Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(2): 3104-3111
Received: 03-01-2018
Accepted: 04-02-2018

Bihon Abera
Department of Chemistry, College of Natural and Computational Sciences, Hawassa University, Ethiopia

Legesse Adane
Department of Chemistry, College of Natural and Computational Sciences, Hawassa University, Ethiopia

Fikre Mamo
Department of Chemistry, College of Natural and Computational Sciences, Hawassa University, Ethiopia

Correspondence
Legesse Adane
Department of Chemistry, College of Natural and Computational Sciences, Hawassa University, Ethiopia

Phytochemical investigation the root extract of *Syzygium guineense* and isolation of 2,3,23-trihydroxy methyl oleanate

Bihon Abera, Legesse Adane and Fikre Mamo

Abstract
Syzygium guineense is one of the species in the genus *Syzygium.* The plant is well known for its use in traditional medicine in several countries of tropical regions of the world (including Ethiopia). Despite its medicinal use, there are no reports on scientific investigation on the roots of this plant species. In the present study, phytochemical screening tests were carried out on solvent (n-hexane, dichloromethane: methanol (1:1) and methanol) extracts that were obtained by subjecting 600 g of plant material to sequential extraction approach. Phytochemical tests, employing standard procedures, revealed the presence of secondary metabolites such as steroids, terpenoids, saponins, flavonoids, tannins alkaloids, phenols, and glycosides in the dichloromethane: methane (1:1) and methanol extracts. But only steroids and terpenoids were detected in the n-hexane extract. Column chromatographic separation of dichloromethane/methanol (1:1) extract led to isolation of compound B1. Spectroscopic (IR, UV and NMR) data and comparison with literature reports indicated that compound B1 to be 2, 3, 23-trihydroxy methyl oleanate. This is the first report of isolation of 2, 3, 23-trihydroxy methyl oleanate from the genus *Syzygium.*

Keywords: 2,3,23-trihydroxy methyl oleanate, arjunolic acid, *Syzygium guineense*, medicinal plants, phytochemical screening/test, crude extract

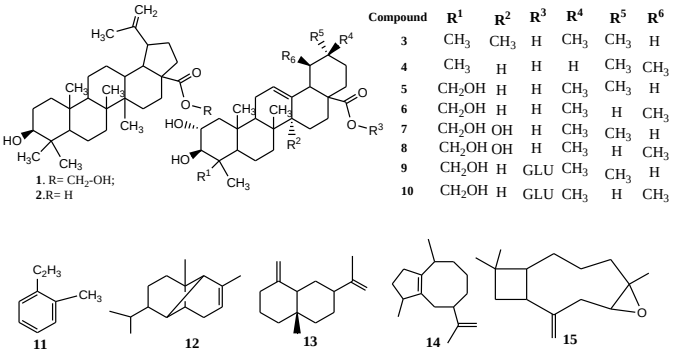
1. Introduction
Syzygium is a genus of flowering plants belonging to the family Myrtaceae comprising of about 1200 species [1, 2]. It is widely distributed spreads across in tropical Africa, sub-tropical and tropical Asia and Australia [3]. Several species such as *Syzygium jambos, Syzygium aqueum* and *Syzygium samarangense* are grown and consumed for their edible fruits. Some are used in traditional medicine to treat inflammation, various allergic disorders, bronchitis, thirst, dysentery and ulcers [4]. Studies also revealed that extracts of different species in the genus *Syzygium* showed antibacterial, antifungal, antioxidant, anti-inflammatory, cytotoxic, anti-HIV, antidiarrheal, anthelmintic, antinociceptive, antivirus and anticancer activities [5-15].
Syzygium guineense is one of the most important species in the genus *Syzygium* (Fig 1). It is a large evergreen flowering plant that is distributed in the tropical regions of such as Australia, Asia and Africa including Ethiopia [16-18]. The plant is also widely distributed in different parts of Ethiopia [19-21], and known by different local names such as "*Dokma*" in Amharic, "*Badeessaa"* in Oromifa, and "*Duwancho"* in Sidama [22]. Its fruits are edible as they are known to have higher nutritional value [23, 24]. There are also reports that mention potential of the tree for family diet and household food security in different parts of Africa including Ethiopia [24-26].

Fig 1: A typical plant of *Syzygium guineense* (Photo taken from Shonie town, Hadya Zone, SNNPR, Ethiopia, November 2016 by Bihon Abera)

In traditional medicine, a liquid material obtained from the pounded bark and roots of *Syzygium guineense* has been reported to act as purgative when mixed with water. The use of its bark for treatment of gastro-intestinal upsets, diarrhea and malaria has been reported in literatures [27, 28]. Reports also revealed the use of other parts of the plant for treatment of various diseases such as tuberculosis, chronic diarrhea, cough, dysentery, malaria, amenorrhea, wounds, ulcers, rheumatism, infections, nasopharyngeal affections, pulmonary troubles and diarrhea [16, 18, 29]. Similar to that of other countries, the plant is being used in traditional medicine by many Ethiopian communities. For instance, oral administration of its fruits and bark are used for treatment of dysentery and diarrhea. Infusion prepared from its leaves, fruits or bark is used for treatment of hypertension [30]. The hot water extract of its fresh bark is used for treatment of stomachache [31, 32]. Dried leaf powder mixed with honey is used to treat leprosy [33]. Other authors also reported traditional medicinal uses of roots, barks and leaves of the plant for treatment of malaria, snake bite, hemorrhoid, gonorrhea, tuberculosis, stomach ache, liver problem and expelling internal worms [34-36].

Reports on the phytochemical screening tests of the leaf extracts of *Syzygium guineense* showed presence of secondary metabolites such as flavonoids, tannins, saponin, alkaloids and cardiac glycosides. These metabolites are believed to be responsible for traditional medicinal uses of the plant or its potential as source of bioactive compounds to be used in drug discovery [37]. For instance, methanolic extract of leaves of *Syzygium guineense* on mice test showed its potential for treatment of snake bites and as antidiabetic agent whereas its ethanolic extract exhibited anti-inflammatory, analgesic activities and antibacterial activities [6, 16, 38]. Studies carried out on *Syzygium guineense* (of Ethiopian origin) also indicated that extracts from its different parts showed antimicrobial [31], antihypertensive [22, 39] and antimalarial [40] activities.

There are also reports that show detection and isolation of compounds from different parts of *Syzygium guineense.* Oladosu *et. al*. (2017) reported isolation of 3-β-hydroxylupane-type isoprenoids: betulinic acid methylenediol ester (1) and betulinic acid (2) (Fig 2) from the chloroform extract of stem bark of *Syzygium guineense* [41]. Isolation of 2-hydroxyoleanolic acid (3), 2-hydroxyursolic acid (4), arjulonic acid (5), asiatic acid (6), terminolic acid (7), 6-hydroxy asiatic acid (8), arjulonic acid 28-β-glycopyranosyl ester (9) and asiatic acid 28-β- glycopyranosyl ester (10) were reported from the leaves of *Syzygium guineense* (Figure 1) [39]. Abok and Manulu (2016) reported detection of twelve compounds from n-hexane extract of leaves of *Syzygium guineense* using TLC and GC-MS analyses. Some of the compounds were 1-ethyl-2-methylbenzene (11), Ylangene (12), decahydro-4a-methyl-1-methylene-7-(1-methylethynyl)-naphthalene (γ-muurolone) (13), 4-dimethyl-7-(1-methylethenyl) azulene (14) and caryophyllene oxide (15) [18] (Figure 2). Noudogbessi1 *et. al.* (2008) also reported detection of several compounds as components of essential oil that was extracted from dried leaves of *Syzygium guineense* [28]. Despite its medicinal use, there are no reports on the phytochemical investigation of the roots of *Syzygium guineense*. In this paper, we report phytochemical screening of root extracts and isolation of compounds from the extracts.



Compound	R^1	R^2	R^3	R^4	R^5	R^6
3	CH_3	CH_3	H	CH_3	CH_3	H
4	CH_3	H	H	H	CH_3	CH_3
5	CH_2OH	H	H	CH_3	CH_3	H
6	CH_2OH	H	H	CH_3	H	CH_3
7	CH_2OH	OH	H	CH_3	CH_3	H
8	CH_2OH	OH	H	CH_3	H	CH_3
9	CH_2OH	H	GLU	CH_3	CH_3	H
10	CH_2OH	H	GLU	CH_3	H	CH_3

Fig 2: The chemical structures of compounds isolated/detected from *Syzygium guineense*

2. Material and Methods

2.1 General experimental material

The IR spectrum of the isolated compound was recorded as KBr pellets using Perk-Elmer BX Infrared Spectrometer in the range of 4000-400 cm^{-1}. The ^{1}H-NMR and ^{13}C-NMR data were recorded using Bruker Avance 400 MHz spectrometer with tetramethylsilane (TMS) as internal standard and DMSO-*d*6 as solvent. Analytical thin layer chromatography (TLC) was carried out using aluminum sheet pre-coated with pre coated 0.2 mm silica gel (60-120) Visualization of spots/compounds on TLC were carried out under UV chamber (at 254 and 365 nm). Column chromatography was performed on silica gel (60-120 mesh size). All the spectral analyses were carried out at The Department of Chemistry, Addis Ababa University, Ethiopia.

2.2 Plant material collection

The roots of *Syzygium guineense* were collected in November, 2016 from Shonie town, Hadya Zone, South Nation Nationalities Peoples' Region (SNNPR), Ethiopia. The area is located about 340 km south of Addis Ababa, and 125 km West of Hawassa University, Ethiopia. The plant species was identified and authenticated by botanist Reta Regassa, Department of Plant Science, Hawassa Teachers' Training College, Ethiopia.

2.3 Preparation and extraction

The collected plant material was chopped to small pieces and dried in an open air for 20 days (without exposure to sun light). The dried plant material was ground using mortar and pestle. The powdered plant material (600 g) was soaked into 3 L n-hexane containing Erlenmeyer flask and subjected to mechanical shaking for 24 hrs. The resulting solution was filtered using Whatmann filter paper. The filtrate was concentrated using rotary evaporator at reduced pressure (and temperature of about 40-45 ^{0}C). The marc was dried at room temperature in an open air and soaked in to 3L dichloromethane/methanol (1:1) for 24 hrs. The mixture was then filtered using Whatmann filter paper and the filtrate was concentrated using rotary evaporator at reduced pressure. The yields all solvent extracts were calculated (Eq. 1).

2.4 Phytochemical screening tests

Phytochemical screening tests were carried out on the crude extracts of n-hexane, dichloromethane/methanol (1:1) and methanol using standard procedures reported in literature [6, 42-44] to detect the presence of secondary metabolites namely

steroids, terpenoids, saponins, flavonoids, tannins alkaloids, phenols and glycosides.

2.4.1 Test for steroids: Two milliliter of acetic anhydride was added to 2 ml of extract that was dissolved in methanol and then 2 ml of H_2SO_4 was added into the test tube containing the mixture [42]. Appearance of a blue-green ring indicates the presence of steroids.

2.4.2 Test for terpenoids: Five milliliter of extract that was dissolved in methanol was mixed with 2 ml of chloroform. Then 3 ml of concentrated H_2SO_4 was carefully added into the test tube containing the mixture in order to get a layer (Salkowski test) [42]. Appearance of a reddish brown coloration indicated the presence terpenoides.

2.4.3 Test for saponins: 0.2 g of crude extract was dissolved in 5 ml of water in test tube and shaken vigorously for 15 minute [43]. Formation of 1 cm layer of foam indicates the presence of saponins.

2.4.4 Test for flavonoids: 3 ml of extract dissolved by methanol was treated with 3 drops of sodium hydroxide solution (Alkaline Reagent Test). Formation of intense yellow color, which becomes colorless on addition of dilute hydrochloric acid, indicates the presence of flavonoids [44].

2.4.5 Test for tannins: 0.2 g of crude extract was boiled in 20 ml of water in a test tube. The solution was filtered and 4 drops of 0.1% ferric chloride ($FeCl_3$) was added into the test tube [6]. Appearance of a brownish green or blue-black coloration confirmed presence of tannins.

2.4.6 Test for alkaloids: One ml of 1% HCl was added into a test tube containing 3 ml of the extract. The mixture was heated for 20 min, cooled and filtered. To 1 ml of the filtrate, 0.5 ml Dragendorff's reagent was added [44]. A red precipitate that confirmed the presence of alkaloids was observed at the end of the test.

2.4.7 Test for phenol (Ferric chloride test): Two milliliter of extracts dissolved by methanol was treated with 4 drops of ferric chloride solution [42]. The formation of green or bluish black color was observed as indicator of the presence of phenols.

2.4.8 Test for glycosides: Two milliliter of chloroform was added in 2 ml of extract dissolved by methanol and then 2 ml H_2SO_4 was added carefully and shaken gently [43]. A color change from orange to reddish brown at interface was observed.

2.5 Column chromatographic isolation of compounds

The dichloromethane/methanol (1:1) crude extract was subjected to column chromatographic separation. The column was then eluted with ethyl acetate: ethanol mixture (with gradual increase in polarity starting from ethyl acetate) employing isocratic approach. A total of 44 fractions (each with 50 mL) were collected and analyzed by TLC. Fractions 9-18 were combined and were subjected for further fractionation using small column and mobile n-hexane: ethyl acetate. The process led to isolation of the compound labeled as compound B1.

3. Results and Discussion

3.1 Percentage yield of crude extracts

As stated in methodology section (Section 2.3), the extraction was conducted in three solvent systems of different polarities. The yield of each crude extract is shown below (Table 1). Out of the three solvents extracts (n-hexane, dichloromethane/methanol (1:1) and methanol), the dichloromethane/methanol (1:1) extract was found to be highest yield. This suggests the root part of *Syzygium guineense* is rich source of medium polar phytochemical constituents. The percent yields of the extracts are calculated using the formula (Eq. 1).

$$\frac{\text{Mass of the extract}}{\text{Mass of the plant material used for extraction}} \times 100\% \quad (1)$$

Table 1: Percentage yield of crude extracts

Solvent used for extraction	Mass of crude extract (g)	% Yield
n-Hexane	2	0.33
Dichloromethane/methanol (1:1)	17	2.83
Methanol	7	1.17

As the given in Table 1, most phytochemicals are extracted by polar solvents (dichloromethane/methanol (1:1) and methanol, thus the chemical constituents of root of *Syzygium guineense* majorly may be polar compounds. The result also indicated that dichloromethane: methanol (1:1) was the best solvent.

3.2 Phytochemical screening tests

Phytochemicals are natural products or plant constituents that are responsible for medicinal properties of plants. Plants of the genus *Syzygium* are known as a rich source of secondary metabolites such as pentacyclic triterpenes and their glycoside derivatives, flavonoids, tannins and other aromatic compounds. Some of these secondary metabolites have been found to show antibacterial, antifungal, anticancer and hepatoprotective activities [21]. In this study, phytochemical screening tests were carried out on n-hexane, dichloromethane: methanol (1:1) and methanol extracts of roots of *Syzygium guineense*. The results revealed the presence of steriods, terpenoids, saponins, flavonoids, tannins, alkaloids, phenol and glycosides in the dichloromethane: methanol (1:1) and methanol extracts whereas only terpenoids and steroids were detected in the n-hexane extract (Table 2). This observation was consistent with previous reports mentioning that different parts of *Syzygium guineense* are rich in secondary metabolites that could be attributed to the medicinal uses of the plant discussed in the introduction section [16, 18, 19, 22, 28-40].

Table 2: The phytochemical screening test results of root of *Syzygium guineense*

Phytochemicals	n-hexane extract	Dichloromethane:methanol(1:1) extract	Methanol extract
Steriods	+	+	+
Terpeniods	+	+	+
Saponins	-	+	+
Flavonoids	-	+	+
Tanins	-	+	+
Alkaloids	-	+	+
Phenol	-	+	+
Glycosides	-	+	+

Note: (+) indicates the presence; (-) indicates absence of particular metabolite

3.3 Structural elucidation of isolated compounds

Compound B1 was obtained from dichloromethane/methanol (1:1) extract (Section 2.5) as white solid with R_f value of 0.3 in n-hexane: ethyl acetate (20:80 %) solvent system. The structure of the compound was determined based on spectroscopic data (IR, ^{1}H-NMR and ^{13}C-NMR) and in comparison with data in literature. The IR spectrum (Appendix 1) of compound B1 showed a broad absorption around 3743 cm^{-1} indicating O-H stretching of alcohols. Strong absorption bands at 1700 cm^{-1} and 2946 cm^{-1} showed the presence of C=O stretching and C-H stretching in sp^3 hybridized carbon, respectively. On the other hand, medium absorption at 1053 cm^{-1} indicates C-O stretching of an ester and a band at 700 cm^{-1} indicated absorption band for CH_2 rocking.

The ^{1}H-NMR spectrum (Appendix 2) of compound B1 showed an intense peak at δ5.17 suggesting the presence of olefinic proton whereas the peaks at δ3.4 and 3.0 indicate methine protons bonded to hydroxyl group bearing carbon atoms. The peak observed at δ3.2 indicates protons on methylene group bearing hydroxyl functional group (CH_2-OH). Moreover, a peak at δ 2.74 indicates proton attached to a carbon that is attached to the olefinic carbon, and a singlet signal at δ3.36 indicates methoxy protons. ^{1}H-NMR spectrum also showed broad peaks at δ4.0 - 4.5 that could be attributed to protons of hydroxyl groups. Six aliphatic methyl signals at were observed at δ0.60 (s), 0.75(s), 0.85 (s), 0.92 (s), 0.97 (s) and 1.10 (s). The multiplet peaks at 0.88-2.2 indicating methine and methylene proton reported for terpenoids in literature [45-47] (Table 3). The peaks at δ67.89 and 76 in the ^{13}C-NMR and DEPT-135 NMR spectra (Appendix 3 and 4) of compound B1 indicated the presence methine groups that bear hydroxyl functional groups. The peaks at δ64.40, δ52.85 and 179.05 indicate hydroxyl methylene (CH_2-OH), one methoxy group bonded to carbonyl functional group and one ester carbonyl group, respectively (Table 4).

The ^{13}C-NMR and DEPT-135 data also confirmed the presence of olefinic carbons at δ121.9 and 144.4 and ten methylene groups (one hydroxyl methylene listed above), seven methyl group (one methoxy), six methine group and eight quaternary carbons. This suggests that compound B1 could be a pentacyclic terpenoids specifically, Olean-12-ene-skeleton [46]. Information obtained from IR, ^{1}H-NMR, ^{13}C-NMR and DEPT-135 spectra indicated that the compound B1 is most probably to be methyl ester of arjunolic acid ester. As the data are more closely related to the literature reported spectral data of arjunolic acid (Table 3 and 4) [47, 48].

Table 3: Comparison of ^{1}H-NMR spectral data of compound B1 with that of arjunolic acid reported in literature [47, 48].

Proton numbers	^{1}H-NMR data of compound B1 (δ)	^{1}HNMR data of arjunolic acid reported in literature [47,48]
2	3.40 (1H,m)	3.31 (1H,m)
3	3.00 (1H,d)	3.04 (1H,d)
12	5.17 (1H,t)	5.17 (1H,brs)
18	2.74 (1H,dd)	2.74 (1H,dd)
23	3.20 (2H,d)	3.17 (2H,d)
24	1.10 (s)	1.54 (s)
25	0.92 (s)	0.97 (s)
26	0.85 (s)	1.06 (s)
27	0.97 (s)	1.18 (s)
29	0.75 (s)	0.87 (s)
30	0.60 (s)	0.74 (s)
31	3.36 (s)	-
1,5,6,7,9,11,15,16, 19, 21,22	0.88-2.2 (m,CH and CH_2 terpenoid protons (20 H)	0.74-1.98 (Terpenoids protons)

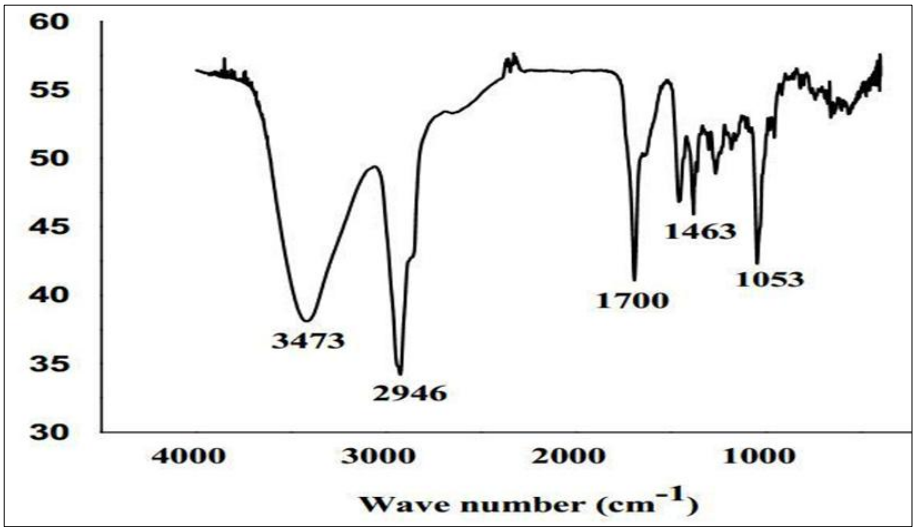
☞**Note**: 4, 8, 10, 13, 14, 17, 20 and 28 are quaternary carbons

The data indicated that similarity between the two compounds (compound B1 and arjunolic acid) except the their difference is the presence of carboxylic acid functional group at carbon 28 at δ 179.04 and the absence peak of methoxy carbon in the ^{13}C-NMR in arjunolic acid but for compound B1. IR spectra also confirmed absence of carboxylic acid functional group in compound B1. ^{13}C-NMR spectrum also suggests the presence methoxy carbon at δ52.85 that is not observed in the case of arjunolic acid (Table 4). Thus, compound B1 is an esterified form of arjunolic acid, and can be proposed to be 2, 3, 23-trihydroxy methyloleanate (Fig 3).

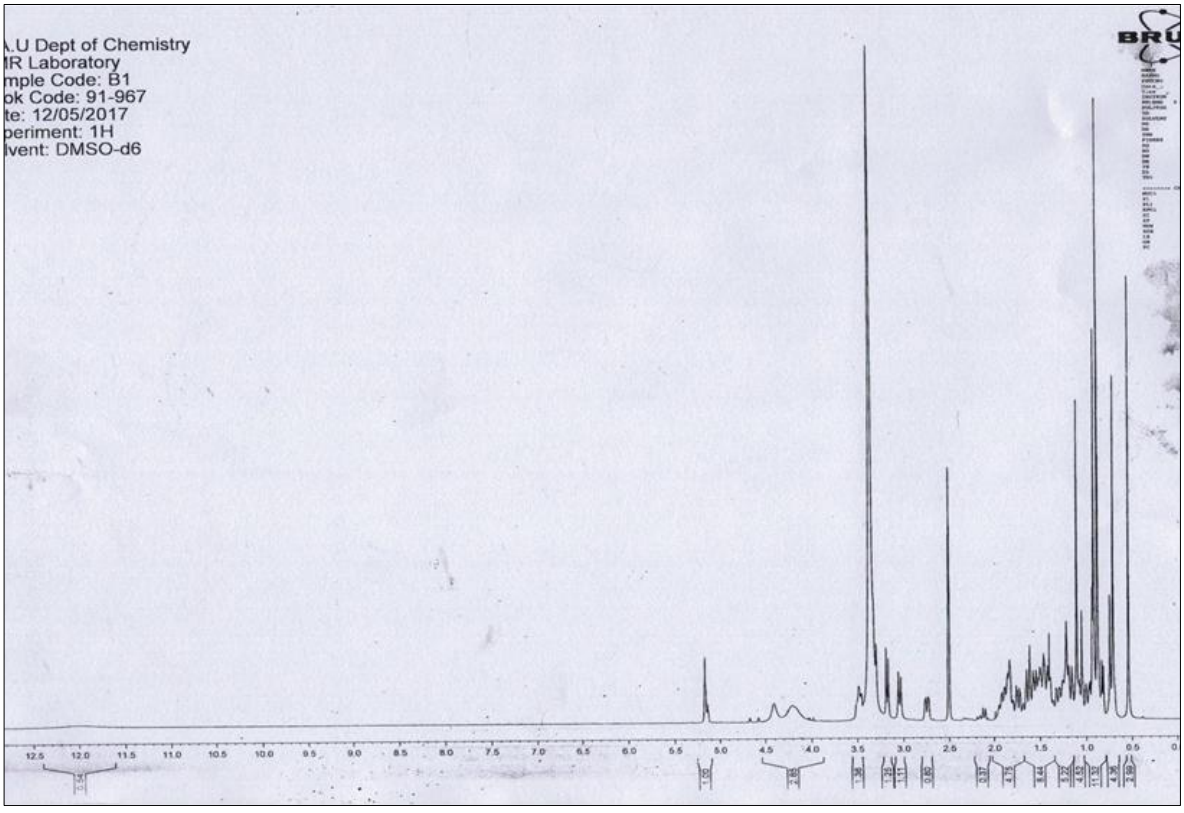
Table 4: The ^{13}C-NMR spectral data of compound B1 and that of arjunolic acid reported in literature [47, 48]

Carbon numbers	^{13}C-NMR data of Compound B1	DEPT-135 data of Compound B1	^{13}C-NMR data reported for arjunolic acid [47, 48]	DEPT-135 data reported for arjunolic acid	Remarks
1	47.15	47.15	47.14	47.14	CH_2
2	67	-	67.89	-	CH
3	76	-	76.04	-	CH
4	43	-	42.96	-	Quaternary
5	47.53	-	47.52	-	CH
6	17.93	17.93	17.93	17.93	CH_2
7	32.57	32.57	32.56	32.56	CH2
8	39.96	-	39.96	-	Quaternary
9	47.29	-	47.27	-	CH
10	37.70	-	37.70	-	Quaternary
11	23.60	23.60	23.60	23.6	CH_2
12	121.90	-	121.95	-	CH
13	144.40	-	144.38	-	Quaternary
14	42.20	-	42.20	-	Quaternary

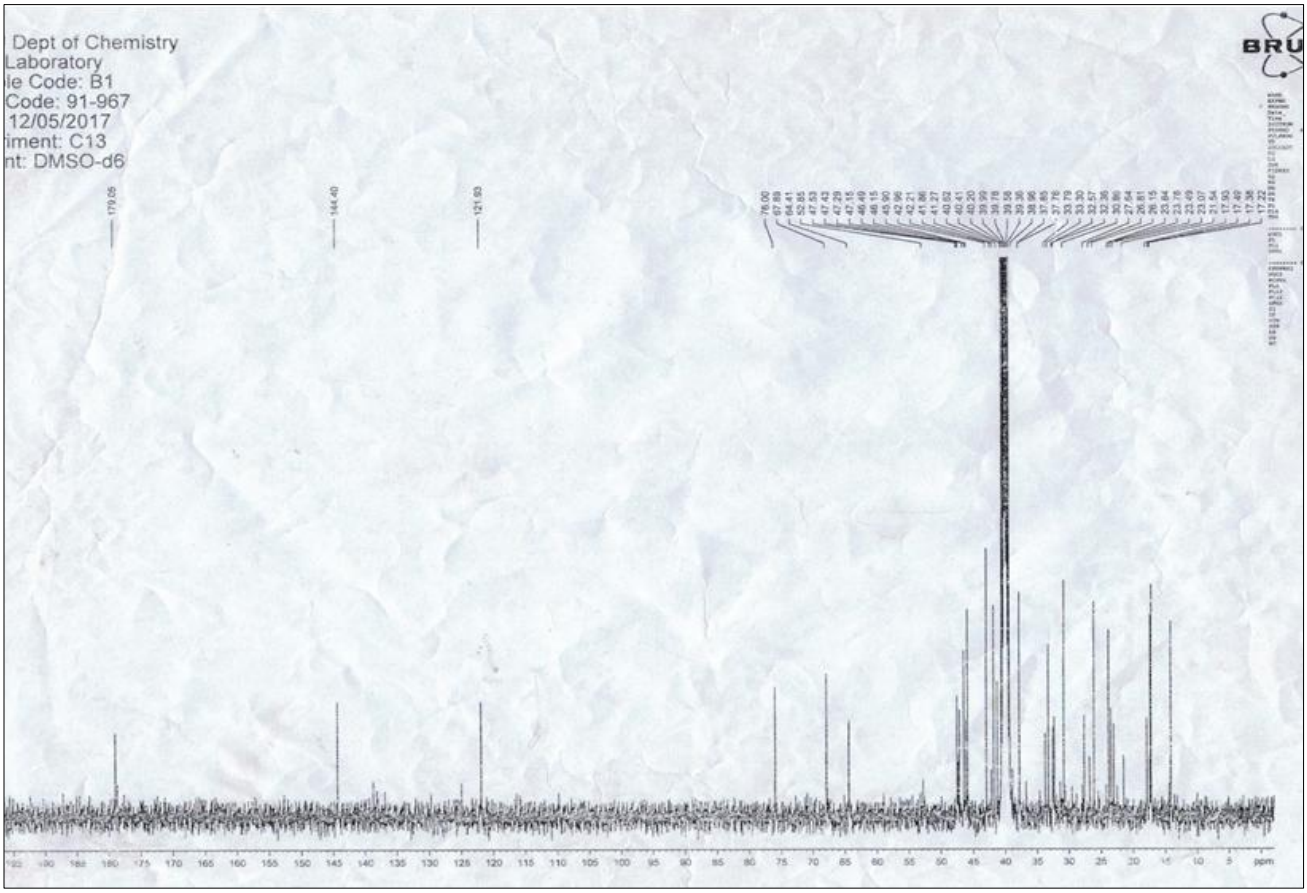
15	27.60	27.60	27.63	27.63	CH_2
16	23.80	23.80	23.80	23.8	CH_2
17	45.90	-	45.90	-	Quaternary
18	41.85	-	41.86	-	CH
19	46.48	46.48	46.48	46.48	CH_2
20	30.86	-	30.86	-	Quaternary
21	33.79	33.79	33.78	33.78	CH_2
22	33.30	33.30	33.56	33.56	CH_2
23	64.4	64.4	64.4	64.4	CH_2
24	14.2	-	14.2	-	CH_3
25	17.38	-	17.37	-	CH_3
26	17.49	-	17.49	-	CH_3
27	26.15	-	26.15	-	CH_3
28	179.05	-	179.04	-	Quaternary
29	33.3	-	33.35	-	CH_3
30	23.7	-	23.78	-	CH_3
31	52.85	-	-	-	OCH_3 (for compound B1)



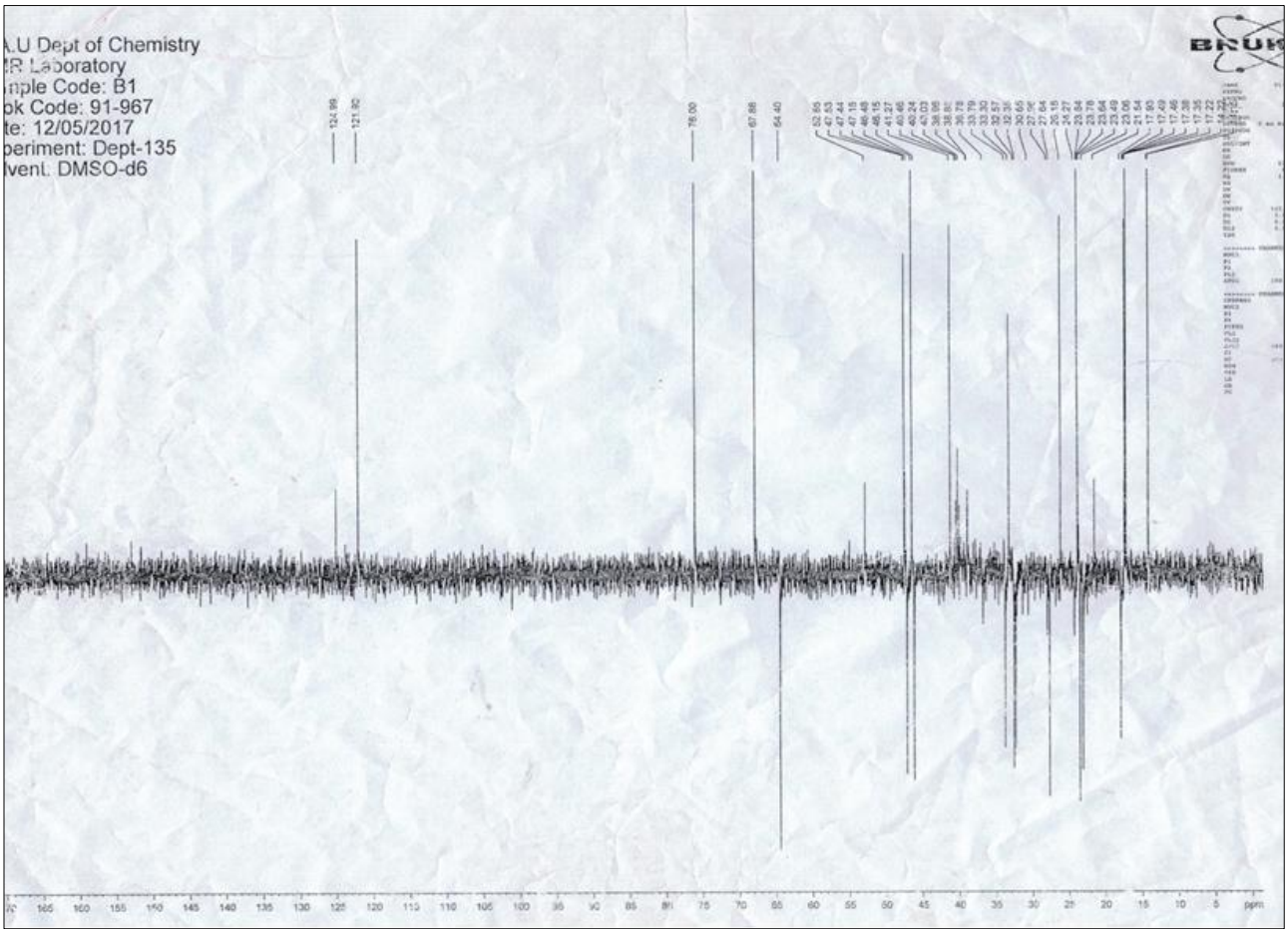
Appendix 1: IR Spectrum of compound B1



Appendix 2: [1]HNMR Spectrum of compound B1



Appendix 3: ^{13}C-NMR Spectrum of compound B1



Appendix 4: DEPT 135- Spectrum of compound B1

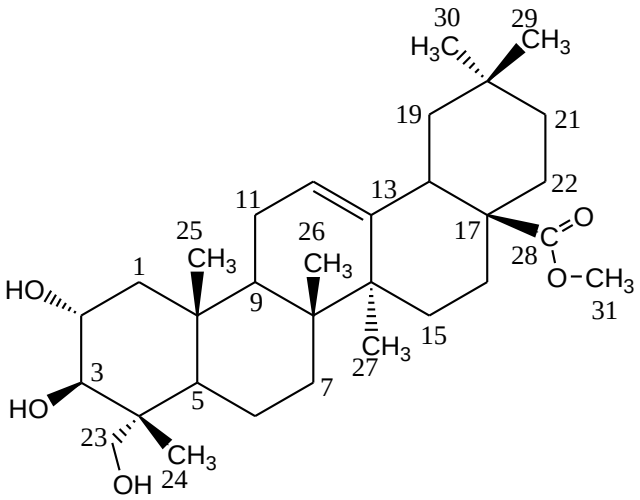


Fig 3: The proposed structure of compound B1 (2, 3, 23-trihydroxy methyloleanate)

4. Conclusion

In this study, phytochemical screening tests were carried out on n-hexane dichloromethane: methanol (1:1) and methanol extracts of roots of *Syzygium guineense*. The results revealed the presence of steriods, terpenoids, saponins, flavonoids, tannins, alkaloids, phenol and glycosides in the dichloromethane: methanol (1:1) and methanol extracts whereas only terpenoids and steroids were detected in the n-hexane extract. The presence of these metabolites could be responsible for medicinal use of the *Syzygium guineense*. Moreover, compound B1 was isolated from dichloromethane: methanol (1:1) extract. Its structure was determined based on spectroscopic data and comparison with literature reports. The compound was proposed to be 2,3,23-trihydroxy methyl oleanate. This is the first report of isolation of 2,3,23-trihydroxy methyl oleanate from the species.

5. Acknowledgement

Bihon acknowledges Ministry of Education, Federal Democratic Republic of Ethiopia (FDRE) for financial support.

6. References

1. Kuphumla Z, Seedroom K. *Syzygium guineense* (Willd). http://pza.sanbi.org/syzygium-guineense (Accessed on August 31, 2017).
2. Anuruk C, Thiti J, Wilart P, Narong N, Puttinan M. Two flavonoids first isolated from the seed of *Syzygium nervosum* and preliminary study of their anticancer and anti-HIV-1 reverse transcriptase activities. Maejo Int. J. Sci. Technol. 2017; 11:58-67.
3. Karunamoorthy K, Jothiramshekar S, Palanisami E, Puthiyapurayil S, Ajay P. Chemical composition, antimicrobial, antioxidant and anticancer activity of leaves of *Syzygium benthamianum* (Wight ex Duthie) Gamble. J. Biol. Active Prod. Nature. 2011; 1:273-278.
4. Nur AB. Chemical composition, antioxidant and antibacterial activity of essential oil from leaf of *Syzygium polyanthum* (Wight) Walp. Faculty of industrial sciences and technology Universiti Malaysia Pahang, 2014.
5. Francine NT, Constant AP, Cabral PB, Romain JN, Bruno MM, Angelo C, *et al*. *In vitro* organo-protective effect of bark extracts from *Syzygium guineense var macrocarpum* against ferric-nitrilotriacetateinduced stress in wistar rats homogenates. BMC Complementary Alternative Med., 2016; 16:315. DOI 10.1186/s12906-016-1263-1
6. Sheila M, Godeliver K, Charles WM, Lucy M. Determination of mineral content, cytotoxicity and anthelmintic activity of *Syzygium guineense* Fruits. Saudi J. Med. Pharmaceut. Sci. 2016; 2:95-99.
7. Tsakala TM, Penge O, John K. Screening of in vitro antibacterial activity from *Syzygium guineense* (Willd) hydrosoluble dry extract. Annal. Pharmaceut. Francaises., 1996; 54:276-279.
8. Ajam MS. *In vitro* antimicrobial and antioxidant activity of aqueous and acetone extracts of *Syzygium grande* (Wight) Walp. leaves. J. Genetic Environ. Resources Conserv. 2014; 2:178-185.
9. Chellam M, Nadarajan S, Das AK, Swamy AS. Chemical profiling of leaf essential oil, antioxidant potential and antibacterial activity of *Syzygium lanceolatum* (Lam.) Wt. & Arn. (Myrtaceae). Free Radicals. Antioxidants, 2016; 6:1-22.
10. Azra K. Phytochemical screening of *Syzygium cumini* seeds. Indian J. Plant Sci. 2014; 3:1-4.
11. Bharathi T, Siddaiah M, Sriharsha SN. Anti-inflammatory activity of methanol extract of *Syzygium alternifolium* in experimental rats. Int. J. Innovations. Pharmaceut. Res. 2012; 3:255-257.
12. Sudhakar A, Ramesh C, Nagaraju N, Vedavathy S, Murthy SR. Pharmacognostical studies on stem and fruit of *Syzygium alternifolium (*Wight) Walp.: An endemic to south eastern Ghats, India. Asian J. Biochem. Pharmaceut. Res. 2012; 2:127-138.
13. Sangeeth KM, Nusrath Y. Antibacterial activity of methanolic extract of *Syzygium alternifolium* leaves. Am. J. Advanced Drug Delivery. 2013; 5:628-634.
14. Appala R, Ganapaty S, Rao PS, Asha JV. Phytochemical screening and analgesic activity of *Syzygium alternifolium* (Wild). Asian J Chem. 2010; 22:2468-2470.
15. Zeba B, Anusha J, Kemala K, Sam JR. Anti-uceleractivity of *Syzygium alternifolium* against ethanol and NSAID induced uceler in rats. Int. J Pharmacol. Pharmacotherapeutics. 2012; 1:18-22.
16. Ezenyi IC, Mbamalu ON, Balogun L, Omorogbe L, Ameh FS, Salawu OA. Antidiabetic potentials of *Syzygium guineense* methanol leaf extract. J Phytopharmacol. 2016; 5:150-156.
17. Teketay D, Tesfaye G, Fetene M. Regeneration of fourteen tree species in Harenna forest, southeastern Ethiopia. Flora, 2002; 197:461-474.
18. Abok JI, Manulu C. TLC analysis and GC-MS profiling of Hexane extract of S*yzygium guineense* Leaf. J Med. Plants Studies. 2017; 5:261-265.
19. Girmay A. Chronic effects of the aqueous leaf extract of *Syzygium guineense* on some blood parameters and histopathology of liver and kidney of mice. MSc Thesis, Addis Ababa University. Addis Ababa, Ethiopia, 2009.
20. Mathewos A. Ethnobotany of spice and condiment plants and the associated indigenous knowledge on management, utilization and conservation of them in and around home gardens in *Loma* and *Gena Bosa* districts (*Weredas*) of *Dawuro Zone*, Southern Ethiopia. Int. J Agri. Innovations Res. 2015; 4:2319-1473.

21. Sisay N. Flora biodiversity assessment in *Bonga*, *Boginda* and *Mankira* forest, *Kaffa*, Ethiopia. A report submitted to PPP Project, Addis Ababa, 2008 (Accessed from www. http://www.kafa-biosphere.com/assets/content-documents/KafaFloral-Survey-Final-Report.pdf. (Accessed on 5 September, 2017).
22. Bizuayehu N. Antispasmodic, antidiarrheal and LD_{50} determination of *Syzygium guineense* in animal models. MSc. Thesis, Faculty of Medicine, Addis Ababa University, Addis Ababa, Ethiopia, 2004.
23. Saka JD, Msonthi JD. Nutritional value of edible fruits of indigenous wild trees in Malawi. Forest Ecol. Manag., 1994; 64:245-248.
24. Baressa A. Ethnobotanical study of wild edible plants in Bule Hora Woreda, Southern Ethiopia. African J. Basic. Appl. Sci. 2016; 8:198-207.
25. Kebu B, Fassil K. Ethnobotanical study of wild edible plants in Dershe and Kucha districts, South Ethiopia. J. Ethnobiol. Ethnomed. 2006; 2:1-9.
26. Ermias L, Zemede A, Ensermu K, Patrick VD. Wild edible plants in Ethiopia: a review on their potential to combat food insecurity. Afrika Focus, 2011; 24:71-121.
27. Omale J, Ebiloma UG, Ogohi DA. Anti-venom studies on *Olax viridis* and *Syzygium guineense* extracts. Am. J Pharmacol. Toxicol. 2013; 8:1-8.
28. Jean PN, Paul Y, Dominique CK, Jean CC, Gilles F. Chemical composition of essential oil of *Syzygium guineense* (Willd.) DC. var. *guineense* (Myrtaceae) from Benin. Records of Nat. Products. 2008; 2:33-38.
29. Kisangau DP, Lyaruu HV, Hosea KM, Joseph CC. Use of traditional medicines in the management of HIV/AIDS opportunistic infections in Tanzania: A case in the Bukoba rural district. J. Ethnobiol. Ethnomed. 2007; 3. doi: 10.1186/1746-4269-3-29.
30. Million, L. Evaluation of acute and subacute toxicity of ethanol extract of *Syzygium guineense* leaves on the histology of the liver and kidney and biochemical compositions of blood in rats. MSc. Thesis. Addis Ababa University, Ethiopia, 2016.
31. Geyid A, Abebe D, Debella A, Makonnen Z, Aberra F, Teka F, *et al*. Screening of some medicinal plants of Ethiopia for their anti-microbial properties and chemical profiles. J Ethnopharmacol. 2005; 97:421-427.
32. Regassa R. Diversity and conservation status of some economically valued indigenous medicinal plants in Hawassa College of Teachers' Education Campus, Southern Ethiopia. Int. J Advanced Res. 2013; 1:308-328.
33. Getaneh G, Zemede A, Abiyu E, Nagappan R. Ethnobotanical study of traditional medicinal plants and their conservation status in Mecha Wereda, West Gojjam Zone of Ethiopia. Int. J. Pharmaceut. Health. Care Res. 2014; 2:137-154.
34. Fisseha M, Talemos S, Abreham A. An ethnobotanical study of medicinal plants in *Amaro* Woreda, Ethiopia. Ethnobotany Res. Applications, 2014; 12:341-354.
35. Gonfa K, Tesfaye A, Ambachew D. Indigenous knowledge on the use and management of medicinal trees and shrubs in *Dale* district, Sidama Zone, Southern Ethiopia. Ethnobotany Res. Applications, 2015; 14:171-182.
36. Tewodros T. Use and management of medicinal plants by people of *Melka Belo Woreda*, East Hararghe, Oromia Region, Ethiopia. MSc Thesis. Haramaya University, Haramaya, 2016
37. Ior LD, Otimenyin O, Umar M. Anti-inflammatory and analgesic activities of the ethanolic extract of the leaf of *Syzygium guineense* in rats and mice. J Pharm. 2012; 2:33-36.
38. Djoukeng JD, Abou ME, Tabacchi R, Tapondjou AL, Boudab H, Lontsi, D. Antibacterial triterpenes from *Syzygium guineense* (Myrtaceae). J. Ethnopharmacol. 2005; 101:283-286.
39. Ayele Y, Urga K, Engidawork E. Evaluation of in vivo antihypertensive and in vitro vasodepressor activities of the leaf extract of *Syzygium guineense* (Willd) DC. Phytother. Res., 2010; 24:1457-1462.
40. Tadesse SA, Wubneh ZW. Tadesse and Wubneh. Antimalarial activity of *Syzygium guineense* during early and established *Plasmodium* infection in rodent models. BMC Complementary and Alternative Med., 2017, *17*:21. DOI 10.1186/s12906-016-1538-6
41. Oladosu IA, Lawson L, Aiyelaagbe O, Emenyonu N, Afieroho O. Anti-tuberculosis lupane-type isoprenoids from *Syzygium guineense* Wild DC. (Myrtaceae) stem bark. Future J Pharmaceut. Sci. 2017. (http://dx.doi.org/10.1016/j.fjps.2017.05.002)
42. Tiwari P, Kumar B, Kaur M, Kaur H. Phytochemical screening and extraction: A review. Int. Pharmaceut. Sci., 2011; 1:98-106.
43. Alebiosu CO. Phytochemical screening, thin-layer chromatographic studies and UV analysis of extracts of *Citrullus lanatus*. J Pharmaceut. Chem. Biol. Sci. 2015; 3:214-220.
44. Wozniak L, Pska SS, Marszałe K. Ursolic acid: A pentacyclic triterpenoid with a wide spectrum of pharmacological activities. Molecules. 2015; 20:20614-20641.
45. Rao VL, Rammohan A, Duvvuru G. A new pentacyclic triterpenoid from *Syzygium alternifolium.* J Chem. Pharm. Res. 2015; 7:123-125.
46. Conrad J, Vogler B, Kalaiber I, Roos G, Walter U, Kraus W. Two triterpene esters from *Terminalia macroptera* bark. Phytochemistry. 1998; 48:647-650.
47. Ramesh SA, Christopher JG, Radhika R, Setty CR, Thankamani V. Isolation, characterisation and cytotoxicity study of arjunolic acid from *Terminalia arjuna*. Natural Products Res. 2012; 26:1549-1552.
48. Eder B, Walmir SG, Lidilhone H, Caroline T, Fernanda RG. Bioactive pentacyclic triterpenes from the stems of *Combretum laxum.* Molecules. 2008; 13:2717-2728.