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***J. Nat. Prod. Plant Resour.*, 2013, 3 (5):1-6**
(http://scholarsresearchlibrary.com/archive.html)

ISSN : 2231 – 3184
CODEN (USA): JNPPB7

Phytochemical investigation of *Sapium ellipticum*

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ABSTRACT

The main objective of this work was to isolate and characterize the natural products from stem of Sapium ellipticum. This powdered plant material was gradiently extracted using petroleum ether, chloroform and acetone using a soxhlet extractor. The chloroform extract was subjected to column chromatography over silica gel using petroleum ether and ethyl acetate as eluents. Three compounds were isolated in the pure form. The IR, ^{1}H NMR, ^{13}C NMR and DEPT-135 spectra of the compounds were recorded. A pair of broad singlet protons appeared in the ^{1}H NMR spectra at δ 4.604 and 4.730(1 H each) for compound 1. Similar peaks at δ4.587 and 4.709(1H each) were seen for compound 2 also. This was indicative of the exomethylene group present in the case of lupane class of triterpenoids. Compound 1 was characterized as Lupeol acetate and compound 2 as Lupeol based upon on the other significant data from ^{1}H NMR, ^{13}C NMR and DEPT-135 spectrum and also from comparison of this obtained data with published values. The ^{1}H NMR spectrum of compound 3 showed characteristic values to that for a plant sterol. The ^{13}C-NMRspectrum of compound 3 showed the presence of 29 carbons with four olefinic carbons at δ 121.9(C-6), 141.0 (C-5), δ 138.5(C-22) and 129.5 (C-23). Compound 3 was characterized as stigmasterol based upon the physical and spectral data. All the compounds are isolated from this plant for the first time.

Key words: Sapium ellipticum, Euphorbiaceae, Lupeol acetate, Lupeol, Stigmasterol

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INTRODUCTION

Plants and plant products were used for treating various illnesses since ancient time. This has resulted in the use and study of several medicinal plants. Man has been using various medicinal plants for curing physical and mental disorders and the knowledge regarding these plants were passed through generations. It has been noted that traditional knowledge on medicinal plants are still very rich among various ethnic communities throughout the world. Several secondary metabolites or natural products isolated from these medicinal plants are useful as clinically active drugs or serve as a drug leads. Bio assay guided fractionation is one the key technique by which compounds with good biological activity has been isolated from medicinal plants. It is seen from literature that more than 50% of the drugs which were used clinically are either natural products or their derivatives. [1-4]

Lot of research has been carried out on isolation of novel natural products and the clue for such searches usually comes from the ethnobotanical use of the medicinal plants. With the combined efforts of botanist, chemists and pharmacologists new drugs can be discovered. Structural elucidation of compounds remain as an important task in this process and has still much to offer, especially when combined with biological tests to provide highly useful leads for drug discovery. Thus structure elucidation has still much to offer, especially when combined with biological tests to provide highly useful leads for drug discovery.

In Ethiopia more than 800 species of plants are used traditionally to treat various illnesss. A wide diversity of medicinal plants is spread throughout the highlands and low lands of Ethiopia. A very high percentage of the population still relies on these medicinal plants for health purposes. Despite the great role of natural products in

industries and primary health care system, little work has so far been done in the country to properly document and promote the associated knowledge of the bioactive compounds existing in the medicinal plants [5].

The genus Sapium Belongs to the family Euphorphiacea and nearly 125 species of this genus are present world wide. The main species of *Sapium* that are found in Ethiopia is *Sapium ellipticum* and *Sapium sebiferum* [6]. *Sapium ellipticum* is widely used as an ethnomedicine in different parts of Africa. The root decoction of *Sapium ellipticum* is used for treating coughs in Kenya. In Tanzania the dried stems is powdered and crushed with water and applied for wounds, pain in chest, head etc. The leaves are used for abdominal swellings and eye diseases. The root decotion is used for malaria. Also the traditional healers in Zambia and Burundi prescribe the stem bark decotion for anemia, fever, guinea worms, elephantiasis and rheumatic problems. The leaves of this plant are traditionally used for the treatment of mumps in Ethiopia by Kaffa people of Bonga zone [7,8].

There are only few reports on the isolation of compounds and biological activity from *Sapium ellipticum.* Antioxidant studies on the methanol extract of *Sapim ellipticum* has been carried out and the extract showed significant free radical scavenging activity in a dose dependent fashion [9]. The leaves of *Sapium ellipticum* was found to be cytotoxic against HeLa cervix adenocarcinoma cells which was almost comparable to the used reference ciplastin [10]. Anti-fungal activity studies on the dichloromethane extracts of *Sapium ellipticum* bark has been carried out in Tanzania and three triterpenoids namely lup-20(29)-en-3-one, β-amyrin and acetyl aleuritolic acid and one steroid β-sitosterol has been isolated from the active fractions [11]. In this paper the isolation and characterization of three compounds from *Sapium ellipticum* is discussed.

MATERIALS AND METHODS

General: Melting point apparatus (Griffin) was used for melting point determination. TLC plates W/UV254, were used to analyze the fractions collected from column chromatography (CC) with visualization under UV at (254 and 366 nm) and exposure to iodine vapor. The IR spectra were recorded on IR Perestinge -21 FT – IR spectrometer. The ^{1}H-NMR, ^{13}C-NMR, DEPT-135, spectra were recorded on Bruker 400 MHz Advance NMR spectrometer with TMS as internal standard. Deuterated chloroform ($CDCl_3$) was used as solvent for recording NMR spectra. CC was performed on silica gel 80-120 mesh size.

Plant material

The stem of *Sapium ellipticum* was collected from Kaffa area, Bonga Zone in November 2011. The botanical identification of the plant was done by Dr. M. Remesh, Botanist of department of Biology in Jimma University and a Voucher specimen (number 002EA) was deposited in the herbarium of Biology Department, Jimma University.

Extraction and isolation

Powdered *Sapium ellipticum* stem (800g) was gradiently extracted for 9 hours until the colorless extract was obtained using Soxhlet extraction method [12]. The solvent used for extraction were petroleum ether, chloroform and acetone. The extracted matters were then filtered through cotton plug and Whatman (No.1) filter paper. The chloroform extract of stems of *Sapium ellipticum* (10 g) was chromatographed on 200 g of silica gel. And was eluted using petroleum ether, increasing the degree of polarity by addition of ethyl acetate. Fractions 10-14 eluted with 100% petroleum ether afforded compound **1** (30mg). Fractions7-10 eluted with 6% ethyl acetate afforded compound **2** (20mg). Fractions 15-20 eluted with 10% of ethyl acetate gave the compound **3**(20 mg).The structures of these compounds were elucidated using spectroscopic analysis as well as by comparing with the spectral data and physical properties of the same compounds that were previously reported in literatures.

RESULTS AND DISCUSSION

The stem bark of *Sapium ellipticum* was ground and extracted with petroleum ether, chloroform and acetone .The $CHCl_3$ crude extract was subjected to column chromatography with an increasing gradient of petroleum ether and ethyl acetate resulting in the isolation of three compounds identified as lupeol acetate (Figure 1), lupeol (Figure 2) and stigmasterol (Figure 3).

Characterization of compound 1

Compound 1 was isolated as white crystalline needles (30mg) on elution with 100% petroleum ether. It showed a melting point of 216-217^{0}c . The IR spectrum of compound **1** showed the presence of ester carbonyl at 1721cm $^{-1}$ (C=O) and exomethylene group at 3019cm^{-1}, 2950cm^{-1}(C-H), 2416cm^{-1}(C=C), 1217cm^{-1} (C-O)and bending at 792cm^{-1}.

The ^{1}H-NMRspectrum (400 MHz, $CDCl_3$) of compound **1** showed the presence of seven methyls at δ 1.70 (3H, *s*, H-30), 1.40 (3H, *s*, H-25),1.27 (3H, *s*, H-28), 1.04 (3H, *s*, H-23), 0.95 (3H, *s*, H-24), 0.86 (3H, m, H-26), 0.80 (3H, *s*, H-27) and an acetate methyl at δ 2.06 (3H, H-2'). A pair of broad singlet protons appeared at δ 4.604 and 4.730 as singlets which was indicative of the exomethylene group, while the doublet of doublet at δ 4.512 (dd, 1H, J=10.4, 7.6Hz) was indicative of the proton attached to the carbon bonded to the acetoxy group. A sextet proton at δ 2.4 was attributed to 19 β-H, which was a characteristic of lupane type triterpenoids [13].

^{13}C-NMRspectrum of compound 1 showed the presence of a carbonyl at δ 171.08 and two sp^2 carbons at δ 151.021 and 109.376. The ^{13}C-NMR signals of various carbons showed the presence of 8 methyl, 11 methylene, 6 methine and seven quaternary carbons, which was also substantiated from DEPT-135 spectrum of compound 1. The ^{1}H-NMR and ^{13}C-NMR data of compound 1(Table 1) indicated a pentacyclic triterpenoid of lupane type and comparison of its physical and spectral data with published values confirmed the identity of compound 1 as lupeol acetate (Figure 1) [13-15].

Characterization of compound 2

Compound 2 was obtained as a white powdery solid (50mg) with a melting point of 213-215 ^{0}C. The IR spectrum of compound 2 showed absorption bands characteristic for the hydroxyl group (3440cm^{-1}). The presence of terminal double bond was confirmed by bands at 2948 cm^{-1}, 2881cm^{-1} (aliphatic C-H stretching), 2249cm^{-1} vinylic stretching,1381cm^{-1} (methyl), 911$cm^{-1}$and 741cm^{-1}. The ^{1}H-NMRspectrum of compound 2 showed that the presence of seven methyl at δ0.779 (3H, d), 0.84(3H, s), 0.962(3H, d), 1.047(3H, s), 1.280(3H, s), 1.404(3H, d) and 1.700(3H, s). Also, a pair of singlets at δ4.587 and 4.709(1H each) was indicatives of terminal isopropenyl moiety or due to the vinylic proton at carbon 29. This indicated that compound 2 belongs to the lupane class of triterpenoids. The presence of a doublet of doublet with one proton intensity at δ 3.2 (dd, J=11.2, 5.2 Hz) was seen which was due to the proton attached to the carbon bearing the hydroxyl group or typical of oxymethine proton at C-3. These chemical shift values lead to the conclusion of β-orientation of the hydroxyl functional at C-3. A one proton intensity doublet of triplet at δ 2.4 was assigned to 19β-H on comparision with literature value [16].

The ^{13}C-NMR spectral assignments of various carbons ofcompound 2were substantiated by the DEPT-135experiments,which revealed the presence of thirty carbon,which includes seven methyls, elevene methylenes, six qauterinary carbons and six methine carbons. Among these the exomethylene carbon appeared at δ 109.33, the quaternary carbon attached to be exomethylene at δ 151.8 and the oxygenated methine at δ 79.01. The chemical shifts of these carbon signals and other physical data were identical to that of lupeol.Based upon this^{1}H-NMR and ^{13}C-NMR data of compound 2 (Table 1) and in comparison of its physical and spectral data with published values confirmed the identity of compound 2 as Lupeol (Figure 2) [13-16].

Characterization of compound 3

Compound 3 was isolated as white powder (50mg) and soluble in chloroform. The IR spectrum of compound 3showed OH absorption band at 3421 cm^{-1} and absorption at 2954 cm^{-1} and 2858 cm^{-1}, due to aliphatic C-H stretching.

The proton NMR spectrum of compound 3showed the presence of six methyl peaks at δ 0.714, 0.839, 1.027, 1.512, 2.03 and 2.294which were assigned to the H-18, H-27, H-29, H-26, H-21 and H-19 protons respectively. It also shows olefinic protons at δ 5.36, 5.02 and 5.16. One proton appeared at δ 5.36 as the doublet represents the endocyclic double bond proton H-6 of compound 3.The other two olefinic protons appeared as two doublets of doublets at δ 5.02 and δ 5.16 which were assigned for the other olefinic protons at H-23 and H-22 respectively. The proton NMR spectral data of compound 3 are in agreement with the reported spectral data for stigmasterol [13,17-19].

The^{13}C-NMRspectrum of compound 3 showed the presence of 29 carbons. From the 13C-NMR and DEPT-135 spectrum of compound 3 it was revealed that the compound has six methyl, 9 methylene, 11 methine and three quaternary carbons. The presence of an endocyclic carbon-carbon double bond of compound 3 was represented by two signals at δ 121.9 and 141.0 of C-6 and C-5. Other olefinic carbons of compound 3 appeared at δ 138.5 and 129.5 for C-22 and C-23 carbons. The similarity of ^{13}C spectral data of compound 3(Table 1) with published data confirmed as the compound was stigmasterol (Figure 3) [13,17-19, 20-22].

SPECTRAL DATA

Lupeol acetate (Figure 1) was isolated as white crystalline needle with melting point 216-217^{0}C. IR: 3019, 2950,2414,1721,1217 and 792 cm^{-1}.^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.704 (3H, *s*, H-30), 1.408 (3H, *s*, H-25) 1.273 (3H, *s*, H-28), 1.048 (3H, *s*, H-23), 0.957 (3H, *s*, H-24), 0.865 (3H, *s*, H-26), 0.805 (3H, *s*, H-27) and the acetate methyl at δ 2.065 (3H, H-2'), 4.604 , 4.730 , 4.512 and δ 2.4.^{13}C-NMR (100 MHz, CDCl3): δ 38.396(C-1),

23.734(C-2), 81.003(C-3), 37.821(C-4), 55.387(C-5), 18.222(C-6) 34.214(C-7), 40.857(C-8), 50.348(C-9), 37.098(C-10), 20.956(C-11) 25.098(C-12), 38.047(C-13) 42.840(C-14), 27.446(C-15), 35.583(C-16), 43.023(C-17), 48.033(C-18), 48.290(C-19), 151.021(C-20), 29.845(C-21), 40.019(C-22), 27.972(C-23), 15.995(C-24), 16.216(C-25), 16.531(C-26), 14.528(C-27), 18.030(C-28), 109.376(C-29), 20.956(C-30), 171.083(C-1'), 21.385(C-2').

Lupeol (Figure 2)was isolated as a white powder with melting point 213-215^{0}C.IR: 3440, 2881, 2249, 1381, 911, 741 cm^{-1}.^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.779, 0.846, 0.962, 1.047,1.280,1.404, 1.700,3.215, 4.587, 4.709, 7.284and 2.4.^{13}C-NMR (100 MHz, $CDCl_3$): δ 38.05(C-1), 25.13(C-2), 79.01(C-3) 38.70(C-4), 55.29(C-5), 18.32(C-6) 34.27(C-7), 40.82(C-8), 50.43(C-9), 37.17(C-10), 20.93(C-11), 27.41(C-12), 38.87(C-13) 38.05(C-14), 42.83(C-15), 35.58(C-16), 43.01(C-17), 48.30(C-18), 47.99(C-19), 150.98(C-20), 29.84(C-21), 40.01(C-22), 28.00 (C-23), 15.38(C-24), 16.13(C-25), 15.98(C-26), 14.55(C-27), 18.01(C-28), 109.33(C-29), 19.31(C-30).

Stigmasterol (Figure 3) was white powdered and its melting point is 142-144^{o}c. IR:3421, 2954, 2858,2553,1469,1379,905,733 cm^{-1}.^{1}H-NMR (400 MHz, $CDCl_3$): δ0.714, 0.839, 1.027, 1.512, 2.03, 2.294,3.53, 5.36, 5.02 and 5.16.^{13}C-NMR (100 MHz, $CDCl_3$): δ 37.28(C-1), 28.92(C-2), 71.81(C-3), 42.30(C-4), 140.7(C-5), 121.7(C-6), 31.9(C-7) 31.66(C-8), 50.14(C-9) 39.68(C-10) 24.37(C-11), 29.18(C-12) 39.7(C-13) 56.77(C-14), 25.41(C-15), 28.25(C-16), 56.06(C-17), 12.273(C-18), 12.073(C-19), 40.50(C-20) 21.22(C-21), 138.32(C-22), 129.28(C-23), 51.24(C-24), 32.42(C-25), 19.82(C-26), 19.02(C-27) 29.16(C-28), 11.88(C-29).

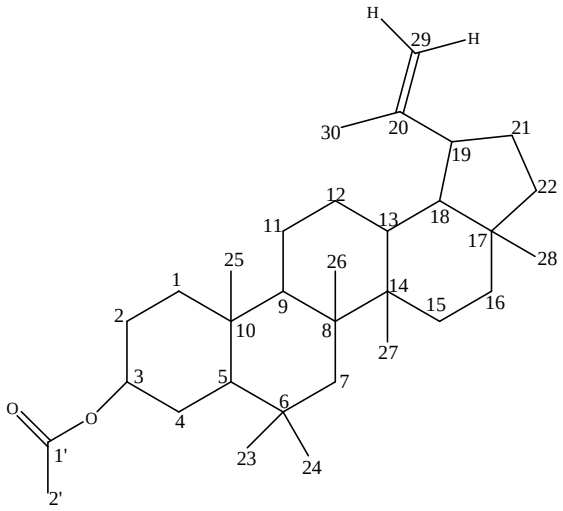

Figure 1 Structure of Lupeol acetate

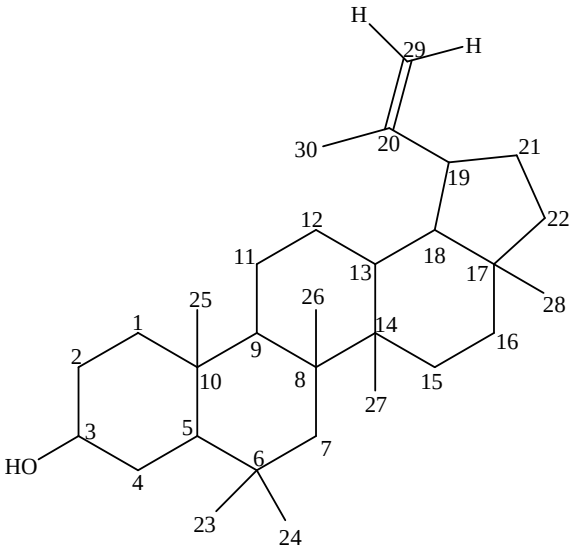

Figure 2 Structure of Lupeol

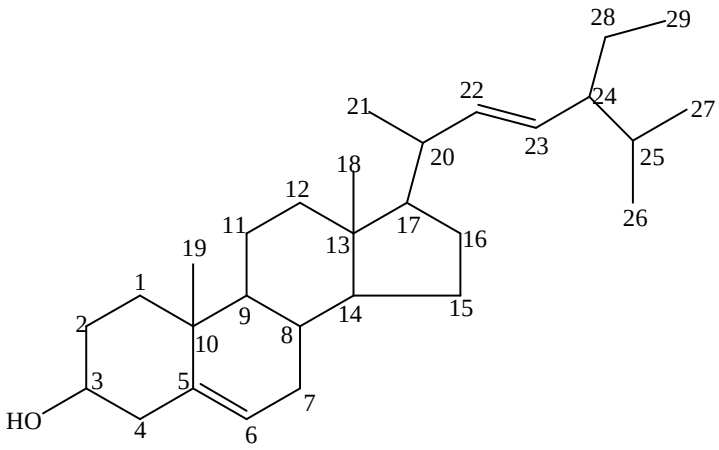

Figure 3 Structure of Stigmasterol

Table1 : ^{13}C NMR data of compounds 1-3

Carbon	Lupeol Acetate	Lupeol	Stigmasterol
1	38.396	38.05	37.28
2	23.734	25.13	28.92
3	81.003	79.01	71.81
4	37.821	38.70	42.30
5	55.387	55.29	140.7
6	18.222	18.32	121.7
7	34.214	34.27	31.9
8	40.857	40.82	31.66
9	50.348	50.43	50.14
10	37.098	37.17	39.68
11	20.956	20.93	24.37
12	25.098	27.41	29.18
13	38.047	38.87	39.7
14	42.840	38.05	56.77
15	27.446	42.83	25.41
16	35.583	35.58	28.25
17	43.023	43.01	56.06
18	48.033	48.30	12.273
19	48.284	47.99	12.073
20	151.021	150.98	40.50
21	29.845	29.84	21.22
22	40.019	40.01	138.32
23	27.972	28.00	129.28
24	15.995	15.38	51.24
25	16.216	16.13	32.42
26	16.531	15.98	19.82
27	14.528	14.55	19.02
28	18.030	18.01	29.16
29	109.376	109.33	11.88
30	20.956	19.31	-
1’	171.083	38.05	-
2’	21.385	25.13	-

CONCLUSION

Sapium ellipticum is a traditionally claimed medicinal plant of Ethiopia. A thorough search of literature revealed that there were only few attempts on phytochemical investigation on *Sapium ellipticum.* Hence phytochemical analysis of this plant has been carried out. Column chromatographic analysis of the chloroform extract has been carried out using petroleum ether and ethyl acetate combinations. Three compounds lupeol acetate, lupeol and stigmasterol were isolated in the pure form and were characterized by using spectroscopic data (IR, ^{1}H-NMR, ^{13}C-NMR and DEPT-135) and by comparing the observed spectral data with literature. All the compounds are isolated from this plant for the first time. The other attempt of any phytochemical investigation from *Sapium ellipticum* and further isolation and purification of other fractions of this plant is recommended which could yield some novel and bioactive compounds.

Acknowledgements
The authors are thankful the Department of Chemistry, Jimma University, Jimma, Ethiopia for the financial support. The authors also acknowledge the help rendered by Dr. M. Remesh, Department of Biology, Jimma University, Jimma, Ethiopia during the identification and collection of the plant material.

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