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Lonchocarpus eriocalyx (Harms) Herb Extract for Use as Painkillers

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Abstract: Today, many herbal preparations are being prescribed as analgesics. In the past decade, there has been a resurgence of interest in traditional systems of medicine which has become a topic of global interest. Indeed, many important drugs in the market have been obtained directly/ indirectly from natural sources, for example: morphine, pilocarpine, quinine and artemisinin among others. *Lonchocarpus eriocalyx* (Harms) belongs to the family Fabaceae and is used traditionally to control fever, headache and general body pain. This plant was studied for presence of secondary metabolites and the antinocieceptive effects. Four lupane-type terpenoids; lupeol (1), friedelin (2) stigmasterol (3), and stigmasterol glucoside (4) were isolated from the ethylacetate (EtOAc) extract of leaves by extensive silica gel chromatography and their structures elucidated by spectroscopic 1D and 2D Nuclear Magnetic Resonance (NMR) as well as comparison with literature data. Acetic acid-induced writhing test in mice was used to study the analgesic effect of the crude extract and isolates with Acetyl-salicylic acid as the positive control (87.37%). After prior intraperitoneal injection (i.p) of the mice with the EtOAc extract (100 mg/Kg) and the isolates (10 mg/kg, p.o.), comparatively less number of writhes were observed implying that the extract and isolates had significant ability to relieve pain. Similarly, a percent inhibition of 50.52, 76.7, 66.47 and 62.24% was observed in EtOAc and compounds 1, 2 and 3 respectively compared to the positive control (87.37%). This research has confirmed the presence of painkillers in this plant and scientifically validates its use in folk medicine. The isolates can be used as templates and derivatised into alternative analgesics to support the existing strategies in the management of diseases. Improved health will enhance productivity both at National and Global levels. Large scale cultivation of this plant for commercial purposes will be an Income Generating Activity (IGA) for the rural poor and supplement the strategies aimed at poverty alleviation.

Keywords: *Lonchocarpus eriocalyx*, Fabaceae, Leaves, Terpenoids, Analgesic Activity

1. Introduction

An analgesic or painkiller is any member of the group of drugs used to achieve analgesia/relief from pain. The commonly used ones are aspirin, paracetamol and morphine among others [1]. Response to pain in animals can be investigated by applying unpleasant stimuli such as (i) thermal (radiant heat as a source of pain), (ii) chemicalirritants such as acetic acid and (iii) physical pressure like tail compression [2]. Today, many herbal preparations are being prescribed as analgesicsas well as anti-inflammatory agents and in the past decade, there has been a resurgence of interest in traditional systems of medicine which has become a topic of global importance [3]. It is estimated that in many developing countries, a largeproportion of the population relies heavily on traditional practitioners and medicinal plants in pain relief implying that phytomedicines have continued to maintain popularity [4]. Indeed, many important drugs in the market have been obtained directly or indirectly from natural sources, for example: morphine, pilocarpine, digitalis, quinine, artemisinin among others [5-7]. *Lonchocarpus eriocalyx* (Harms) belong to the family Fabaceae commonly known as

Leguminosaeis used to control fever, headache and diarrhea and also as an insecticide (Ceres *et al*., 1981). Crude extract of the root bark of *L. eriocalyx*exhibited antiplasmodial activity against *Plasmodium ovale* [9]. Chromatographic separation of extract from the plant yielded lupeol (1), which showed good antiplasmodial activity [10]. This plant was studied for presence of secondary metabolites and the antinocieceptive effects. This communication reports the isolation of lupeol (1), friedelin (2) stigmasterol (3), and stigmasterol glucoside (4). Compounds 2-3 are reported from the plant for the first time. Also reported here-in are analgesic properties of theisolated compounds.

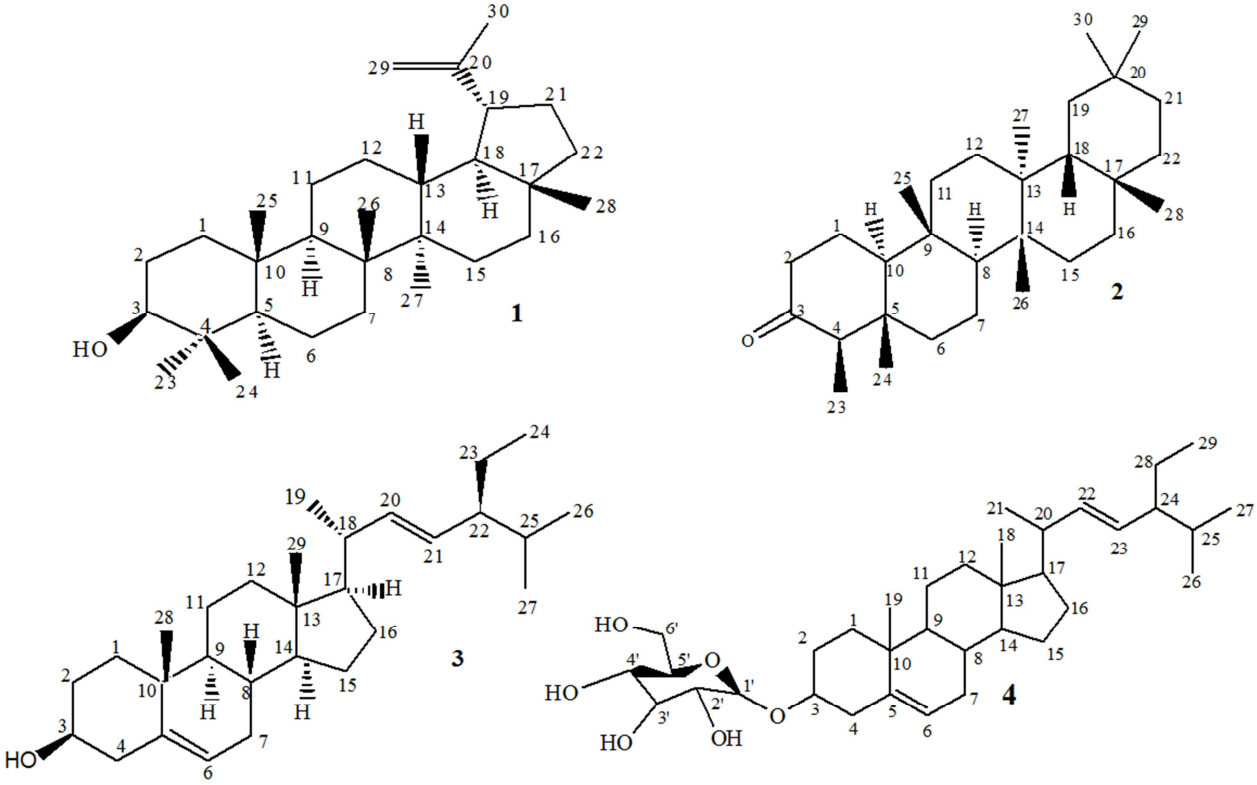

Figure 1. *Structures of Compounds 1, 2, 3 and 4.*

2. Materials and Methods

2.1. Experimentation, Solvents and Fine Consumables

Melting points were determined using Gallenkamp melting point apparatus and are uncorrected. The NMR data were measured in $CDCl_3$ and $CDCl_3$-DMSO-d_6 on a JOEL NMR instrument operating 600 and 150 MHz, respectively. Some NMR analyses were done using Brucker AM 300 spectrometer operating at 300 and 75 MHz, respectively. TMS was used as internal standard. The mass spectral data were obtained using a Varian MAT 8200 A instrument. Column chromatography was performed using silica gel 60 (0.063 - 0.200 mm, Merck) while thin layer chromatography (TLC) was performed using silica gel 60 F_{254} (Merck) pre-coated plates. All solvents used were of analytical grade.

Collection of Plant materials

Leaves of Lonchocarpus eriocalyx were collected fromEmbu-Mbeere (Lat: 0.5833° S and Long: 37.6333° E) where it naturally occurs. The plant materials were authenticated at the Herbarium of the Museums of Kenya where voucher specimens are be preserved (Angeline Ochung' and Regina Ochieng' No. 2013/58).

2.2. Extraction and Isolation

The air dried and pulverized leaves (1.5 kg) of the plant was soaked sequentially in *n*-hexane (3×3 L), EtOAc (3×3L) and MeOH (3×3L), each lasting four days at room temperature. The extracts were separately filtered and evaporated under reduced pressure to afford yellowish (1 g), brown (106 g) and reddish-brown (2 g) extracts of *n*-hexane, EtOAc and MeOH extracts, respectively. The extracts were kept at 4°C for phytochemical and analgesic activity studies.

Fractionation of EtOAc extract

Ethylacetate extract (30 g) was adsorbed onto silica gel and subjected to column chromatography (2.5 x 60 cm, SiO_2 240 g, pressure≈1 bar) using *n*-hexane-CH_2Cl_2 gradient (increment 10%) up to 100% CH_2Cl_2 and elution concluded with 100% ethyl acetate, collecting 20 ml each. The process afforded sub-fractions (I-V) as determined by TLC profiles [solvent systems: *n*-hexane-CH_2Cl_2 (1:3, 1:2) and CH_2Cl_2-MeOH, 95:5 and 90:10)]. The sub-fraction I (fractions 1-10) showed no spot and solvent was recovered. Sub-fraction II (fractions 15-60, 20 g) gave a single spot R_f 0.83 (eluent: *n*-hexane-EtOAc, 2:3) which upon evaporation of solvent followed by crystallization in CH_2Cl_2-MeOH of ratio 1:1 mixture afforded compound 1as white needle-like crystals

(55 mg). Sub-fraction III (fractions 61-86, 5 g) showed two spots of R_f values 0.82 and 0.62 (eluent: *n*-hexane-EtOAc, 2:3) which upon repeated chromatographic separation afforded a further 1 (45 mg) and 2 (50 mg), respectively. Sub-fraction IV (fraction 93-103, 7.4 g) showed two major spots R_f 0.48 and 0.65 (eluent: CH_2Cl_2-MeOH, 97:3) and upon evaporation of solvent, followed by crystallization gave compounds3 and 4,64 and 35 mgrespectively. Fractions 104-180 constituted sub-fraction V (5 g) and was further purified by medium pressure chromatography (2.5×50 cm, SiO_2 150 g, pressure≈1 bar) to give further compounds 3 and 4 in 50 mg and 30 mg, respectively.

Compound 1: White needle-like crystals $C_{30}H_{50}O$, (100 mg), R_f 0.83, mp 216-218°C (lit. 214-215°C [11-13]*.

Table 1. *^1H (300 MHz) and ^{13}C (75 MHz) NMR ($CDCl_3$) data for Compound 1.*

C#	^1H Multiplicity, (*J* in Hz)	^1H (Multiplicity, (*J* in Hz)	^{13}C	^{13}C*
1	-	-	38.7	38.7
2	-	-	27.2	27.4
3	3.20 (1H, dd, *J* = 11.4, 4.8)	3.23 (1H, dd, *J* = 11.5, 4.7)	77.7	79.0
4	-	-	38.7	38.9
5	-	-	54.0	55.5
6	-	-	18.6	18.5
7	-	-	34.3	34.2
8	-	-	41.7	40.9
9	-	-	49.2	50.5
10	-	-	37.5	37.2
11	-	-	21.3	21.0
12	-	-	37.9	38.1
13	-	-	38.0	38.1
14	-	-	41.6	42.9
15	-	-	28.6	27.1
16	-	-	35.9	35.5
17	-	-	42.9	43.0
18	3.10 (1H, dd, *J* = 11.0, 5.0)	3.15 (1H, dd, *J* = 11.5, 5.3)	49.2	48.3
19	-	-	48.0	48.0
20	-	-	149.7	151.0
21	-	-	30.0	29.9
22	-	-	39.6	40.0
23	0.76 (3H, s)	0.77, s	27.3	28.0
24	0.79 (3H, s)	0.80, s	14.9	15.5
25	0.83 (3H, s)	0.82, s	15.9	16.1
26	0.94 (3H, s)	0.95, s	16.0	16.0
27	1.02 (3H, s)	1.10, s	15.0	14.8
28	0.76 (3H, s)	0.75, s	18.0	18.0
29	4.57 (1H, d, *J* = 0.4, H_α-29) 4.67 (1H, d, *J* = 0.5, H_β-29)	4.60 (d, *J* = 0.5, H_α.29) 4.70 (d, *J* = 0.6, H_β-29)	108.1	109.0
30	1.20 (3H, s)		19.5	19.7

Compound 2: White crystals, $C_{30}H_{50}O$ (50 mg), R_f 0.62, mp 254-256°C (lit. 252-254°C [15]*.

Table 2. *^1H (300 MHz) and ^{13}C (75 MHz) NMR ($CDCl_3$) data for Compound 2.*

C #	^1HMultiplicity, (*J* in Hz)]	^1H*Multiplicity, (*J* in Hz)]	^{13}C	^{13}C*
1	1.76 (dd, *J* = 13.0, 7.5)	1.82 (dd, *J* = 12.8, 8.0)	22.5	22.3
2	2.25 (H_α d, *J* = 6.8) 2.72 (H_β, dd, *J* = 13, 7.5)	2.54 (d, *J* = 7.0) 2.76 (dd, *J* = 13.5, 8.0)	41.6	41.5
3	-	-	213.4	213.2
4	2.27, (q, *J* = 5.4)	2.37, m	58.5	58.2
5	-	-	42.4	42.2
6	1.62 (dd, *J* = 11.4, 5.2)	1.56 (dd, *J* = 11.5, 5.6)	41.4	41.3
7	1.31, m	1.31	36.3	35.8
8	1.39, m	1.39	53.4	53.1
9	-		37.7	37.5
10	1.39, m	1.30	59.8	59.5
11	1.56, m	1.56	33.5	33.2
12	1.56, m	1.56	30.8	30.5
13	-	-	41.8	41.2
14	-	-	41.6	41.0
15	1.31, m	1.31	30.3	30.2
16	1.31, m	1.31	35.9	36.8
17	-	-	30.8	30.0
18	1.39, m	1.39	43.1	42.8
19	1.45, m	1.45	35.7	35.5

C #	^{1}HMultiplicity, (*J* in Hz)]	^{1}H*Multiplicity, (*J* in Hz)]	^{13}C	^{13}C*
20	-	-	29.9	29.4
21	1.31, m	1.29	33.1	32.8
22	1.31, m	1.31	39.6	39.3
23-Me	1.20, (d, *J* = 6.8)	1.11	7.1	6.8
24-Me	1.02, m	1.04	14.9	14.7
25-Me	0.74, s	1.04	20.5	21.0
26-Me	0.91, s	1.04	18.9	18.8
27-Me	1.03, s	2.04	18.5	18.8
28-Me	1.07, s	1.04	32.1	32.1
29-Me	0.88, s	0.99	32.4	31.7
30-Me	0.89, s	0.99	32.7	31.8

Compound 3: White needle-like crystals, $C_{29}H_{48}O$, (114 mg), R_f 0.48, mp m p 166-168°C (lit. 165-166°C [15-17]*.

Table 3. *^{1}H (300 MHz) and ^{13}C (75 MHz) NMR ($CDCl_3$) data for Compound 3.*

C#	^{1}H Multiplicity, (*J* in Hz)	^{13}C	^{13}C*	C#	^{1}HMultiplicity, (*J* in Hz)	^{13}C	^{13}C*
1	1.43 (d, *J* = 3.2)	37.3	37.3	16	-	28.3	28.6
2	1.64 (d, *J* = 5.6)	31.7	31.7	17	-	56.1	56.1
3	3.52 m	71.8	71.8	18	1.02 s	11.7	12.1
4	2.19 (d, *J* = 9.8)	39.8	42.4	19	0.78, s	19.3	20.6
5	-	140.8	140.8	20	5.19 (dd, *J* = 14.8, 5.5)	129.2	129.4
6	5.35 (d, *J* = 5.2)	121.7	121.9	21	4.96 (dd, *J*=15.5, 6.6)	138.1	138.3
7	1.52 (d, *J* = 12.4)	31.9	31.9	22	1.25 9d, *J* = 0.93)	45.6	45.7
8		31.7	31.9	23	1.10, s	23.1	23.1
9		50.1	50.2	24	0.68, s	11.9	12.0
10		36.5	36.6	25	0.78, s	29.2	29.3
11		21.1	21.0	26	0.84 (d, *J* = 6.8)	19.0	19.6
12		37.2	39.8	27	0.78, s	19.6	20.0
13		42.3	42.3	28	1.25, s	19.4	20.1
14		56.8	56.8	29	0.81, s	12.0	12.1
15		24.3	24.4				
3-OH	3.52 (m)						

ESI-MS (rel. int): *m/z*412, 367, 271, 255, 189, 175, 161, 133, 121, 105, 95, 81, 69, 41. IR ν_{max} (KBr) cm^{-1}: 3373, 2940, 2867, 1641, 1457, 1381, 1038.
Compound4: Colourless needles, $C_{35}H_{58}O_6$, (65 mg), R_f 0.63, mp 290-292°C (lit. 289-290°C [18-20)]*.

Table 4. *^{1}H (300 MHz) and ^{13}C (75 MHz) NMR ($CDCl_3$,) data for Compound 4.*

C#	^{1}H (Multiplicity,(*J* in Hz)	^{13}C	^{13}C*	C#	^{1}H (Multiplicity,(*J* in Hz)	^{13}C	^{13}C*
1	-	37.2	36.7	19	0.96, s	19.3	19.1
2	-	28.9	29.1	20	-	35.8	35.7
3	-	78.1	78.6	21	1.03 (d, *J* = 6.4)	18.7	18.7
4	-	41.2	42.1	22	5.10 (dd, *J* = 15.2, 8.8)	137.5	33.5
5	-	140.8	140.0	23	-	127.3	25.6
6	5.13 (dd, *J* = 4.7, 1.7) 2.77 (H-7$_\alpha$, dd, *J* =12.3, 2.6)	121.9	121.5	24	-	45.5	45.5
7	*J* = 11.7, 2.4) 2.37 (H-7$_\beta$, dd *J* = 11.5, 11.3)	31.1	31.4	25	5.06 (dd, *J* = 15.2, 8.8)	27.3	28.7
8	2.01, m	31.8	31.5	26	0.92 (d, *J* = 6.8)	19.2	18.7
9	1.99, m	50.2	49.8	27	0.90 (d, *J* = 6.8)	18.7	18.4
10	-	36.5	36.3	28	0.78, m	25.9	22.6
11	-	20.2	20.2	29	0.88 (t, *J* = 7.0)	12.3	12.3
12	-	39.9	38.2	1’	4.95 (d, *J* = 7.9)	101.4	100.7
13	-	42.3	41.9	2’	4.40, (t, *J* = 7.9, 8.8)	74.0	73.2
14	-	56.4	56.4	3’	4.80, m	76.8	76.2
15	-	23.5	23.8	4’	4.44, m	70.5	70.0
16	-	28.4	27.8	5’	2.81, m	76.1	75.2
17	-	56.4	55.7	6’	4.87 (H-6’$_\alpha$, dd, *J* = 11.7, 2.4) 4.82 (H-6’-$_\beta$, dd *J* = 11.7, 5.3)	62.1	61.4
18	0.83 (s)	11.8	11.3				

ESI-MS (rel. int): *m/z*574, 603.3{M+ (C_2H_5), (100), 601.3 (20), 583 (10). IR ν_{max} (KBr) cm^{-1}: 3392 (OH), 2931-2868 (aliphatic stretch, 1641 (C=C) stretch), 1432 (CH_2-stretch) 1369 (isopropyl stretch), 1256, 1164, 1061, 1017.

2.3 Analgesic Effect in the Hot Plate Test

The modified method of Eddy and Leimbach, 1953 [21]was used. Groups of mice (5 per group) of either sex (17–30 g) were used as test organisms. The mice were initially screened by placing the animals in turn on a hot plate (Electrothermal Eng. Ltd) set at 55±1°C and animals which failed to lick the hind paw or jump within 15s were discarded (nociceptive responses). Eligible animals were divided into five groups of five each and pre-treatment reaction time for each mouse was determined before drug treatment so that each animal served as its own control. The times until the animals licked the paw, flutter any of the paws or jump was taken as reaction time and were recorded with aid of an inbuilt stopwatch. Mice in the different groups were then treated with normal saline water [10 ml/kg, per oral (p.o)], the ethylacetate extract of the leaves of *L. eriocalyx* (100 mg/kg, p.o), together with compounds 1, 2, 3 and 4 (100 mg/kg) and morphine (10 mg/kg, s.c). The latency was recorded after 30 and 60 min following oral administration of extracts (100mg/kg), normal saline (10 ml/kg) and subcutaneous administration of morphine (10 mg/kg). A post-treatment cut off time of 30 s was used to avoid paw tissue damage [22].

$$\%\ \text{Inhibition} = \frac{[\text{Post} - \text{treatment Latency}] - [\text{Pre} - \text{treatment Latency}]}{[\text{Cut} - \text{off Time} - \text{Pre} - \text{treatment Latency}]} \times 100$$

2.4 Acetic Acid (Chemical-Induced) Writhing Method

Abdominal writhes consist of contraction of the abdominal muscle together with a stretching of the hind limbs, induced by intra-peritoneal injection (i.p) in mice of acetic acid (0.8% solution in normal saline, 0.1 ml/10 kg), the nociceptive agent [23]. Ethylacetate extract of leaves of *L. eriocalyx* (100 mg/kg, p.o) and compounds 1, 2, 3 and 4 (100mg/kg, p.o) were administered to mice (animals fasted overnight and divided into five groups of six animals each) 60min before (i.p) of acetic acid (0.6%, v/v in normal saline, 10 ml/kg, i.p). Normal saline was used as the control. The number of writhes (characterized by contraction of the abdominal musculature and extension of the hind limbs) was counted for 30 min at 5 min interval of intra-peritoneal injection of acetic acid [24]. Statistical analysis results obtained were expressed as mean±standard error of mean (SEM) or standard deviation (SD). The data were analyzed using one way ANOVA followed by Bonferroni posttests and Dunnett's multiple comparison tests. Values were considered significant when $P \leq 0.05$.

$$\text{Inhibition}\ (\%) = \frac{\text{Number of Writhes [Control]} - \text{Number of Writhes [Treatment]}}{\text{Number of Writhes [Control]}} \times 100$$

3. Results and Discussion

Repeated column chromatography separation of the ethylacetate extract of the leaves of *L. eriocalyx* (30 g) yielded four compounds (1-4): Figure 1. Compound 1, white needle-like crystals; with a molecular formula $C_{30}H_{50}O$ evidenced by a molecular ion at *m/z* 426 [M^+] gave a positive stable violet ring with Libermann- burchard test indicating a triterpenoid or steroid skeleton [25]. The ^1H and ^{13}C NMR spectra had signals consistent with a pentacyclic lupane-type triterpene with olefinic protons of an exocyclic double bond at δ 4.57 and 4.67 (2H, m, H_a-29 and H_β-29). The latter signals were confirmed by the appearance in the ^{13}C NMR of olefinic carbons at δ 149.7.0 for C-20; extremely downfield due to the electron donating effect of the methyl group at C-30 and another signal at δ 108.1 for C-29 further upfield. Both ^1H and ^{13}C NMR showed the signal typical of hydroxymethine proton (H-3) at δ 3.20 (dd, J = 11.0, 4.8 Hz) and carbon (C-3) δ 77.7 respectively whichwas attributed to a proton geminal to alcoholic group [26]. Seven singlet signals of tertiary methyl protons at δ 0.76 (2×CH_3), 0.79, 0.83, 0.94 and 1.02, 1.20 (intergrating for 3H each) with corresponding ^{13}C NMR signals at 27.3, 14.9, 16.0, 15.9, 15.0, 18.0 and 19.5respectively as deduced from the HMQC which were in agreement with the structure of Lupeol. The confirmation of this structure was accomplished through extensive analysis of the 2D NMR experiments of COSY, HMBC and HMQC [13].

Comparison of ^1H NMR and ^{13}C NMR spectra of compound 1 with those of Lupeol previously isolated from *Lonchocarpus sericeus*, *Holarrhena floribunda*were all in agreement with the structure of lupeol (Fotie *et al*., 2006, Lutta *et al*., 2008, Correa *et al*., 2009). IR ν_{max} (KBr) cm^{-1}: 3315, 2900, 1650, 1462, 1190, 1037, 997, 681 which were typical of the functional groups in lupeol. The identity of this compound as Lupeol has been confirmed by Co-TLC with authentic specimen [13].

Compound 2 (50 mg) was obtained as white crystals mp; 254-256°Cwith a molecular formula $C_{30}H_{50}O$. The ^1H, ^{13}C NMR had a signal for H-1a at δ 1.76 and H-2b at δ 2.27 (dd) integrating for 1 proton each. A signal for H-4 was observed as a multiplet integrating for one proton at d 2.25 while H-6 was at δ 1.62 (dd) with corresponding C-2 and C-6 at δ 41.6 and 41.8 respectively [14]. Eight signals typical of methyl protons were also observed at δ 0.74, 0.88, 0.89, 0.91, 1.02, 1.03 and 1.07, 1.20 with corresponding ^{13}C NMR signals at δ 32.4, 32.7 for Me-29 and Me-30, 7.1 for Me-23, 18.9 for Me-26. Signals typical of methyne carbons were also observed at δ 58.5, 53.4 and 59.8 for C-4, C-8 and C-10 while quartenary carbon signals were observed at δ 29.9 and 30.0 for C-20 and C-17. Also observed were eleven signals typical of methylene carbons at δ 18.2, 22.5, 29.9, 32.7, 33.05, 35.3,

35.6, 35.9, 36.3, 39.5, 41.6. The C=O carbon was confirmed by signal at 213.4 typical of a ketonic carbon [14]. Mass spectral fragmentation pattern was quiet typical of pentacyclic ring structure, containing seven methyl groups in rings other than A (*m/z* 341) [14]. The mass peak at *m/z* 273 indicated that the carbonyl oxygen is present in ring A, B or C. Another peak at m/z 302 was proof for the presence of three methyl groups in ring E[11]. In the mass spectrum peak at *m/z* 341 originated by the loss of ring A, the peak at *m/z* 302 by the loss of ring E, and the peak at m/z 273 by the loss of ring D and E [14].

Compound 3 was isolated as white needle-like crystals and the ESI-MS indicated a molecular ion peak at *m/z* 412 suggesting a molecular formula of $C_{29}H_{48}O$, (114 mg), mp; 166-168°C The ^{1}H and ^{13}C NMR spectra had a multiplet signal for H-3 at δ 3.52 for the oxymethine proton which and suggested the presence of an α-proton typical of sterols hydroxylated at C-3 [17]. (A δ_C signal at 71.8 in the oxygenated aliphatic region confirmed the presence of the oxymethine carbon [17]. A signal for H-6 typical of olefinic proton of appeared at δ 5.35 (d, *J* = 5.2 Hz) while two other olefinic protons appeared upfield at δ 5.01 (dd, *J* = 15.2, 6.6 Hz) and 5.40 (dd *J* = 12, 6.0 Hz) which were confirmed by carbon-carbon double bond resonances at δC signals at 129.2 (C-20) for a disubstituted carbon and 138.1 (C-21) respectively; and this confirmed the presence of two double bonds. Another olefinic carbon signal appeared downfield at 140.8 (C-5) typical of a trisubstitution compared to a δ_C signals at 121.7 for C-6. The presence of six methyl protons was confirmed by signals at 0.69, 0.78 ($2\times CH_3$), 0.93, 1.02 and 1.25 with corresponding ^{13}C NMR signals at δ 19.3, 11.9, 19.4, 19.6, 19.0 and 12.0 respectively [15, 16]. The appearance of six methyl signals suggested the presence of a sterol. ^{13}C NMR spectra showed the presence of 29 carbon atoms which included six methyls, nine methylenes, eleven methines and three quartenary carbons in total. This compound was identified as stigmasterol based on spectral data as well as comparison with information contained in literature [15-17].

Compound 4 was obtained as colourless needles (65 mg) with molecular formula of $C_{35}H_{58}O_6$, mp; 290-292°C which was consistent with *m/z* of 574. Its ^{1}H NMR spectrum showed olefinic proton H-6 at d 5.13 ppm (dd, *J* = 4.7, 1.7 Hz) as a double doublet because the two equivalent adjacent protons at (H-7) due to its close proximity of19-Me group. Another set of olefinic protons resonated as two doublets of doublet at δ 5.10 (H-22, dd, *J* = 15.2, 8.8 Hz) and δ 5.06 (H-23, dd, *J* = 15.2, 8.8 Hz) which represented *trans* olefinic protons plus adjacent methine proton [27]. The protons of its sugar moiety resonated at δ 2.81-4.95 ppm. This compound almost completely corresponded to the data for stigmasterol with the exception of the signals between H δ 2.81-4.95 ppm typical for a sugar moiety δ [27]. The ^{13}C NMR spectrum of compound 4 revealed 35 carbon signals in the molecule. The olefinic carbon resonances at δ 121.9 (C-6), 137.5 (C-22), and 127.3 (C-23) were observed for the methine carbons, as well as a signal at δ 140.8 represented the C-5 quarternary carbon of the sterol moiety. A signal typical of an anomeric carbon at δ 101.4 (C-30) indicated the presence of a single monosaccharide moiety. Four other sugarcarbons resonated at δ 74.0 (C-2'), 76.75, 76.8 (C-3', 70.5 (C-4') and 76.1 (C-5') as well as the methylene resonance at δ 62.1 (C-6'), respectively of the *β-D*-glucopyranoside [28]. The presence of anomeric proton (H-30) was evident by a signal at δ 4.95 with diagnostic *J*-value of 7.9 Hz (H-30, 1H, d, *J* = 7.9 Hz) and this reflected that the proton is the axial-axial to H-31 which means glucopyranoside moiety binds to the sterol moiety at β-position [27]. Extensive interpretation of the spectral data coupled with comparison with spectroscopic data contained in literature led to unequivocal identity of the structure of compound 4 (Ahmad *et al*., 2012). The relationship in the bonding structure was proven through long-range correlation of ^{1}H and ^{13}C of HMBC spectrum. The existence of long-range correlations of protons at δ4.95 (H-1') with a carbon at δ78.1 (C-3) and 76.1 (C-5') indicates that the group of glucose is bound to C-3 (oxy carbon sp3) [18-20].

3.1 Analgesic Effect in the Hot Plate Test

The analgesic effect of the crude extracts of the leaves *Lonchocarpus eriocalyx* and compounds isolated were studied in mice using hot plate-induced pain. Preliminary results showed that the pretreatment latency for morphine (2.9±0.15 secs) was quite comparable to that of crude extract (100 mg/Kg) at the zero minute whose values were 3.1±0.15 and 3.0±0.01for EtOAc and DCM respectively implying that they delayedinfliction of pain more/less with the same magnitude as the standard drug just as the instant time of administration. Similarly, the EtOAc extract had a significant effect in delaying the pain within 30 minutes which was quite comparable to that of morphine (10 mg/Kg) meaning longer post treatment latency. Generally, the crude extracts significantly increased reaction time for nociception from the beginning to 60 minutes post treatment. However, the effects of the crude extracts (100 mg/kg) were significantly ($P<0.05$) lower than those produced by morphine in the same tests. Lupeol (1) and friedelin (2) delayed incubation of pain from the beginning to 60 minutes after which the effect was insignificant. Lupeol and friedelin had longer latency compared to the crude extracts from the beginning to 60 minutes suggesting that purity enhanced the efficacy of the compounds. Both the extracts and isolates exhibited significant analgesic effect as shown in Table 5.

3.2 Acetic Acid (Chemical-Induced) Writhing Method

Acetic acid-induced writhing test in mice was also used to study the analgesic effect of the crude extracts and the isolates. After intraperitoneal injection with the crude extracts of the leaves of *L. eriocalyx*comparatively less number of writhes was observed (contraction of abdominal muscles together with stretching of the hindlimbs) implying that the extracts had significant ability to relieve pain. A percent inhibition of 50.52, 76.7, 66.47 and 62.24% was observed in

ethylacetate, compounds 1, 2 and 3 respectively.

Similarly, the total number of writhes of compounds 1, 2 and 3 observed were 14.7±2.63, 19.7±2.08 and 21.3±2.50 respectively. Results are summarized in Table 1.

Table 5. *Effects of crude extracts and pure compounds of Lonchocarpus eriocalyx on hot plate-induced pain and acetic acid-induced writhing in mice.*

Dose Treatment (100 mg/kg)	Pretreatment latency (s)	Pain threshold (time lapse after treatment)								Writhing response	
		Post treatment latency (s) and% inhibitions									
	0 min	30 min		60 min		90 min		120 min		Total no. of writhes	%Inhibition
	sec	sec	% Inb	sec	% Inb	sec	% Inb	sec	% Inb		
Vehicle	3.4±0.15	3.4±0.15	NS	5.2±0.14	6.76	5.8±0.20	9.02	5.8±0.15	9.02	h	0.00
n-hexane	3.9±0.01	5.0±0.1	4.51	30.5±0.20	40.50	11.2±0.20	26.51	6.9±0.20	13.32	23.7±2.54	37.69
EtOAc	3.1±0.15	6.4±0.13	6.04	12.3±0.31	35.04	11.0±0.25	22.92	8.5±0.20	14.33	24.3±1.49	50.52
DCM	3.0±0.01	4.4±0.04	8.23	11.5±0.25	32.16	7.4±0.20	25.13	6.1±0.21	13.55	27.3±2.51	36.05
MeOH	3.5±0.12	4.8±0.10	7.33	12.0±0.31	33.18	12.6±0.20	28.52	6.9±0.25	11.98	34.7±2.63	53.70
Cpd 1	3.1±0.12	6.8±0.10	10.33	17.0±0.31	53.18	20.6±0.20	56.52	9.5±0.25	30.98	14.7±2.63	76.70
Cpd 2	3.2±0.12	6.8±0.13	7.97	15.1±015	38.09	16.4±0.10	41.94	7.5±0.13	38.31	19.7±2.08	66.47
Cpd 3	3.2±0.15	4.8±0.10	5.29	8.0±0.10	13.5	5.8±0.22	8.51	7.3±0.23	31.90	21.3±2.50	62.24
Cpd 4	3.6±0.11	4.1±0.14	6.97	8.1±015	27.31	8.4±0.12	19.94	7.5±0.13	22.61	35.3±4.53	45.44
Morphine (10 ml/kg)	2.9±0.15	7.2±0.15	15.86	20.4±0.20	64.57	23.3±0.57	75.27	15.8±0.35	47.6	NT	
Acetyl-salicylic acid										10.9±1.10	87.37

Values are mean±SEM (n = 6). NS = non significant ($p < 0.05$) vs. control (one-way ANOVA followed by Bonferroni posttests). NT = Not tested.

4. Conclusion

1. The results obtained show that the extract and isolates had significant ability to relieve pain.
2. This implies that the plant possess analgesic properties with varying potencies in hot plate-induced pain and acetic acid-induced writhing in mice. From the present findings, it can be concluded that the ethylacetate and dichloromethane extracts of this plant materials have got analgesic properties within 30 minutes of administration whose mechanisms need to be investigated further.

5. Recommendations

1. Further research should be done to ascertain the mechanism of drug action.
2. Concoctions from the three plants can be used as herbal remedies in health-care systems since the ethnomedical information has been confirmed by the positive results in bioassay analysis of both the crude extracts and isolates.
3. This ethnomedical information should be documented fro dissemination and stored as part of Kenya/African/Global medicinal plants database
4. Large-scale cultivation of these plants should be done while conservation of plants already there should be encouraged to avoid their extinction.

6. Suggestions for Further Studies

1. More tests should be carried out to evaluate the crude extracts from these plants for any broad spectrum bioactivities.
2. Structural modification should be done on the isolated compounds to test if this can improve activity.
3. Studies should also be carried out on the active isolates to test any synergy, antagonism and mechanism of action.

The result obtained confirms the folkloric information contained in literature that this plant has anti-inflammatory activity and is also used in managing fever and authenticates its use as a herbal remedy.

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