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*Full Length Research Paper*

# Analgesic and anti-inflammatory properties of *Oxyanthus unilocularis*

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***Oxyanthus unilocularis*** **is a Rubiaceae used in traditional medicine for the treatment of arthritis and pain. The purpose of this study was to evaluate the antinociceptive and anti-inflammatory properties of *O. unilocularis.* Antinociceptive properties of both the leaf and stem bark extracts of this plant were studied using the pressure-induced pain, writhing, tail flick and formalin test methods. Carrageenan-induced hyperalgesia was used to further evaluate the analgesic properties of the plant extracts while the anti-inflammatory properties were studied using the carrageenan-induced acute inflammatory method. Oral pretreatment with the leaf and bark extracts of *O. unilocularis* significantly increased pain threshold in all nociceptive models used as well as inhibited carrageenan-induced paw oedema in the rat. These results confirm that *O. unilocularis* has both analgesic and anti-inflammatory properties thus validating its ethnomedicinal uses.**

**Key words:** *Oxyanthus unilocularis*, analgesic, hyperalgesia, anti-inflammatory, pain.

## INTRODUCTION

Pain is one of the most common reasons for which people seek medical attention, thus, analgesics are among the most commonly prescribed medications in clinical practice (Cuartero et al., 2006). However, the more common analgesics are falling out of favour because of their toxic side effects on chronic use; the NSAIDs induce gastric ulcerations (Wallace, 2000) while acetaminophen causes liver damage (Bower et al, 2007). Consequently, it is important to search for analgesics which are able to manage pain of various etiologies and yet have less adverse side effects. Plants have been used for a very long time to treat different types of diseases. Over 80% of people living in developing countries depend on medicinal plants for the treatment of disease (Kitula, 2007). One of such plants is *Oxyanthus unilocularis*. *O. unilocularis* is a Rubiaceae (Aburi Botanica Gardens, 2002; Adomou, 2005) which is widely distributed in the forest under storey, fringing forest and secondary jungles of savanna (McCullough et al., 2007) regions stretching from Sierra Leone to west Cameroon, and across the Congo basin to Uganda. It is a shrub which may grow to over 8 m high. Some communities in this broad area use the leaf, bark or roots of this plant for the treatment of arthritis, swellings, pain, mild to moderate depression as well as for cutaneous and subcutaneous parasitic infestations (Aluka, 2008). The therapeutic virtues of this plant seem to be intuitively known by the great apes which consume small amounts of the plant from time to time (Idani et al., 1994) and use its branches as a preferred honey probe (Irvine, 1961; Deblauwe, 2006). Although the Oxyanthus plants have been known for a very long time and form part of the ethnopharmacy in areas where they are found, yet no pharmacological investigation of the acclaimed properties have been done. Thus the aim of this study was therefore to validate the antinociceptive and anti-inflammatory properties of *O. unilocularis* using different

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nociception models and the classical carrageenan paw inflammatory model.

## MATERIALS AND METHODS

### Drug

Aspirin (as Disprin from Reckitt Benckiser Pharmaceuticals, South Africa), Indomethacin (Fluka, Biochemika), Celecoxib (Pfizer, South Africa), Morphine (Bodene PTY Limited, South Africa) and plant extract were dissolved in distilled water while carrageenan and formalin (Sigma Chemical Co, St Louis, USA) were dissolved in physiological saline.

### Animal

Swiss mice (25 - 35 g) and wistar rats (180 - 220 g) were housed at 22 ± 2°C under a 12 h light/12 h dark cycle and with access to food and water *ad libitum.* Animals were moved from the animal holding facility to the laboratory 1 h prior to testing for acclimatization. For each experiment a different set of animals was used. The number of animals was five per treatment group. Approval for these studies was obtained from the Ethical Committee of the Walter Sisulu University, Reference No: Ethics 0009-07.

### Plant extract

The leaves and bark from the stems of *O. unilocularis* were collected in Yaoundé – Cameroon in October 2007. It was identified in the Botany Department of the University of Yaoundé I and authenticated in the National Herbarium in Yaoundé where a voucher specimen was deposited (No: Nguembou 665). The leaves and bark were air-dried and ground then macerated in methanol at room temperature for seven days. After filtration, the solvent was removed by rotatory evaporation under reduced pressure, yielding the leaf extracts of *O. unilocularis* (OUL) and the stem bark extract of *O. unilocularis* (OUB). Preliminary Phytochemical studies of the extracts showed the presence of flavonoids and triterpenoids. Both extracts were prepared in 5% DMSO solution to increase their solubility in water.

### Acetic acid-induced abdominal writhing test

Abdominal constriction was induced in mice as described by Du et al. (2007) with some modifications. Separate groups of five mice each received oral doses of the following: OUL (150 mg/kg), OUB (150 mg/kg), morphine (10 mg/kg), and aspirin (100 mg/kg) 60 min prior to intraperitoneal injection with acetic acid. Control animals received similar volumes of 5% DMSO which was the vehicle. One hour post treatment with the various drugs, mice received intraperitoneal injections of 1% v/v acetic acid. Pairs of mice were placed in clear glass boxes and the number of contortions of the abdominal muscles together with stretching, were counted cumulatively over a 30 min period. Mean number of abdominal contractions was calculated per experimental group.

### Tail flick test

The antinociceptive effects of OUL, OUB and reference drugs, expressed as the latency for rat to flick its tail after exposure to a source of radiant heat, were evaluated. Mice were placed in a restrainer which allowed the tail to hang out freely from the posterior end. The restrainer was placed on the Tail Flick analgesia meter (Ugo Basile, model: 37450-001) with a portion of the posterior one third length of the tail occluding a slit over a photocell for radiant heat stimulation generated by a power lamp mounted in a reflector. Animals flicked the tail in response to pain caused by the radiant heat applied to the tail (Zhou et al., 2006). The apparatus is made in such a way that when the operator depresses the start button, the timer is activated and signal is automatically cut-off when the tail is moved away from the window slit, thus, recording the tail flick latency (Verri et al., 2005). A 16 s cut-off time was set in order to prevent tissue damage. Before the experiments, baseline tail flick latencies of all animals were determined; animals with response time of more than 10 s were excluded from the study. The tail-flick response was measured at 1, 2 and 3 h after oral administration of OUL (150 mg/kg), OUB (150 mg/kg), morphine (10 mg/kg), aspirin (100 mg/kg) or 5% DMSO. Results were expressed as the difference between post-treatment tail flick latency and pre-treatment tail flick latency.

### Pressure test

The paw pressure test was carried out using the Randall and Selitto paw withdrawal method as described by Aley and Levine (1999). Pressure was measured in gram force using an Ugo Basile Analgesy Meter (Model: 37215) by applying an increasing force to the right hind paw of rats until they reacted either by paw withdrawal or squealing. Thresholds for nociception were determined for each animal at the start of the experiment. Animals received one of the two extracts or reference drugs while control animals received a 5% DMSO solution. Pain thresholds were measured 1, 2, 3, and 4 h after administration of drugs. Each animal served as its own control. Results were expressed as the percentage of pain inhibition.

### Formalin test

In the formalin test, mice were adapted in open transparent observation chambers for 60 min before trials. Five groups of five mice were used for these tests with each group receiving one of the following: OUL (150 mg/kg), OUB (150 mg/kg), morphine (10 mg/kg), and aspirin (100 mg/kg) orally 60 min before formalin injection. Control animals received a similar volume of 5% DMSO. Formalin (20 µl of a 2.5% solution in saline) was injected subcutaneously into the dorsal surface of the right hind paw of mice using a microsyringe with a 30-gauge needle (Baamone et al., 2000; Uchida et al., 2008) one hour after treatment with the various drugs. Each mouse was immediately returned to the observation chamber after formalin injection. The number of times mice licked/bit the injected paw was measured for 30 min post-formalin injection and considered as an indication of pain. The number of times the paw was licked from 0 - 5 min post injection was considered as the first phase response while, the second phase was taken as the number of times paw was licked/bitten from 20 - 30 min. Antinociceptive activity was expressed as the difference between the number of times of licking/biting of control animals and the pretreated animals.

### Carrageenan-induced thermal hyperalgesia

Both male and female mice weighing 25 - 35 g were used in these experiments. Mice were lightly restrained and the plantar surfaces of their hind paws were exposed to a source of radiant heat set to and IR intensity of 25. Animals with latencies higher than 10 s were

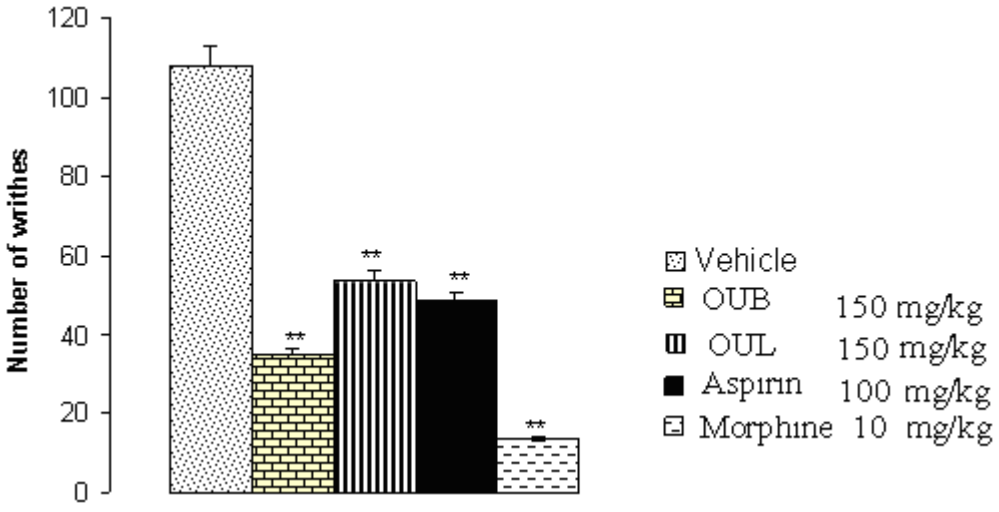


**Figure 1.** Antinociceptive effect of oral pre-treatment with OUB and OUL on acetic acid induced writhing in mice. Results are expressed as the mean number of writhes during the first thirty minutes following acetic acid injection ± SEM. n = 5. (** $p < 0.01$ when results were compared to results from the control group).

not included in the study. Animals were dosed orally with the various test drugs and one hour later the left hind paw was injected subplantarly with 0.05 ml normal saline while the right hind paw was injected with 0.05 ml of 0.5% carrageenan solution in normal saline. Paw withdrawal latencies were determined bilaterally 15, 60, 120 and 180 min after carrageenan injection. Results were expressed as the difference in latencies between the left and the right hind paws for each pre-determined time (paw withdrawal latency of untreated hind paw – paw withdrawal latency of treated hind paw).

#### Carrageenan-induced paw edema test in rats

Baseline right hind paw volumes were determined, using a plethysmometer (Ugo Basile: Model 7140) for all rats prior to the oral administration of the different test drugs: OUL (150 mg/kg), OUB (150 mg/kg), morphine (10 mg/kg), aspirin (100 mg/kg) or 5% DMSO. Inflammation was induced in the right hind paw of rats by subplantar injection of 0.1 ml/rat of 1.0% carrageenan in saline 30 min post treatment. Paw volumes were again determined 1, 2, 3 and 4 h after carrageenan injection. Inflammation was calculated as paw volume at time t – paw volume before the carrageenan injection.

#### Statistical analysis

The statistical package, GraphPad Instat® was used to analyse all results. ANOVA followed by Dunnett's *post hoc* test was used for analysis of data. For comparisons between treated and control groups, $p < 0.05$ was considered significant. Values are expressed as mean ± S.E.

## RESULTS

### Acetic acid-induced abdominal writhing test

Intraperitoneal injection of acetic acid induced abdominal writhing episodes in mice (Figure 1). Morphine, aspirin, OUB and OUL, significantly ($p < 0.01$) reduced the number of contortions in mice compared to the vehicle-treated group. In this nociceptive model OUB and OUL significantly ($p < 0.01$) reduced the number of writhing episodes. Aspirin also reduced the number of abdominal contortions though not as much as OUB. Morphine on the other hand, significantly ($p < 0.01$) reduced abdominal contractions better than aspirin, OUB and OUL, respectively.

### Tail flick test

In Figure 2, it is seen that the difference in the tail flick reaction time of the control animals was lower than 0.5 s throughout the experimental period while OUL had significant ($p < 0.01$) antinociceptive effects 1 and 2 h post treatment. Moreover, the effects of OUL were comparable to results obtained with morphine 1 and 2 h after treatment. Morphine showed significant analgesic effects ($p < 0.05$) from the first to the third hour after treatment. OUB on the other hand, had a reaction profile which was very similar to that of aspirin, both of which failed to reach significance

### Paw pressure test

The oral administration of OUL significantly ($p < 0.05$) increased pain threshold during the first and second hours post treatment comparably to morphine. However, during the third hour, OUL analgesic effects greatly decreased and was no longer significant. Although, the

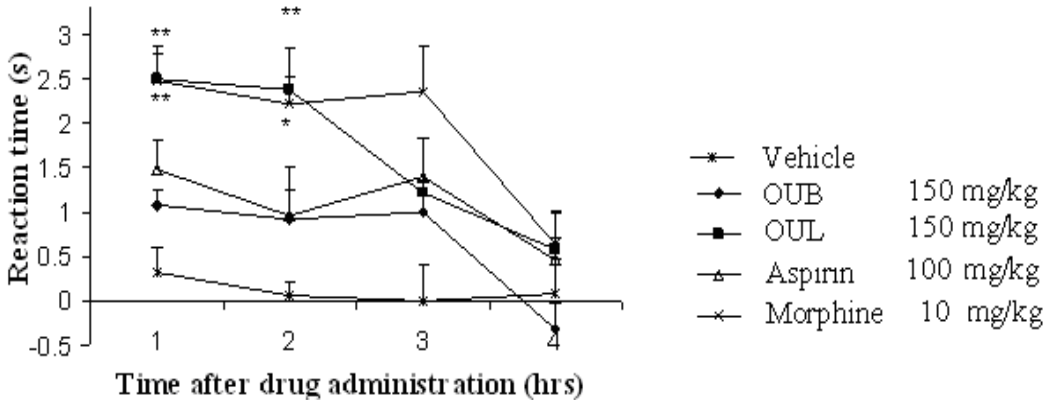


**Figure 2.** Effect of plant extracts and reference compounds on tail flicking response induced by radiant heat. Values are mean reaction time in seconds ± SEM for 5 animals. *p < 0.05, and **p < 0.01.

effect of morphine was greater during the third hour, it however, failed to reach significance as well (Figure 3).

**Formalin test**

Both OUB and OUL showed significant ($p < 0.05$; $p < 0.01$) inhibition of both the early and late phases of the formalin induced nociceptive responses, respectively. Aspirin on the other hand, had no effect during the first 5 min of this test, although, it was significant ($p < 0.01$) during the 20 - 30 min period (Figure 4). As expected, morphine (10 mg/kg) greatly reduced the licking time in both phases of this test. The injection of formalin into rat hind paws elicited the characteristic biphasic nociceptive agitation response. The first or early phase began immediately after formalin injection and lasted for approximately 5 min. The first phase was followed by a 6 - 10 min interval, during which the rat was relative quiet and exhibited little nociceptive agitation activities. The second phase or late phase began approximately 10 - 15 min after formalin injection and in these experiments was measured from 20 - 30 min after formalin injection.

**Carrageenan-induced thermal hyperalgesia**

The dose of carrageenan used produced marked redness with only slight inflammation. The difference in paw withdrawal latencies between the treated and untreated paws in the control group, showed a time dependent decrease in paw withdrawal latencies of the right paw (Figure 5). There was no significant difference in reaction time in all groups 15 min after carrageenan injection. The extract of OUB, OUL, celecoxib and indomethacin significantly ($p < 0.05$) reduced thermally-induced pain in inflamed tissue 60 min post carrageenan injection. During the second hour (120 min) however, celecoxib alone significantly ($p < 0.05$) reduced pain and continued to inhibit pain 180 min after carrageenan injection. Although, indomethacin and both OUB and OUL failed to protect against inflammatory pain 120 min after carrageenan challenge, yet their effects become once more significant ($p < 0.05$) 180 min and beyond.

**Carrageenan induced paw edema**

The extracts of OUL significantly ($p < 0.05$) attenuated paw swelling during the first three hours following the injection of carrageenan (Table 1). OUB decreased paw swelling though results were not significant. Indomethacin and celecoxib both significantly ($p < 0.05$) inhibited paw swelling 3 h post treatment.

## DISCUSSION

The present study was aimed at investigating the analgesic and anti-inflammatory properties of the leaf and bark extracts of *O. unilocularis*. We have been able to show that these extracts have both analgesic and anti-inflammatory properties, thus, validating some of its traditional uses.

The antinociceptive effects were studied using five distinct *in vivo* models of analgesia: The acetic acid-induced writhing, tail flick, pressure-induced, formalin and carrageenan-induced hyperalgesia tests while inflammation was studied using the carrageenan induced paw edema model. The methanolic extracts of OUL and OUB, showed significant analgesic properties in all models studied.

In the writhing test model, OUB and OUL significantly inhibited pain comparably to aspirin. Morphine however, showed stronger analgesic effects which were significantly different from the results produced by aspirin

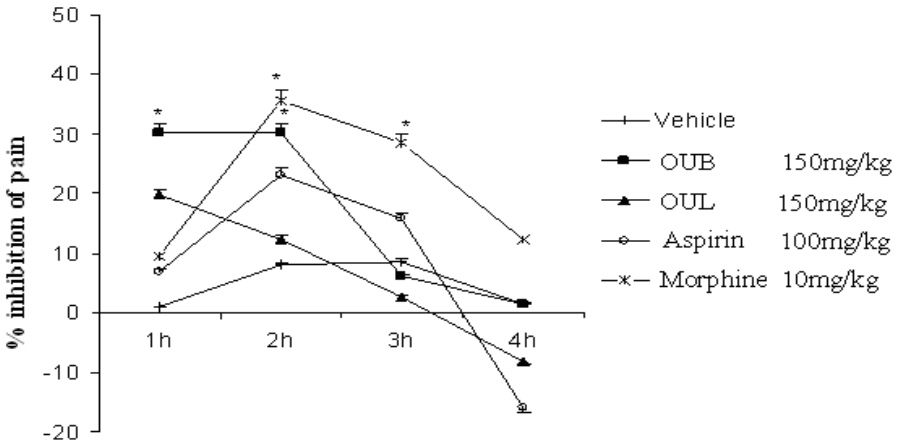


**Figure 3.** Analgesic effect of oral treatment with OUL and OUB on pressure-induced pain in rats. Results are shown as mean ± SEM, n=5. (*p < 0.05).

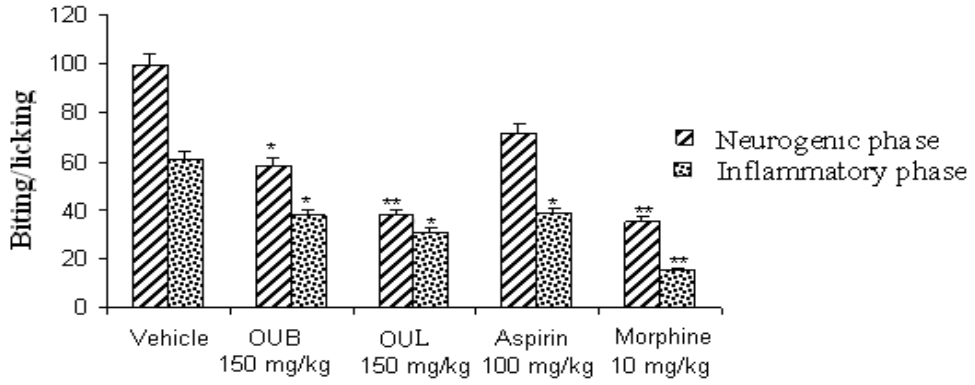


**Figure 4.** Analgesic effect of OUL and OUB on formalin induced pain. Results are expressed as the mean ± SEM, n=5. (* p < 0.05; **p < 0.01) compared to control group.

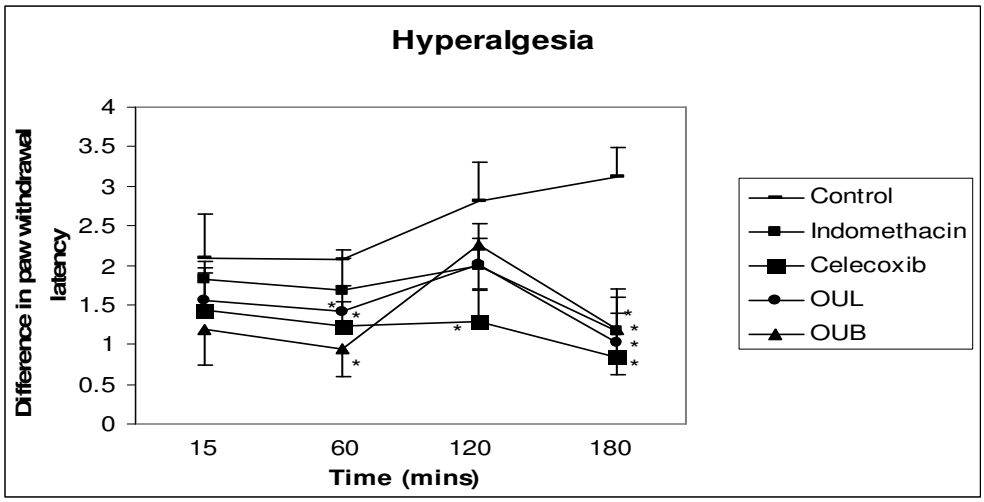


**Figure 5.** Anti-hyperalgesia effects of OUL and OUB. Results are expressed as the difference between the paw withdrawal latencies of the untreated versus treated paws. Each result point represents the mean ± SEM, n=5. (* p < 0.05) compared to control group.

**Table 1.** Anti-inflammatory effect of oral pre-treatment with OUB and OUL on carrageenan induced paw oedema.

| | | Inflammation (ml) | | | |
|---|---|---|---|---|---|
| **Drugs** | **Doses (mg/kg)** | **1 h** | **2 h** | **3 h** | **4 h** |
| Vehicle | - | 0.17±0.4 | 0.25±-.06 | 0.21±0.09 | 0.18±0.03 |
| OUL | 150 | 0.04 ±0.01* | 0.10±-.02* | 0.08±0.01* | 0.14±0.01 |
| OUB | 150 | 0.12±0.08 | 0.25±0.8 | 0.12±0.05 | 0.12±0.08 |
| Celecoxib | 20 | 0.14±0.04 | 0.18±0.07 | 0.11±0.08* | 0.12±0.08 |
| Indomethacin | 10 | 0.21±0.09 | 0.16±0.06 | 0.10±0.05* | 0.18±0.05 |

Results are shown as the mean ± SEM of change in paw volumes (paw volume at $V_x$-$V_0$, where $V_x$ = paw volume at 1, 2, 3 or 4h after carrageenan injection and $V_0$ = paw volume at start of experiment before carrageenan injection) n = 5. (* $p < 0.05$).

and both OUB and OUL. The abdominal injection of acetic acid induces the release of mediators of pain such as prostaglandins and other cytokines, meaning that OUB and OUL may be acting by inhibiting the actions of cyclocoxygenase (COX) which is responsible for producing prostaglandins from arachidonic acids. Previous reports however, show that the acetic acid-induced abdominal writhing test in mice, is a nonspecific test which responds to both central and peripheral analgesia (Meotti et al., 2005; Le Bar et al., 2001; Malec et al., 2008). It is therefore used for screening analgesic properties. This explains why both morphine and aspirin produced significant pain inhibition. OUB and OUL both significantly reduced the writhing nociceptive behaviour in mice indicating that these extracts had either central or peripheral or both types of analgesic properties. To clarify the analgesic effects of OUB and OUL we performed the tail flick tests.

In the tail flick test, OUB and OUL prolonged the paw withdrawal latencies to thermal stimulation in mice. OUL significantly increased paw withdrawal latencies 1 and 2 h post treatment while aspirin failed to induce significant analgesic effect. Unlike the writhing test, thermally-induced pain responds better to centrally acting agents (Chakraborty et al., 2004) such as narcotics. In this model of pain, morphine which acts centrally significantly ($p < 0.05$) increased pain threshold in mice. The tail flick test involves the transmission of pain from the periphery via C fibres to the spinal cord. OUB and OUL could therefore be acting by inhibiting the transmission of pain via C fibres to the CNS. To further confirm our findings, the pressure induced pain model was used. In this model of pain, OUL significantly increased the ability of animals to withstand pressure-induced pain indicating a more central acting mechanism of the extract. This test is important for differentiating between central opioid-like analgesics and peripheral analgesics (Jones et al., 2005). According to previous studies, aspirin an NSAID, is ineffective against pain due to sensory nerve stimulation (Moore et al., 1995) which explains why aspirin did not elicit analgesic effects in these two models. These results suggest the possible central action of these extracts.

In the formalin test, both OUL and OUB showed significant analgesic properties. In the first phase, OUL inhibited pain comparably to morphine, while in the second phase, OUB, OUL, morphine and aspirin all significantly inhibited pain. Furthermore, the effects of morphine were significant even when compared with the effects of the other drugs used. Compared to other traditional pain-evaluating models, which consist of brief stimuli of threshold intensity, the formalin test involves moderate to long-lasting pain. Importantly, the formalin nociception is associated with tissue injury and is therefore believed to more closely resemble clinical pain in comparison to other tests that use mechanical or thermal stimuli (dos Santos et al., 2006; Xie et al., 2005). In this test, the nociceptive response was in two distinct phases involving different mechanisms. The first phase (neurogenic pain) which began immediately after formalin injection resulted from the direct chemical stimulation of myelinated and unmyelinated nociceptive afferent fibres, mainly C fibres which can be suppressed by opioid analgesic drugs like morphine (Viegas et al., 2008). The second phase noted 20 - 30 min after formalin injection resulted from the release of inflammatory mediators in the peripheral tissues as well as facilitation of synaptic transmission at the spinal level (Hao et al., 2001). This later phase was reported to be sensitive to the action of the majority of NSAIDs (Hwang et al., 2008). In this study, OUB and OUL inhibited the licking response of mice in both phases of the formalin test, suggesting that its antinociceptive effects could be through central and peripheral mechanisms. The present results suggest that the management of pain by this plant may be by either peripheral pain inhibition or by inhibition of inflammatory mediators. To confirm this, the thermal hyperalgesia test using Carrageenan was performed.

Carrageenan injection decreased the latency of the paw withdrawal latency in the ipislateral paw in comparison to the untreated contralateral paw. OUB and OUL significantly reduced inflammatory pain 60 and 180 min post carrageenan injection. Celecoxib on the other hand, significantly reduced inflammatory pain from 60 min post carrageenan injection to beyond 180 min post

carrageenan injection. Indomethacin protected against this type of pain only at 180 min post injection. Inflamed tissue has sensory neurons which are sensitized by the mediators of inflammation during the inflammatory process induced by carrageenan (Verri et al., 2007). Thus, inflamed tissue is more susceptible to pain than non-inflamed tissue. OUB and OUL may therefore induce their analgesic effects by inhibiting the release of the mediators of inflammation.

Carrageenan injection resulted in paw volume increase which was inhibited significantly by OUL from 1 - 3 h post carrageenan injection. Celecoxib and indomethacin significantly reduced inflammation 3 h post injection. The plant extract was mostly active in the first phase of the carrageenan response phase. Carrageenan injection causes an increase in paw volume 1 h after carrageenan injection and maximal paw volumes are attained 4 - 6 h after injection. The early phase of oedema resulting from carrageenan injection commonly corresponds with the early exudative stage of inflammation, which is an important process in inflammatory pathology (Gupta et al., 2003, Lee et al., 2006). This second phase is reportedly due to the liberation of bradykinin, prostaglandins and kinins in injected paw tissue accompanied by leukocyte migration (Pinheiro and Calixto, 2002). Although, the cyclooxygenase and lipoxygenase pathways are both involved in the inflammatory process, the inhibition of cyclooxygenase is more effective in inhibiting carrageenan-induced inflammation than lipoxygenase inhibitors (Giuliano and Warner, 2002). The present results suggest that OUL suppresses the first phase of carrageenan-induced paw oedema, thus, confirming an NSAID-like property of the extract. The present study showed that the leaf and stem bark extracts of *O. unilocularis* have both analgesic and anti-inflammatory properties thus validating the traditional use of this plant in pain management and arthritis.

## ACKNOWLEDGEMENT

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### REFERENCES

Aburi Botanical Gardens (2002). Conservation and cultivation of medicinal plants in Ghana. Medicinal Plant Workbook.

Adomou AC (2005). Vegetation patterns and environmental gradients in Benin. Implications for biogeography and conservation. PhD thesis Wageningen University, Wageningen.

Aley KO, Levine JD (1999). Role of protein kinase A in the maintenance of inflammatory pain. J. Neurosci. 19: 2181-2186.

Aluka I (2008). Useful Plants of West Tropical Africa: Oxyanthus unilocularis Hiern (family Rubiaceae). Available from: http://www.aluka.org/action/showMetadata?doi=10.5555/AL.AP.UPWTA.4_961&pgs= [Accessed 25 September 2008].

Baamone A, Alverez-Vega M, Hidalgo A, Memendez L (2000). Effects of intraplantar morphine in the mouse formalin test. Jpn. J. Pharmacol. 83:154-156.

Bower WA, John M, Margolis HS, Williams IT, Bell BP (2007). Population-based surveillance for acute liver failure. Am. J. Gastroenterol. 102: 2459-2463.

Chakraborty A, Devi RKB, Rita S, Sharatchandra K, Singh TI (2004). Preliminary studies on antiinflammatory and analgesic activities of Spilanthes acmella in experimental animal models. Int. J. Pharmacol. 36: 148-150.

Cuartero M, Carrie H, Malik U, Romic U, Tasios B, Wu M (2006). A comparison of frequently prescribed analgesics at the University of Toronto for post operative pain following dental surgery: an evidence-based study of the literature. Community Dentistry 1: 1-12.

Deblauwe I (2006). New evidence of honey-stick use by chimpanzes in Southeast Cameroon. PAN. 13: 1-4.

dos Santos MD, Camila M, Lopes NP, de Souza GEP (2006). Evaluation of the anti-inflammatory, analgesic and antipyretic activities of the natural polyphenol chlorogenic acid. Biol. Pharm. 29: 2236-2240.

Du J, Yu Y, Ke Y, Wang C, Qian ZM (2007). Ligustilide attenuates pain behavior induced by acetic acid or formalin. J. Ethnopharmacol. 112: 211-214.

Giuliano F, Warner TD (2002). Origins of Prostaglandin E2: Involvements of Cyclooxygenase (COX)-1 and COX-2 in Human and Rat Systems. J. Pharmacol. Exp. Ther. 303: 1001-1006.

Gupta M, Mazumder UK, Gomathi P, Selvan VT (2006). Antiinflammatory evaluation of leaves of *Plumeria acuminate*. BMJ. Compl. Alter. Med. 6: 36-41.

Hao S, Takahata O, Iwasaki H (2001). Antinociceptive interaction between spinal clonidine and lidocaine in the rat formalin test: an isobolographic analysis. Anesth. Analg. 92: 733-738.

Hwang HJ, Kim O, Kim CJ, Lee HJ, Him I, Yin C, Yang Y Hahm DH (2008). Antinociceptive effect of amygdalinniolated from *Prunus armeniaca* on formalin-induced pain in rats. Biol. Pharm. Bull. 31: 1559-1564.

Idani G, Kuroda S, Kano T, Asato R (1994). Flora and vegetation of Wamba forest, central Zaire with reference to bonobo (Pan paniscus) foods. Tropics 3: 309-332.

Irvine FR (1961). Woody plants of Ghana, with special reference to their uses. Oxford University Press p. 697.

Jones CK, Peters SC, Shannon HE (2005). Efficacy of duloxetine, a potent and balanced serotonergic and noradrenergic reuptake inhibitor, in inflammatory and acute pain models in rodents. J. Pharmacol. Exp. Ther. 312: 726-732.

Kitula RA (2007). Use of medicinal plants for human health in Udzungwa Mountains Forests: a case study of New Dabaga Ulongambi Forest Reserve, Tanzania. J. Ethnobiol. Ethnomed. 3: 7-10.

Le Bar D, Gozariu M Cadden SW (2001). Animal models of nociception. Pharmacol. Rev. 53: 597-652.

Lee JY, Jang YW, Kang HS, Moon H, Sim SS, Kim CJ (2006). Anti-inflammatory Action of Phenolic Compounds from *Gastrodia elata* Root. Arch. Pharm. Res. 29: 849-858.

Malec D, Mandryk M, Fidecka S (2008). Interaction of memantine and ketamine morphine- and pentazocine-induced antinociception in mice. Pharmacol. Rev. 60: 149-155.

McCullough J, Alonso LE, Naskrecki P, Wright HE, Osei-Owusu Y (2007). A rapid biological assessment of the Atewa range forest reserve, Eastern Ghana. The RAP Bulletin of Biological Assessment Series.

Meotti FC, Luiz AP, Pizzolatti MG, Kassuya CAL, Calixto JB, Santos ARS (2005). Analysis of the antinociceptive effect of the flavonoid myricitrin. Evidence for a role of the L-arginine-nitric oxide and protein kinase C pathways. JPET 10: 1124-1158.

Moore UJ, Marsh VR, Ashton CH, Seymour RA (1995). Effects of peripherally and centrally acting analgesics on somato-sensory evoked potential. Br. J. Clin. Pharmacol. 40: 111-117.

Pinheiro RM, Calixto JB (2002). Effect of the selective COX-2 inhibitors, celecoxib and rofecoxib, in rat acute models of inflammation. Inflamm. Res. 51: 603-610.

Uchida S, Hirai K, Hatanaka J, Hanato J, Umegaki K, Yamada S (2008).

Antinociceptive effects of StJohn's worth, *Harpagophytum procumbens* extract and grape seed proanthocyanidins extract in mice. Biol. Pharm. Bull. 31: 240-245.
Verri Jr WA, Cunha TM, Poole S, Ferreira SH, Cunha FQ (2007). Cytokine inhibitors and pain control. Rev. Bras. Reumatol. 47: 341-353.
Verri Jr, WA, Molina RO, Schivo IRS, Cunha TM, Parada CA, Poole S, Ferreira SH Cunha FQ (2005). Nociceptive effects of subcutaneously injected interleukin-12 is mediated by endothelin (ET) acting on ETB receptors in rats. J. Pharmacol. Exper. Ther. 315: 609- 615.
Viegas Jr C, Alexandre-Moreira S, Fraga M, Barreiro EJ, Bolzani VS, de Miranda ALP (2008). Antinociceptive profile of 2,3,6-trisubstituted piperidine alkaloids: 3-o-acetyl-spectaline and semi-synthethic derivative of (-)-spectaline. Chem. Pharm. Bull. 56: 407-412.
Wallace JL (2000). How do NSAIDs cause ulcer disease? Best Prac. Res. Clin. Gastroenterol. 14: 147-159.
Xie YF, Wang J, Huo FQ, Jia H, Tang JS (2005). Validation of a simple automated movement detection system for formalin test in rats1 Acta. Pharmacol. Sinica 26: 39-45.