

Antinociceptive Ecdysteroids and Other Constituents of *Palisota hirsuta* K. Schum (Commelinaceae)

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ABSTRACT

The root of *Palisota hirsuta* is used in Ghana and other West African countries for the treatment of various disease conditions such as rheumatoid arthritis (also in other inflammatory and painful conditions), infertility in females, anaemia, and dysentery. The current study sought to evaluate the anti-nociceptive effect of the hydroethanolic root extract and compounds isolated from *Palisota hirsuta*. Mice were used for studying the antinociceptive activity of *P. hirsuta* extracts and isolates at doses of 10-300 mgkg⁻¹p.o and 10- 30 mgkg⁻¹ respectively using the Formalin Induced paw licking model. The total crude extract, methanolic and petroleum ether fractions showed analgesic activity in a dose-dependent manner for both the early and late phases. Three isolates, a fatty acid mixture, 20-hydroxyecdysone and an uncharacterized ecdysteroid (PH V) were obtained from the methanol fractions with significant activity. 20-hydroxyecdysone exhibited a dose-dependent inhibition of the nociception for both the early and late phases; 71.39±9.19% and 89.19±3.81% respectively. PH V showed significant activity between the early and late phases of inhibition as compared to the reference drug morphine. The present study has given scientific credence to the use of the roots of *P. hirsuta* for the mitigation of pain and established its antinociceptive constituents.

INTRODUCTION

Pain is a normal manifestation in everyday life and serves as a vital defensive function. However, uncontrolled pain can dramatically diminish quality of life (Page *et al.*, 1997). It is an unpleasant sensation that can be either acute or chronic and is as a consequence of complex neurochemical process in the peripheral and central nervous system (Richard *et al.*, 2012). Pain and inflammation is the most common manifestation of diseases that affect millions of people worldwide (Bhatia *et al.*, 2013). According to a WHO report, about 70-80% of the world's populations rely on non-conventional medicines mainly from herbal sources in their primary health care. Plants are still the reservoirs for obtaining drugs and drug leads (Newman and

Cragg, 2007) and are also a good source of anti-nociceptive compounds; morphine from *Papaver somniferum* L. (Papaveraceae).

Palisota hirsuta is used traditionally for its pain-relieving and antiseptic effects. The plant juice and crushed pieces of twigs are used as a poultice for fractures, bruises, joint pains, nail ulcers and lymph gland inflammation. The whole plant decoction is used for urethral problems. Leaf preparations are used against lice (Bellomaria and Kacou, 1995). Boakye-Gyasi *et al.*, (2014), demonstrated that oral administration of an ethanolic leaf extract of *Palisota hirsuta* attenuates pain-related behavior in vincristine-induced neuropathic pain model. Sarpong *et al.*, (2013) also showed that the roots of *P. hirsuta* and its constituents, mainly 20-hydroxyecdysone, show anti-inflammatory and antioxidant activities. However, the root of *Palisota hirsuta* and its compounds have not being thoroughly evaluated for analgesic activity as suggested by folklore medicine. Therefore, the present study aimed at evaluating the possible anti-nociceptive activity of the

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hydroethanolic root extracts, isolate and characterise the antinociceptive constituents.

MATERIALS AND METHODS

Plant collection and processing

Palisota hirsuta roots were collected from Esaase-Bontefufuom in the Amansie West district of Ashanti Region of Ghana, in May 2014. The plant was authenticated by Dr George Henry Sam of the Department of Herbal Medicine, Kwame Nkrumah University of Science and Technology; a voucher specimen was kept in the Faculty of Pharmacy and Pharmaceutical Sciences' Herbarium (No. FP 1008). The roots were washed and sun-dried for seven (7) days and then milled into coarse powder.

Extraction

The powdered roots of *P. hirsuta* (2 kg) was then Soxhlet extracted with 4 litres of 70% ethanol. The crude extract (crystallized, PHC), was then concentrated with a rotary evaporator (R-114, Buchi, Switzerland) at a temperature of 55 °C under vacuum to give a yield of 200 g (11.69%). 100 g of the crude extract (PHC) was dissolved in 80% methanol and defatted with Petroleum ether (BDH) 40-60 °C to afford an extract, PHE and the remaining methanol soluble extract as PHM.

Chromatographic fractionation and isolation

The methanol extract (60 g) was adsorbed onto silica gel and loaded onto a glass column (80 cm x 5 cm) packed with 400 g of silica gel (60-120 ASTM). The column was then eluted using gradient mixtures of petroleum ether/ethyl acetate (80:20), petroleum ether/ethyl acetate (50:50), ethyl acetate (100%), ethyl acetate/methanol (80:20), ethyl acetate/methanol (50:50), ethyl acetate/methanol (20:80) and methanol (100%). One hundred and fifty eluates were collected and bulked into four fractions (F1-4). The fractions were further column chromatographed according to the procedure described by Sarpong *et al.*, 2013 to afford fraction PH₁ (an oil containing mixtures of fatty acids), compound PH IV (20-hydroxy ecdysone) and an uncharacterized compound PH V.

Experimental

NMR and MS were employed as the techniques to identify the structure and identity of compounds in this project/study. ¹H NMR, ¹³C NMR and 2D NMR (COSY, NOESY, HMQC and HMBC) spectra were obtained on a Bruker 400 MHz instrument. One bond 1 H-¹³C connectivities were determined with HSQC while two- and three-bond 1 H-¹³C connectivities were determined by HMBC experiments. Chemical shifts were reported in δ (ppm) using the solvent (DMSO). GC-MS was also utilized to resolve the compounds which were mixtures of fatty acids and for that matter difficult to separate with silica gel.

Anti-Nociceptive Activity of Extracts and Compounds

Drugs: Formalin was obtained from BDH, Poole, England and Morphine hydrochloride was purchased from Phyto-Riker Pharmaceuticals, Accra, Ghana.

Animals: Mice [Imprinting Control Region (ICR)] (30-40g) were purchased from Centre for Scientific Research into Plant Medicine Akwapim Mampong, Ghana and housed in the animal facility of the Department of Pharmacology, Kwame Nkrumah University of Science and Technology (KNUST). The animals were housed in groups of six in stainless steel cages (34×47×18 cm) with soft wood shavings as bedding, fed with normal commercial pellet diet (GAFCO, Tema), given water *ad libitum* and maintained under laboratory conditions (temperature 24-28 °C, relative humidity 60-70%, and 12 hour light-dark cycle). All procedures and techniques used in these studies were in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH, Department of Health and Human Services publication no. 85 - 23, revised 1985). All protocols used were approved by the Department of Pharmacology Ethics Committee, KNUST, Kumasi.

Formalin induced nociception

The formalin test first described by Dubuisson and Dennis, (1977) was carried out as described by Malmberg and Yaksh, (1995) with a few modifications. Each animal was assigned and acclimatized to one of 20 formalin test chambers (a Perspex chamber 15 × 15 × 15 cm) for thirty minutes before formalin injection (Wilson *et al.*, 2002). The mice were then pre-treated with the test drugs (30 min for *i.p.* route and 1 hr for oral route) before intraplantar injection of 10 µl of 5 % formalin. The animals were immediately returned individually into the testing chamber. A mirror angled at 45° below the floor of the chamber allowed a complete view of the paws. The behaviour of the animal was then captured over a 60 minute period for analysis by a camcorder (Everio™ model GZ-MG1300, JVC, Tokyo, Japan) placed in front of the mirror. Pain response was scored for 60 min, starting immediately after formalin injection.

The first phase of the formalin test was defined conservatively as 0-10 minutes and the second phase 10-60 minutes post formalin injection (Wilson *et al.*, 2002). Nociceptive behaviour was quantified by counting the incidents of spontaneous biting/licking of the injected paw (Zochodne *et al.*, 2001) using the public domain software JWatcher™ Version 1.0 (University of California, Los Angeles, USA and Macquarie University, Sydney, Australia available at <http://www.jwatcher.ucla.edu/>). Nociceptive score was determined for each 5-mins time block in each phase by measuring the amount of time spent biting/licking the injected paw. The product of the frequency and duration of biting/licking was used as nociceptive score. Mice were randomly selected for one of the following study groups: Group I: Morphine (1, 3, and 10 mg kg⁻¹), Group II: *P. hirsuta* extract, PHC (30, 100 and 300 mg kg⁻¹), Group III: Vehicle treated control, Group IV: Methanol fraction, PHM (30, 100 and 300 mg kg⁻¹), Group V: Petroleum Ether Fraction, PHE (30, 100 and 300 mg kg⁻¹), Group VI: PH IV (1, 10 and 30 mg kg⁻¹), Group VII: PH V (1, 10 and 30 mg kg⁻¹).

Statistical Analysis

The statistical analyses were performed by ANOVA, followed by Newman-Keuls *post hoc* tests using GraphPad Prism

for Windows version 5 (GraphPad Software, San Diego, USA). The results were expressed as the mean \pm S.E.M to show variation in groups. Differences are considered significant when $P \leq 0.05$.

RESULTS

Anti-nociceptive Assay

Formalin-induced nociception

Formalin administration produced a typical pattern of flinching and licking behaviour (Figures 1 & 2), produced a marked and dose-related inhibition of both phases of formalin-induced nociception first phase ($P < 0.0001$) and second phase ($P < 0.0001$). The highest dose produced maximal inhibitions of $40.89 \pm 24.10\%$ and $66.25 \pm 32.08\%$ for the early and late phases of the crude extract PHC. For the pet-ether fraction, PHE the highest dose caused maximal nociceptive inhibition of $45.02 \pm 29.81\%$ and $80.50 \pm 4.628\%$. The maximal inhibitions caused by the highest dose of the methanol fraction PHM was $31.72 \pm 15.29\%$ and $61.0 \pm 41.97\%$ for the two phases.

Considering the isolates, the pattern of inhibition was not regular for the isolated compounds. For instance, with PH I, the maximal inhibitions caused by the doses (phase 1: 10 mg kg^{-1} and phase 2: 30 mg kg^{-1}) were $55.08 \pm 14.90\%$ and $63.57 \pm 25.91\%$. The highest dose (30 mg kg^{-1}) caused maximal inhibitions of nociception of $71.39 \pm 9.192\%$ and $89.19 \pm 3.816\%$ for PH IV and $61.34 \pm 11.07\%$ and $82.89 \pm 3.978\%$ and for PH V (Figure 2). Similarly, morphine ($1-10 \text{ mg kg}^{-1}$, *i.p*) produced marked inhibition of both the neurogenic ($P < 0.0001$) and inflammatory ($P < 0.0001$) pain phases (Figure 1a-b). Morphine, reduced the duration of formalin evoked nociceptive behavior by a maximum percentage of $92.49 \pm 4.679\%$ in the early phase and $95.16 \pm 5.497\%$ in the late phase of the formalin test (Figure 1a-b). Examining the results obtained in the formalin test, it was deduced that; PHC, PHE, PHM, PH I, IV, V and morphine significantly reduced the time spent in licking the injured paw, an indication that these compounds possess strong anti-nociceptive or analgesic properties.

Identification of the compounds

The fatty acid mixture PH-1 was subjected to GC/MS. Compounds were identified by their mass spectra, interpretation of their fragmentation patterns and comparing retention times of their peaks to those of standard compounds analysed previously in literature (National Institute of Standards and Technology (NIST), 2011). The fatty acid components were identified as hexanoic acid, stearic acid and eicosanoic acid as previously reported by Sarpong *et al.*, (2013).

PH IV was isolated as an off white amorphous solid with a melting point of $240-242^\circ\text{C}$ [Literature: $237.5-239.5^\circ\text{C}$] (Hikino and Hikino, 1990). It showed positive Liebermann's test. The Electron Spray Ionization Mass Spectrometer (ESI-MS) revealed a molecular ion peak $[\text{M-H}]^+$ at m/z of 479 which corresponded to the molecular formula, indicating six degrees of unsaturation, ascribed to α , β -unsaturated ketone and four steroid rings (Yun-

Song *et al.*, 2007).

The $^1\text{H-NMR}$ revealed the presence of five methyl singlets at δ 0.76 (H-18), 0.83 (H-19), 1.06 (H-21), 1.05 (H-26) and 1.07 (H-27). The relatively downfield resonance of the last three signals revealed the presence of hydroxyl groups at C-20 and C-25 positions (i.e. OHd and OHf respectively). Other hydroxyl groups occurred at C-2, C-3, C-22 and the quaternary carbon C-14 (i.e. OHa, OHb, OHe and OHc respectively). The presence of the OH groups caused the methine protons H-2, H-3 and H-22 to occur more downfield (at δ 3.52, 3.76 and 3.11) relative to other methine protons at δ 2.19 (H-5), 3.02 (H-9) and 2.23 (H-17). The cluster of signals in the region 1.09-2.01 ppm, revealed the presence of methylene protons of the steroid framework.

The $^{13}\text{C-NMR}$ spectrum revealed the presence of twenty-seven (27) carbon resonances. Five methyl signals occurred at δ 17.6 (C-18), 24.3 (C-19), 21.4 (C-21), 30.8 (C-26) and 30.5 (C-27). A carbonyl (ketone) group occurred at δ 203.13 (C-6). The signals at 165.89 and 120.94 ppm were assigned to the olefinic carbons C-8 and C-7 respectively. The presence of 27 carbon resonances including five methyl signals suggested that PH IV may be an analogue of cholesterol. Other carbons present in the spectrum included five quaternary carbon signals at δ 83.5, 76.28, 69.17, 47.35 and 38.1. The signals occurring more downfield at δ 83.5, 76.2 and 69.17 were assigned to the oxygenated carbons C-14, C-20 and C-25 respectively. Other oxygenated carbon resonances occurred at δ 76.8 (C-22), 67.25 (C-2), and 67.0 (C-3). Eight methylene carbon signals occurred at δ 37.1, 32.0, 21.4, 31.34, 29.5, 20.6, 26.6 and 41.9 ppm which were assigned to C-1, C-4, C-11, C-12, C-15, C-16 C-23 and C-24. Three methine signals occurred at δ 50.6 (C-5), 33.5 (C-9) and 49.2 (C-17). All of the protonated carbons were assigned by heteronuclear single quantum correlation (HSQC) experiment.

In the HMBC spectrum, the olefinic proton H-7 had 3J correlations with C-5, C-9, and C-14. The proton signal H-5 had 2J correlation with the carbonyl C-6. This suggested the presence of a 7-en-6-one chromophore, characteristic of ecdysteroids (Mamadaliyeva, 2012). H-5 also coupled to C-9 (3J); H-19 to C-5 (3J), C-9 (3J) and H-9 to C-8 (3J) in HMBC. The methyl proton singlet δ 0.78 (H-18) had 2J correlation with C-13 and 3J correlation with C-14 and C-17. The hydroxyl signal at δ 4.62 (OH) coupled in HMBC to both C-14 (2J) and C-13 (3J). Also the proton H-17 had a 2J and 3J correlations with C-13 and C-18; H-17 (3J) with C-14 in long range correlation. The hydroxyl signal OHf had 3J correlations with C-26 and C-27 and 2J correlation with C-25. The methyl singlet H-26 also coupled to C-27 (3J) and C-25 (2J). H-27 also couples to C-26 (3J), C-25 (2J) and C-24 (3J). Also OHd coupled to C-20 (2J), C-22 (3J) and C-17 (3J); methyl singlet H-21 had 2J and 3J correlation with C-20 and C-17 respectively. OHe also coupled in HMBC to C-23 (3J), C-20 (3J) and C-22 (2J). This established the partial side chain structure. 3J correlation of OHd and H-21 with C-17 suggested that the side chain was attached to the steroidal nucleus at C-17.

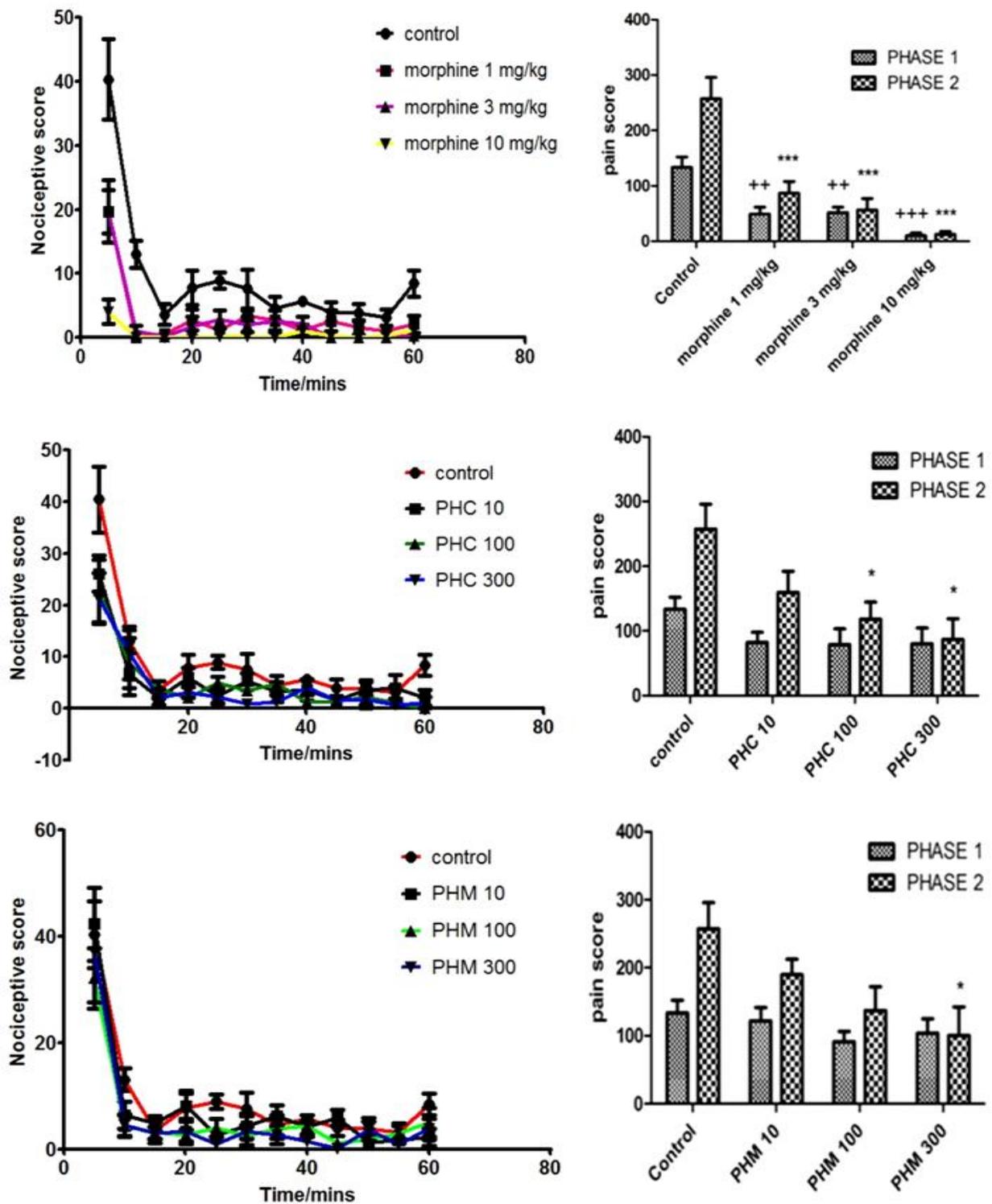


Fig. 1: Effect of Morphine (a-b) (1-10 mg kg⁻¹ *i.p.*), PHC (10-300 mg kg⁻¹ *i.p.*, c-d), PHM (10-300 mg kg⁻¹ *i.p.*, e-f) on the time course of formalin induced pain in mice. Nociceptive/pain scores are shown in 5 min blocks up to 60 min post formalin injection. Each point represents Mean \pm S.E.M (n = 5). * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ compared to respective controls (two-way repeated measures ANOVA; the AUC (total response) for phase 1 and phase 2. Each column in b represents the mean \pm S.E.M. + $P \leq 0.05$, ++ $P \leq 0.01$, +++ $P \leq 0.001$ (one-way ANOVA followed by Newman-Keuls *post hoc* test).

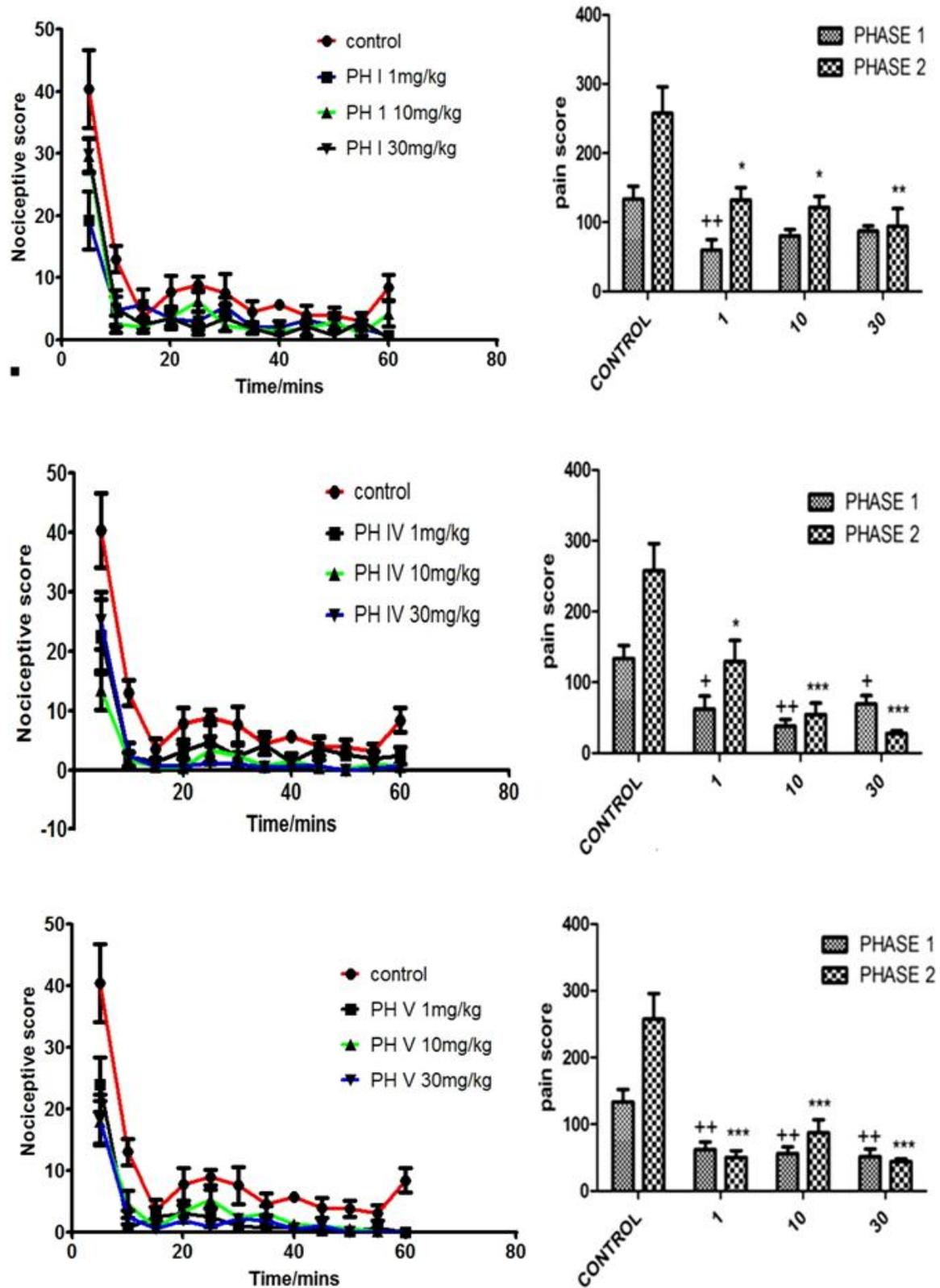
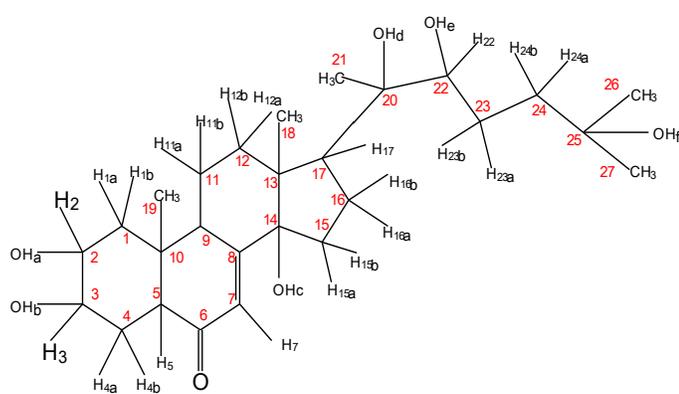
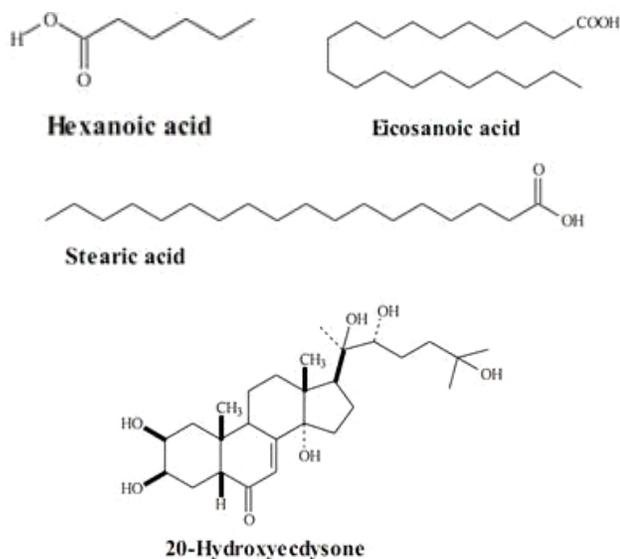


Fig. 2: Effect of PH I (1-30 mg kg⁻¹ i.p.a-b), PH IV (c-d) and PH V (e-f) (1-30 mg kg⁻¹ i.p.) on the time course of formalin induced pain in mice. Nociceptive/pain scores are shown in 5 min blocks up to 60 min post formalin injection. Each point represents Mean ± S.E.M (n = 5). *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 compared to respective controls (two-way repeated measures ANOVA; the AUC (total response) for phase 1 and phase 2. Each column in b represents the mean ± S.E.M. +P ≤ 0.05, ++P ≤ 0.01, +++P ≤ 0.001 (one-way ANOVA followed by Newman-Keuls *post hoc* test).



The $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ resonances of PH IV, all agreed with that published for 20-hydroxyecdysone, reported in the roots of *Palisota hirsuta* (Sarpong *et al.*, 2013). PH V was not characterized due to ample NMR data. The preliminary phytochemical screening revealed PH V to be a steroid and the molecular masses and the fragmentation pattern from the GC/MS spectral analyses suggested it to be an ecdysteroid.



DISCUSSION

In the formalin test, the early phase is considered to be due to direct activation of nociception neurons by formalin, whereas the late phase reflects pain generated in acutely injured tissue (Hunskar and Hole, 1987; Tang *et al.*, 2007). The licking response induced by formalin, results from a combination of peripheral input and spinal cord sensitization (Tjolsen *et al.*, 1992). The intraplantar injection of formalin, releases prostaglandin 2 (PGE_2), nitrous oxide (NO) and kinins in the spinal cord (Tjolsen *et al.*, 1992). Taking this into account, the anti-nociception of *Palisota hirsuta* could be dependent on either peripheral or central sites of action or both. Centrally acting drugs, such as opioids, inhibit both phases of pain by equally inhibiting the effect produced by prostaglandins released at this level in response to inflammation (Ferreira, 1981; Hunskar and Hole, 1987; Shibata *et*

al., 1989) and by endogenous opioids through their action on the central nervous system. It has been demonstrated (Tjolsen *et al.*, 1992) that the late phase in formalin test depends on an inflammatory reaction in peripheral tissue. Peripheral acting drugs such as diclofenac (Rosland *et al.*, 1990) which block prostaglandin synthesis, reduce nociception mostly in the late phase but can also affect the early stage (Ortiz *et al.*, 2008). In fact, the anti-nociceptive effects of the extracts and compounds, exhibited in the formalin test, suggest an involvement at both central and peripheral levels, which further implies that the extract and the isolates possess not only anti-nociceptive but also anti-inflammatory activity. This result therefore agrees with the anti-inflammatory activity of *P. hirsuta* established by Sarpong *et al.*, (2013).

From Figure 1a-f and 2a-f, there is the indication of maximal inhibitions or reductions of the licking time in the early and late phases, respectively. Similarly, morphine (1-10 mg kg^{-1} , *i.p.*) produced marked inhibition of both the neurogenic ($P < 0.0001$) and inflammatory ($P < 0.0001$) pain phases (Figure 1a-b). Morphine, reduced the duration of formalin evoked nociceptive behavior by a maximum percentage of $92.49 \pm 4.67\%$ in the early phase and $95.16 \pm 5.49\%$ in the late phase of the formalin test (Figure 1a and b). The analgesic activity of 20-hydroxyecdysone (PH IV) was very much comparable to the standard drug morphine. The anti-nociceptive actions of the fractions and various isolates of the roots of *Palisota hirsuta* are being reported for the first time. From the results obtained, the anti-nociceptive properties of *Palisota hirsuta* are not in doubt due to the steroidal nature of the compounds found in the plant. Thus this research has shown that the root extract and its steroidal compounds have profound analgesic effects. However, the anti-nociceptive action is not dose dependent for the extract, fractions and the isolates used for the study. Woode *et al.*, (2009), previously demonstrated the antinociceptive effect of *P. hirsuta* leaf extract. They highlighted that the analgesic effect of *P. hirsuta* might partially or wholly be due to the stimulation of peripheral opioid receptors through the activation of the nitric oxide-cyclic GMP-ATP-sensitive K^+ ($\text{NO}/\text{cGMP}/\text{K}^+$ ATP)-channel pathway without tolerance induction after chronic administration. This gives scientific credence to the use of the whole plant for treatment of pain and inflammatory conditions in folklore medicine.

Phytoecdysteroids have received attention as anabolic steroids. Chermnykh *et al.*, (1988) compared the anabolic activities of 20-hydroxyecdysone and that of metandrostenolone and found the former to increase protein and carbohydrate metabolism and was non-toxic to the reproductive organs. Another ecdysteroid, ecdysterone, has also been reported to exhibit potent oxytocic activity in the guinea pig uterus assay besides inhibiting nicotine and serotonin-induced contractions during guinea pig ileum assay (Parameswaran *et al.*, 2001) and may have some implication in the folkloric use to treat infertility. There is ample evidence of the varied biological activities of the ecdysteroids especially 20-hydroxyecdystone, however this is the first time the anti-nociceptive activity has been established.

CONCLUSION

The roots of *Palisota hirsuta* contain ecdysteroids which are responsible for the treatment of pain. This also supports the claims of traditional medicine practitioners. The ethanolic root extract, methanolic and petroleum ether fractions and the isolates (fatty acids and 20-hydroxy ecdysone) of *Palisota hirsuta* have potent anti-nociceptive/analgesic activities.

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REFERENCES

- Bellomaria B and Kacou P. Plants et Medicine Populaire d'Agboville(Cote D'Ivoire). Fitoterapia LXVI, 1995: 117-141.
- Bhatia D, Bejarano T, Novo M. Current interventions in the management of knee osteoarthritis. J Pharm Bioallied Sci. 2013; 5(1):30-38.
- Boakye-Gyasi E, Kasanga EA, Biney RP, Abotsi WKM, Mensah KB and Woode E. Ameliorative Effects of Ethanolic Leaf Extract of *Palisota hirsuta* K. Schum (Commelinaceae) on Vincristine-Induced Neuropathic Pain in rats. Journal of Applied Pharmaceutical Science, 2014; 4 (11): 035-041
- Chermnykh NS, Shimanovsky NL, Shutko GV, Syrov VN. The action of methandrostenolone and ecdysterone on the physical endurance of animals and on protein metabolism in the skeletal muscles. Farmakol. Toksikol., 1988; 51(6):57-60.
- Dubuisson D and Dennis SG. The formalin tests: a quantitative study of the analgesic effects of morphine, meperidine and brain stem stimulation in rats and cats. Pain, 1977; 4: 161-74.
- Ferreira SH. Local control of inflammatory pain. Agents Actions, 1982; 11(6-7): 636-638.
- Hikino H, and Hikino, Y. Arthropod moulting hormones. Progress in Chemistry of Organic Natural Products, 1990; 28:256-312.
- Hunnskaar S, and Hole K. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain, 1987; 30(1): 103-114.
- Malmberg AB and Yaksh TL. Cyclooxygenase inhibition and the spinal release of prostaglandin E2 and amino acids evoked by paw formalin injection: a microdialysis study in unanaesthetized rats. J. Neurosci, 1995; 15: 2768-2776.
- Mamadaliyeva, NZ. Phytoecdysteroids from Silene plants: distribution, diversity and biological (antitumour, antibacterial and antioxidant) activities. Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas, 2012; 11(6):474-497.
- Newman DJ, Cragg GM. Natural Products as sources of New Drugs over the last 25 years. J Nat Prod., 2007; 70 (3):461-77
- Ortiz MI, Lozano-Cuenca J, Granados-Soto V, Castañeda-Hernández G. Additive interaction between peripheral and central mechanisms involved in the antinociceptive effect of diclofenac in the formalin test in rats. Pharmacol Biochem Behav., 2008; 91(1):32-7.
- PageCP, Curtis MJ, Sutter MC, Walker MJA, Hoffman BB. 1997. Integrated Pharmacology, Mosby Inc.; London. p. 306-310.
- Parameswaran, P.S.; Naik, C.G.; Gonsalves, C.; Achuthankutty, C.T. Isolation of 2 deoxyecdysterone, a novel oxytocic agent, from a marine Zoanthus sp. J.Indian Inst.Sci., 2001; 81: 169-173.
- Richard F, Michelle AC, Luigi XC. Opioids.2001. In: Richard AH, Pamela CC. (edited) Lipincott's Illustrated Reviews. Lipincott Williams and Wilkins Philadelphia, 2009; 4th ed:159.
- Rosland JH, Tjolsen A, Maehle B, Hole K. The formalin test in mice: effect of formalin concentration. Pain, 1990; 42(2): 235-242.
- Sarpong FM, Fleischer TC, Annan K, Amponsah IK, Dickson RA and Mensah MLK. Anti-inflammatory and anti-oxidant ecdysteroid and Fatty acids from the roots of *Palisotahirsuta* K.Schum (Commelinaceae). International Journal of Pharmacognosy and Phytochemistry, 2013; 28(2):1159-1165.
- Shibata M, Ohkubo T, Takahashi H, Inoki R. Modified formalin test:characteristic biphasic pain response. Pain, 1989; 38(3): 347-352.
- Tang L, Chen Y, Zili Chen Z, Blumberg PM, Kozikowski AP and Wang ZJ. Anti-nociceptive pharmacology of N-(4-chlorobenzyl)-N-(4-hydroxy-3-iodo-5-methoxybenzyl)thio urea,a high-affinity competitive antagonist of the transient receptor potential vanilloid 1 receptor. J Pharmacol Exp Ther, 2007; 321(2): 791-798.
- Tjolsen A, BergeOG, Hunnskaar S, Rosland JH, Hole K. The formalin test: an evaluation of the method. Pain, 1992; 51(1): 5-17.
- Wilson SG, Chesler EJ, Hain H, Rankin AJ, Schwarz JZ, Call SB, Murray MR, West EE, Teuscher C, Rodriguez-Zas S, Belknap JK, Mogil JS. Identification of quantitative trait loci for chemical/inflammatory nociception in mice. Pain, 2002; 96(3): 385-91.
- Woode E, Boakye-Gyasi E, Ainooson GK, Ansah C and Duwiejua M. Anti-Nociceptive Effects and the Mechanism of *Palisota hirsuta* K. Schum. Leaf Extract in Murine Models. Int. J. Pharm., 2009; 5: 101-113.
- Yun-Song W, Ying-Tong D, Hong-Ping H, Liang-Bo L, Yang L, Jian-Bo G, Xin F, Ning-Chuan K, Shun-Lin L, Hua-Jie Z and Xiao-Jiang H. Isolation, X-ray crystallography and computational studies of calyphaphinone, a new alkaloid from *Daphniphyllum calycillum*. Org. Lett., 2007; 9(7): 1355-1358.
- Zochodne DW, Sun H, Li X-Q. Evidence that nitric oxide- and opioid-containing interneurons innervate vessels in the dorsal horn of the spinal cord of rats. J. Physiol., 2001; 532(Pt 3):749-758.

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