INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY, BIOLOGY AND CHEMISTRY

Research Article

# Phytochemical and Antinociceptive studies on the leaf extract of *Ochna schweinfurthiana* (Ochnaceae)

**ZYY Ibrahim*[1], AM Musa[3], MI Abdullahi[2], A Uba[2], AJ Yusuf[2], MG Magaji[4], IM Aliyu[4] and J. Ya'u[4]**

[1]Department of Pharmacognosy and Ethnopharmacy, Usmanu Danfodiyo University, Sokoto, Nigeria.

[2]Department of Pharmaceutical and Medicinal Chemistry, Usmanu Danfodiyo University, Sokoto, Nigeria.

[3]Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria, Nigeria.

[4]Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria.

**ABSTRACT**

Pain is a symptom of almost all medical conditions. The anti-nociceptive activity of the methanol extract of *Ochna schweinfurthiana* leaves obtained through maceration was evaluated using (Acetic acid - induced abdominal writhing and tail flick models in mice. The extract significantly (P<0.01) inhibited acetic acid - induced writhing in mice at the highest (200mg/kg), median (100mg/kg) and low (50 mg/kg) doses with 84.30%, 80.81% and 53.49% inhibition respectively. Inhibition at 200 mg/kg and 100 mg/kg were higher in comparison with ketoprofen (10mg/kg), the standard drug which had 61.05% inhibition. In the tail-flick model, though with a slower onset of action, the extract at the graded doses (200, 100 and 50 mg/kg) significantly (P<0.05) attenuated pain response in a similar manner observed with the standard drug piroxicam (10mg/kg). The observed activity was dose-dependent in both models and also time dependent in the tail-flick model. Preliminary Phytochemical screening revealed the presence of glycosides, steroids/terpenes, flavonoids, tannins, saponins and carbohydrates. The result showed that the methanol leaf extract of *O. schweinfurthiana* contains bioactive principles with analgesic property, rationalising its use in folklore medicine for the management of pain.

**Keywords:** *Ochna schweinfurthiana,* Anti-nociceptive, Acetic acid-induced, Tail-flick

## INTRODUCTION

Pain is associated with majority of health conditions. It is an unpleasant or distressing localised sensation caused by the stimulation of certain sensory nerve endings called nociceptors, irrespective of the site of nerve stimulation[1]. Major drugs used clinically for management of pain are the opioids including; morphine, codeine[2] or synthetic Non-steroidal anti-inflammatory drugs (NSAIDs) such as salicylates and aminopyrine). Opioid analgesics otherwise called narcotic analgesics are natural or synthetic compounds that produce morphine-like effects[3]. The NSAIDs are cyclo-oxygenase (COX) inhibitors that work to inhibit prostaglandin synthesis, the chemical that promotes inflammation and pain[4]. Most of these analgesics only relieve pain temporarily but do not provide permanent cure. Moreover, they are accompanied by severe side effects among which are renal failure, dependency, gastro-intestinal tract disturbance etc[5]. Unavailability and high cost is

another limitation of these analgesics especially in developing countries

Since time immemorial, natural products have played a very important role in health management and disease prevention through the practice of traditional medicine[6]. Today, medicinal plants serve as the source of almost 50% of the therapeutic agents in clinical use whether as drugs or as lead compounds[7]. Several analgesic agents have been isolated from plants including morphine from Opium poppy[8], aspirin from Willow tree bark[9] among others. However, a large number of medicinally useful plants have not been explored or are used traditionally with no scientific basis[10].

*Ochna schweinfurthiana* F. Hoffm. (Ochnaceae) is a small tree or shrub that grows to about 4m, mainly in the savannah woodland from Guinea to North and South Nigeria and across West to Central Africa and Asia[11]. It is commonly known as the Brick-red Ochna, in Hausa Language as Jan-taru and Hiéké in Yoruba[11]. Species in the genus Ochna have a long history of use as herbal remedies in African and Asian folkloric medicine[12]. For example, *O. lanceolata* is used as an abortifacient and in the treatment of gastric and menstrual disorder[13], *O. squarrosa* as digestive tonic and for asthma treatment[14]. *O. pumila* is used for treatment of lumbago, ulcer, snake bite and epilepsy. Several preparations (powdered form and decoction) of the leaves and/or root of *Ochna schweinfurthiana* have found a general use as antimicrobial (wound dressing, eye infection), analgesic, anti-inflammatory and anthelmintic agents[11]. The leaf is also used as a laxative, enema and febrifuge. Research conducted on members in this genus revealed them to have significant analgesic and anti-inflammatory activities[15]. Other pharmacological activities include antimicrobial[16], cytotoxic[17], anti-malarial[18]. Except for antimicrobial activity[19], there is no report of scientific validation for the acclaimed uses of the plant in traditional medicine. This study was aimed at investigating the analgesic property of the crude methanol leaf extract of *Ochna schweinfurthiana.*

## MATERIALS AND METHOD

### Plant material

The plant sample of *Ochna schweinfurthiana* was obtained from Samaru, Zaria-Nigeria in June, 2013. It was identified and authenticated at the Herbarium Unit, Department of Biological Sciences, Ahmadu Bello University Zaria by comparing with herbarium reference voucher specimen (Number 900229).

### Experimental animals

Locally bred adult Swiss albino mice of either sex (15-30 g body weight) were acquired from Animal House Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria, Nigeria. The animals were fed with laboratory diet and water *ad libitum* and maintained under standard conditions in clean cages at room temperature. All experimental procedures were approved by the Animal Right and Ethics Community of the University.

### Drugs and Chemicals

Analytical grade laboratory reagents and chemicals, Ketoprofen (Lek, Slovenia), Piroxicam (Pfizer, Pakistan) and CME (50, 100 and 200 mg/kg) were used for the study.

### Extraction

Fresh leaves of *Ochna schweinfurthiana* were removed and shade-dried at room temperature for 4 weeks and pulverized. The pulverized material was labelled and stored for further use. The powdered material (300g) was macerated with methanol for ten (10) days with occasional shaking. The filtrate was concentrated in-vacuo using rotary evaporator at 45$^{o}$C affording a yield of 36g (12.0%) which was subsequently referred to as crude methanol extract (CME).

### Preliminary Phytochemical Screening

Standard methods described by Trease and Evans[20] were used to test the extract for the presence of secondary metabolites.

### Screening for Analgesic Studies

#### *Acetic Acid-induced writhing*

The method described by Koster*et al*[21] was employed. Twenty five (25) animals were divided into 5 groups containing 5 mice each. Groups 1, 2 and 3 (test groups) received the graded doses of CME at 50, 100 and 200 mgkg$^{-1}$ respectively. Group 4 (positive control) received the standard drug, ketoprofen at 10 mg/kg while group 5 (negative control) was treated with 10 ml/kg normal saline intra-peritoneal. 0.6% aqueous acetic acid solution was administered *i.p.* to each mouse 30 minutes after treatment of all groups. After an interval of 5 minutes, the mice were observed for specific contraction of abdomen for the next 15-20 minutes.

#### *Mechanical (Tail-Flick) Model*

The method adopted by Okolo*et al*[10] was used. Mice were grouped into 5 containing 5 mice each. An analgesiometer with 100g weight was used to apply pain in the form of pressure. The middle region of each animal's tail was positioned under the fulcrum of the device and the extension was pressed to apply the pressure. Readings were taken pre-treatment (0

reading), after which graded doses of the extract of 50, 100 and 200 mg/kg, 10 mg/kg standard drug (piroxicam) and 0.2 ml/kg BW normal saline were administered intraperitoneally to groups 1-5 respectively. Further readings were taken 30 minutes post-treatment and repeated 3 times with 30 minutes interval. The pain threshold was calibrated in cm and noted as the time when the mouse showed any sign of discomfort (tail pulling, tail flicking or squeaking).

## RESULTS AND DISCUSSION

Phytochemical screening of the crude methanol leaf extract of *O. schweinfurthiana* gave result as presented in Table 1.

Treatment with, 50, 100 and 200 mg/kg of the extract significantly ($P<0.01$) reduced the number of acetic acid-induced writhes with inhibitions of 53.49%, 80.81% and 84.30% respectively. The activity of the extract against acute pain at the median and highest doses was higher when compared to the inhibitory activity of the standard drug, ketoprofen 61.05% (Table 2).

Acetic acid-induced writhing model is very sensitive, compared to other methods, in detecting anti-nociceptive effect of compounds[22] and other analgesic agents that act on peripherally mediated pain. Abdominal writhing are manifested through sensitisation of chemosensitive nociceptors by prostaglandins because prostanoids particularly $PGE_2$ and $PGF_{2\alpha}$[23,24] and lipoxygenases[25,26] have been found in the peritoneal fluids after administration of acetic acid[27]. Of recent, nociceptive effect of acetic acid has also been associated with the release of cytokines like TNFα by resident macrophages and mast cells[28]. NSAIDs relieve pain by suppressing the formation of pain inducing substances such as prostaglandins and bradykinin[29, 30] in the peripheral tissues[31]. Thus, reduction of acetic acid induced abdominal writhes by the graded doses of the extract in a similar manner to ketoprofen; a known peripherally acting analgesic suggests that the anti-nociception is peripherally mediated.

The extract increased the mean pain latency in a dose and time dependent manner after pain was mechanically stimulated. Activity 120 minutes post-administration of 200 mg/kg of extract was the same with piroxicam, the positive control. Overall activities of all doses of the extract were similar to that of the standard analgesic used (Table 3).

Tail flick methods are used primarily to evaluate analgesics acting through central mechanism[32]. Although the mechanism of action of some central analgesics is controversial, they are thought to inhibit sensitisation of the opioid receptors and other pain-related neuronal inputs such as enkephalins and endorphins that is, they act as CNS depressants[33].

**Table 1**
**Phytochemical Screening of the Crude Methanol Extract (CME)**

| Constituent | Observation |
|---|---|
| Carbohydrates | + |
| Anthraquinones | - |
| Steroids/Terpenes | + |
| Glycosides | + |
| Saponin | + |
| Tannins | + |
| Flavonoids | + |
| Alkaloids | - |

**+ = present, - = absent**

**Table 2**
**Effect of crude methanol extract of *Ochna schweinfurthiana* on acetic acid-induced writhing in mice**

| Treatment (mg/kg) | Mean No. of writhes ± SEM | Percentage Protection (%) |
|---|---|---|
| Normal Saline | 34.40±2.02 | |
| CME 50 | 16.00±1.00** | 53.49 |
| CME 100 | 6.60±1.69** | 80.81 |
| CME 200 | 5.40±2.02** | 84.30 |
| Ketoprofen 10 | 13.40±1.69** | 61.05 |

Result is expressed as mean ± SEM; n=5; **P<0.01

**Table 3**
**Effect of methanol extract of *Ochna schweinfurthiana* on mechanically stimulated pain in mice**

| | Mean Pain Threshold ± SEM | | | | |
|---|---|---|---|---|---|
| **Time (min)** | 0 | 30 | 60 | 90 | 120 |
| **Treatment(mg/kg)** | | | | | |
| Normal saline | 2.18±0.49 | 1.52±0.53 | 1.76±0.40 | 1.80±0.49 | 1.88±0.50 |
| CME (200) | 1.70±0.66 $^{ns}$ | 2.18±0.44 $^{ns}$ | 2.74±0.54 $^{*}$ | 2.64±1.27 $^{**}$ | 4.82±0.26 $^{**}$ |
| CME (100) | 1.70±0.54 $^{ns}$ | 1.86±0.34 $^{ns}$ | 3.02±0.82 $^{ns}$ | 4.30±0.35 $^{ns}$ | 4.64±1.19 $^{**}$ |
| CME (50) | 1.40±0.37 $^{ns}$ | 1.80±0.21 $^{ns}$ | 2.68±0.87 $^{ns}$ | 2.74±0.19 $^{ns}$ | 4.46±0.69 $^{**}$ |
| Piroxicam (10) | 1.52±0.40 $^{ns}$ | 2.70±0.70 $^{**}$ | 3.56±1.18 $^{**}$ | 4.44±0.58 $^{**}$ | 4.82±0.84 $^{**}$ |

n=5; P > 0.05 = ns; P < 0.01 = $^{**}$; P < 0.05 = $^{*}$

The action of centrally mediated analgesics is also known to involve inhibition of a biphasic nociceptive response[34]. In this study, Piroxicam and all administered doses of the extract produced time and dose dependent analgesia. The mean pain latencies of all groups at 0 minutes were not statistically different from the control. Analgesia was observed in group treated with 200 mg/kg of the extract 60 minutes post-treatment. Significant activity was however not observed at 100 mg/kg and 50 mg/kg until 90mins after administration. The standard drug, piroxicam showed faster onset (30 minutes) and higher activity than the test groups. However 120 minutes post-treatment, there was significant (P<0.01) anti-nociception in all test groups, analogous to the positive control. This implies that all doses of the extract have significant activity against pain with slower onset of action.

Analgesic effects of flavonoids[15], tannins[35, 36] and saponins[37, 38, 39] have been reported. The ability of the extract to exhibit its analgesic effect may be due to the synergistic effect of phytochemical constituents such as flavonoids, saponins etc detected in the plant.

## CONCLUSION

Due to adverse effects associated with analgesic agents in current clinical use, management of chronic pain still remains a challenge for the medical community. This necessisates continuous search into more potent and safer analgesic agents. Despite the use of *O. schweinfurthiana* to manage a wide range of diseases, their is no scientific proof for most of the acclaimed effects. In this study, the crude methanol leaf extract of the plant was evaluated for anti-nociceptive activity. The results provided experimental evidence validating its use in folklore medicine againts pain associated conditions.

## REFERENCES


1. Youngson RM, Collins Internet-linked Dictionary of Medicine. Harper-Collins Publishers, Westernhill Road, Bishopbriggs, Glasgow. 2005
2. Kapur D, Centrally acting non-opioid analgesic drugs, Current anaesthesia and critical care. 2002; 13(6): 328-333
3. Howland RD, Mycek MJ, Lippincott's Illustration Review: Pharmacology. Harvey RA, Champe PC, (eds.) Lippincott Williams and Wilkins publishers London. 2006; pp. 157-168
4. Insel PA, Analgesic–antipyretic and anti-inflammatory agents and drugs employed in the treatment of gout. In: Hardman, J.G. and Limbird, L.E., eds, Goodman. 1996; pp: 17-25
5. Jachak SM, Saklani A. Challenges and opportunities in drug discovery from plants. Curr Sci. 2007; 92(9):1251-1257
6. Kinghorn AD. Plants as sources of medicinally and pharmaceutically important compounds, Phytochemical resources for medicine and agriculture. 1992; 75-95
7. Dewick PM. Medicinal Natural Products: A biosynthetic approach (3rd Ed.). Chichester: Wiley, 2009; pp. 10
8. Jaffe JH, Martin JW. Opioid Analgesics and Antagonists. In: Gilman AG, Rall TW, Nies AS, Taylor P, (Eds). The Pharmacological Basis of Therapeutics, Permagon Press, New York, USA. 1990; pp 638-680
9. Buchner A. Rigatelli's antipyretic and an alkaloid substance discovered in Willow bark, Repertorium fur die Pharmacie. 1828; 29:405
10. Okolo CO, Johnson PB, Abdulrahman EM, Abdu-Aguye I, Hussaini IM. Analgesic effect of Irvingia gabonensis stem bark extract. J Ethnopharmacol, 1995; 45(2):125-129
11. Burkill HM, The Useful Plants of West Tropical Africa, Royal Botanic Gardens, Kew. 1985; 4:445
12. Bandi AKR, Lee D, Tih RG, Gunasekar D, Bodo B. Phytochemical and Biological Studies

of Ochna Species,. Chemistry and Biodiversity. 2012; 9:251- 271
13. Muthukumaraswamy S, Mohan VR, Kumaresan S. Pharmacognostic studies on the trunk bark of Ochhna lanceolata. J. Med. Arom. Plant Sci. 2014;4(1):93- 100.
14. Kirtikar KR, Basu BD. Indian Medicinal Plants, Periodical Book Agency, New Delhi, 1980; pp. 515
15. Anuradha V, Srinivas PV, Rao RR, Manjulatha K, Purohit MG, Rao JM. Isolation and synthesis of analgesic and anti-inflammatory compounds from Ochna squarrosa L. Bioorg. Med. Chem. 2006; 14(20): 6820- 6826
16. Ndoile MM. Structure, synthesis and biological activities of biflavanoids isolated from Ochna serrulata. PhD Thesis. 2012; pp 20-137
17. Sriram S, Chozhan J, Meenaa V, Kavitha SJ, Sasikumar C. Evaluation of Antibacterial Activity of Ochna obtusata against Selected Pathogens. Adv Bio Tech. 2011; 11(6):39-41
18. Reutrakul V, Ningnuek N, Pohmakotr M. Anti-HIV-1 flavonoid glycosides from Ochna Integerrima. Planta medica. 2007; 73(7): 683-688
19. Abdullahi MI, Iliya I, Haruna AK, Sule MI, Musa AM, Abdullahi MS. Preliminary phytochemical and antimicrobial investigations of leaf extracts of Ochna schweinfurthiana (Ochnaceae). Afr J Pharm Pharmacol. 2010; 4(2):83-86.
20. Trease GE, Evans WC. Textbook of Pharmacognosy, 12th (Ed) Balliers Tindall, London. 1983; pp. 343-384
21. Koster R, Anderson M, Beer EJ. Acetic acid for analgesic screening, Federation Proceeds. 1959; 18:412-416
22. Shumaia P, Md. Abu Shuaib R, Md. Abdul K, Most. Afia A, Tahmida S et al. Phytochemical screening and studies of analgesic potential of Moringa oleifera Lam. Stem bark extract on experimental animal model. Int J Phytopharm. 2014; 4(5):128-131
23. Deradt R, Jongney S, Deivaicee F, Falhout M. Release of Prostaglandins E and F in an algogenic reaction and its inhibition. Euro J Pharmacol 1980; 61(1):17-24.
24. Chandra-Shekhar N, Jayasree T, Sheikh U, Rohit D, Manohar VS, Shankar J. Evaluation of Antinociceptive Activity of Aqueous Extract of Bark of Psidium guajava in Albino Rats and Albino Mice. J Clin Diag Res, 2014; 8(9):1-4
25. Levini JD, Lau W, Kwait G, Goetzl EJ. Leukotriene produces hyperalgesia that is dependent on the polymorphonuclear leucocytes. Science. 1984; 255(4663):743-745
26. Dhara AK, Suba V, Sen T, Pal S, Chaudhuri AK. Preliminary studies on the anti-inflammatory and analgesic activity of methanolic fraction of the root of Tragia involucrate. J. Ethnopharm, 2000; 72(1-2):265-268
27. Magaji GM, Mohammed A, Yaro AH, Tanko Y, Danjuma IK. Preliminary analgesic and anti-inflammatory activities of methanolic stem bark extract of in Securinega virosa rodents. Biol Env Sci J Trop, 2008; 5(1):131-135
28. Ribeiro RA, Vale ML, Thomazzi SM, Paschaolato AB, Poole S, Ferreir SH. Involvent of resident macrophages and mast cells in the writhung nociceptive response induced by zamosan and acetic acid in mice, Eur J Pharmacol, 2000; 387(1):111-118
29. Hirose K, Jyoyama H, Koojiima Y. Antiniciceptive and Antipyretic Activities of Pongamia pinnata Leaves, Drug Res, 1984; 34:280
30. Prabha MN, Ramesh, CK, Kuppast IJ, Mankani KL. Studies on anti-inflammatory and analgesic activities of Euphorbia tirucalli L. Latex, Int J Chem Sci, 2008; 6(4):1781-1787
31. Jain PS, Mallipedi S, Belsare DP, Mandal SC, Pal SC, Badgujar VB. Analgesic Activity of Stem Bark of Kigelia pinnata Linn. Indian Drugs. 2007; 44(1): 63-65
32. Vogel HG. Analgesic, anti inflammatory and anti pyretic activity: Drug discovery and evaluation in pharmacological assays. J. Pharm Res, 2008; 983-1113
33. Brune K. Pharmacology of centrally acting analgesics: an introduction. Postgrad Med J, 1987; 63(3):5-7
34. Mohammad AR, Mohammed I, Tajmira T, Qamrul A, Md. Ruhul K, Ridwan BR. Analgesic, anti-inflammatory and anxiolytic activities of Sarcochlamys pulcherrima gaudich. Carib J Sci Tech, 2014; 2:295-300
35. Ahmadiani A, Hosseiny J, Semnanian S, Javan M, Saeedi F, Kamalinejad M, Saremi S. Antinociceptive and anti-inflammatory effects of Elaeagnus angustifollia fruit extract. J. Ethnopharm, 2000; 72(1-2): 287-293
36. Yerima M, Magaji MG, Yaro AH, Tanko Y, Mohammed MM, Analgesic and anti-inflammatory activities of the methanolic leaves extract of Securinega virosa (Euphorbiaceae). Nigerian Journal of Pharmaceutical Sciences. 2009; 8(1):47-53
37. Choi J, Jung H, Lee K, Park H. Antinociceptive and Anti-inflammatory effects of saponin and

sapogenin obtained from the stem of Akebia quinata. Journal of Medicinal Food. 2005; 8(1): 78-85

38. De Araujo PF, Coelho-de-Souza AN, Morais SM, Ferreira SC, Leal-Cardoso JH. Antinociceptive effects of the essential oil of Alpinia zerumbet on mice. Phytomedicine. 2005; 12(6-7):482-686
39. Reanmongkol W, Subhadirasakul S, Thienmontree S, Thanyapanit K, Kalnaowakul J, Sengsui S. Antinociceptive activity of the alkaloid extract from Kopsia macrophylla leaves in mice, Songklanakarin Journal of Science and Technology. 2005; 27(2):509-516