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Macaranga barteri stem bark extract exerts anti-inflammatory and anti-hyperalgesia activity in murine models.

Evelyn Asante-Kwatia,[1*] Yakubu Jibira,[2] Abraham Y. Mensah,[1] Deborah Osei-Sarfoh[1]

ABSTRACT

This study was undertaken to evaluate the anti-inflammatory, anti-hyperalgesia and antioxidant activity of the hydro-alcoholic stem bark extract of *Macaranga barteri* (MBE). The carrageenan-induced foot oedema and Hargreaves thermal hyperalgesia models in rats were used to examine the anti-inflammatory and anti-hyperalgesic effects respectively. The 2, 2-diphenyl-2-picrylhydrazyl hydrate (DPPH) free radical scavenging and total antioxidant capacity assays were used to determine the antioxidant activity. In a curative protocol, MBE (30, 100, 300 mg kg^{-1}, *p.o.*) dose dependently and significantly inhibited carrageenan-induced foot oedema by 37.01 ± 13.08, 53.01 ± 9.87 and 64.11 ± 9.05% respectively (ED_{50} = 89.37 ± 7.52 mg kg^{-1}). The extract further attenuated cutaneous hyperalgesia by prolonging paw withdrawal latencies towards an external heat stimulus with an ED_{50} of 105.5 ± 4.22 mg kg^{-1}. MBE was found to possess a total antioxidant capacity of 531.62 ± 10.98 mg g^{-1} dry weight (Gallic acid equivalent) and scavenged DPPH free radicals with an IC_{50} of 19.45 ± 1.46 µg/mL. The results provide the first report on the anti-inflammatory, analgesic and antioxidant activity of *M. barteri* stem bark and partly justify its traditional use in the management of inflammation and pain. This could be attributed to phytochemicals such as tannins, terpenoids, sterols, coumarins and flavonoids which were identified in preliminary phytochemical studies of the stem bark.

Keywords: *Macaranga barteri*, inflammation, analgesia, Hargreaves, carrageenan, antioxidant

*Correspondence to:
Evelyn Asante-Kwatia, Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, College of Health Sciences, Kwame Nkrumah University of Science & Technology, Kumasi.
eamireku@knust.edu.gh

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INTRODUCTION

Inflammation is a complex defensive and protective response of living tissues to injury, irritation or infection and is accompanied by typical symptoms of pain, swelling, redness and fever.[1] Although defensive in nature, the complexity of events as well as the mediators released during an inflammatory process often result in the induction or aggravation of painful disease conditions such as rheumatoid arthritis, osteoarthritis, inflammatory bowel disease, colitis and hepatitis. These diseases can cause disability, reduce the quality of life and impose a huge social and economic burden on individual victims and the society as a whole.[2] The current medications available for the treatment of inflammatory conditions include non-steroidal anti-inflammatory drugs (NSAIDs), disease modifying anti-rheumatic drugs (DMARDs), opioids and corticosteroids which are unfortunately associated with adverse effects limiting their use in several cases. Due to this, the search for other alternative effective treatment options has occupied many research groups for the past years.[3]

Macaranga barteri Müll.-Arg is a common species of the family Euphorbiaceae distributed in the arid forests and grassy savannahs of tropical Africa. In Ghana, it is locally called '*opam kokoo*' (Akan) or '*opesare*' (Akyem) alluding to the plant's habit of occurring in depleting forests.[4] Ethnobotanical surveys report the use of the powdered leaves and bark or a decoction of these parts as a vermifuge and febrifuge in Nigerian and Congolese folk medicine. In Sierra Leone, the leaf decoction is used to treat sexually transmitted infection, as a laxative and haematinic. Together with other *Macaranga* species, it is also used to relieve persistent cough, bronchitis, stomatitis, gastric ulcer, dysentery, swelling and arthritis.[5] In previous scientific reports, the leaf extract of *M. barteri* showed remarkable antimicrobial, antioxidant and anti-diabetic activity as well as the ability to protect the gastric mucosa against gastric injury.[6-8] Moreover, a bioactive polyoxygenated ellagitannin isolated from the stem bark called macabarterin significantly inhibited the production of superoxides in a human neutrophil

[1] Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, College of Health Sciences, Kwame Nkrumah University of Science & Technology, Kumasi
[2] Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, College of Health Sciences, Kwame Nkrumah University of Science & Technology, Kumasi

respiratory burst assay indicating potential anti-inflammatory activity.[9] Consequently, it suggests that the stem bark may be a potential source of therapeutic agents in the treatment of inflammatory conditions. There is however no report on the anti-inflammatory or analgesic effects of *M. barteri* stem bark. This study therefore investigated the anti-inflammatory activity of the hydro-ethanolic stem bark extract of *M. barteri* and its ability to attenuate cutaneous hyperalgesia. The total antioxidant capacity and free radical scavenging activity of the stem bark extract were also investigated.

MATERIALS AND METHODS

Plant collection and authentication

The stem bark of *M. barteri* was collected from a semi-arid forest in Kwahu Asakraka, a town in the Eastern Region of Ghana in August, 2018. Verification of the plant material was carried out at the Herbal Medicine Department, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Ghana by Dr. George Henry Sam. A voucher specimen with the code KNUST/HM/2017/M016 was deposited at the herbarium of the faculty.

Preparation of extract

The stem barks were cleared of foreign material, cut into pieces, air dried and ground into fine powder. One kilogram of the powdered stem bark was extracted by cold maceration with 70 % ethanol for 72 h. The extract obtained was concentrated on a rotary evaporator under reduced pressure and temperature and further dried in the hot air oven at 50 °C to obtain a dark brown powdery extract subsequently referred to as MBE or 'the extract' in this study. A yield of 10.4 %$^{w}/_{w}$ was obtained.

Phytochemical Screening

Various classes of plant secondary metabolites were screened for following previously established methods.[10]

Drugs and chemicals

Diclofenac sodium, dexamethasone and morphine hydrochloride were obtained from Phyto-Riker, Accra, Ghana and Carrageenan from Sigma-Aldrich Inc., St. Louis, MO. USA. The extract was reconstituted in normal saline (NaCl, 0.9%) for oral administration.

Animals

Male Sprague–Dawley rats weighing approximately 200 ± 50 g were bought from Noguchi Memorial Institute for Medical Research, University of Ghana and transferred to the animal house of the Pharmacology Department, Kwame Nkrumah University of Science and Technology (KNUST). The animals were kept in groups of five in stainless steel cages with wood-shavings as bedding. Animals had access to local commercial rodent chow (Agricare Ltd, Kumasi, Ghana) and water *ad libitum*. The laboratory conditions were maintained at a temperature of 25 ± 1°C, relative humidity 60–70%, and 12-h light-dark cycle. The National Institute of Health Guidelines for the Care and Use of Laboratory Animals (Directive 2010/63/EU; Animal Care and Use Committee, 1998) were followed during experimental procedures. Approval was also sought from the Pharmacology Department Ethics Committee, KNUST.

Acute toxicity test

The acute toxicity testing was performed to determine the doses of extract that will be safe for administration to rats without causing any adverse effect or death. The assay was performed following a previously described method.[11] Briefly, male and female Sprague-Dawley rats were grouped into four with five rats in each group. After an overnight fast (water *ad libitum*), the rats were orally administered 300, 1000 and 3000 mg kg^{-1} of extract or normal saline (0.9%; 10 mL/kg) and monitored closely for gross behavioral changes or death for 0, 15, 30, 60, 120 and 180 minutes and also 24 h and 14 days after extract administration.

Carrageenan-induced rat paw edema

The anti-inflammatory activity of MBE was evaluated by the carrageenan-induced paw oedema assay in rats with few modifications.[12] Briefly, inflammation was induced in the right hind paw by injection of 100 µL of carrageenan suspension (2% $^{w}/_{v}$) into the sub-plantar tissue after the baseline reading of the foot diameter was taken for all animals. The various groups of animals received saline (10 mL kg^{-1} of 0.9 % NaCl *i.p*), MBE (30-300 mg kg^{-1}, *p.o*), diclofenac (10-100 mg kg^{-1}, *i.p*) or dexamethasone (1-10 mg kg^{-1}, *i.p*) 1 h for *o.p.* and 30 min for *i.p.* post-carrageenan injection. Increase in foot volumes was then recorded by measuring the foot diameters with an electronic calliper (model: Z22855, Milomex Ltd., Bedfordshire, UK) at hourly intervals for six hours. The percentage change in paw oedema from time 0 h was calculated using the equation $[(D_t - D_0)/D_0 \times 100]$ where D_0 is the paw diameter pre carrageenan administration (time 0 h) and D_t is the paw diameter post carrageenan

administration (time *t* h). Each test result was calculated as a mean of three repeated measurements.

Carrageenan-induced thermal hyperalgesia

The anti-hyperalgesic effect of MBE was evaluated using the Hargreaves thermal hyperalgesia model with few modifications.[13] On the day of experiment, the animals (five in each group) were individually placed in Plexiglas cages with a plain glass underneath and were allowed to acclimatize to the testing chamber for 20 min. The baseline paw withdrawal latency (PWL) for each animal towards a radiant heat source was determined before the experiment. Inflammation was evoked by a subcutaneous intra-plantar injection of 100 µL carrageenan (2 %$^{w}/_{v}$) into the right hind paw. The groups of animals then received vehicle (10 mL kg^{-1} of 0.9% NaCl, *i.p*), MBE (30-300 mg kg^{-1}, *p.o*), diclofenac (10-100 mg kg^{-1}, *i.p*) or morphine (1-10 mg kg^{-1}, *i.p*), 1 h for *o.p.* and 30 min for *i.p.* post-carrageenan injection. Hyperalgesia was assessed by placing the inflamed paw above a radiant heat stimulus and the PWL was measured with an Analgesia Meter (Model 336, IITC Life Science Inc., Woodland Hills, CA, USA) actuated with an automatic timer. The heat source was set to a low intensity and a cut-off time of 25 s was set to prevent damage to paw tissues. The paw withdrawal latency (PWL) was defined as the time required for the rat to lift the paw away from heat source. The PWLs were determined every hour for 5 h, starting 2 h after carrageenan injection. Each test was calculated as a mean of three repeated measurements.

Antioxidant Activity

2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay

The radical scavenging effect of *M. barteri* stem bark extract (MBE) was determined following a previously described method.[14] MBE (100 µL each) at a concentration range of 500 to 7.8 µg mL^{-1}, was mixed with 300 µL of DPPH (20 mg L^{-1} in methanol). The mixture was incubated for 30 min in the dark, after which the absorbance of residual DPPH was measured at 517 nm on a Cecil CE 7200 spectrophotometer, (Cecil Instrument Limited, Milton Technical Centre, England). Gallic acid and methanol were used as positive and negative controls respectively. The experiments were carried out in triplicate and results presented as the mean of three values. The percentage free radical scavenging activity was calculated according to the following equation: % DPPH inhibition = $[(Abs_{sample} - Abs_{control})/ Abs_{control} \times 100]$.

Where 'Abs sample' and 'Abs control' are absorbance of sample and control respectively.

Total Antioxidant Capacity (TAC)

The total anti-oxidant capacity of MBE was determined using the phosphomolybdenum method as previously described.[15] Briefly, 100 µL of serially diluted MBE extract (500 - 7.8 µg mL^{-1}) was added to 1 mL of a reagent solution consisting of Ammonium molybdate (4 mM), disodium hydrogen phosphate (28 mM) and sulphuric acid (6 mM) and incubated at 95°C for 90 minutes. The cooled mixture (200 µL) was transferred into a microtitre plate and the absorbance at 695 nm was measured. A standard calibration curve was generated from Gallic acid (500 - 7.8 µg mL^{-1}) and used as the reference standard (Figure. 6). The experiments were performed in triplicate and expressed as Gallic acid equivalent (GAE) in mg/g of dried extract.

Statistical analysis

All data were presented as the mean ± standard error mean (S.E.M, $n = 5$). Raw data for the carrageenan-induced inflammation and thermal hyperalgesia tests was calculated as the percentage change in maximum possible effect (% MPE). Two-way repeated measures analysis of variance (ANOVA) was used to analyze the time-course curves followed by Dunnett's *post hoc* test. The total anti-inflammatory or analgesic score for the treatment group was calculated as the area under the curve (AUC). The differences in AUCs were compared using 1-way ANOVA complemented Newman–Keuls test with drug treatment as a between-subjects factor. An iterative computer least squares method, with the following non-linear regression (3-parameter logistic) equation was used to evaluate the ED_{50}s: $Y = a + (b - a)/ 1 + 10^{Log\ ED_{50} - X}$

Where, X denotes logarithm of dose and Y represents response. Y starts at 'a' (the bottom) and goes to 'b' (the top) with a sigmoid shape. GraphPad Prism for Windows version 8.0 (GraphPad Software, San Diego, CA, USA) was used for all statistical analyzes and a *P* value less than 0.05 was considered statistically significant.

RESULTS

Phytochemical screening

Phytochemical analysis of the stem bark of *M. barteri* revealed major classes of secondary metabolites presented on Table 1.

Acute toxicity

Administration of 3000 mg of MBE resulted in no toxic signs, changes in gross behaviour or death during the study period and after 14 days of observation.

Table 1 **Phytochemical screening of *M. barteri* stem bark**

Secondary metabolite	Results
Tannins	+
Saponins	-
Alkaloids	+
Flavonoids	+
Coumarins	+
Sterol	+
Triterpene	+

(+): detected, (-): not detected

Table 2 **Percentage inhibition of carrageenan induced paw oedema**

	MBE			DICLO			DEXA		
Dose (mg kg^{-1})	30	100	300	10	30	100	1	3	10
% Inhibition	37.01 ± 13.08	53.01 ± 9.87	64.11 ± 9.05	52.78 ± 6.95	57.93 ±12.50	64.67 ± 4.35	49.89 ± 10.04	57.54 ± 14.39	67.93 ± 3.40
	**	***	****	****	****	****	***	****	****

Data is presented as mean ± S.E.M. (n =5); ****P < 0.0001; *** P < 0.001; **P < 0.01, *P < 0.05 compared to vehicle-treated group

Table 3 **IC_{50} of MBE and Gallic acid in DPPH radical scavenging assay**

Sample	IC_{50} ± SD µg/mL
MBE	19.45 ± 1.46
Gallic acid	7.32 ± 1.36

Inhibition of carrageenan-induced paw oedema

Figure 1 shows the effect of MBE, dexamethasone and diclofenac on carrageenan-induced rat paw oedema observed over a period of 6 hours. From the time course curves (Figure. 1 A, B, C), injection of 100 µL of 2 %$^{w}/_{v}$ carrageenan resulted in moderate inflammation which caused an increase in paw volume peaking at about 2 -3 h, after which a slow decrease in oedema was observed. Two-way ANOVA statistics (*treatment* × *time*) revealed that the administration of MBE (30, 100, 300 mg kg^{-1}) significantly ($F_{(3, 16)}$ = 17.4, p < 0.0001) reduced the increase in paw volume in a dose-dependent manner. The total oedema recorded for each treatment group was expressed in arbitrary units as the area under curve (AUC) of the time-course curves (Figure. 1 D, E, F). The percentage reduction of total paw oedema for all treatment groups is presented on Table 2. Diclofenac (10, 30,100 mg kg^{-1}) significantly ($F_{(3, 16)}$ = 23.74, p < 0.0001) suppressed the increase in paw volume dose dependently. The effect of dexamethasone (1, 3, 10 mg kg^{-1}), was more significant than diclofenac ($F_{(3, 16)}$ =21.14, p < 0.0001). A non-linear regression analysis of the dose-response curves (Figure. 2) gave an ED_{50} value of MBE as 89.37 ± 7.52 mg kg^{-1} which was about 5 times less potent than diclofenac (ED_{50} = 17.52 ± 4.28 mg kg^{-1}) and about 49 times less potent than dexamethasone (ED_{50} = 1.82 ± 2.28 mg kg^{-1}).

Inhibition of inflammation-induced thermal hyperalgesia

Subcutaneous intra-plantar injection of 2 %$^{w}/_{v}$ carrageenan into the right hind paws of rats resulted in inflammation accompanied by cutaneous hyperalgesia to thermal stimuli approximately 1 h after carrageenan injection, peaked around 2-3 h and subsided afterwards. In the negative control group, hyperalgesia was observed as shorter paw withdrawal latency towards the thermal heat stimulus compared with baseline measurements. On the contrary, administration of MBE (30–300 mg kg^{-1} *p.o.*), significantly ($F_{(3, 16)}$ = 26.31; p < 0.0001) and dose dependently attenuated the thermal hyperalgesia being recorded as a longer paw withdrawal latency compared to the vehicle treated group (Figure. 3 A, D). The anti-nociceptive effect produced by all treatment groups was calculated as percentage maximum possible effect (% MPE) and also expressed in arbitrary units as the area under curve (AUC) of the time course curves (Figure. 3 D, E, F). Diclofenac (10-100 mg kg^{-1}, *p.o.*) and morphine

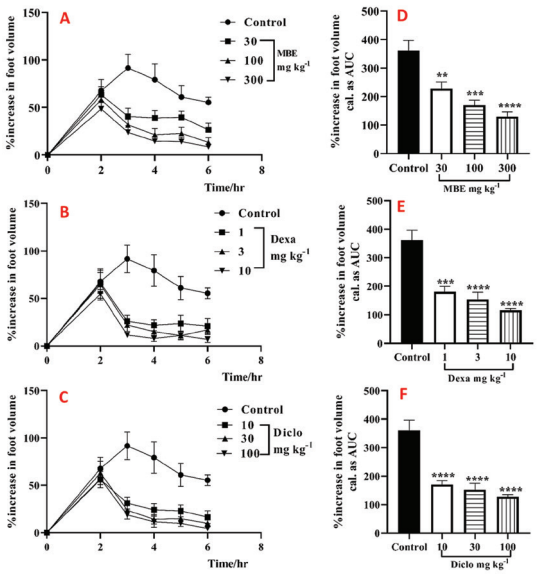

Figure 1 Effect of MBE (30 - 300 mg kg^{-1} *p.o.*), diclofenac (10 – 100 mg kg^{-1} *i.p.*) and dexamethasone (1– 10 mg kg^{-1} *i.p.*) on the time course curves (A, B, C) and total oedema response (D, E, F) in carrageenan-induced paw oedema in rats. Data is presented as mean ± S.E.M. (n =5); ****P < 0.0001; *** P < 0.001; **P < 0.01, *P < 0.05 compared to vehicle-treated group (1-way ANOVA followed by Bonferroni's post hoc test).

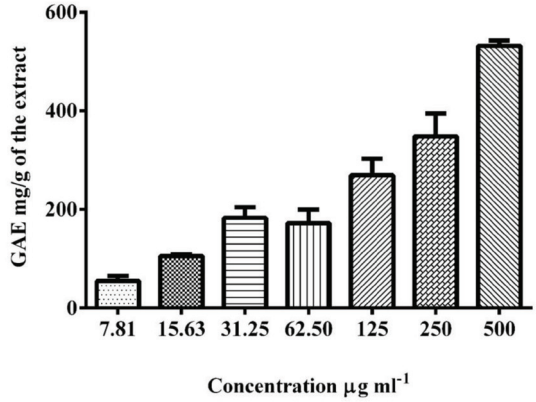

Figure 2 Dose response curves for MBE (30-300 mg kg^{-1} *p.o.*), diclofenac (10-100 mg kg^{-1} *i.p.*) and dexamethasone (1-10 mg kg^{-1} *p.o.*) on carrageenan induced thermal hyperalgesia in rats.

(1–10 mg kg^{-1}, *i.p.*) also dose-dependently inhibited the hyperalgesia (Figure. 3 B, E: diclofenac: $F_{(3, 16)}$ = 50.04, $p < 0.0001$; Figure. 3 C, F: morphine: $F_{(3, 16)}$= 41.55; $p < 0.0001$), but were more potent than MBE. From non-linear regression analysis of the

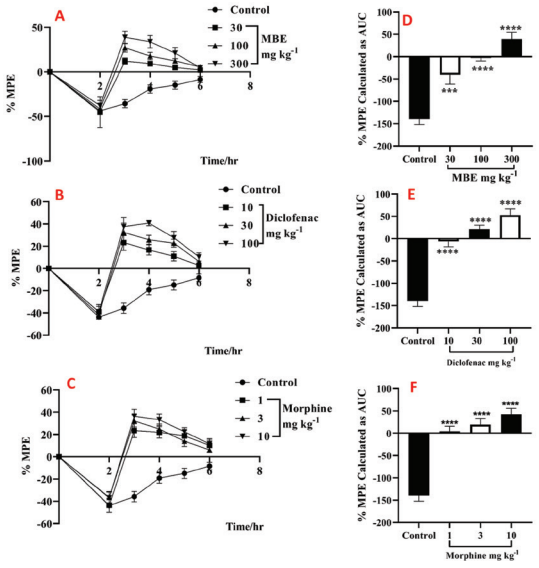

Figure 3 Effect of extract (30 - 300 mg kg^{-1} *p.o.*), diclofenac (10 – 100 mg kg^{-1} *i.p.*) and morphine (1– 10 mg kg^{-1} *i.p.*) on the time course curves (A, B, C) of carrageenan-induced thermal hyperalgesia in rat in the Hargreaves-model and the AUC (D, E, F). Data is presented as mean ± S.E.M. (n = 5); ****P < 0.0001; *** P < 0.001; **P < 0.01, *P < 0.05 compared to vehicle-treated group (1-way ANOVA followed by Bonferroni's post hoc test). [black circles = MBE; black squares = Diclo; black triangles = Dexa]

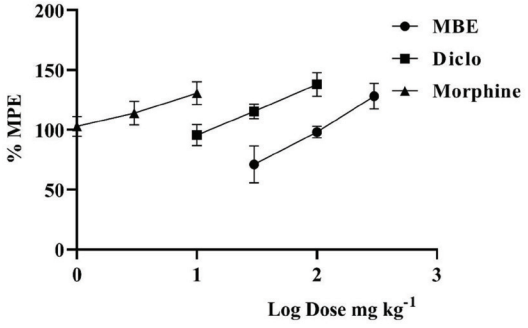

Figure 4 Dose response curves for extract (30-300 mg kg^{-1} *p.o.*), diclofenac (10-100 mg kg^{-1} *i.p.*) and morphine (1-10 mg kg^{-1} *p.o.*) on carrageenan induced thermal hyperalgesia in rats. [black circles = MBE; black squares = Diclo; black triangles = Morphine]

dose-response curves (Figure. 4), the ED_{50} value for MBE was determined to be 105.5 ± 4.22 mg kg^{-1} which was less potent than diclofenac (ED_{50} =

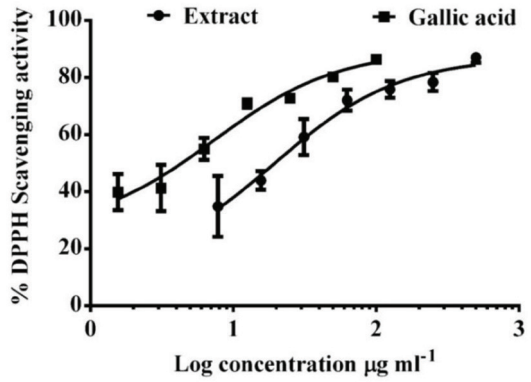

Figure 5 Percentage DPPH scavenging activity against log concentration of extract and Gallic acid. [black squares = Gallic acid; black circles = MBE/extract]

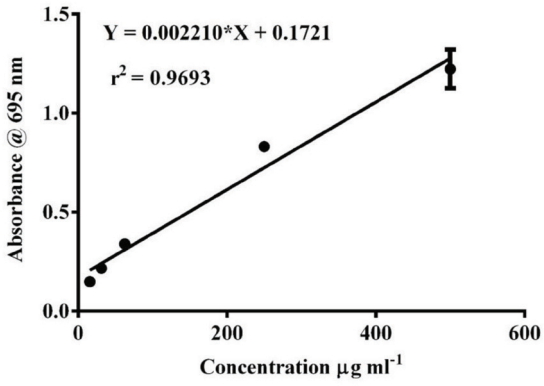

Figure 6 Standard calibration curve for absorbance of Gallic acid at concentration range of 500-7.8 µg mL^{-1}

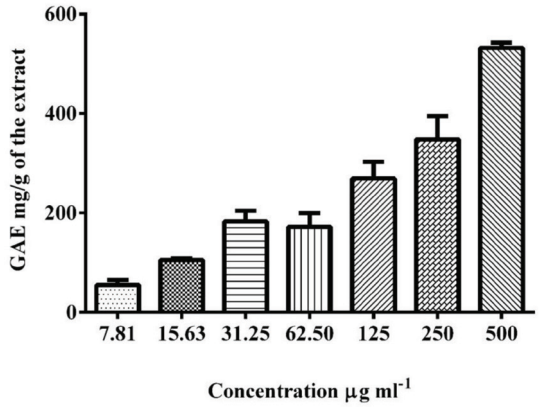

Figure 7 Increasing TAC with increasing concentration of MBE

32.13 ± 3.21 mg kg^{-1}) and morphine (ED_{50} = 3.68 ± 1.81 mg kg^{-1}).

Antioxidant Activity

In the DPPH radical scavenging assay, MBE exhibited a maximum radical scavenging effect of 87.04 ± 1.84 % corresponding to an IC_{50} value of 19.45 µg mL^{-1}. The effect was concentration dependent (Figure. 5). The reference compound, Gallic acid was about thrice as more effective as the extract with 86.68 ± 0.90 % radical scavenging effect at 100 µg mL^{-1} and an IC_{50} of 7.32 µg mL^{-1} (Table 3).

The total antioxidant capacity was measured as milligram of Gallic acid equivalent (GAE) per gram of the dried extract. The extract showed increasing total antioxidant capacity with increasing concentration (Figure. 7). MBE had a GAE of 531.62 ± 10.98 mg g^{-1} dry weight of the extract which implies that a gram of the extract would have an antioxidant capacity equivalent to that of about 531 mg of Gallic acid.

DISCUSSION

The stem bark of *Macaranga barteri* is used traditionally for the treatment of conditions characterized by inflammation and pain in African folk medicine.[9] This study investigated the anti-inflammatory, anti-hyperalgesia and anti-oxidant effects of the 70% hydro-alcoholic extract of the stem bark.

In a curative treatment regimen, oral administration of MBE caused a significant dose-dependent reduction in carrageenan-induced rat paw oedema validating the use of the plant in treatment of inflammatory conditions. The development of oedema following the injection of carrageenan has been shown to be a biphasic event beginning with the release of mediators such as serotonin, histamine and bradykinin (1-3 h) which causes increased permeability of post-capillary venules, exudation of fluids to the site of injury and eventually visible oedema of the affected tissue.[16] Subsequently, inducible isoforms of cycloygenase (COX-2) and nitric oxide synthase (iNOs) cause the release of prostaglandins (PGs) and NO respectively sustaining the oedema in the latter phase of inflammation (3-5 h). Though the mechanism of action of MBE is yet to be established, this study reveals that *M. barteri* stem bark extract possibly acts through the inhibition of the production and/or action of one or more of these inflammatory mediators.

The nociceptive threshold of inflamed paws in control and MBE-treated animals was further determined by the Hargreaves method which uses radiant heat as a thermal stimulus applied to unrestrained animals.[17] MBE (30, 100 and 300 mg kg^{-1}) resulted in an increased paw withdrawal latency corresponding to a decreased sensitivity to pain compared to the vehicle treated group. Inflammation-induced thermal hyperalgesia has been proposed to result from cytokine (tumour necrosis factor-α (TNF-α)) production followed by the induction of interleukins (IL-1β, IL-6 and

IL-8) and subsequent production of COX products such as PGE_2 and sympathomimetic amines. These pro-inflammatory mediators cause an increased sensitivity of sensory neurons at the affected site to noxious thermal or mechanical stimuli.[18] According to earlier studies, paw withdrawal response in inflammation-induced thermal hyperalgesia may be attenuated either by drugs that act peripherally (conventional COX-2 inhibitors) and/or spinally.[19]

In previous phytochemical analysis, the stem bark of *M. barteri* was found to contain ellagic acid, 3-*O*-methylellagic acid 4-*O*-β-D-ylopyranoside, 3-*O*-methylellagic acid, gallic acid, methyl gallate, scopoletin and a polyoxygenated ellagitannin, macabarterin.[9] The presence of some of these compounds may be responsible at least in part for the observed pharmacological effects. Ellagic acid is well known for its anti-inflammatory and anti-nociceptive effects linked to its ability to decrease the levels of malondialdehyde (MDA), iNOS, and COX-2 in the oedema paw, subsequently attenuating the formation of cytokines (TNF-α, IL-1β), NO and PGE_2 in the inflamed paw tissue.[20] Macabarterin was found to inhibit the production of superoxides produced in a human neutrophil respiratory burst assay suggesting potential anti-inflammatory effect.[9] The anti-inflammatory and anti-hyperalgesic effects of scopoletin is also well established and has been linked to the inhibition of PGE, TNF- α, IL-1β and IL-6 and suppression of the expression of COX-2.[21] The demonstration of free radical scavenging ability and antioxidant capacity is consistent with previous works on the leaves of *M. barteri*.[22]

In the genus, Macaranga, *M. peltata*, *M. conifera* and *M. denticulata* have been reported to possess anti-inflammatory, anti-arthritic and anti-nociceptive activities.[23-25] The present results on *M. barteri* stem bark extract is the first evidence of its anti-inflammatory and anti-nociceptive activities and give scientific justification to its use in traditional medicine, complementing existing information on the pharmacological reports of the genus Macaranga.

CONCLUSION

The anti-inflammatory, anti-nociceptive and antioxidant activity of *M. barteri* stem bark demonstrated in this work is worth reporting. This report validates the stem bark of *M. barteri* as a potential anti-inflammatory and analgesic agent justifying its traditional uses in the treatment of painful inflammatory conditions. Investigation of its mechanisms of action, toxicity and other bioactive constituents are considered in further work.

CONFLICT OF INTEREST

Authors have no conflict of interest to declare.

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