHB fraction, while ABEB fraction contained fatty acid esters and clionasterol. Clionasterol has also been detected in the essential oil of *Silphium trifoliatum* and *Silphium integrifolium* used by the American Indians (Kowalski, 2008). Boonyaratavej et al. (1990) isolated lupeol, β-amyrin and β-sitosterol from the petroleum spirit extract of *Bridellia ferruginea*, while in this study lupeol, lup-

20(29)-en-3-one, β-amyrin and α-amyrin acetate were identified in the BFHB fraction of *Bridellia ferruginea* leaf extract. The effectiveness of *Bridellia ferugginea* in the treatment of gestational type-2 diabetes has been linked to the constituents such as terpenoids, steroids and alkaloids (Taiwo et al. 2012). Similarly Olajide et al. (2012) established that the constituents of *B. ferruginea* extracts are a source of new therapeutic substances for neuro inflammatory and neurodegenerative conditions. The clinical effect of extracts from *Alstonia boonei* was associated with the constituents (Opoku and Akoto 2015). Some of the variations in compounds obtained in this study as compared to previous report could be as a result of differences in geographical location, season, soil types as well as influences of other environmental and anthropogenic factors.

Table3
Chemical composition of fraction BFHB from *Bridelia ferruginea*

Peak no	RT	%RA	Component
1	28.55	0.17	2-Pentadecanone,6,10,14-trimethyl
2	29.54	0.59	Hexadecanoic acid, methyl ester
3	29.95	0.44	n-Hexadecanoic acid
4	31.39	1.19	9-octadecanoicacid, methyl ester (E)
5	31..45	0.05	Octadecanoic acid, methyl ester
6	31.67	0.32	Pentadecanal
7	33.12	3.90	1-Cyclohexene-1-butanol,2,6,6-trimethyl
8	35.16	2.16	4,4,6a,6b,8a,11,11,14b-octamethyl-1 4,4a,5,6,6a, 6b,7,8,8a, 9,10,11, 12, 12a, 14,14a,-octadecahydro-2H-picen-3-one
9	35.36	4.27	17-pentatriacontene
10	35.62	5.31	Beta-amyrin
11	36.13	6.48	Lup-20(29)-en-3-one
12	36.60	10.39	Lupeol
13	37.47	19.05	Alpha-amyrin acetate
14	39.71	45.63	Methyl-5,9,23-nonacosatrienoate

RT indicates retention time on the column in minutes. %RA indicates percentage relative area (peak area relative to the total peak area).

4. Conclusion

GC-MS analysis of chromatographic fractions from the leaf extract of *Alstonia boonei* and *Bridelia ferruginea* was carried out to determine the phyto-constituents responsible for some of the reported folkloric use of the plants. The result confirms the presence of fatty acid esters and triterterpenoids as the major constituents in the leaf extracts of *Alstonia boonei* and *Bridelia ferruginea.* The fractions from *Alstonia boonei* extract contain more of fatty acid esters while *Bridelia ferruginea* extract contains more of pentacyclic triterpenoids. Owning to the presence of numerous triterpenes in *Bridelia ferruginea*, it may be a plant of better pharmaceutical importance than *Alstonia boonei*. Generally, the compounds in both plants may find applications in herbal supplements, pharmaceutical and bio-pesticides formulations, while the relative abundance of the constituents could be relevant biomarkers for the authentication of the plant species.

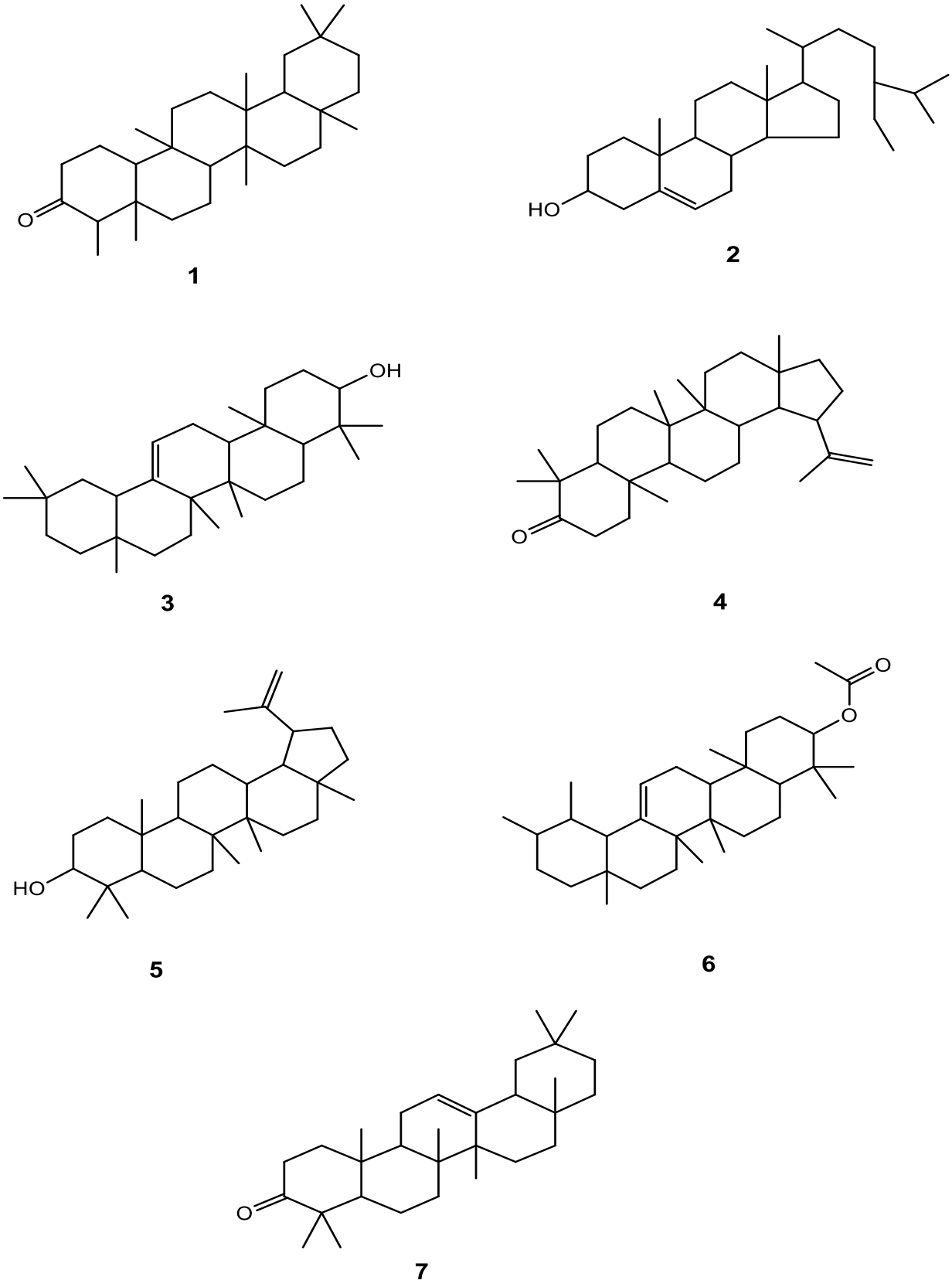

Figure 1

Tetracyclic and pentacyclictriterpenoids obtained from the chromatographic fractions of *Alstonia boonei* and *Bridelia ferruginea* leaf extracts.

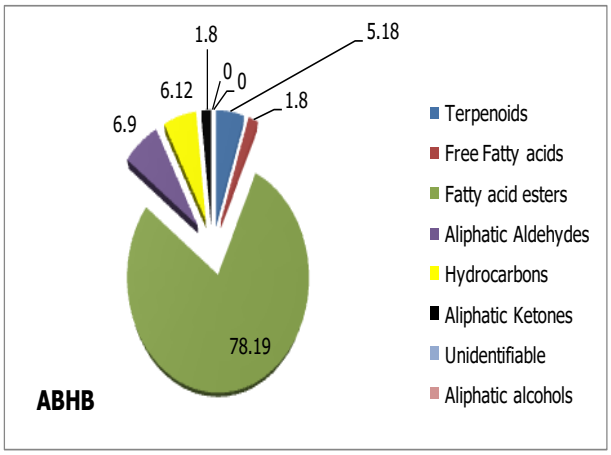

Figure 2

Percentage composition of the classes of compounds in ABHB

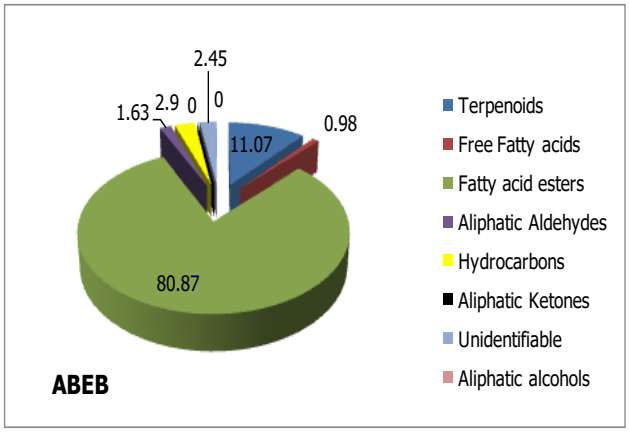

Figure 3

Percentage composition of the classes of compounds in ABEB

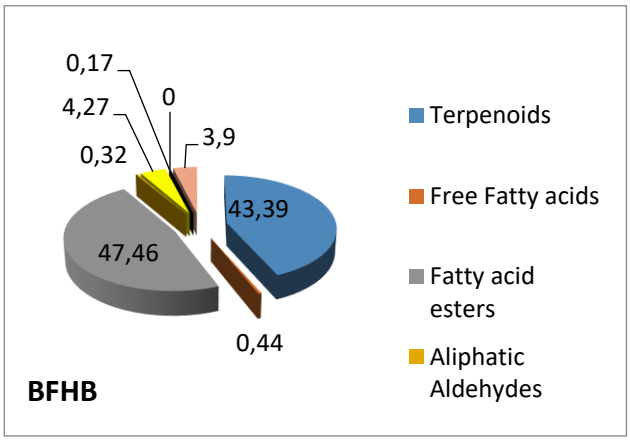

Figure 4

Percentage composition of the classes of compounds in BFHB

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