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ORIGINAL ARTICLE

Anti-inflammatory and anti-oxidant activities of *Mallotus oppositifolius* (Geisel) methanol leaf extracts

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Abstract The methanol leaf extract of *Mallotus oppositifolius* was evaluated for anti-inflammatory activity in rats and mice using acute and chronic anti-inflammatory models with acetylsalicylate acid (aspirin) as the reference drug. The antioxidant activity was done in vitro using ferric reducing antioxidant power (FRAP) and 1,1-diphenyl-2-hydrazyl (DPPH) spectrophotometric assays. The extract dose dependently and significantly reduced paw edema volume in rats induced by carrageenan ($p < 0.01$), decreased croton oil-induced ear inflammation ($p < 0.05$), inhibited cotton pellet-induced granuloma in mice and reduced the rat paw thickness in formalin-induced arthritis.

1. Introduction

Inflammation is the response of living tissues to injury. It is a complex pathophysiological process mediated by a variety of molecules produced by leukocytes, macrophages and mast cells as well as by enzyme activation and mediator release which bring about tissue breakdown and edema formation as a result of extravasation of fluid and proteins and accumulation of leukocytes at the inflammatory site (White, 1999; Katzung, 2004). Inflammation is typically a protective mechanism that is triggered in response to noxious stimuli; trauma or infection to protect the body and to facilitate recovery process, however, if unchecked leads to chronic inflammatory disorders.

A large number of steroids and non-steroidal anti-inflammatory drugs (NSAIDS) are available in the market

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for the treatment of inflammation (Rang et al., 2003). However, despite their great number, their therapeutic efficacy seems to be hampered because they are often associated with serious adverse side effects which include: gastro-intestinal irritation, ulcers, bone marrow depression, metabolic disorders, hypertension etc. (Wolfe and David, 1999).

Oxidative stress is an important factor in the genesis of most pathologies, ranging from cancer to cardiovascular and degenerative diseases (Parthasarathy et al., 2001). In order to protect the body against the consequences of oxidative stress, an efficacious approach consists in improving the antioxidant nutrition. Antioxidants from natural sources have a higher bioavailability and therefore higher protective efficacy than synthetic antioxidants (Benedetti et al., 2004).

It is therefore of utmost importance to search for less toxic anti inflammatory and antioxidant drugs and medicinal plants have been documented to have advantage in toxicity considerations based on their long term use and one might expect bioactive compounds obtained from such plants to have low animal and human toxicity (Fabricant and Farnsworth, 2001). Also the use of herbal remedies for arthritis treatment has been gaining momentum in recent years (Chrubasik et al., 2007).

Mallotus Oppositifolius (Geisel) (MO) belongs to the family *Euphobiaceae*. It is a shrub or small tree that grows up to 13 m high and widespread across tropical Africa to Madagascar (Burkill, 1985). In Nigeria the plant is known as ''Kafar Mutuwaa'' by Hansas (Northern Nigeria) ''Nne Okpo Kirinya'' by Igbos (Eastern, Nigeria) and ''Ija'' by Yorubas (Western Nigeria). The folkloric uses include the use of leaves for the treatment of parasitic, eye and kidney infections, as diuretic, pain killers, treatment of paralysis, spasm, headache and swellings. Decoction of the root is used for anemia, pneumonia and as aphrodisiac, and the stick is chewed for oral hygiene and teeth cleaning (Burkill, 1985; Idu et al., 2007). The antifungal, antibacterial and antimalarial activities of the ethanolic and aqueous leaf extracts of *M. oppositifolius* have been reported and the phytochemical screening showed the presence of anthocyanins, flavonoids, saponins steroids and tannins (Chukwujekwu et al., 2005; Adekunle and Ikumapayi, 2006).

In consideration of the claimed efficacy of *M. oppositifolius* in Nigerian folk medicine for the treatment of many diseases which may be caused by oxidative stress and swelling (edema), we investigated the methanol leaf extract for anti-inflammatory and antioxidant activities.

2. Materials and methods

2.1. Plant collection and identification

The leaves of *M. oppositifolius* were collected from the botanical garden of the University of Nigeria Nsukka, Enugu State, Nigeria and were identified by a botanist, Mr. A. Ozioko of Bioresources Development and Conservation Programme (BDCP) Aku road, Nsukka, Enugu state. A voucher specimen number VPP/UNN/075/2010 was deposited in the University herbarium.

2.2. Extraction of plant material

Cold maceration was for the extraction. The leaves were air dried at room temperature (27 °C) and pulverized into a coarse powder using a laboratory mill. 500 g of the plant material was extracted in 80% methanol for 48 h with manual intermittent shaking at 2 h interval. The extract was then filtered using Whatman No 1 filter papers and later concentrated *in vacuo* using rotary evaporator at 40 °C and 210 millibar. It was then freeze dried with lyophilizer to preserve the extract in cases of power outage. The yield of the extract was determined and the extract was stored in a refrigerator at 10 °C until the time of use (6 weeks).

2.3. Animals

Mature albino Wistar rats (150–200 g) and mice (25–30 g) of both sexes obtained from the laboratory facilities of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka were used for the experiment. The animals were kept in stainless steel cages at the temperature of between 27 and 30 °C and relative humidity of about 50–55% with lighting period averaging 12 h a day. The animals were given clean drinking water and provided with standard pelleted feed (Vital feed® Nigeria) *ad libitum*. The study protocol was approved by the University of Nigeria Nsukka ethical committee and ethical guidelines governing the use of live animals for the conducts of experiment as stipulated by Zimmerman (1983) and Ward and Elsea (1997) were strictly observed.

2.4. Anti-inflammatory studies

2.4.1. Carrageenan-induced rat paw edema

Paw edema was induced by the method of Winter et al. (1962). 30 rats of mixed sexes were divided into 5 groups of 6 rats each. Group 1 (negative control) rats received 10 ml/kg normal saline, group 2 (positive control) rats were given 100 mg/kg acetylsalicylate acid (ASA) or Aspirin while groups 3,4, and 5 rats received 50, 100 and 200 mg/kg of *M. oppositifolius* (MO) extract respectively all by gastric gavage. Thirty minutes prior to administration of the extract and drug paw edema was induced in the rats by injecting 0.1 ml of 1% carrageenan in physiological solution into the subplantar aponeurosis of the left hind paw of each of the rats. The right hind paw served as control. The paw volume of the rats was measured 4 h after carrageenan injection by the mercury displacement method using a plethysmograph.

2.4.2. Croton oil-induced ear inflammation

The method described by Brooks et al. (1985) was employed for this study. 30 mature albino Wistar mice of mixed sexes, divided into 5 groups of 6 mice each were treated as follows: 50, 100 and 200 mg/kg of *M.* oppositifolius (MO) extract were given to groups 3, 4 and 5 mice while normal saline (10 mg/kg) and 100 mg/kg ASA were given to the negative (group 1) and positive (group 2) control groups respectively by gastric gavage 30 min before croton oil application. Croton oil irritant solution (0.1 ml) was applied externally to the outer surface of the right ear of each mouse. Four hours after the application of croton oil the mice were sacrificed by cervical dislocation and 7 mm punches were made in the ear

with a cork borer. Each ear disk was weighed and compared with the control.

2.4.3. Cotton pellet-induced granuloma

Wistar albino mice of both sexes were divided into 5 groups of 6 mice per group. Two sterilized and autoclaved cotton pellets weighing 10 mg each were implanted subcutaneously into both sides of the dorsal area of each mouse (D'Arcy et al., 1960). Group 1 (negative control) rats received 10 ml/kg normal saline, group 2 (positive control) rats received 100 mg/kg ASA while groups 3, 4 and 5 rats received 50, 100 and 200 mg/kg respectively of MO extract all by gastric gavage for 7 days. On the 8th day the animals were sacrificed and the pellets together with the granuloma tissues were carefully removed, dried in the oven at 60 °C, weighed and compared with the control.

2.4.4. Formalin-induced arthritis

24 mature albino rats were randomly divided into 4 groups of 6 each. Initially, the left hind paw thickness of the rats was measured using venire caliper. Chronic inflammation/arthritis was induced by the injection of 0.02 ml of 2.5% freshly prepared formaldehyde solution into the sub plantar area of the hind paw of the rats (Sen and Nag-Chaudhari, 1991). The rats were pretreated per os using gastric gavage prior to formaldehyde injection as follows: group 1 (negative control) rats were given 10 ml/kg normal saline, group 2 (positive control) rats received 100 mg/kg ASA while groups 3 and 4 received 50 and 100 mg/kg of MO extract respectively. The rats were treated for 7 days. The left hind paw thickness of each of the rats was measured daily before each treatment from day 2.

2.5. Anti oxidant study

2.5.1. DPPH antioxidant assay

The free radical scavenging activity of *M.* oppositifolius (MO) extract was analyzed by DPPH photometric assay (Mansor et al., 2001). 2 ml of test extract at concentrations ranging from 100 to 400 μg/ml was each mixed with 1 ml of 0.5 mM DPPH in methanol. Absorbance at 517 nm was taken after 30 min incubation in the dark at room temperature using a spectrophotometer. The experiment was done in triplicates and the percentage antioxidant activity was calculated as follows:

$$\%\text{Antioxidant Activity}[\text{AA}] = 100 - [\{(\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}}) \times 100\}/\text{Abs}_{\text{control}}].$$

where 1 ml methanol + 2 ml extract were used as blank while 1 ml 0.5 mM DPPH solution + 2 ml methanol was used as control. Ascorbic acid was used as reference standard.

2.5.2. FRAP

Total antioxidant activity was measured by ferric reducing antioxidant power (FRAP) assay (Benzie and Strain, 1999).

Sample (100 μl) of different concentrations of the extract (10, 50, 100, 200 and 400 μg/ml) was mixed with 3 ml of working FRAP reagent. The absorbance was immediately taken at 593 nm at 0 min. Thereafter samples were placed at 37 °C in water bath and absorbance was measured after 4 min with a spectrophotometer. Ascorbic acid (100 μl) was used as standard. FRAP value of the samples was calculated using the formula below.

$$\text{FRAP value} = \frac{\text{change in absorbance from } 0-4\text{min} \times \text{FRAP value of standard}}{\text{change in absorbance of standard form } 0-4\text{min}} \quad (2)$$

3. Data analysis

Results were presented as mean ± SEM and analyzed using one way analysis of variance (ANOVA). The differences between the means were tested using Post HoC Duncan and the value of $P < 0.05$ was considered statistically significant.

4. Results

4.1. Anti-inflammatory studies

4.1.1. Carrageenan-induced paw edema

The extracts of *M. oppositifolius* exhibited a dose dependent and significant ($P < 0.01$) reduction in the paw edema volume of rats induced by carrageenan, reducing the paw edema volume from 0.61 ± 0.01 ml in the negative control rats to 0.20 ± 0.02 ml at the dose of 200 mg/kg representing 67.2% inhibition of edema formation. This effect of the extract at the dose of 200 mg/kg was better than the reference drug aspirin (ASA) at 100 mg/kg (Table 1).

4.1.2. Croton coil-induced ear inflammation

The reference drug and MO extract at the doses of 100 and 200 mg/kg significantly ($P < 0.05$) reduced the mice ear granuloma from 13.0 ± 5.0 mg in the normal saline (negative control) treated group to 11.0 ± 2.0 mg by ASA and to 9.8 ± 2.0 mg at the dose of 200 mg/kg of the extract 4 h after croton oil application. MO extract at the dose of 50 mg/kg had a slight increase of the ear inflammation as shown in Table 1.

4.1.3. Cotton pellet-induced granuloma

The methanol leaf extract of MO and the reference drug ASA significantly ($P < 0.001$) inhibited the development of granulomatous tissue induced by cotton pellets in mice when compared to the negative control group. The highest activity was observed at the dose of 200 mg/kg of MO which was better than the reference drug ASA (Table 1).

4.1.4. Formalin-induced arthritis

Fig. 1 shows the result of the effect of MO extract on formalin-induced paw thickness of rats. The formalin-induced pedal edema formation was inhibited significantly ($P < 0.001$) by MO extract when compared to the negative control group. The reduction was dose dependent. ASA also exerted

Table 1 Effects of *Mallotus oppositifolius* extract on carrageenan-induced paw edema in rats, croton-oil induced ear inflammation and cotton pellet-induced granuloma in mice.

Drug	Carrageenan-induced rat paw edema volume (ml)	% reduction of paw edema	Croton oil-induced ear inflammation in mice (mg)	Cotton pellet-induced granuloma in mice (mg)
Normal saline 10 ml/kg	0.61 ± 0.01	0	13.0 ± 5.0*	70.0 ± 4.0
Aspirin 100 mg/kg	0.21 ± 0.02**	65.6	11.0 ± 2.0*	48.0 ± 2.0***
MO extract 50 mg/kg	0.39 ± 0.04**	36.1	13.5 ± 4.0	52.3 ± 1.8***
MO extract 100 mg/kg	0.22 ± 0.01**	63.9	10.9 ± 3.4*	45.3 ± 1.5***
MO extract 200 mg/kg	0.20 ± 0.02**	67.2	9.8 ± 2.0*	29.2 ± 1.3***

* $P < 0.05$, when compared to negative control.
** $P < 0.01$, when compared to negative control.
*** $P < 0.001$ when compared to negative control.

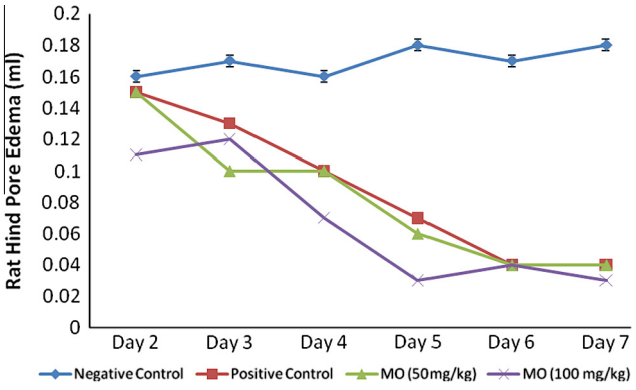

Figure 1 Effect of *Mallotus oppositifolius* on formalin-induced arthritis.

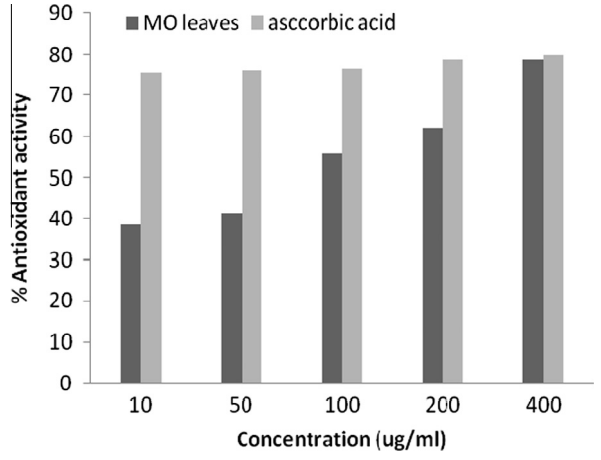

Figure 2 Percentage antioxidant activity of *Mallotus oppositifolius* using DPPH photometric assay.

inhibiting action on the paw edema formation which was comparable to MO extract on day 6 as shown in Fig. 1.

4.2. Antioxidant assay

4.2.1. DPPH

Fig. 2 shows the antioxidant activity of *M. oppositifolius* and ascorbic acid standard. The result showed that MO extract caused a concentration dependent percentage increase of antioxidant activity from 38% at the concentration of 10 μg/ml to 78% at the concentration of 400 μg/ml. The ascorbic acid standard had a better antioxidant activity of 73% at the concentration of 10 μg/ml and 82% at 400 μg/ml.

4.2.2. FRAP

The total antioxidant result estimated through ferric reducing power assay is presented in Fig. 3. The result also showed that MO extract had a concentration dependent ferric reducing power over time with the highest value of 1.92 at the concentration of 400 μg/ml.

5. Discussion

This study evaluated the anti-inflammatory activity of *M. oppositifolius* in laboratory animals using acute and chronic inflammatory models and the antioxidant property evaluated in vitro using 1,1-diphenyl- 2-picrylhydrazyl (DPPH) spectrophotometric assay and ferric reducing antioxidant power (FRAP).

Carrageenan-induced rat paw edema model is a popular and widely accepted model for the study of anti-inflammatory activity of compounds which assesses the degree of inflammation and efficacy of test drugs especially at the acute stage (Alluri et al., 2009). Induction of edema in the paw of rats following subplantar injection of carrageenan results from the release of serotonin, histamine and prostaglandin-like substances (Vinegar et al., 1969; Ratheesh and Helen, 2007). From the results, the extract significantly ($p < 0.01$) reduced the paw edema volume dose dependently in the treated rats just like the standard reference drug ASA and this may be

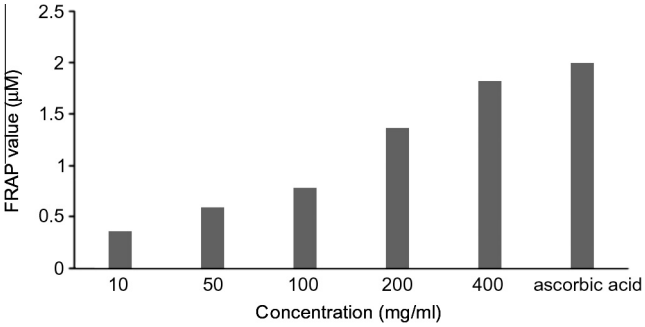

Figure 3 Antioxidant activity of *M. oppositifolius* using FRAP method.

due to the inhibition of the mediators of inflammation such as serotonin, histamine and prostaglandin by the extract (Amberkar et al., 2011). Lipoxygenase inhibitors also play an important role in carrageenan-induced paw edema. In this experiment the ability of *M. oppositifolius* methanol extract to reduce paw edema volume may also be attributed to its inhibitory activity on lipoxygenase enzyme.

Croton oil-induced inflammation is also a model for the evaluation of drugs and extracts against acute inflammation. Severe vasodilatation, edematous changes of skin and inflammatory cell infiltration which are typical signs of acute inflammation are observed after topical application of croton oil or xylene (Ighodaro et al., 2010), therefore croton oil offers a model for exudative type of inflammation (Tonussi and Ferreire, 1994). Although the chemical mediators of this type of inflammation are not known, it is thought that protein synthesis is necessary for the formation of granuloma and kinin is said to also play a role in granuloma formation as it not only causes vasodilatation but also increases vascular permeability in the early stages of inflammation (Shivaji et al., 2000). In this study, *M. oppositifolius* extract significantly inhibited the development of granulomatous tissue in the mice ear at doses of 100 and 200 mg/kg which suggests the antiphlogistic effects of the extract. This may have occurred as a result of extract's effect on protein synthesis or anti-kinin activity.

Cotton pellet-induced granuloma is widely used for the assessment of the transudative, exudative and proliferative components of chronic inflammation. The absorption of the surrounding fluid by the cotton pellet influences the wet weight of the granuloma (Raju et al., 2005). *M. oppositifolius* methanol extract dose dependently reduced the weight of granuloma formed as measured on day 8 suggesting that MO may be effective in the management of chronic inflammation.

Evaluation of the ability of drugs or extracts to inhibit formalin-induced edema in rats is recognized as the most suitable procedure for screening anti-inflammatory and ant arthritic drugs or compounds. Formalin-induced arthritis is a model which evaluates agents with likely anti-proliferative activity (Ighodaro et al., 2010). Formalin produces localized inflammation and pain when injected into the paw of rat which is biphasic comprising of early neurogenic component and a later tissue mediated response (Greenwald, 1991). The result of the formalin-induced arthritis study showed both dose and time dependent reduction of the paw thickness by both the extract and the reference drag confirming the anti-proliferative activity of the extract and therefore suggests that *M. oppositifolius* methanol extract may be effective in the management of arthritis.

DPPH and FRAP spectrophotometric assay methods are employed for the investigation of antioxidant activities of natural compounds (Sreedhar et al., 2010). In this study, the percentage antioxidant activity of *M. oppositifolius* extract in DPPH was increased in a concentration dependent manner comparing favorably with the ascorbic acid standard at the concentration of 400 μg/ml. Also the FRAP value increased with increase in concentration of the extract. According to Ramdas and Seema (2010), substances that increase FRAP value and percentage antioxidant activity in DPPH spectrophotometric assay is assumed to have antioxidant activity. It is therefore not out of place to state that *M. oppositifolius* has demonstrated good antioxidant activity from our results and it can be suggested that the plant may be useful in maintaining health and preventing degenerative diseases such as cancer, diabetes, coronary heart disease, mountain sickness that are exacerbated by the generation of reactive oxygen species (ROS) in the body as suggested by Bailie et al. (2009).

Adekunle and Ikumapayi (2006) reported the presence of tannins, flavonoids, saponins and alkaloids in the leaf extract of *M. oppositifolius* and it has been observed that many flavonoids and alkaloids exhibit anti-inflammatory effects (Martini et al., 2004). It can therefore be suggested that the presence of the reported phytochemical constituents including the flavonoids and alkaloids may have contributed to the observed anti-inflammatory and antioxidant activities.

6. Conclusion

The results of this study have shown that the leaves of *M. oppositifolius* have demonstrated significant level of anti-inflammatory and antioxidant activities in the models used establishing the ethnopharmacological basis for its use in Nigerian traditional medicine for the treatment of edema and some degenerative diseases. However, more work is required for the isolation and characterization of the active principles responsible for these activities.

References

Adekunle, A.A., Ikumapayi, A.M., 2006. Anti-fungal property and phytochemical screening of the crude extracts of *Funtumia elastica* and *Mallotus oppositifolius*. West Indian Med. J. 55 (4), 219–223.

Alluri, V.K., Chundi, B.M., Drdda, S., Krishanu, S., Golakoti, T., 2009. Anti-Inflammatory activity of Vitex Leuoxylon L. Bark extracts against Feud's complete adjuvant- induced arthritis in Sprague Dawley rat. Am. J. Infect. Dis. 5 (2), 68–73.

Amberkar Mohanbabu Vittalrao, Tara Shanbhag, Meena Kumari, K., Bairy, K.L., Smita Shenoy, 2011. Evaluation of anti-inflammatory and analgesic activities of alcoholic extract of Kaempferia galanga in rats. Indian J. Physiol. Pharmacol. 55 (1), 13–24.

Bailie, J.K., Thompson, A.A., Irving, J.B., Bates, M.G., Sutherland, A.I., MacNee, W., Maxwell, S.R., Webb, J., 2009. Oral antioxidant. Supplementation does not prevent acute mountain sickness: double blind randomized placebo-controlled trial. OJM. Int. J. Med. 102 (5), 341–348.

Benedetti, S., Benvenah, F., Pagharami, S., Francegh, S., Stephano, S., Canestrari, F., 2004. Antioxidant properties of a novel phycocyania extract from the blue–green alga Aphanizomenonflos-aquae. Life sci. 75, 2353–2362.

Benzie, F.F., Strain, Z.J., 1999. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and Ascorbic acid concentration. Methods Enzymol. 299, 15–23.

Brooks, R.R., Bonk, K.R., Decker, K.E., Miller, K.E., 1985. Anti-inflammatory activity of parnoxin administered orally and topically to rodents. Agents Actions 16, 269–376.

Burkill, H.M. 1985. The useful plants of west tropical Africa, vol. 2. Royal botanical Gardens, 969.

Chrubasik, J.E., Roufogalis, B.D., Chrubasik, S., 2007. Evidence of Effectiveness of herbal anti-inflammatory drugs in the treatment of painful osteoarthritis and chronic low back pain. Phytother. Res. 21, 675–683.

Chukwujekwu, J.C., Van Staden, J., Smith, P., 2005. Antibacterial and anti-malarial activities of some Nigerian medicinal plants. S. Afr. J. Bot. 71 (3), 46–63.

D'Arcy, P.F., Haward, E.M., Muggleton, P.W., Townseed, S.B., 1960. The anti-inflammatory action of griseofulvin in experimental animals. J. Pharm. Pharmacol. 12, 659–665.

Fabricant, D.S., Farnsworth, N.R., 2001. The value of plants used in traditional medicine for drug discovery. Eviron. Health Prospect. Suppl. 109 (1), 69–76.

Greenwald, R.A., 1991. Animal models for evaluation of arthritic drugs. Methods find. Exp. Clin. Pharmacol. 13, 75–83.

Idu, M., Osemwegie, O.O., Odia, E.A., Onyibe, H.I., 2007. A survey of Indigenous flora used by folk medicine Practitioners in Yola council area of Adamawa state, Nigeria. Plant Arch. 7 (2), 517–521.

Ighodaro, I., Ching, F., Aigbe, E., 2010. Anti-inflammatory activity of aqueous fruit pulp extract of Hunteria umbellata K. Schum in acute and chronic inflammation. Acta Poloniae-Pharmaceutica-Drug Res. 67 (1), 81–85.

Katzung, B.G., 2004. Basic and Chemical pharmacology, nineth ed. McGraw Hill, London.

Mansor, L.L., Fabio, S.M., Gilda, G.L., Alexandre, S.R., Tereza, C., dos Santos, Suzana, G.L., Cintia, S.C., 2001. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother. Res. 15, 127–130.

Martini, N.D., Katerere, D.R., Ellof, J.N., 2004. Biological activity of five antibacterial flavonoids from Combretus erythrophylum (Combretaceae). J. Ethnophormacol. 93, 207–212.

Parthasarathy, S., Khan-Marchant, N., Penumeteha, M., santanam, N., 2001. Oxidative stress in cardiovascular disease. J. Nuclear Cardiol. 8 (3), 379–389.

Raju, I., Moni, M., Subramanian, V., 2005. Anti-inflammatory and antioxidant activities of Cassia fistula (Linn) bark extracts. Afr. J. Trad. Complimentary Altern. Med. 2 (1), 70–85.

Ramdas, P., Seema, M., 2010. Antioxidant activity and antimutagenic effect of phenolic compounds in Fermonia limomia (L) Swingle-fruit. Int. J. Pharm. Pharm. Sci. 2 (4), 68–73.

Rang, H.P., Dale, M.M., Ritter, J.M., Moore, P.K., 2003. Pharmacology, fifth ed. Elsevier Science Ltd., New Delhi, India.

Ratheesh, M., Helen, A., 2007. Anti-Inflammatory activity of Ruta graveolens (Linn) on carrageenan-induced paw edema in Wistar male rats. Afr. J. Biotechnol. 6 (10), 1209–1211.

Sen, T., Nag-Chaudhari, A.K., 1991. Anti-inflammatory evaluation of Plunchea indicia roots extract. J. Ethnopharmacol. 33, 135–141.

Shivaji, B., Tapas, K.S., Suvra, M., Prabhash, C.D., Sridhar, S., 2000. Assessment of the anti-inflammatory effects of Swerta Chirata in acute and chronic experimental models in male albino rats. Indian J. Pharmacol. 32, 21–24.

Sreedhar, V., Ravindra, L.K., Gopal, N.M., Nath, M.S., 2010. In vitro antioxidant activity and free radical scavenging potential of roots of Vitex trifoliate. Res. J. Pharm., Biol. Chem. Sci. 1 (4), 1036–1044.

Tonussi, C.R., Ferreire, S.H., 1994. Mechanism of diclofenic analgesia: direct blockage of inflammatory sensitization. Eur. J. Pharmacol. 251, 173–179.

Vinegar, R., Franx, J.F., Selph, J.H., Johnson, P.R., Venable, A.L., Mckenzie, K.K., 1969. Biphasic development of carrageenan-induced edema in rats. J. Pharmacol. Exp. Ther. 106, 96–103.

Ward, J.W., Elsea, J.R., 1997. In: Garrette, Edward R., Hirtz, Jean L. (Eds.), . In: Animal case and use in drug fate and metabolism. Methods and techniques, Vol. 1. Marcel Dekker, New York, pp. 372–390.

White, M.J., 1999. Mediator of inflammation and inflammatory Process. J. Allergy Clin. Immunol. 103, 378–S381.

Winter, C.A., Risley, E.A., Nuss, G.W., 1962. Carrageenan-induced edema in hind paw of rat as an assay for anti-inflammatory drugs. Proc. Soc. Exp. Biol. Med. 111, 544–547.

Wolfe, M.M., David, R.L., 1999. Lichtenstein gastrointestinal toxicity of Non-steroidal anti-inflammatory drugs. N. Engl. J. Med. 340, 1888–1899.

Zimmerman, M., 1983. Ethical guidelines for investigation of experimental pain in conscious animals. Pain 16 (2), 109–110.