International Journal of Secondary Metabolite
2024, Vol. 11, No. 4, 710–721
https://doi.org/10.21448/ijsm.1404324
journal homepage: https://dergipark.org.tr/en/pub/ijsm
Research Article

The isolation of bioactive compounds from *Warburgia ugandensis* bark: A report of albicanyl acetate, caseamemin and β-sitosterol from *Warburgia* species

Zelalem Gizachew[1*], Chiristopher Suh[2]

[1]Bio and Emerging Technology Institute, Addis Ababa, Ethiopia
[2]Country Engagement Officer and Central Africa Regional Coordinator Technologies for African Agricultural Transformation (TAAT), Kinshasa, Congo

ARTICLE HISTORY
Received: Dec. 13, 2023
Accepted: Apr. 02, 2024

KEYWORDS
Warburgia ugandensis,
Albicanyl acetate,
Caseamemin,
Isolation,
Phytotoxic activity.

Abstract: *Warburgia ugandensis,* which is one of the indigenous species of Ethiopia, is known for its wide range of biological activities. A series of drimane sesquiterpenoids have been isolated from the stem bark of the plant. However, there is no report on the herbicidal potential of the plant against invasive weeds like *Parthenium hysterophorus*. In this study, the herbicidal potential of *W. ugandensis* against the *P. hysterophorus* weed was investigated. Following the bioassay protocol, muzigadial as powerful phytotoxic compound together with other eight compounds were isolated from the EtOAc soluble portion of the ethanol extract of the bark of the plant. These compounds were identified using different physical and spectroscopic methods. The isolated compounds are albicanyl acetate (35), caseamemin (36), β-sitosterol (37), muzigadial (38), cinnamolide-3β-acetate (39), ugandensidial (40), 11α-hydroxy muzigadiolide (41), polygodial (42) and 9-deoxymuzigadial (43). The first three compounds are new to the species *W. ugandensis.* Furthermore, two other compounds namely heptacosanol (44) and hentriacontane (45) were also isolated from this species. In summary, the purpose of this study, to the best of my knowledge, is to provide the three initially identified compounds from the plant material and provide information on the plant's potential utility in agricultural applications.

1. INTRODUCTION

Warburgia ugandensis Sprague (Canellaceae), one of Ethiopia's indigenous species, is an evergreen and aromatic perennial plant (Maroyi, 2014) with a characteristic aromatic and pungent bark. Traditional healers have used the plant to treat various ailments since antiquity, despite its multiple uses as timber, poles, charcoal, firewood, ornaments, shade, and resin (Kairu *et al*., 2013). The plant is a remedy for stomachache, headache, constipation, toothache, common cold, internal wound, and malaria (Kipkore *et al*., 2014; Maroyi, 2014). In addition to these, it is also reported that the plant is used to prevent diarrhea, cough, sexually transmitted diseases, snake bites, bronchial infections, fever, oral thrush, muscle pain, urinary tract infections, constipation, weak joints, and measles (Okello & Kang, 2021).

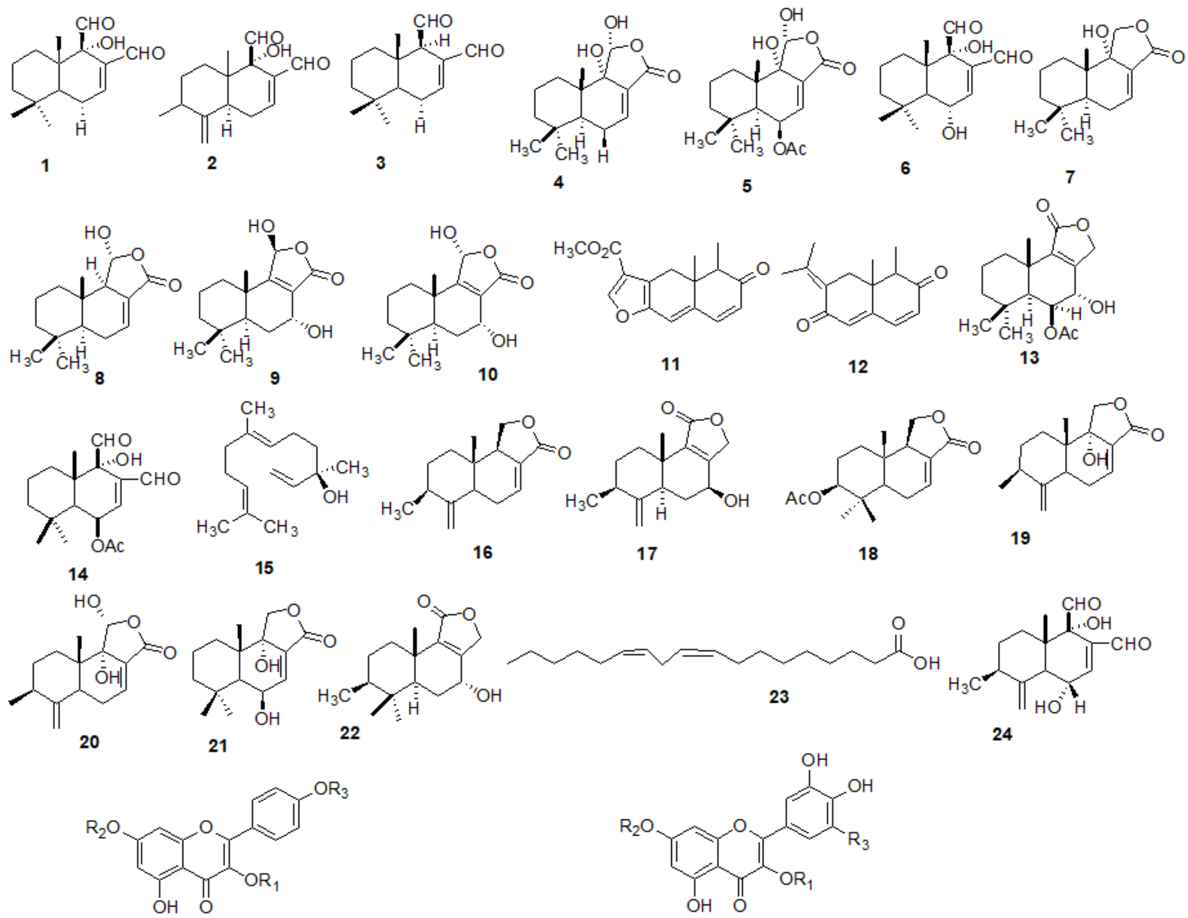
*CONTACT: Zelalem Gizachew ✉ zelalemgizachew2013@gmail.com Bio and Emerging Technology Institute, Biotechnology center, Plant Biotechnology, Addis Ababa, Ethiopia

e-ISSN: 2148-6905

In a study done by Wube *et al*, the antimicrobial activity of the plant was reported (Wube et al., 2005; Okello & Kang, 2021). The antileishmanial activity of different extracts of *W. ugandensis* was studied, and it was reported that the nonpolar extract of the plant displayed strong activity against *Leishmania major* promastigotes (IC_{50} value of 9.95) and amastigotes (IC_{50} value of 8.65) at minimum inhibition concentrations of 62.5 µg/ml (Ngure *et al*., 2009). *In vitro* pharmacological studies on this plant have revealed its insecticidal potential against maize weevils (Opiyo, 2021) and molluscicids (Maroyi, 2014).

Based on a number of biological activities reported, several Drimane-type sesquiterpenes and flavonoids (Figure 1) have been isolated from various parts of the plant (Arot Manguro, Ugi, Hermann, *et al*., 2003; Wube *et al*., 2005). Widely applicable dialdehydic compounds: warburganal (**1**), muzigadial (**2**) and polygodial (**3**) and different sesquiterpene compounds: ugandenial A (**4**), 9α, 11α-dihydroxy,6β-acetyl-cinnamolide (**5**), mukaadial (**6**), 9α-hydroxycinnamolide (**7**), and dendocarbins A, L, and M (**8, 9** and **10**) were isolated using different chromatographic and spectroscopic methods.

The first phytochemical investigation of *W. ugandensis* led to the isolation of drimane-type sesquiterpenoids from the heartwood of the plant. These are warburgin (**11**), warburgiadione (**12**), ugandensolide (**13**) and ugandensidial (**14**) (Brooks, 1969). Another study on the stem bark of *W. ugandensis* conducted by Gonfa, T. led to the isolation of four sesquiterpenes, namely nerolidol (**15**), muzigadial (**2**), ugandensidial (**14**), and cinnamolide-3*β*-acetate (**18**) (Gonfa *et al*., 2020).



25 R_1 = xylosyl (1→ 2) glucose, R_2 = H, R_3 = Me
26 R_1 = rhamnose, R_2 = R_3 = glucose
27 R_1 = R_2 = R_3 = glucose
30 R_1 = R_2 = R_3 = H
31 R_1 = rhambose, R_2 = R_3 = H
32 R_1 = glucose, R_2 = R_3 = H

28 R_1 = [rhamnosyl (1→6)] [glucosyl (1→ 2)]-glucoside, R_2 =rhamnose, R_3 = H
29 R_1 = H, R_2 = rhamnose, R_3 = H
33 R_1= R_2 =H, R_3 =OH
34 R_1= R_2 = R_3 = H

Figure 1. Previously isolated compounds from *Warburgia* species.

In the interest of searching for antibacterial sesquiterpenoids, numerous compounds were reported from *W. ugandensis*. The compounds isolated are 4(13),7-coloratadien-12,11-olide (**16**), and 7β-hydroxy-4(13),8 coloratadien-11,12-olide (**17**), together with nine known sesquiterpenes, i.e., cinnamolide-3β-acetate (**18**), muzigadial (**2**), muzigadiolide (**19**), 11α-hydroxy muzigadiolide (**20**), pereniporin-B (**21**), 7α-hydroxy-8-drimen-11,12-olide (**22**), 6α,9α-dihydroxy-4(13),7-coloratadien 11,12-dial (**23**), and linoleic acid (**24**) (Rabe & Staden, 2000). Flavonol glycosides (**25-29**) together with known flavonols like kaempferol (**30**), kaempferol-3-rhamnoside (**31**), kaempferol-3-glucoside (**32**), myricetin (**33**), and quercetin (**34**) were reported from the leaf methanol extracts of *W. stuhlmannii* and *W. ugandensis* (Arot Manguro, Ugi, Hermann, *et al*., 2003; Arot Manguro, Ugi, Lemmen, *et al*., 2003). Even though many bioactive components for various applications were reported from *W. ugandensis*, the scope is still there to identify bioactive compounds from this plant for different applications, including the agricultural sector. Thus, aiming to study the herbicidal potential of the plant led to the isolation of three previously unreported compounds from this plant.

2. MATERIAL and METHODS

2.1. Plant Material

Warburgia ugandensis bark (Figure 2) was collected from Bale Robe, Oromia region, south-central Ethiopia, which has a latitude of 6° 44' 59.99'' N and a longitude of 40° 14' 60.00'' E. It is the area found at an altitude of 2,492 meters and 430 kilometers far away by road from Ethiopia's capital, Addis Ababa. The specimen of the plant was deposited at the National Herbarium Department of Biology, Addis Ababa University Herbarium, with voucher number 97-41A, and its identity was determined by a plant taxonomist, Dr. Melaku.

Figure 2. Stem bark of *W. ugandensis* tree.

2.2. Materials Used for Purification and Spectroscopic Analysis

The compounds reported in this study were isolated using two sizes of column chromatography on silica gel and aluminum oxide (neutral), medium size and small size, which can carry 80 g and 12 g of silica gel, respectively. Fractions collected from CC were purified by Sephadex LH-20. Preparative thin-layer chromatography was run on a 1.0 mm thick layer of silica gel. The silica gel used for the CC is 60-120 mesh particle size. TLC was performed on precoated plates (Silica gel 60 F254, 230-400 mesh, Merck) and aluminum oxide plates; melting points are uncorrected; detection by UV light at 254 and 366 nm and spray reagents vanillin-H_2SO_4; IR: KBr disk or neat and measured on a Perkin Elmer 1600 and Pye Unicam Infrared spectrophotometer SP3-300. UV spectra were measured on a Shimadzu UV-VIS recording spectrophotometer, a UV-160 spectronic genesys spectrophotometer; ^{1}H and ^{13}C NMR were recorded, in $CDCl_3$, DMSO, $(CD_3)_2CO$, and CD_3OD using the solvent peak as reference (chloroform: δ_H 7.2 and δ_C 77.2, DMSO, δ_H 2.5 and δ_C 39.5, deuterated acetone: δ_H 2.05 and δ_C 29.5 and 205.5 methanol: δ_H 3.3 and δ_C 49.0). Chemical shift values were reported in δ (ppm) units, the solvent signals as internal references; ^{1}H, ^{13}C, and 2D-NMR spectra were obtained on a Jeol F X 90 Ω spectrophotometer at 90 and 22.5 MHz; a Jeol JNM-EX400 instrument at 400 MHz and 100 MHz; and a Bruker Ultrashield TM 400 spectrometer at 400 and 100 MHz with TMS and solvents as internal standard, and δ values are given in ppm relative to TMS internal standard. EIMS was obtained on a Finnigan MAT 95Q and VG Quattro quadrupole mass spectrometer (70 eV).

2.3. Extraction Procedure

The bark of the plant was ground with a manual grinder into a fine powder. The powder (150 g) was extracted with ethanol (500 mL) to afford 15 g of red-like jelly material. It was then partitioned with EtOAc and methanol. The EtOAc soluble part (4 g) was adsorbed on silica gel (60-120 mesh) and subjected to silica gel (80 g) column chromatography. The column was eluted with an n-hexane: EtOAc solvent system by increasing polarity to afford 12 combined fractions.

Fraction 5 was collected from CC using n-hexane: EtOAc (9:1) and white powder was precipitated on the surface of the vial and labeled as compound **35** (27 mg). The remaining part was concentrated using rotary vapor, and 800 mg was obtained. From this fraction, 155 mg was taken and applied on PTLC using n-hexane: EtOAc (9:1) as a mobile phase, which gave compound **36** (15 mg). From the remaining part of fraction 5, 400 mg was applied on small-size silica gel (10 g) column chromatography, and eight subfractions were collected. Subfraction 2 (60 mg) was further purified by PTLC to give compound **37** (20 mg).

2.3.1. *Physical and spectral data for compound 35*

White solid (27 mg); soluble in $CHCl_3$; mp 130-136^oC; R_f 0.62 (mobile phase hexane: EtOAc, 2:1); UV (EtOH) λmax nm: no absorbance; IR υ_{cm-1}: 3453 (OH stretching), 1736, and 1680 (*α*, *β* unsaturated C=O stretching), 1736 (acetate group), 1370 (geminal dimethyl stretching), 1230 and 1024 (C-O stretching and bending); ^{1}H-NMR (400 MHz, $CDCl_3$), chemical shift δ in ppm, coupling constant *J* in Hz: δ_H 3.53 (1H, *m*, H-3), 5.37 (1H, *t*, *J* = 2 Hz, H-6), 0.93 (3H, *d*, *J* = 6.8 Hz, H-19), 0.84 (3H, *d*, *J* = 2 Hz, H-24), 0.84 (3H, *d*, *J* = 2 Hz, H-26), 0.82 (3H, *d*, H-27), 0.70 (3H, *s*, H-28), and 1.03 (3H, *s*, H-29); ^{13}C NMR (100 MHz, CDC_3): δ_C 37.26 (C-1), 31.68 (C-2), 71.84 (C-3), 42.32 (C-4), 140.76 (C-5), 121.75 (C-6), 31.92 (C-7), 31.98 (C-8), 50.14 (C-9), 36.53 (C-10), 21.11 (C-11), 39.78 (C-12), 42.34 (C-13), 56.78 (C-14), 26.05 (C-15), 28.28 (C-16), 56.05 (C-17), 36.17 (C-18), 19.43 C-19), 33.95 (C-20), 24.33 (C-21), 45.84 (C-22), 23.07 (C-23), 12.01 (C-24), 29.15 (C-25), 19.86 (C-26), 19.05 (C-27), 18.80 (C-28), and 11.89 (C-29).

2.3.2. *Physical and spectral data for compound 36*

Jelly material (15 mg); soluble in $CHCl_3$; R_f 0.50 (mobile phase hexane: EtOAc, (5:1); no absorption in the UV-Vis region; IR υ_{cm-1}: 2923 (C-H stretching), 1735 (acetate unit), 1641 and 1461 cm-1 (exocyclic double bond stretching and bending), 1230 (C-O stretching), 1376 cm-1 (geminal dimethyl stretching); ^{1}H-NMR (400 MHz, $CDCl_3$), chemical shift δ in ppm, coupling constant *J* in Hz: δ_H 1.25, 1.68 (2H, *m*, H-1), 1.45, 1.52 (2H, m, *qt*, H-2), 1.09 (1H, *m*, H-5), 2.06, 2.41 (2H, *m*, H-7), 2.09 (1H, *m*, H-9), 4.53 (1H, *s*, H_a-12), 4.87 (1H, *d*, H_b-12), 4.2 (1H, *dd*, *J*= 11.2, 9.2 Hz, H_a-11), 4.3 (1H, *dd*, *J*= 11.2, 3.6 *Hz*, H_b-11), 0.84 (3H, *s*, H-13), 0.77 (3H, *s*, H-14), 0.71 (3H, *s*, H-15), and 2.07 (3H, *s*, H-17); ^{13}C NMR (100 MHz, CDC3): δ_C 39.03 (C-1), 19.17 (C-2), 41.92 (C-3), 33.94 (C-4), 55.06 (C-5), 23.90 (C-6), 37.60 (C-7), 146.83 (C-8), 54.73 (C-9), 38.97 (C-10), 107.15 (C-11), 61.58 (C-12), 33.64 (C-13), 21.76 (C-14), 15.11(C-15), 171.41 (C-16), 21.12 (C-17).

2.3.3. *Physical and spectral data for compound 37*

Brown jelly material (20 mg); soluble in $CHCl_3$; R_f 0.5 (mobile phase hexane: EtOAc, (5:1); UV (EtOH) λmax nm: 298; IR υ_{cm-1}: 2916 and 1478 cm^{-1} are due to the C-H stretching and bending, 1376 cm^{-1} geminal dimethyl stretching, 1205 cm^{-1} C-O stretching; ^{1}H-NMR (400 MHz, $CDCl_3$), chemical shift *δ* in ppm, coupling constant J in Hz: δ_H 5.12 (1H, *m*, H-3), 2.14

(2H, *m*, H-4), 6.40 (1H, *d*, *J* =2.8Hz, H-5), 6.49 (1H, *d*, *J* = 2.8 Hz, H-7), 1.62 (3H, *s*, H-9), 2.14 (3H, *s*, H-10), 2.71 (2H, *t*, *J* = 6.4 Hz, H-1'), 1.74 &1.81 (2H, *td*, H-2'), 2.01 (2H, *m*, H-5'), 1.75 (2H, *m*, H-6'), 5.12 (1H, *m*, H-7'), 1.78 (2H, *m*, H-9'), 2.09 (2H, *m*, H-10'), 5.12 (1H, *m*, H-11'), 1.61 (3H, *s*, H-13'), 1.28 (3H, *s*, H-14'), 1.62 (3H, *d*, *J* = 3.6 Hz, H-15'), 1.70 (3H, *s*, H-16'); ^{13}C NMR (100 MHz, CDC_3, δ in ppm: δc 147.89 (C-2), 124.21 (C-3), 22.20 (C-4), 134.95 (C-4a), 112.63 (C-5), 121.20 (C-6), 115.71 (C-7), 127.30 (C-8), 145.92 (C-8a), 16.0 (C-9), 15.87 (C-10), 22.51 (C-1'), 31.42 (C-2'), 75.31 (C-3'), 39.71 (C-4'), 26.78 (C-5'), 39.71 (C-6'), 124.33 (C-7'), 135.10 (C-8'), 39.71 (C-9'), 26.62 (C-10'), 124.43 (C-11'), 131.31 (C-12'), 25.69 (C-13'), 24.0 (C-14'), 16.0 (C-15'), and 16.68, (C-16').

3. RESULTS and DISCUSSION

Generally, the EtOAc soluble portion of the EtOH extract of the bark of *W. ugandensis* resulted in the first report of the isolation of albicanyl acetate, caseamemin, and *β*-sitosterol together with other 6 known dialdehydic and drimane-type compounds (Figure 3) (Gizachew, 2019). These are muzigadial (38), cinnamolide-3β-acetate (39), ugandensidial (40), 11α-hydroxy muzigadiolide (41), polygodial (42), and 9-deoxymuzigadial (43). In the course, 1-heptacosanol (44) and Hentriacontane (45) were also isolated.

Different physical and spectroscopic examinations, along with a comparison of the results with related compounds in the literature, were carried out to elucidate the structures of the compounds. The NMR data of each compound is depicted as supporting material for this manuscript.

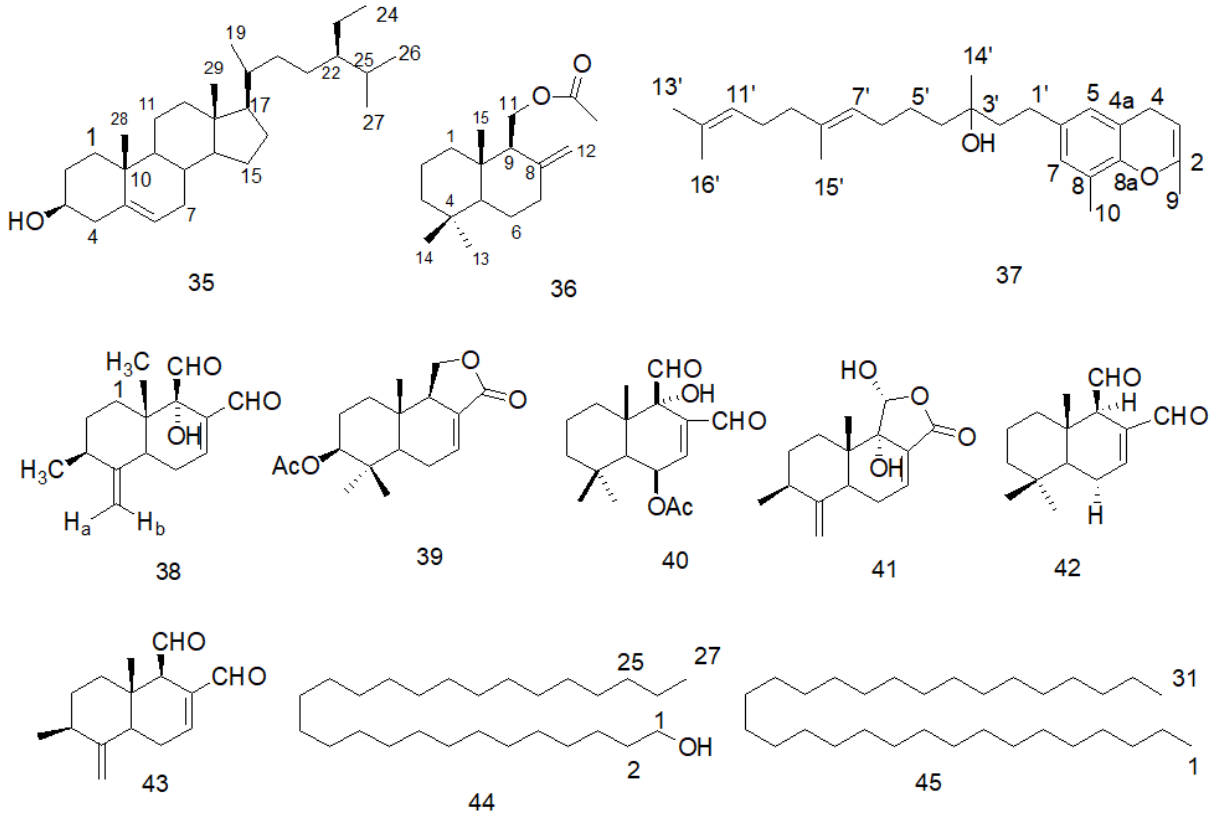


Figure 3. Compounds isolated from the stem bark of *W. ugandensis*.

3.1. Characterization of Compound 35

Compound **35** was isolated as a white solid (27 mg) from fraction five. The TLC profile developed using a hexane: EtOAc (9:1) solvent system and vanillin as a spraying agent showed a purple single spot (Rf 0.62). Compound **35** melts at 130-136°C (lit. 134-136°C) (Chaturvedula & Prakash, 2012). The UV-Vis spectrum (in ethanol) showed no absorption band from the 600-200 nm wavelength region.

The ^{1}H-NMR spectrum showed the most downfield signal for one olefinic proton at δ_H 5.36 and the other signals appeared at 3.53 as a multiplet for a proton corresponding to the proton connected to the C-3 hydroxyl group. The ^{1}H-NMR spectrum revealed the presence of 6 methyl groups, of which three are methyl singlets that appeared at δ_H 0.82 (3H, *s*), 0.70 (3H, *s*), 1.03 (3H, *s*), and the other three are observed as methyl doublets at δ_H 0.84 (3H, *d*), 0.86 (3H, *d*), and 0.93 (3H, *d*).

From the ^{13}C-NMR spectrum, the downfield signals that appeared at δ_C 140.7 and 121.7 were interpreted as the marker signals for olefinic carbons of *β*-sitosterol. The signal that appeared at δ_C 71.8 was assigned to the oxygenated carbon of the sterol (C-3). The remaining signals of both the ^{1}H and ^{13}C-NMR data of compound **35** were compared with literature values of *β*-sitosterol (Table 3) and found a good agreement (Chaturvedula & Prakash, 2012).

Table 1. ^{1}H and ^{13}C-NMR spectral data comparison of compound **35** with literature values of β-sitosterol.

^{13}C & ^{1}H-NMR data of compound **35**			Lit. value of β-sitosterol (Chaturvedula & Prakash, 2012)	
1	37.2		37..5	
2	31.6		31.9	
3	71.8	3.53 (1H, *m*)	72.0	3.53 (tdd, 1H, J = 4.5, 4.2, 3.8 Hz)
4	42.3		42.5	
5	140.7		140.9	
6	121.7	5.36 (1H, *t*, 2.8)	121.9	5.36 (t, 1H, J = 6.4 Hz)
7	31.9		32.1	
8	31.9		32.1	
9	50.1		50.3	
10	36.5		36.7	
11	21.1		21.3	
12	39.7		39.9	
13	42.3		42.6	
14	56.7		56.9	
15	26.0		26.3	
16	28.2		28.5	
17	56.0		56.3	
18	36.1		36.3	
19	19.4	0.93 (3H, *d*, 6.8)	19.2	0.93 (d, 3H, J = 6.5 Hz)
20	33.5		34.2	
21	24.3		26.3	
22	45.8		46.1	
23	23.0		23.3	
24	12.0	0.84 3H, *t*)	12.2	0.84 (t, 3H, J = 7.2 Hz)
25	29.1		29.4	
26	19.8	0.84 (3H, *d*, 6.8)	20.1	0.83 (d, 3H, J = 6.4 Hz)
27	19.0	0.82 (3H, *d*, 6.8)	19.1	0.81 (d, 3H, J = 6.4 Hz)
28	18.8	0.70 (3H, *s*)	19.0	0.68 (s, 3H)
29	11.8	1.03 (3H, *s*)	12.0	1.01 (s, 3H)

Chemical shifts are reported in parts per million ($CDCl_3$), and ***J*** values are in Hertz.

The data generated from the characterization of compound **35** agreed well with the literature report on *β*-sitosterol. To my knowledge, there has been no report made on the isolation of β-sitosterol from Warbirgia species previously. The compound is known for its antinociceptive, anxiolytic & sedative effects, analgesic, immunomodulatory, antimicrobial, anticancer, anti-inflammatory, and hepatoprotective (Babu & Jayaraman, 2020).

3.2. Characterization of Compound 36

This compound was isolated from fraction five using hexane: EtOAc (9:1) as eluent. Fraction five (100 mg) was applied on PTLC, which led to the isolation of compound **36** (15 mg) as a jelly-like material. The TLC profile of this compound was developed using a hexane: EtOAc (5:1) solvent system and 1% vanillin as a spraying agent and showed a single purple spot (R_f 0.5). The UV-Vis spectrum of the compound showed no absorption band from 600-200 nm, indicative of the absence of conjugated chromophore. The IR spectrum of compound **36** exhibited bands at 2923 cm^{-1}(C-H stretching), and 1735 (acetate unit). The other band observed at 1641 cm^{-1} is due to the presence of C-C stretching of the exocyclic double bond. Geminal dimethyl stretching is observed at 1376 cm^{-1}. The signal that appeared at 1230 cm^{-1} is due to C-O stretching in the molecule.

The ^{1}H-NMR spectrum of compound **36** displayed four terminal methyl protons, each appearing at δ_H 0.71 (3H, *s*), 0.77 (3H, *s*), 0.84 (3H, *s*), and 2.07 (3H, *s*). The two most downfield singlet signals that appeared at δ_H 4.87 (1H, *s*), and 4.53 (1H, *s*) were indicative of the presence of an exocyclic double bond (H-12). The other downfield signals that appeared as a doublet of a doublet at δ_H 4.20 (1H, *dd*, J = 11.2, 9.2 Hz), and 4.34 (1H, *dd*, J = 11.2, 3.6 Hz) were due to the diastereotopic protons on oxygenated carbons (H-11). The HH-COSY experiment revealed the correlation of protons of exocyclic double bonds with H-7 and H-9. The protons were assigned to the carbon with the help of HSQC and HMBC. The oxygenated protons were only correlated with protons that appeared at 2.07 (H-9).

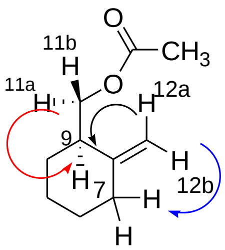


Figure 4. Showed the HH-COSY correlation.

The ^{13}C-NMR spectrum together with DEPT-135 revealed the presence of four methyls, seven methylenes, two methines, and four quaternary carbon atoms. The downfield signal that appeared at δ_C 171.4 together with a methyl signal that appeared at δ_C 21.1 was evident for the presence of an ester functional group. The signals observed at δ_C 146.8, and 107.1 in consistency with the proton NMR experiment were suggestive of the presence of an exocyclic double bond. The methylene signal that appeared at δ_C 61.5 was evident for the presence of one oxygenated carbon. The remaining methylene signals appeared at δ_C 39.0, 19.1, 41.9, 23.9, and 37.6. The two methine carbons appeared at δ_C 55.0 and 54.7. Both ^{1}H and ^{13}C spectral data of this compound were found in good agreement with the literature report on albicanyl acetate (Dumdei, E.J., *et al*, 1997).

This compound was previously reported from the skin extracts of *Cadlina luteomarginata* (Dumdei, *et al*, 1997). However, this is the first report from *W. ugandensis*. Albicanyl acetate is known as a potent fish antifeedant biological activity compound (Barrero *et al*., 1995).

Table 2. NMR data comparison of compound **36** with literature values of albicanyl acetate.

Experimental data of compound **36**			Literature data of albicanyl acetate (Dumdei, E.J., *et al* 1997)	
	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR
1	39.0	1.25 (1H, *m*), 1.68 (1H, *m*)	39.0	1.25 (1H, *m*, H-eq), 1.68 (1H, *m*)
2	19.1	1.45, 1.52 (2H, m, *qt*)	19.2	1.45, 1.52 (2H, *m*, *qt*)
3	41.9		41.9	
4	33.9		33.5	
5	55.0	1.09 (1H, *m*)	55.1	1.09 (1H, *m*)
6	23.9		23.9	
7	37.6	2.06 (1H, *m*), 2.41 (1H, *qd*)	37.8	2.06 (1H, *m*), 2.36 (1H, *m*)
8	146.8		146.8	
9	54.7	2.09 (1H, *m*)	54.7	2.09 (1H, *m*)
10	38.9		38.9	
11	61.5	4.2 (1H, *dd*), 4.3 (1H, *dd*)	61.6	4.14 (1H, *dd*), 4.29 (1H, *dd*)
12	107.1	4.53 (1H, *s*), 4.87 (1H, *d*)	107.1	4.47 (1H, *s*), 4.81 (1H, *s*)
13	33.6	0.84 (3H, *s*)	33.6	0.84 (3H, s)
14	21.7	0.77 (3H, *s*)	21.7	0.77 (3H, s)
15	15.1	0.71 (3H, *s*)	15.1	0.71 (3H, s)
16	171.4		171.4	
17	21.1	2.07 (3H, s)	21.1	2.07 (3H, s)

s = singlet, *dd* = doublet of doublet, *qd* = quartet of doublet, *m* = multiplet

3.3. Characterization of Compound 37

Compound **37** (20 mg) was obtained as a jelly material from fraction 5. The TLC profile of the compound developed using the hexane: EtOAc (4:1) solvent system showed a single spot at *Rf* values of 0.5 visualized after spaying with 1% vanillin in sulfuric acid. The UV-Vis spectral analysis showed the presence of a conjugated chromophore with λmax 298 nm. In the IR spectrum of compound **37**, the broadband observed at 3429 cm^{-1} is suggestive of the presence of the OH group in the molecule. The sharp peak that appeared at 2916 is due to the C-H stretching. The peaks at 1607 and 1478 cm^{-1} are indicative of the presence of the aromatic ring. The other band displayed at 1376 cm^{-1} is a characteristic of the presence of geminal dimethyl stretching. The band at 1205 cm^{-1} is due to the C-O stretching.

The ^{1}H-NMR spectrum is suggestive of the presence of aromatic groups in the molecule due to the two signals that appeared at δ_H 6.49 (1H, *d*, *J= 3.2*), and 6.40 (1H, *d*, *J= 2.8*). The presence of other olefinic protons is confirmed by the proton signals observed at δ_H 5.08-5.14 (3H, *m*). The triplet signal at δ_H 2.71 integrated for two protons is due to two methylene protons of the chain appended to the aromatic ring., Six terminal methyl protons are observed in the ^{1}H-NMR spectrum, of which three methyl protons appear to be overlapped at δ_H 1.61 (9H, *s*). The remaining terminal methyl protons are shown at δ_H 1.70 (3H, s), 1.28 (3H, *s*), and 2.14 (3H, *s*).

Two-band carbon signals are observed in the olefinic (aromatic) and aliphatic regions in the ^{13}C-NMR spectrum. There are twelve carbon signals observed in the olefinic region (from δ_C 112.6 up to 147.8). Only five of them (δ_C 112.6, 115.7, 124.2, 124.3, and 124.4) are methine carbons, and the rest seven (δ_C 121.1, 127.2, 131.2, 134.9, 135.1, 145.9, and 147.8) are quaternary carbons. The ^{13}C-NMR spectrum together with Dept-135 showed one oxygenated quaternary carbon at δ_C 75.3. All NMR data of compound **37** are compared with the literature values of caseamemin previously isolated from the stem of *Casearia membranacea*, and found to be in close agreement (Chang *et al*., 2003).

Table 3. NMR spectral data comparison of compound **37** with literature values.

	Experimental data of compound **37**		Literature data of caseamemin (Chang *et al*., 2003)	
	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR
2	147.8		147.7	
3	124.2	5.12 (1H, *m*)	124.2	5.13 (1H, *m*)
4	22.2	2.14 (2H, *m*)	22.1	2.14 (2H, *m*)
4a	134.9		135.0	
5	112.6	6.40 (1H, *d*, *J* =2.8Hz)	112.6	6.38 (1H, *d*, *J* = 3 Hz)
6	121.2		121.2	
7	115.7	6.49 (1H, *d*, *J* = 2.8 Hz)	115.6	6.48 (1H, *d*, *J* = 3 Hz)
8	127.3		127.4	
8a	145.9		146.0	
9	16.0	1.62 (3H, *s*)	16.0	1.60 (3H, *s*)
10	15.9	2.14 (3H, *s*)	15.9	2.13 (3H, *s*)
1’	22.5	2.71 (2H, *t*, *J* = 6.4 Hz)	22.5	2.69 (2H, *t*, *J* = 6.6 Hz)
2’	31.4	1.74 &1.81 (2H, *td*)	31.3	1.74 &1.81 (2H, *td*)
3’	75.3		75.3	
4’	39.7		39.7	1.53 & 1.64 (2H, *m*)
5’	26.7	2.01 (2H, *m*)	26.7	2.04 (2H, *m*)
6’	39.7	1.75 (2H, *m*)	39.7	1.69 (2H, *m*)
7’	124.3	5.12 (1H, *m*)	124.3	5.08 (1H, *m*)
8’	135.1		135.1	
9’	39.7	1.78 (2H, *m*)	39.7	1.69 (2H, *m*)
10’	26.6	2.09 (2H, *m*)	26.6	2.08 (2H, *m*)
11’	124.4	5.12 (1H, *m*)	124.4	5.10 (1H, *m*)
12’	131.3		131.3	
13’	25.7	1.61 (3H, *s*)	25.7	1.59 (3H, *d*, *J* = 0.8 Hz)
14’	24.0	1.28 (3H, *s*)	24.0	1.26 (3H, *s*)
15’	16.0	1.62 (3H, *d*)	16.0	1.60 (3H, *s*)
16’	16.7	1.70 (3H, *s*)	17.7	1.68 (3H, *d*, *J* = 0.8 Hz)

Chemical shifts are reported in parts per million ($CDCl_3$), and ***J*** values are in Hertz.

The previous report showed that this compound was isolated from the stem of *Casearia membranacea* and exhibited cytotoxicity activity (Chang *et al*., 2003). This is the first time to been reported from the Warburgia species.

3.4. Phytotoxic Activity of Isolated Compounds

Parthenium hysterophorus weed seeds were prevented from germinating when exposed to a 0.05 mg/mL concentration of the crude ethanol extract of *Warburgia ugandensis* bark (Gizachew, 2019). The crude ethanol extract was further partitioned with hexane, chloroform, and methanol. Each fractionation was screened for its phytotoxic activity. The hexane and chloroform soluble portions showed impressive phytotoxic activity with 100% parthenium seed germination inhibition at 0.05 mg/mL concentration. However, the methanol-soluble part showed minimum seed germination inhibition (20%) as it is shown in Figure 5.

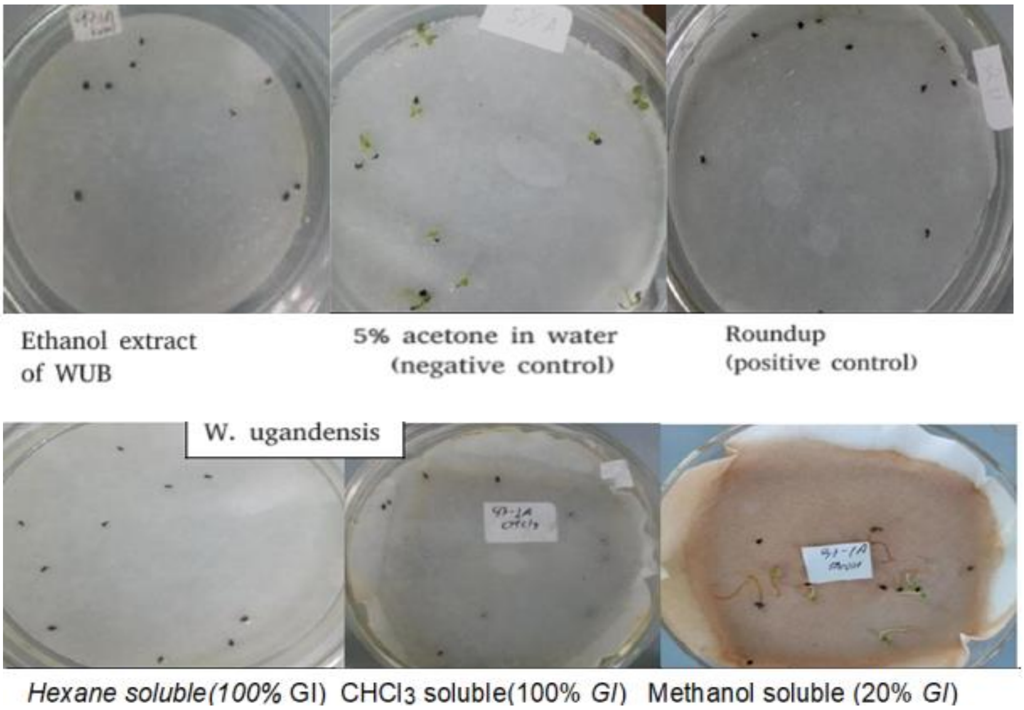


Figure 4. Results of the *in vitro* phytotoxicity of the ethanol extract and its soluble component in hexane, chloroform, and methanol against the germination of parthenium seeds.

So, from the medium polar fraction of the ethanol extract, a total of 11 compounds were isolated. The NMR spectra of these compounds are depicted in the supporting material of this manuscript. Six of the isolated compounds were subjected for their phytotoxic activities both *in vitro* and *in vivo*. The phytotoxic study of other compounds was not studied due to their amount for the bioassay. From the compounds screened for their herbicidal activity, muzigadial showed the highest activity with 100 and 95% germination inhibition (*in vitro*) and seedling growth inhibition (*in vivo*), respectively. Cinnamolide-3*β*-acetate is the second bioactive compound against the target weed, with 96 and 91% seed germination and seedling growth inhibition, respectively. For reference, the commercial herbicide Roundup® used as a positive control also achieved 100% seed germination inhibition at the same concentration as muzigadial and Cinnamolide-3*β*-acetate. Hence, these two compounds could be considered to be used as future generations of organic-based herbicides. However, additional herbicide parameters and field validation have to be studied.

Table 4. Summary of *In vitro* and *in vivo* results of pure compounds from *W. ugandensis.*

Compound name	*% GI (In vitro) 0.05 mg/mL*	*%GI (In vivo) 1 mg/mL*
Muzigadial	100	95
Cinnamolide-3*β*-acetate	96	91
Ugandensidial	100	87
11α-hydroxy muzigadiolide	66	40
Heptacosanol	59	66
Hentriacontane	55	18
Roundup® (+ve control)	100	97
5%acetonein water (-ve control)	0	0

4. CONCLUSION

Generally, the EtOAc soluble portion of the EtOH extract of the bark of *W. ugandensis* resulted in the first report of the isolation of albicanyl acetate, caseamemin, and *β*-sitosterol from this species together with other 6 known dialdehydic and drimane-type compounds that indicate the plant is not exhaustively studied and there is still a possibility to find other new compounds from it.

Muzigadial is isolated as the most phytotoxic compound against Parthenium weed. This compound may be utilized as an organic herbicide to fight the weed if other herbicide requirements are investigated and satisfied.

Acknowledgments

The author would like to acknowledge Addis Ababa University for providing all necessary materials and chemicals for this research study. Ambo Agricultural Research Center is also kindly acknowledged for the bioassay study. I would like to thank Dr. Yadessa Melaku for his guidance, supervision throughout this study, and editing of the manuscript.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Zelalem Gizachew: Investigation, carrying out both the chemistry and bioassay experiments, data analysis, and writing the original draft manuscript. **Chiristopher Suh:** Focused on Manuscript editing and analyze the data

Orcid

Zelalem Gizachew https://orcid.org/0000-0002-2504-4347
Chiristopher Suh https://orcid.org/0000-0002-1141-2719

REFERENCES

Arot Manguro, L.O., Ugi, I., Hermann, R., & Lemmen, P. (2003). Flavonol and drimane-type sesquiterpene glycosides of *Warburgia stuhlmannii* leaves. *Phytochemistry*, *63*(4), 497–502.

Arot Manguro, L.O., Ugi, I., Lemmen, P., & Hermann, R. (2003). Flavonol glycosides of *Warburgia ugandensis* leaves. *Phytochemistry*, *64*(4), 891–896.

Babu, S., & Jayaraman, S. (2020). An update on β-sitosterol: A potential herbal nutraceutical for diabetic management. *Biomedicine and Pharmacotherapy*, *131*, 110702.

Barrero, A.F., Manzaneda, E.A., Altarejos, J., Salido, S., Ramos, J.M., Simmonds, M.S., & Blaney, W.M. (1995). Synthesis of biologically active drimanes and homodrimanes from (-)-sclareol. *Tetrahedron*, *51*(27), 7435–7450.

Brooks, C.J.W., & Draffan, G.H. (1969). Sesquiterpenoids of wazwurgza species-i warburgin and warburgiadione. *Tetrahedron*, *25*, 2865–2885.

Chang, K.C., Duh, C.Y., Chen, I.S., & Tsai, I.L. (2003). A cytotoxic butenolide, two new dolabellane diterpenoids, a chroman and a benzoquinol derivative formosan Casearia membranacea. *Planta Medica*, *69*(7), 667–672.

Chaturvedula, V.S.P., & Prakash, I. (2012). Isolation of Stigmasterol and ?-Sitosterol from the dichloromethane extract of Rubus suavissimus. *International Current Pharmaceutical Journal*, *1*(9), 239–242.

Dumdei, E.J., Kubanek, J., Coleman, J.E., Pika, J., Andersen, R.J., Steiner, O.R., Clardy, J. (1997). New Terpenoid Metabolites from the Skin Extracts, an Egg Mass, and Dietary Sponges of the Northeastern Pacific Dorid Nudibranch *Cadlina luteomarginata*. *Canadian Journal of Chemistry*, *75*(6), 773–789.

Gizachew, Z. (2019). *Plant derived compounds against the widespread weed: Parthenium hysterophorus* [Unpublished doctoral dissertation]. Addis Ababa University.

Gonfa, T., Fisseha, A., & Thangamani, A. (2020). Isolation, characterization and drug-likeness analysis of bioactive compounds from stem bark of *Warburgia ugandensis* Sprague. *Chemical Data Collections*, *29*, 1-15.

Kairu, A., Gichuki, N., Kanya, J., & Kindt, R. (2013). Disappearing medicinal plants in mt . kenya forests , kenya : a case study of east african green heart (*Warburgia ugandensis* sprague). *Top Class Journal of Herbal Medicine*, *2*(7), 159–165.

Kipkore, W., Wanjohi, B., Rono, H., & Kigen, G. (2014). A study of the medicinal plants used by the Marakwet Community in Kenya. *Journal of Ethnobiology and Ethnomedicine*, *10*(1), 1-22.

Maroyi, A. (2014). The genus Warburgia: A review of its traditional uses and pharmacology. *Pharmaceutical Biology*, *52*(3), 378–391.

Ngure, P.K., Tonui, W.K., Ingonga, J., Mutai, C., Kigondu, E., Ng'ang'a, Z., Rukunga, G., & Kimutai, A. (2009). In vitro antileishmanial activity of extracts of *Warburgia ugandensis* (Canellaceae), a Kenyan medicinal plant. *Journal of Medicinal Plants Research, 3*(2), 061–066.

Okello, D., & Kang, Y. (2021). Ethnopharmacological Potentials of *Warburgia ugandensis* on Antimicrobial Activities. *Chinese Journal of Integrative Medicine*, *27*(8), 633–640.

Opiyo, S.A. (2021). *Insecticidal* Drimane Sesquiterpenes from *Warburgia ugandensis* against Maize Pests. *American Journal of Chemistry*, *11*(4), 59–65.

Rabe, T., & Staden, J. van. (2000). Isolation of an antibacterial sesquiterpenoid from *Warburgia salutaris*. *Journal of Ethnopharmacology*, *73*, 171–174.

Wube, A.A., Bucar, F., Gibbons, S., & Asres, K. (2005). Sesquiterpenes from *Warburgia ugandensis* and their antimycobacterial activity. *Phytochemistry*, *66*(19), 2309–2315.