Anti-Inflammatory And Anti-Nociceptive Effects Of The Methanol Root Extract Of *Maniophyton fulvum* Muell Arg. (Eupherbiaceae) In Albino Mice

Udoh AE,[1] Udobre AS[2*] and E. I., Etim[2]
1 Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Nigeria.
2 Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, University of Uyo, Nigeria.

ABSTRACT

Maniophyton fulvum is a herbaceous plant used in Akwa Ibom State, Nigeria to treat pains, frequent stooling and swellings. In this study, the methanol root extract of the plant was investigated for its phytochemical constituents, safety profile, anti-inflammatory and anti-nociceptive properties. The result of the phytochemical screening revealed that the plant contained flavonoids and terpenes. The median Lethal Dose (LD_{50}) was 948.68 mg/kg. At a dose of 284.60 mg/kg the extract exhibited a significant ($p<0.05$) anti-inflammatory effect against oedema induced by egg-albumin and xylene. The dose also exhibited a significant ($p<0.05$) anti-nociceptive effect against acetic acid-induced writhing movement and formalin-induced hind paw licking in mice. The presence of flavonoids and terpenes in the plant is believed to cause the reduction in the inflammation and pain in the mice.

Keywords: Anti-inflammatory, Anti-nociceptive, Methanol Root Extract, *Maniophyton fluvum*

1.0 INTRODUCTION

Maniophyton fluvum (Eupherbiaceae) is popular herb among the local traditional medicine practitioners in the south-south region of Nigeria. In Nigeria, the plant (Ikon-Ikon, Annang name) is used to treat pains, diarrhea, swellings, stomach-ache, cough and lungs problems (Burkill, 1994). The red stem sap is used to treat insanity,heart, ear and tooth problem, wounds, dysentery, pile, painful menstruation and skin infection such as herpes (Okoli *et al* 2003).

The stem decoction is used for gonorrhoea; the leaf sap for throat infection, the powdered dry leaf is sprayed on sores and the seeds for blood disorders (Essien, 2005).

In Nigeria *maniophyton fluvum* is used traditionally to treat swellings and pains.An array of drugs are available in the market to treat swellings and pains but only very few of them are free from toxicity.

It is believed that the results of this research may lead to the discovery and development of new anti-inflammatory and analgesic drugs with low toxicity and higher therapeutic value.

Inflamation is the response of a living tissue to injury, often caused by invading parasites. It is characterized by increased blood flow in the tissue causing increasing temperature, redness, swelling and pain (Usifoh *et al*, 2016). Pain is an unpleasant feeling and emotional experience that is related to real or potential tissue damage (Usifoh*et al*, 2016).Analgesic or pain killer is any member of the group of drugs used to achieve analgesia (Harper, 2001).

MATERIALS AND METHOD

The roots of *maniophyton fulvum* were collected a forest in Obot Akara Local Government of Akwa Ibom State, Nigeria. The plant was identified by Dr. (Mrs.) Margaret Bassey, a taxonomist in the department of botany, University of Uyo. The specimen copy with identification number UUH – 31 (K) where UUH stands for University of Uyo Herbarium.

Extraction

The root of the plant was washed, cut into small pieces, shade-dried then pulverized. The powder (530g) was extracted with ethanol (50%) for 72hours. The extract was filtered, the filtrate evaporated in water bath at 40^0C. The dry extract (53.58g) was used for the research work.

The phytochemical screening of the extract was carried out using the standard method (Sofowora, 1993 and Trease and Evans, 2002).

The experimental animals were 108 albino mice mean weight (20.0±1.4) of either sex obtained from the University of Uyo Animal house. Ethical approval for the use of the animal was obtained from the College of Health Sciences Animal Ethics Committee, University of Uyo.

The median lethal dose (LD_{50}) of the extract was determined by injecting the extract by intraperitoneal route using Lorke's (1983) method.

Egg-Albumin Induced Inflammation

Inflammation was induced in mice by the injection of egg albumin (0.1ml, 1% in normal saline) into the sub planar tissue of the right hind paw (Akah*et al* 1994; Okokon *et al* 2010). The linear circumference of the injected paw was measured before and 0.5, 1, 2, 3, 4 and 5hrs after the administration of the phlogistic agent. The root extract and Acetyl salicylic acid,ASA (100mg/kg orally) were administered to groups (n=6) of 24h fasted mice 1h before the induction of inflammation.

* Corresponding author's Email: aniefiokudobre@yahoo.com Phone: +2348027672367

Control group received 10ml/kg of distilled water orally. Edema (sign of inflammation) was assessed as the difference in paw circumference between the control and 0.5, 1, 2, 3, 4 and 5hrs post administration of phlogistic agent (Okokon and Nwafor, 2010). The eodema was measured with vernier calipers.

Xylene induced inflammation

Inflammation was induced in mice by topical administration of drops of xylene at the inner surface of the right ear. The xylene was left to act for 15mins. The root extract (94.87, 189.74 and 284.60mg/kg i.p), dexathasone (4mg/kg) and distilled water (0.2ml/kg) were orally administered to mice in the various groups (n=6) of mice 1h before the induction of inflammation. The animals were sacrificed under light anaesthesia and the left ears cut off. The difference in the weights of the ear was taken as the oedema induced by the xylene (Okokon and Nwafor, 2010 Mbagwu *et al*., 2007; Okokon *et al* 2010).

Acetic Acid Induced Writhing movement in Mice

Writhing movement (abdominal constrictions consisting of the contraction of abdominal muscles together with the stretching of hind limbs . It is a sign of pain resulting from injection of acetic acid (Okokon and Nwafor 2010). The animals were divided into 5 groups of 6 mice per group. Group 1 served as negative control and received 10ml/kg of normal saline, while groups 2, 3 and 4 were pre-treated with doses of extract intraperitoneally, and group 5 received 100mg/kg of acetyl salicylic acid. After 50 minutes, 0.2ml of 2% acetic acid was administered intraperitoneally (i.p) to all the groups except group 1 The number of writhing movements was counted for 30 minutes. Antinociception (analgesia) was expressed as the reduction of the number of abdominal constrictions between control animals and mice pretreated with extracts.

Formalin- Induced Hind Paw Licking in Mice

The procedure adopted was as described by (Okokon and Nwafor 2010). The animals were injected with 20µL of 2.5% formalin solution (0.9% formaldehyde) made up in phosphate butter solution (PBS concentration: NaCl 137millimole (Mm), KCl 2.7Mm and phosphate buffer, 10Mm) under the surface of the right hind paw. The amount of time spent licking the injected paw was timed and considered as indication of pain. Adult albino mice (20 – 25g) of either sex randomized into five groups of 6 mice each were used for the experiment. The animals in group 1 (negative control) received 10ml/kg of normal saline, groups 2 – 4 received 94.87, 189.74 and 284.60mg/kg doses of the root extract respectively, while group 5 received 100mg/kg of acetyl salicylic acid (ASA) 30 minutes before being challenged with buffered formalin. The responses were measured for 30mins (first and second phases) after formalin injection.The first phase occured 10min following formalin injection.This corresponds to the period of acute pain.After a quiet period of 10min, the second phase which correspond to the period of chronic or persistent pain.

Statistical analysis and data evaluation

Data obtained from this work were analyzed statistically using Students't-test and ANOVA (One way) followed by a post test (Tukey-Kramer multiple comparison test). Differences between means were considered significant at 5% level of significance ($P \leq 0.05$).

RESULT

Table 1: Effect of *Manniophyton fulvum* root extract on egg-albumin induced oedema in mice.

TREATMENT/	**AVERAGE INFLAMMATION (mm) ± SEM**					
DOSE (mg/kg)	**0.5hr**	**1hr**	**2hr**	**3hr**	**4hr**	**5hr**
CONTROL	4.40 ± 0.22	4.78 ± 0.21	4.52 ± 0.38	4.26 ± 0.12	4.14 ± 0.14	3.82 ± 0.10
Extract						
94.87	3.55 ± 0.26	4.05 ± 0.10	2.95 ± 0.31b	2.63 ± 0.08	1.81 ± 0.07	1.04 ± 0.05
189.74	3.88 ± 0.15	2.53 ± 0.12	1.54 ± 0.10	1.22 ± 0.01	0.84 ± 0.01	0.55 ± 0.05
284.60	3.81 ± 0.31	2.09 ± 0.03	1.67 ± 0.29	1.53 ± 0.08	1.13 ± 0.05	0.46 ± 0.05[a]
ASA 100	3.35 ±0.31	2.24 ± 0.35	1.55 ± 0.43	1.31 ± 0.54	0.72 ± 0.22	0.41 ± 0.01

Data are expressed as mean ± SEM.Significant at [a]P<0.05, compared to control. n = 6.

Table 2: Effect of root of *Manniophyton fulvum* on xylene-induced ear oedema in mice

TREATMENT/ DOSE (mg/kg)	WEIGHT OF RIGHT EAR (g)	WEIGHT OF LEFT EAR (g)	INCREASE IN EAR WEIGHT (g)	% INHIBITION
Control (normal saline 0.2ml)	0.078 ± 0.01	0.038 ± 0.00	(105.26)	
Extract 94.87	0.056 ± 0.01	0.038 ± 0.01	(57.89)	45.00
189.74	0.057 ± 0.01	0.034 ± 0.00	(34.21)	67.50
284.60	0.047 ± 0.01	0.037 ± 0.01	(16.21) [a]	85.00
Dexamethasone 4.0	0.045 ± 0.01	0.037 ± 0.01	(21.62)	80.00

Figures in parenthesis indicate % increase in ear weight. Significant at [a]P< 0.05 when compared with control. n = 6

Table 3: Effect of root extract of *Manniophyton fulvum* on acetic acid induced writhing in mice

TREATMENT/ DOSE (mg/kg)	TIME INTERVALS						
	5	10	15	20	25	30	TOTAL
CONTROL	8.00 ± 1.21	10.00 ± 1.38	15.50 ± 1.42	17.23 ± 1.16	16.50±0.62	14.50±0.95	81.73±6.74
Extract 94.87	5.25 ± 0.85	8.25 ± 0.73	10.00 ± 1.18	15.24±1.06	12.22±1.20	12.06±0.72	63.02±4.09
189.74	5.00 ± 0.40	8.50 ± 0.80	7.50 ± 0.76	8.03±0.55	6.50±1.04	4.50±0.39	40.03±2.23
284.60	5.50 ± 0.64	4.74 ± 0.47	6.75 ± 0.88	4.50±0.38	5.00±1.15	3.70±0.34	30.19±3.49[a]
ASA 100	6.50 ± 0.64	5.50 ± 0.00	2.25 ± 0.47	4.25±0.47	2.75±0.85	3.00±0.70	24.25±2.75

Data are expressed as mean ± SEM. Significant at [a]P< 0.05, compared to control n = 6.

Table 4: Effect of *Manniophyton fulvum* extract on formalin- induced hind paw licking in mice.

TREATMENT/ DOSE (mg/kg)	TIME INTERVALS (minutes)						
	5	10	15	20	25	30	TOTAL
CONTROL	36.00±0.11	30.5±0.42	18.50±0.44	16.00±0.22	12.24±0.14	8.00±0.15	120.24±2.48
Extract 94.87	26.23±0.88	17.22±0.45	15.00±0.48	14.25±0.27	10.50±0.25	7.63±0.21	90.83±2.54
189.74	22.00±0.91	16.75±0.12	14.94±0.21	12.50 ±0.49	7.75±0.36	7.14 ±0.36	81.08±1.82
284.60	12.0±1.08	12.50±0.23	8.50±0.14	8.25 ±0.12	7.75±0.51	5.50 ± 0.22	54.50±3.96[a]
ASA 100	10.50±0.43	7.50±0.34	5.00± 0.69	5.00 ± 0.22	3.75 ±0.12	3.00±0.92	34.75±0.43

Data are expressed as mean ± SEM. Significant at [a]p<0.05; compared to control. n=6

DISCUSSION

The methanol root extract of *Maniophyton fluvum* produced physical signs of toxicity such as writhing movement, restlessness, increased mobility, decreased respiratory rate, death depending on the dose.

The medium lethal dose (LD_{50}) was estimated to be 948.68mg/kg i.p

The phytochemcial screening revealed that the extract contained polyphenolic compounds (Flavonoids and terpenes). Flavonoids demonstrates its anti-inflammatory activity by blocking cyclooxygenase and lipooxygenase (Carlo, *et al*, 1999) and analgesic activities by blocking cycloxygenase, lipooxygenase and phospholipase A_2 and C. (Nwafor *et al* 2007).

Administration of root extract of *Manniophyton fulvum* on egg albumin – induced oedema in mice caused a significant (p<0.05) dose-dependent anti-inflammatory effect against oedema caused by egg albumin. The effect was comparable to that of standard drug, ASA (100 mg/kg) at the highest dose of the root extract (284.60mg/kg Tables1). The standard drug ASA caused more reduction. The extract caused the reduction probably by blocking two mediators (histamine and 5-HT) released by egg albumin (Nwafor *et al*, 2007).

Anti-inflammatory effect of the extract against xylene-induced ear oedema in mice is shown in Table 2. The extract exerted a dose-dependent significant (p<0.05) anti-inflammatory effects when compared to control. These reductions were incomparable to that of the standard drug, dexamethasone (4.0 mg/kg).The extract caused the reduction probably by blocking the enzyme phospholipase A_2. This enzyme is involved in the pathophysiology of xylene-induced inflammation.

The administration of extract (94.87 – 284.60 mg/kg) demonstrated a dose-dependent reduction

in acetic acid-induced writhing movement in mice. The reductions were statistically significant ($p<0.05$) relative to control and comparable to that of the standard drug, ASA (Table 3). .Acetic acid causes inflammation by increasing capillary permeability (Amico-Roxas *et al*, 1984, Nwafor *et al*, 2007).

The stem extract exhibited a dose-dependent effect on formalin-induced hind paw licking(sign of pain) in mice. This inhibition was significant relative to the control ($p<0.05$) and comparable to that of the standard drug, ASA at the highest dose, 284.60 mg/kg (Table 4).

Formalin induces two types of pains which occur in two phases (0 to 5min and 15 to 30min) respectively after formalin injection. The ability of the extract to inhibit both phases of formalin-induced hind paw licking suggests that it has both central and peripheral analgesic activities. It also suggests that the extract inhibited bradykinins, substance p, histamine and prostaglandins which are the mediators of these pains.
(Yi-Yu, *et al*, 2008).

CONCLUSION

The result obtained from this study confirmed that the ethanol extract of *maniophyton fluvum* contained polyphenolic compounds and these compounds are believed to be responsible for the anti-inflammatory and analgesic activities of the plant extract.

REFERENCES

Akah, P. A, and Nwanibe, A., (1994). Evaluation of Nigerian Traditional Medicine I. Plants used in Rheumatic (Inflammatory) Disorders. *J. Ethnopharmacol*, 42: 179-182.

Amico-Roxas, M., Caruso A., Trombadore, S., Scifo R., and Scapagnme, U. (1984). Gangliosides Antinociceptive Effects in Rodents. Achieves of *International Pharmacodynamics and Therapeutics* 272: 103-117.

Burkill, H. M. (1994). The useful Plants of West Tropical Africa, 2nd Edition. Royal Botanic Gardens, Kew, Richmond: United Kingdom, 636 p.

Carlo, D. G. Mascolo, N. Capasso, F. (1999).Flavonoids, Old and New Aspecys of a Class of Natural Therepeutic Drugs. *Life Science* 65:337-353.

Essien, E. E. Nia, R. Paper, D. H., Franz, Muguanza, M. and Hohmann, G. (2005).“Antioxidant and Anti-Inflammatory Activity of *Manniophyton fulvum”. Act Horticulture* 678:97-101.

Harper D, (2001). Online Enzymology Dictionary. Analgesia Retrieved. 102-108

Lorke, D. (1983). A New Approach to Practical Acute Toxicity Test. *Arch Toxicol 54: 393-404.*

Mbagwu, H. O. C., Anene, R. A., and Adeyemi O. O. (2007). Analgesic, Antipyretic and Anti-Inflammatory Properties of *Mezoneuron benthamianum Bail* Caesalpiniaccea. .*Nigeria quarterly Journal of hospital medicine.* 17 (1): 35-41.

Nwafor, P. A. Jacks, T. W., and Ekanem, A. U. (2007). Analgesic and Anti-Inflammatory effect of methanolic extract of *Pavsinystalia mecroceras* stem bark in rodents. *Journal of pharmacology 3: 86-90.*

Okokon, J. E., and Nwafor, P. A. (2010). Anti-Inflammatory, analgesic and antipyretic activities of ethanolic root extract of *Croton zambesicus*”. *Pakistan Journal of Pharmaceutical Sciences.*23 (4): 383:390.

Okoli, I. C. Ebere, C. S. Uchegbu, M. C., Udah, A. and Ibeawuchi, I. I., (2003). A Survey of the Diversity of Plants Utilized for Small Ruminant Feeding in South-Eastern Nigeria. *Agriculture, Ecosystems and Environment* 96 (1-3): 147:154.

Sofowora, E. A. (1993). Medicinal Plants and Traditional Medicine in Africa, 2nd Edition. Spectrum Books Limited, Ibadan, Nigeria, pp. 1-88.

Trease, G. E., and Evans, W. C. (2002). Pharmacognosy; London.BailliereTindall: 220-253, 401-404; 527-534.

Usifoh Cyril, Udobre Aniefiok, Isang Mandu and Essien Grace (2016). Phytochemcial and some Biological Activities of the stem Bark Extract of *Pentaclethra macrophylla* Benth. *European Journal of Biomedical and Pharmaceutical Sciences*, India 3 (9) 538-541.

Yi Yl, Zong HA, Lu Zm, XuHy Zhang Xm, Dou Wf and Xu ZH (2008). Analgesic and Antiinflammatory effects of the dry matter of culture broth of *Termitomyces albuminosus* and its extracts. *Journal of Ethnopharmacology* 120: 432-436.