ISSN: 0974-2115
www.jchps.com Journal of Chemical and Pharmaceutical Sciences

Isolation of a mixture of Phytosterol compounds from the *n*-Hexane extract of *Jatropha lagarinthoides* (Sond) collected from Zebediela sub-region in Limpopo province, South Africa

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ABSTRACT

The study aimed to isolate and identify the active principles from the leaves of *Jatropha lagarinthoides*. The dried leaves powder of *Jatropha lagarinthoides* was extracted with *n*-Hexane by cold maceration and subjected to silica gel 60 column chromatography that afforded isolation of a fraction (CPD1JH) with free radical scavenging activity and R_f value of 0.54. Mass spectrum of CPD1JH showed a molecular ion peak at m/z 412 which correspond to molecular formula $C_{29}H_{48}O$. ^{1}H-NMR (acetone) spectrum of CPD1JH showed a multiplet signal at δ 3.36, 3.31 and an olefinic proton at δ 5.37. Other olefinic protons appeared as multiplets at δ 5.17, 4.12 and δ 406. Methyl protons appeared at δ 1.03, 0.89, 0.85 - 0.82. The isolated fraction also tested positive for both steroid and alcohol. Based on the chemical and spectroscopic characteristics of the isolated fraction, CPD1JH was concluded to be a mixture of two phytosterol compounds, Stigmasterol and β-Sitosterol.

KEY WORDS: *Jatropha lagarinthoides*, *n*-Hexane extract, cold maceration, silica gel 60 column chromatography, Stigmasterol, β-Sitosterol.

1. INTRODUCTION

Jatropha lagarinthoides belongs to the family Euphorbiaceae and is mainly found in Magaliesberg and Eastern parts of South Africa that include Limpopo and Mpumalanga provinces, growing in well-drained soil. It is also widely distributed in the wild and cultivated areas of Central America, South America, Africa and India. The leaves are smooth with five lobes spanning 10-15 cm. The plant grows optimally between 15- 40^{o}C and is affected mostly by lower temperatures than high altitudes. The leaves of *J. lagarinthoides* are used by traditional healers in Limpopo province of South Africa for the treatment of stomach complications, especially when food poisoning is suspected (Gololo, personal communication). However, little is known about the active ingredients possessed by the leaves of this plant.

The seeds of *Jatropha lagarinthoides* are used to produce some insecticides and as medicine to relieve constipation, as well as the production of jatropha oil. The leaves may also be used to massage strained muscles and also as antimalarial agents. Previous studies indicated that some members of the *Jatropha* genus possess biological activities such as antimicrobial, antioxidant, anti-inflammatory and anticancer properties. Phytochemical analysis of a related plant species, *Jatropha curcas*, revealed the presence of phenolic, flavonoid and saponin compounds. In this study, a steroid fraction was isolated from the *n*-Hexane leaf extract of *Jatropha lagarinthoides* using column chromatography. The fraction was identified to be a mixture of two sterol compounds (Stigmasterol and β-Sitosterol) on the basis of chemical properties and spectroscopic data, as well as in comparison of the spectroscopic data reported in the literature. To our knowledge, isolation of this compounds from the leaves of *Jatropha lagarinthoides* is reported here for the first time.

2. MATERIALS AND METHODS

Plant material: The leaves of *Jatropha lagarinthoides* were collected from Bolahlakgomo village in Limpopo province, South Africa. The plant was scientifically identified by Dr. Bronwyn Eagan, Taxonomist in the Department of Botany (University of Limpopo) (specimen voucher number: UNIN 11120). The dried powdered leaves (1.2 kg) was extracted with *n*-Hexane by cold maceration and followed by evaporation of the solvent using a rotavapor (Buchi Labotec rotavapor model R-205) and the residue was dried under a stream of air.

Isolation and purification of the compound: The *n*-Hexane leaf extract (15 g) of *J. lagarinthoides* was fractionated on a silica gel 60 column with gradient elution using *n*-Hexane: Chloroform. The obtained fractions were concentrated by evaporating the solvents and small amounts loaded on a TLC plate followed by run using a mobile phase comprising Benzene: Ethanol: Ammonia [(BEA, 90:10:1 v/v/v)]. The dried TLC plate was then sprayed with 0.2 % DPPH in methanol to identify the fraction with free radical scavenging activity. Fraction 4, obtained with *n*-Hexane: Chloroform (2:8, v/v) solvent system, was eluted on a silica gel column using the *n*-Hexane: Chloroform (2:8, v/v) as solvent system. Elution fractions in test tubes 70-104 were pooled together and re-eluted using the same solvent system. After re-elution fractions in test tubes 14-19 were combined to obtain a fraction named CPD1JH (0.16 g) with free radical scavenging activity and R_f value of 0.54 (*n*-Hexane: Chloroform, 1:4 v/v).

Structure elucidation of CPD1JH:

Spectroscopic techniques: 1H-NMR, 13-NMR, COSY-NMR, gHMBC-NMR, GC-MS and UV-Vis techniques were used to obtain data for structural elucidation of CPD1JH. The proton, carbon and 2D NMR were recorded using $Acetone_d$ as solvent on a Varian-400 MHz NMR spectrometer (Shimadzu) at Tshwane University of Technology, Arcadia Campus (South Africa). Mass spectrum was recorded on GC-MS (Shimadzu QP 2010SE) and UV-Vis was obtained using a Varian *Cary* 50 Conc UV-Vis spectrophotometer (Lasec) at Sefako Makgatho Health Sciences University, Ga-Rankuwa (South Africa).

Test for steroid (Salkowski reaction): Few crystals of CPD1JH were dissolved in chloroform and few drops of concentrated sulphuric acid were added. Formation of a reddish color in the upper chloroform layer indicated positive result for steroid.

Test for alcohol: Four gram of ceric ammonium nitrate was dissolved in 10 ml of 2 N HNO_3 with mild heating. Few crystals of CPD1JH were dissolved in 0.5 ml of dioxane and the solution was added to 0.5 ml of ceric ammonium nitrate reagent with further addition of 1 ml dioxane. Formation of a yellow to red color upon shaking indicated the presence of an alcoholic hydroxyl group.

3. RESULTS AND DISCUSSION

The GC-MS, UV, ^{1}H-NMR and ^{13}C-NMR spectral data of CPD1JH are presented in Table.1. Mass spectrum of CPD1JH showed a parent molecular ion peak at m/z 412, which corresponds to the molecular formula $C_{29}H_{48}O$. This molecular formula is equivalent to that of a known phytosterol compound, Stigmasterol. From the UV spectrum of CPD1JH, λ_{max} values were found at 256 and 251 nm. The two UV λ_{max} values are reported in the literature for Stigmasterol and β-Sitosterol, respectively. ^{1}H-NMR spectrum showed multiplet proton signal at δ 3.36 and also revealed the presence of signals for Olefinic protons at δ 5.37, 5.17, 4.12 and 4.06. The proton signal at δ 3.36 is in the chemical shift region that is characteristic to the H-3 of a sterol moiety appearing as a triplet of double doublet for known sterol compounds. Methyl proton signals were showed at δ 1.03, 0.89 and 0.85-0.82.

^{13}C-NMR spectrum of CPD1JH showed twenty nine carbon signal revealing the presence of methyl carbons, alkene carbons and quaternary carbons. The spectrum also showed carbon signal in the chemical shift region recognizable for sterol compounds at δ 138.9 (C-5), 124.2 (C-6), 135.6 (C-20), 129 (C-21), 22 (C-19) and 19.5 (C-27). The sterol nature of CPD1JH was suggested from the ^{1}H-NMR, ^{13}C-NMR and MS data which was supported by the positive reaction to the steroid and alcohol tests. The ^{1}H-NMR and ^{13}C-NMR signals for all protons and carbons were assigned on the basis of COSY and gHMBC correlations and presented as Table 2. The chemical and spectroscopic data of the isolated fraction are consistent to those reported in the literature for sterol compounds [11, 13]. In this study, the NMR spectra of the isolated fraction were obtained using $acetone_d$ which accounts for the slight differences in the chemical shift values of atom signals compared to those reported in the literature whose spectra were obtained using $CDCl_3$.

The presence of two recognizable UV λ_{max} values at 256 and 251 earlier reported for Stigmasterol and β-Sitosterol, respectively suggest that CPD1JH is a mixture of the two sterol compounds. The mass spectrum of CPD1JH also showed recognizable fragments with m/z 395, 342, 271, 245, 175, 161, 149, 121, 109, 95, 81 and 55 corresponding to those reported in the literature for the two compounds. This could be the case considering that literature have shown that β-Sitosterol is difficult to obtain in pure form. The two compounds only differ with the presence of C-22/C-23 double bond in Stigmasterol and C-22/C-23 single bond in β-Sitosterol, which will afford them the same R_f value on the TLC plate.

Table.1. Spectroscopic data of CPD1JH isolated from the n-hexane extract of *J. lagarinthoides*

Spectroscopic Technique	Data
UV λmax (nm)	256, 251
GC-MS (m/z)	412, 395, 368, 342, 298, 271, 257, 245, 232, 189, 175, 161, 149, 121, 109, 95, 81, 67, 55
^{1}H-NMR (δ ppm)	5.37, 517, 4.12, 4.06, 3.36, 3.31, 1.03, 0.89, 0.85-0.82
^{13}C-NMR (δ ppm)	138.9, 135.6, 129, 124.2, 70, 58.6, 57.1, 49, 39.9, 39.8, 39, 38, 37.3, 36.5, 32.1, 32, 31.9, 29.2, 25.0, 24.0, 22.0, 19.5, 19, 14.3

Table.2. ^{1}H and ^{13}C NMR ($Acetone_d$) chemical shift values (ppm) of compound CPD1JH compared with the ones from the literature. (Assignments made on the basis of COSY and HMBC correlations).

Position	^{1}H NMR Experimental ($Acetone_d$)	^{1}H NMR Literature($CDCl_3$)	^{13}C NMR Experimental ($Acetone_d$)	^{13} C NMR Literature ($CDCl_3$)
1			37.3	37.6
2			32.1	32.1

3	3.36/3.31	3.51 (1H)	70	72.1
4			39	42.4
5	5.37	5.31 (1H)	138.9	140.1
6			124.2	121.8
7			31.9	31.8
8			31.9	31.8
9			49	50.2
10			36.5	36.6
11			22.0	21.5
12			39.8	39.9
13			39.9	42.4
14			58.6	56.8
15			24.0	24.4
16			29.2	29.3
17			57.1	56.2
18			38.0	40.6
19	0.89	0.91 (3H)	22.0	21.7
20	4.12/4.06	4.98 (1H)	135.6	138.7
21	5.17	5.14 (1H)	129	129.6
22			-	46.1
23			25.0	25.4
24	0.85	0.83 (3H)	15.3	12.1
25			32	31.94
26	0.82	0.82 (3H)	29	29.6
27	0.82-0.85	0.80 (3H)	19.5	19.8
28	0.82-0.85	0.71 (3H)	19	18.9
29	1.03	1.03 (3H)	14.3	12.2

4. CONCLUSION

From the chemical reactions and spectroscopic data, it could be concluded that CPD1JH is a mixture of two phytosterol compounds, Stigmasterol and β-Sitosterol, which have a higher proportion of Stigmasterol. The study has thus identified some of the active ingredients possessed by the leaves of *Jatropha lagarinthoides*.

5. ACKNOWLEDGEMENT

The authors would like to acknowledge Dr Bronwyn Eagan for assistance with identification of the plant species, the Department of Chemistry and the Department of Biochemistry, Sefako Makgatho Health Sciences University for facilities to carry out the study.

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