Lonchocarpus eriocalyx (Harms) Herb Extract for Use as Painkillers

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To cite this article:
Angeline Atieno Ochung, Phillip Okinda Owuor, Lawrence Arot Manguro, Ishola Ismael. Lonchocarpus eriocalyx (Harms) Herb Extract for Use as Painkillers. Pharmaceutical Science and Technology. Vol. 4, No. 1, 2020, pp. 17-24. doi: 10.11648/j.pst.20200401.13

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 or 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 antinociceptive 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) chemical irritants such as acetic acid and (iii) physical pressure like tail compression [2]. Today, many herbal preparations are being prescribed as analgesics as 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 large proportion 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 antinociceptive 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 the isolated compounds.

![Figure 1. Structures of Compounds 1, 2, 3 and 4.](image)

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 CDCl₃ and CDCl₃-DMSO-d₆ 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 F₂₅₄ (Merck) pre-coated plates. All solvents used were of analytical grade.

Collection of Plant materials

Leaves of Lonchocarpus eriocalyx were collected from Embu-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, SiO₂ 240 g, pressure=1 bar) using *n*-hexane-CH₂Cl₂ gradient (increment 10%) up to 100% CH₂Cl₂ 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-CH₂Cl₂ (1:3, 1:2) and CH₂Cl₂-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 Rₗ 0.83 (eluent: *n*-hexane-EtOAc, 2:3) which upon evaporation of solvent followed by crystallization in CH₂Cl₂-MeOH of ratio 1:1 mixture afforded compound 1as white needle-like crystals
Sub-fraction III (fractions 61-86, 5 g) showed two spots of Rf 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 Rf 0.48 and 0.65 (eluent: CH₂Cl₂-MeOH, 97:3) and upon evaporation of solvent, followed by crystallization gave compounds 3 and 4,6 and 35 mg respectively. Fractions 104-180 constituted sub-fraction V (5 g) and was further purified by medium pressure chromatography (2.5×50 cm, SiO₂ 150 g, pressure=1 bar) to give further compounds 3 and 4 in 50 mg and 30 mg, respectively.

**Table 1.** ^1H (300 MHz) and ^13C (75 MHz) NMR (CDCl₃) data for Compound 1.

| C# | H Multiplicity, (J in Hz) | M Multiplicity, (J in Hz) | □C | □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 | 131.0 |
| 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, H₉-29) | 4.60 (d, J = 0.5, H₉-29) | 108.1 | 109.0 |
| 30 | 4.67 (1H, d, J = 0.5, H₉-29) | 4.70 (d, J = 0.6, H₉-29) | 19.5 | 19.7 |

Compound 2: White crystals, C₁₆H₁₇O₂ (50 mg), Rf 0.62, mp 254-256°C (lit. 252-254°C [15]*).

**Table 2.** ^1H (300 MHz) and ^13C (75 MHz) NMR (CDCl₃) data for Compound 2.

| C # | H Multiplicity, (J in Hz) | M Multiplicity, (J in Hz) | □C | □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 (H₆, d, J = 6.8) | 2.54 (d, J = 7.0) | 41.6 | 41.5 |
| 3  | 2.72 (H₉, d, J = 13.7, 5.5) | 2.76 (dd, J = 13.5, 8.0) | 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 |
Table 3. $^1H$ (300 MHz) and $^{13}C$ (75 MHz) NMR (CDCl$_3$) data for Compound 3.

| C# | $^1H$ Multiplicity (J in Hz) | $^1H$ Multiplicity (J in Hz) | $^1C$ | $^1C^*$ |
|----|-----------------------------|-----------------------------|------|--------|
| 1  | 1.43 (d, J = 3.2)           | 37.3                        | 37.3 | 16     |
| 2  | 1.64 (d, J = 5.6)           | 31.7                        | 31.7 | 17     |
| 3  | 3.52 m                      | 71.8                        | 71.8 | 18     |
| 4  | 2.19 (d, J = 9.8)           | 39.8                        | 42.4 | 19     |
| 5  | -                           | 140.8                       | 140.8| 20     |
| 6  | 5.35 (d, J = 5.2)           | 121.7                       | 121.9| 21     |
| 7  | 1.52 (d, J = 12.4)          | 31.9                        | 31.9 | 22     |
| 8  | -                           | 31.7                        | 31.9 | 23     |
| 9  | -                           | 50.1                        | 50.2 | 24     |
| 10 | 36.5                        | 36.6                        | 36.6 | 25     |
| 11 | 21.1                        | 21.0                        | 21.0 | 26     |
| 12 | 37.2                        | 39.8                        | 39.8 | 27     |
| 13 | 42.3                        | 42.3                        | 42.3 | 28     |
| 14 | 56.8                        | 56.8                        | 56.8 | 29     |
| 15 | 24.3                        | 24.4                        | 24.4 |         |

ESI-MS (rel. int.): m/z=214, 367, 271, 255, 189, 175, 161, 133, 121, 105, 95, 81, 69, 41. IR $v_{max}$ (KBr) cm$^{-1}$: 3373, 2940, 2867, 1641, 1457, 1381, 1038.

Table 4. $^1H$ (300 MHz) and $^{13}C$ (75 MHz) NMR (CDCl$_3$) data for Compound 4.

| C# | $^1H$ Multiplicity (J in Hz) | $^1C$ | $^1C^*$ |
|----|-----------------------------|------|--------|
| 1  | -                           | 37.2 | 36.7   |
| 2  | -                           | 28.9 | 29.1   |
| 3  | -                           | 78.1 | 78.6   |
| 4  | -                           | 41.2 | 42.1   |
| 5  | -                           | 140.8| 140.0  |
| 6  | 5.13 (dd, J = 4.7, 1.7)     | 121.9| 121.5  |
| 7  | 2.37 (dd, J = 11.5, 11.3)   | 31.1 | 31.4   |
| 8  | 2.01, m                     | 31.8 | 31.5   |
| 9  | 1.99, m                     | 50.2 | 49.8   |
| 10 | -                           | 36.5 | 36.3   |
| 11 | -                           | 20.2 | 20.2   |
| 12 | -                           | 39.9 | 38.2   |
| 13 | -                           | 42.3 | 41.9   |
| 14 | -                           | 56.4 | 56.4   |
| 15 | -                           | 23.5 | 23.8   |
| 16 | -                           | 28.4 | 27.8   |
| 17 | -                           | 56.4 | 55.7   |
| 18 | 0.83 (s)                    | 11.8 | 11.3   |

ESI-MS (rel. int.): m/z=257.4, 603.3 [M+ (C$_2$H$_5$)], (100), 601.3 (20), 583 (10). IR $v_{max}$ (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 15 s 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 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 15 s 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 (100 mg/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].

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

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% v/v in normal saline, 10 ml/kg, i.p). The latency was recorded after 30 and 60 min following oral administration of extracts (100 mg/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].

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<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

Repeted 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+]. The latter signals were confirmed by the appearance in the 13C NMR spectra of Lupeol. The identity of this compound was confirmed by Co-TLC with authentic specimen [13].

Both 1H and 13C NMR spectra of compound 1 with those of Lupeol previously isolated from Lonchorcarpus 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 νmax (KBr) cm⁻¹: 3315, 2900, 1642, 1190, 937, 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].

Comparison of 1H NMR and 13C NMR spectra of compound 1 with those of Lupeol previously isolated from Lonchorcarpus 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 νmax (KBr) cm⁻¹: 3315, 2900, 1642, 1190, 937, 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°C with a molecular formula C_{30}H_{50}O. The 1H, 13C NMR had a signal for H-1a at δ 1.76 and H-2b at δ 2.27 (dd) corresponding to 1 proton each. A signal for H-4 was observed as a multiplet integrating for one proton at δ 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 13C 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,
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 \( C_{35}H_{43}O_5 \) (114 mg), mp; 166-168°C. The \( ^1H \) and \( ^13C \) NMR spectra had a multiplet signal for H-3 at \( \delta \) 3.52 for the oxymethine proton which and suggested the presence of an \( \alpha \)-proton typical of sterols hydroxylated at C-3 [17]. (A \( \delta_c \) signal at 71.8 in the oxygenated aliphatic region confirmed the presence of the oxymethylene carbon [17]. A signal for H-6 typical of olefinic proton of appeared at \( \delta \) 5.35 (d, \( J = 5.2 \) Hz) while two other olefinic protons appeared upfield at \( \delta \) 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 \( \delta_C \) 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 trisubstituted proton to a \( \delta_c \) signals at 121.7 for C-6. The presence of six methyl protons was confirmed by signals at 0.69, 0.78 (2×CH₃), 0.93, 1.02 and 1.25 with corresponding \( ^13C \) NMR signals at \( \delta \) 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. \( ^13C \) 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 stigmasteryl 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 \( C_{35}H_{33}O_6 \), mp; 290-292°C which was consistent with \( m/z \) of 574. Its \( ^1H \) NMR spectrum showed olefinic proton H-6 at \( \delta \) 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 \( \delta \) 5.10 (H-22, dd, \( J = 15.2, 8.8 \) Hz) and \( \delta \) 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 \( \delta \) 2.81-4.95 ppm. This compound almost completely corresponded to the data for stigmasterol with the exception of the signals between H \( \delta \) 2.81-4.95 ppm typical for a sugar moiety [27]. The \( ^13C \) NMR spectrum of compound 4 revealed 35 carbon signals in the molecule. The olefinic carbon resonances at \( \delta \) 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 \( \delta \) 140.8 represented the C-5 quartenary carbon of the sterol moiety. A signal typical of an anumeric carbon at \( \delta \) 101.4 (C-30) indicated the presence of a single monosaccharide moiety. Four other sugarcarbons resonated at \( \delta \) 74.0 (C-2’), 76.75, 76.8 (C-3’), 70.5 (C-4’) and 76.1 (C-5’) as well as the methylene resonances at \( \delta \) 62.1 (C-6’), respectively of the \( \beta \)-D-glucopyranoside [28]. The presence of anomeric proton (H-30) was evident by a signal at \( \delta \) 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 \( \beta \)-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 \( ^1H \) and \( ^13C \) of HMBC spectrum. The existence of long-range correlations of protons at \( \delta \)4.95 (H-1’) with a carbon at \( \delta \)78.1 (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±0.01 for EtOAc and DCM respectively implying that they delayed infliction 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) | Post treatment latency (s) and % inhibitions | Writhing response |
|---------------------------|--------------------------|---------------------------------------------|---------------------------------------------|-------------------|
|                           | 0 min        | 30 min   | 60 min   | 90 min   | 120 min   | Total no. of writhes | % Inhibition |
|                           | sec          | sec      | sec      | sec      | sec       |                        |              |
| 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±0.15| 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±0.15 | 27.31     | 8.4±0.12      | 19.94         | 7.5±0.13 | 22.61 | 35.3±4.53 | 45.44 |
| Morphine (10 mg/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           |
| Acetylsalicylic acid       |             |          |          |          |          |                        |              |

Values are means±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 for 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.

Acknowledgements

The authors wish to thank the National Commission for Science, Technology and Innovation (NACOSTI) for providing funds that enabled this research to be done. Kenya Medical Research Institute (KEMRI) is sincerely thanked for carrying out bioassay analysis. We also wish to thank Mr. Simon Mathenge for identifying the plant.

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