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Antinociceptive effect of the ethanol extract of the stem bark of *Musanga cecropioides* in mice

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Abstract
Musanga cecropioides R. Apud Tedlie (Cecropiaceae), also known as umbrella tree is one of the medicinal plants used in Nigeria for pain and inflammation. The stem bark was extracted with absolute ethanol and screened for analgesic activities. The screening for analgesic properties was done using: acetic acid induced writhing; formalin-induced pain, tail immersion and hot plate methods all in mice. Mice were divided into five groups of five each. The first group in all models served as control and received normal saline (0.9 % NaCl), the next three groups received either 50 or 100, 200 and 400 mg/kg of the extract. The 5th group was given a reference drug either pentazocine (10 mg/kg) or aspirin (100 mg/kg). The extract at 50,100 and 200 mg/kg showed a dose dependent significant reduction of the number of writhes ($p<0.05$, $p<0.05$ and $p<0.0001$ respectively) compared with the control. The extract also showed a non-dose dependent significant reduction of the paw licking time in the formalin-induced pain test with more prominence in the second phase. A significant increase in the mean reaction time of mice in the tail immersion and hot plate tests ($p<0.0001$) was also produced by the extract in comparison with the control. This effect however was not dose dependent. The results suggest that the plant has significant peripheral and central analgesic effects, which may not be dependent on dose. Inhibition of the synthesis of prostaglandins may account for its peripheral analgesic effect, while its action on central receptors may account for its central analgesic activities.

Keywords: Writhes; Neurogenic pain; Peripheral pain; *Musanga cecropioides*

INTRODUCTION

Herbal medicine sometimes referred to as herbalism or botanical medicine is the use of herbs for their therapeutic or medicinal value. The world health organization estimates that 4 billon people, 80 % of the world population presently use herbal medicine for some aspects of primary health care, based its cheapness and low incidence of side effects (Narayana *et al*., 1998).

Musanga cecropioides R. Apud Tedlie (Cecropiaceae) is an ever green straight-stemmed rapidly growing tree up to 20 m tall with an umbrella-shaped crown. The plant is locally known as "*oghohoon*" amongst the Binis in Edo state, "*aga*" amongst the Yorubas and "*ooroo*" in the Igbo speaking part of Nigeria (Schnell, 1950).

Musanga cecropioides is believed to have analgesic properties. It is used for the management of pain, asthenia, loss of appetite and in the induction of painless child birth (Adjanohoun *et al.*, 1989).

It is also used as an expectorant and dehydrant (Bouquet and Debray, 1974). The root bark is chewed with kola nuts as a cough

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cure and the bark is tied onto wounds where it is supposed to effect a rapid healing (Bouquet, 1969). The aqueous and ethanol extracts of the stem bark have been screened for hypotensive, hypoglycaemic and oxytocic properties (Ayinde *et al.,* 2003; 2006).

In this study, the biological activity of the ethanol extract of the plant material was evaluated for analgesic effect to examine the claims made of its effects in pain therapy in folk medicine (Adjanohoun *et al.,* 1989).

Ethanol was the extracting solvent because the plant is used locally by soaking in gin which is an alcoholic drink.

EXPERIMENTAL

Plant material. The stem bark of *Musanga cecropioides* was collected from Oluku, Ovia North East local government area, Edo state of Nigeria in June, 2008. The plant was identified by Dr B. A. Ayinde of the Department of Pharmacogonosy, Faculty of Pharmacy, University of Benin, Benin City. Botanical authentication of the plant was confirmed at the Forestry Research Institute of Nigeria (FRIN) Ibadan, Nigeria where a voucher specimen (No FHT 106428) was deposited for future reference. Immediately after collection, the bark was cut into pieces and air-dried for one week in the laboratory after which they were further dried for 3 days in an oven maintained at 40 ºC. The barks were pulverized using an impact mill (Christy and Morris Ltd. UK. Model 474/54).

The powdered bark (300 g) was extracted by maceration using absolute ethanol. The filtration was done using a funnel and filter paper (Whatman filter paper). After filtration, the filtrate obtained was concentrated *in* v*acuo* in a rotary evaporator giving a yield of 5.53 %w/w. The extract was stored in sample bottle, labeled and kept in a refrigerator maintained at 4 ºC before use.

Drugs and chemicals. The drugs and chemicals used include pentazocine (Sigma-Aldrich, UK), acetylsalicylic acid (Sisbu Xierkang Pharm Co. Ltd, India), absolute ethanol (Sigma Aldrich, UK), acetic acid (Sigma-Aldrich, UK), and formalin (Sigma-Aldrich, UK).

Animals. Swiss albino mice weighing 20-30 g of either sex fed on standard diet (Bendel Feeds and Flour Mill Ltd., Ewu, Nigeria) were obtained from the Animal House, Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin. The animals were allowed free access to feed and water. They were maintained in the laboratory for a minimum period of 14 days prior to experimentation. Ethical approval for the study was obtained from the Faculty of Pharmacy, University of Benin, Ethical Committee on the use of Animals for Experiments. Animals were handled according to standard protocols for the use of laboratory animals. (National Institute of Health, USA: Public Health Service Policy on Humane care and use of Laboratory animals, 2002)

Acetic acid induced writhing. The method of Koster *et al.,* 1959 was used. Mice (25) were randomly selected into five groups of five mice each. Group one served as control (normal saline 10 ml/kg, orally), groups 2, 3, and 4 received the ethanol extract (50, 100 and 200 mg/kg respectively, orally) while group 5 received acetylsalicylic acid (100 mg/kg *i.p*). This was done 30 min prior to the intraperitoneal injection of acetic acid (10 ml/kg of 0.6 %v/v in normal saline). These doses were picked after an initial preliminary study. The extract was dissolved in distilled water. The numbers of writhes were counted immediately after acetic acid administration for 30 minutes.

Formalin-induced pain test. The method of Shibata *et al.,* 1989 was used. Formalin (20 µl of 1 % solution) was injected subcutaneously into the right hind paw of the mice. The time (in seconds) spent in licking responses of the

injected paw were taken as an indicator of pain response. Responses were measured for 5 min after formalin injection (first phase, neurogenic pain) and 15-30 min after formalin injection (second phase, inflammatory pain). Mice (25) were placed in five (5) groups of five (5) mice each. Extract (100, 200 and 400 mg/kg, orally) and pentazocine (10 mg/kg, *i.p*) were administered 30 min prior to formalin injection. Control animals received normal saline (10 ml/kg). These doses were picked after an initial preliminary study.

Hot plate test. Mice were divided into five groups of five each after initial screening. The cut-off time/end point for mice was 2 mins. After which they were removed and screened out of the experiment if there was no response. The extract at doses of 100, 200 and 400 mg/kg was given to the first three groups orally via an oro-gastric syringe. The fourth group received the reference drug, pentazocine (10 mg/kg) intraperitoneally, while the 5^{th} group served as control and received normal saline orally (10 ml/kg). These doses were picked after an initial preliminary study. The animals were dropped gently on a hot plate (Ugo Basile Hot/Cold plate, model 35100) maintained at 55 ± 0.5°C (Tuner, 1965). This was done before the administration of drug/extract and at 30, 90 and 150 min following administration. The time in seconds for the mouse to either jump or lick its hind paw was taken as the reaction time.

Tail immersion test. A water bath regulated to a temperature of 55.0 ± 1.0°C to obtain an animal reaction (time in seconds for the mice to flick their tail) was used; this is a modification of the method as described by Janssen *et al.*, 1963.

Mice were also divided into five groups of five each after initial screening. The extract at 100, 200 and 400 mg/kg were given to the first three groups orally via an oro-gastric syringe. The fourth group received the reference drug, pentazocine (10 mg/kg) intraperitoneally, while the 5^{th} group served as control and received normal saline orally (10 ml/kg). These doses were picked after an initial preliminary study. The tails of the mice (1 cm) were immersed into the water bath prior to treatment with extract, pentazocine and normal saline according to the different groups and again at 30, 90 and 120 minutes following treatment.

Statistical analysis. All data are expressed as mean ±SEM (standard error of mean) and where applicable, the data were compared using student's t-test with Graph pad instant version 2.05a software. The level of significance was set at $P < 0.05$.

RESULTS

Acetic acid induced writhing. Table 1 shows the effect of the extract on acetic acid induced writhing. All doses of the extract significantly ($p<0.05$, $p<0.05$, $p<0.0001$ respectively for 50, 100 and 200 mg/kg) inhibited the writhes induced by acetic acid, in mouse in comparison with the controls. This inhibitory effect however was most significant with the 200 mg/kg dose of the extract with mean number of writhe of 13.60. Acetylsalicylic acid had the greatest inhibitory effect, though the inhibitory effect of the 200 mg/kg dose compares well with it.

Formalin induced pain. The results are presented in Figures 1 and 2. Fig 1 shows the effect of the extract on the first phase of pain induced by formalin. The extract at all doses when compared with the control significantly ($p<0.05$) inhibited the first phase (neurogenic pain). Fig 2 shows the effect of the extract on the second phase (inflammatory pain). This reveals that the extract also significantly ($p<0.05$) inhibited this phase at all doses when compared with the controls. Pentazocine, had the greatest inhibitory effect in 2^{nd} phase of pain induction by formalin ($p<0.0001$). In the first phase there was no

significant difference in the inhibitory effect produced by the extract and pentazocine, however in the second phase, pentazocine had a greater inhibitory effect, though the inhibitory effect of the 400 mg/kg dose compares well with pentazocine.

Hot plate test. Figure 3 shows that the different doses of the extract increased the mean reaction time of the mice on the hot plate, but this effect was however most significant ($p<0.0001$) with the 200 mg/kg dose, pointing to a non-dose dependent effect. Its increase of the mean reaction time was most prominent in the 90 and 150th min.

At the 90th min, all doses of the extract, including pentazocine significantly increased the mean reaction time. The effect of the 200 mg/kg dose was greater than that of pentazocine. At the 150th min, all doses of extract and pentazocine also significantly increased the mean reaction time, here again the 200 mg/kg dose produced a higher response.

Table 1: The effects of the ethanol extract of the stem bark of *M. cecropioides*, normal saline and aspirin on acetic acid-induced writhing test in mice.

Treatment (mg/kg)	No of writhes (per 30 minutes)	Percentage inhibition (%)
Control (normal saline)	33.0 ± 1.98	-
M.C(50)	16.25 ± 1.11 ^{a,ab}	50.75
M.C (100)	17.75 ± 2.39 ^{a,ab}	46.10
M.C (200)	13.60 ± 1.89 ^{b,ab}	58.79
Aspirin (100).	8.60 ± 0.51 ^{b}	73.94

Values are mean number of writhes ± SEM (standard error of mean). (n = 5, per group).
$^{a}P<0.05$, $^{b}P<0.0001$ significantly different from control and $^{ab}P<0.05$ significantly higher than aspirin.
M.C- Musanga cecropioides

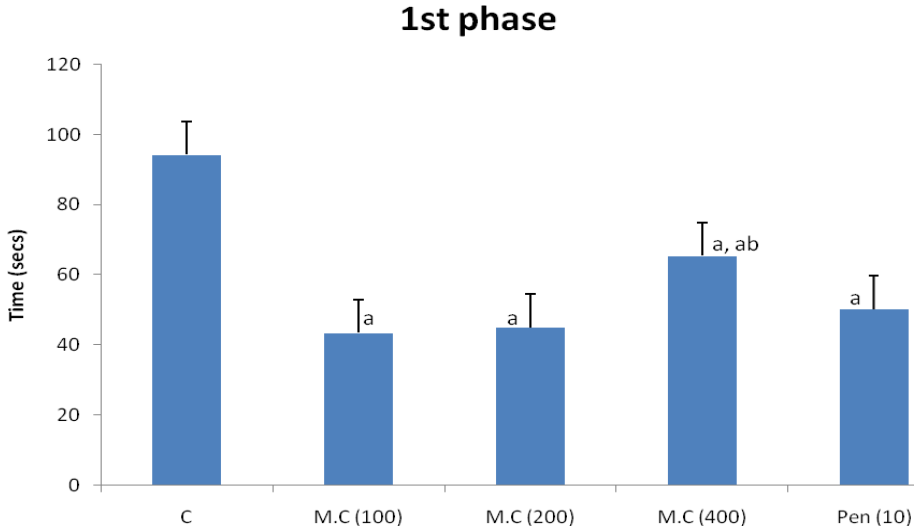

Fig 1: Effect of the extract of *Musanga cecropioides* (M.C) on the first phase of formalin induced pain compared to the control (C) and pentazocine (Pen) treated groups.
Values are mean reaction time in seconds ± SEM (n = 5 per group). $^{a}P<0.05$ significantly different from the control group (C) and $^{ab}P<0.05$ significantly higher than the pentazocine group.

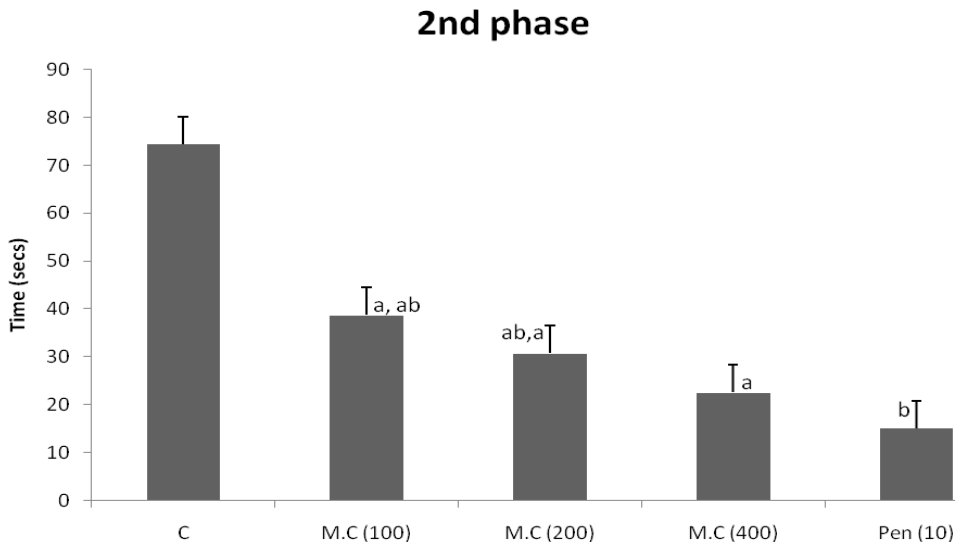

Fig 2: Effect of the extract of *Musanga cecropioides* (M.C) on the second phase of formalin induced pain compared to the control (C) and pentazocine(Pen) treated groups.
Values are mean reaction time in seconds ± SEM (n = 5 per group). [a]P<0.05, [b]P<0.0001 significantly different from the control group and [ab]P<0.05 significantly higher than the pentazocine treated group.

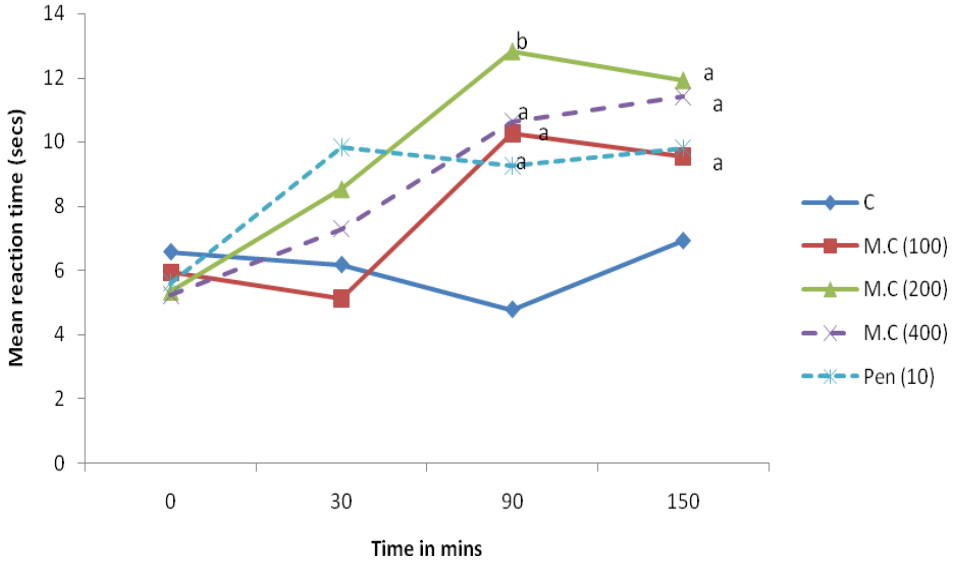

Fig 3: Effect of the extract of *Musanga cecropioides* (M.C) on hot plate reaction time compared to the control (C) and pentazocine (Pen) treated groups. Values are mean reaction time in seconds ± SEM (n = 5 per group). [a]P<0.05, [b]P<0.0001 significantly different from the control group.
The 0 min indicates the mean reaction time on the hot plate prior to any administration.

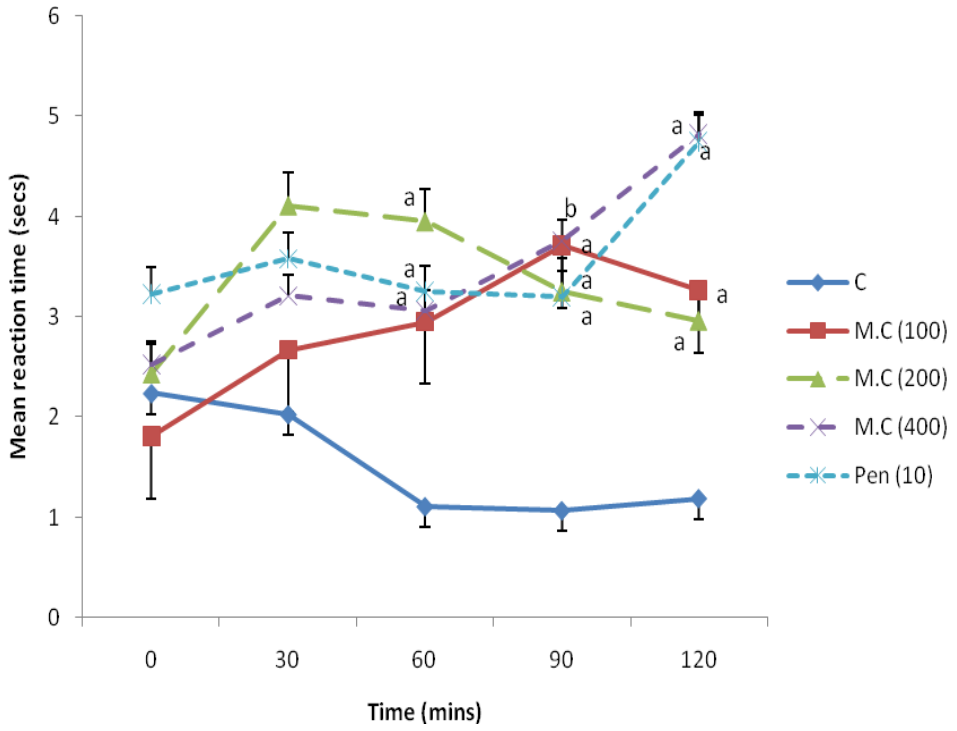

Fig 4: Effect of the extract of *Musanga cecropioides* (M.C) on tail immersion compared to the control (C) and pentazocine (Pen) treated groups. Values are mean reaction time in seconds ± SEM (n = 5 per group). [a]P<0.05, [b]P<0.0001 significantly different from the control group.
The 0 min indicates the mean reaction time on the water bath prior to any administration.

Tail immersion. Figure 4 represent results of tail immersion experiments. Evaluation of the results points to the fact that the highest dose (400 mg/kg) of the extract significantly ($p<0.001$) increased the mean reaction time of the mice compared with the control group. The increase in the mean reaction of mice was noted from the 60 to 120th min. At the 60th min, 200 mg/kg dose had the highest effect, better than the increase in mean reaction time induced by pentazocine. At the 90th min, pentazocine had the highest prolongation of the mean reaction time, with the effect produced by 100 mg/kg close to that of pentazocine. Hence the effect of the extract compares well with pentazocine. At the 120th min both pentazocine and the 400 mg/kg dose produced the highest and similar prolongation of the mean reaction time.

DISCUSSION

In the acetic acid induced writhing test, it was observed that the 200 mg/kg dose of the extract produced significant ($p<0.0001$) inhibition of acetic acid induced writhes compared with the control.

Writhes can be described as a wave of constriction and elongation passing caudally along the abdominal wall with twisting of the trunk and extension of the hind limbs in mice. This is due to the nociceptive property of acetic acid (Singh *et al.*, 1996). The results show that the extract has ability to inhibit writhes induced by acetic acid. It was also noted that the inhibitory effect of the highest dose (200 mg/kg) of the extract was as significant as that of aspirin ($p<0.0001$), the reference standard drug used. This may thus suggests that the extract possesses analgesic activity, which could be attributed to a

peripheral mechanism, since the responses via acetic acid are thought to involve local peritoneal cells and mediated by the prostaglandins pathways (Ronaldo *et al.*, 2000). This is believed to be through the inhibition of the synthesis and release of prostaglandins. The abdominal constricting response induced by acetic acid is a sensitive procedure to establish peripherally acting analgesics; hence the analgesic effect of the extract may be peripherally mediated (Koster *et al*., 1959).

In the formalin induced pain test, a significant decrease in the mean reaction time in mice was observed for both the first and second phases. The effect of the extract on the first phase (neurogenic pain) was however not dependent on dose though it was as significant as that of pentazocine.

In second phase (inflammatory pain), there was a significant reduction in the time spent licking the paw, this effect was noted to be dose dependent, though not as significant as that of pentazocine ($p<0.0001$).

Formalin produces pain by two phases: neurogenic pain, which involves the release of substance P and inflammatory pain which involves the release of histamine, serotonins, bradykinins and prostaglandins (Shibata *et al.,* 1989). The ethanol extract of *Musanga cecropioides* seems to have inhibited both phases suggesting that the extract may act as both a narcotic analgesic and a non steroidal anti-inflammatory drug.

A careful evaluation of both figures 1 and 2 shows that the effect of the extract was more prominent in the second phase than in the first phase. This observation can be used to confirm that the analgesic effect of the extract could be peripherally mediated via the inhibition of the release of prostaglandins, histamine, bradykinins and other inflammatory mediators (Koster *et al.,* 1959). Its inhibitory effect on the second phase also points to a central analgesic property. It can thus be inferred that the plant could be effective in inflammatory pain as in rheumatoid arthritis and in central pain.

On tail immersion, figure 4 points to a significant increase in the mean reaction time of mice at all doses compared with the control. This effect was more significant at 400 mg/kg dose ($p<0.0001$). Increase in reaction time simply suggests that the extract possesses some central analgesic activity. Furthermore, it is known that centrally acting drugs elevate the pain threshold of mice towards heat (Adeyemi *et al*., 2004).

In the hot plate test, the extract at all doses significantly increased the time spent by the mouse on the hot plate ($p<0.05$), compared with the control (Fig 3). This effect was more prominent with the 200 mg/kg dose, suggesting a non-dose dependent effect. Increase in the reaction time just as it was in the tail immersion test is a pointer to the fact that the extract does have central analgesic property.

Thus from both the hot plate and tail immersion experiments, increased mean reaction times of mice can be equated with stress tolerance capacity of mice. This indicates as earlier stated the possible involvement of a higher centre (Vogel and Vogel, 1997). It may therefore be said that the extract possesses narcotic like activity and thus hence may be useful in the management of neurogenic pain.

The activity showed by this extract is of considerable importance and justified its use in pain as suggested in folklore medicines.

Conclusion

This study provides a scientific basis for the folkloric use of *Musanga cecropioides* in the management of pain. The extract possesses analgesic activity that could be employed as an alternative treatment to orthodox medicine. Its analgesic effect is both peripherally and centrally mediated. Further

study is needed to identify the chemical constituents responsible for its effect.

REFERENCES

Adeyemi OO, Okpo SO and Okpaka O (2004). The analgesic effect of the methanolic extract of *Acanthus motanus*. *J. Ethnopharmacol* 90:45-48.

Adjanohoun EJ, Adjakidjè V, Ahyi MRA, Ake Assi L, Akoègninou A, d'Almeida J Apovo F, Boukef K, Chadare M, Cusset G, Dramane K, Eyme J, Gassita J N,Gbaguidi N, Goudote E, Guinko S, Houngnon P, Issa LO, Keita A, Kiniffo HV, Kone-Bamba D, Musampa Nseyya A, Saadou M, Sodogandji T, De Souza S, Tchabi A, Zinsou DC and Zohoun T (1989). Contribution aux études ethnobotaniques et floristiques en République Populaire du Bénin. Agence de Coopération Culturelle et techniques. Paris, 895

Ayinde BA, Onwukaeme DN and Nworgu ZAM (2006). Oxytocic effects of the water extract of *Musanga cecropiodes* R. Brown stem bark. African *Journal of Biotechnology* 5(14):1350-1354.

Ayinde BA, Omogbai EKI and Onwukaeme DN (2003). Pharmacognostic characteristics and Hypotensive effect of the stem bark of *Musanga cecropioides*. *West African Journal of Pharmacology and Drug Research* 19: 2-4.

Bouquet A (1969). The Useful Plants of West Tropical Africa. Vol 1.

Bouquet A, Debray M (1974). The Useful Plants of West Africa. Plantes Medicinales, (1): 146-147.

Janssen PAJ, Neimegeers CJE and Dony JGH (1963). The inhibitory effect of fentenyl and other morphine-like analgesics on the warm water induced tail withdrawal reflex in rats. *Arznei mittel forschung* 13: 502-507.

Koster R, Anderson M and De Berar EJ (1959). Acetic acid for analgesic screening. Federation Proceedings 18:412-416.

Narayana DBA, Katayar CK and Brindavanan WB (1998). Original system search, research or re-search IDMA Bulletin 29 (17): 413-416.

Ronaldo AR, Mariana HV, Sara MT, Adriana BPP, Steve P, Ferreira SH and Fernado QC (2000). Involvement of resident macrophages and most cells in the writhing nociceptive response induced by zymosan and acetic acid in mice. *Eur J. Pharmacol* 387: 111-118.

Schnell KW (1950). Wood Plants of Ghana. London; Oxford University Press, 257.

Shibata M, Ohkubo T, Takahashi H and Inoki R (1989). Modified formalin test, characteristic biphasic pain response. *Pain* 38: 755-759.

Singh S, Majumadar DK and Rehan HMS (1996). Evaluation of anti-inflammatory potential of fixed oil of *Ocimum santum* (Holy Basil) and its possible mechanism of action. *J. Ethnopharmacol*. 54: 19-26.

Turner RAC (1965). Screening Methods in Pharmacology. Academic Press. New York, 158.

Vogel HG and Vogel WH (1997). In D*rug* Discovery and Evaluation. Pharmacological Assays Springer Verlag, Germany, 368-370.