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Phytochemical Composition and Analgesic Property of Ethanolic Leaf Extract of *Maesobotrya barteri*

Dikioye Emmanuel Peters[1*], Emmanuel Onyebuchi Ezendiokwere[1*], Uche Chinedu Njoku[1], Ikehide Friday[1] and Matthew Owhonda Wegwu[1]

[1]University of Port Harcourt, Choba, Rivers State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors DEP and MOW designed and supervised the study. Authors EOE, UCN and IF managed the literature searches, performed the statistical analysis, wrote the protocol and the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study was carried out to identify the phytochemical composition of *Maesobotrya barteri,* and effect on infra-red induced pain in mice.
Methodology: Twenty albino mice were selected and randomly placed in 5 groups of 4 animals each. Pain was induced by infra-red heat. Group 1 was treated with distilled water, Group 2 received 100 mg/kg bwt of aspirin. Extract doses of 500 mg/kg, 800 mg/kg and 1000 mg/kg bw were given to groups 3, 4 and 5 respectively. The time taken for the animals to flick their tail off the I.R window was noted in seconds and taken as the reaction time.
Results: The plant contains alkaloids, flavonoids, steroids, carbohydrates, cardenolides and saponins. Flavonoids were the most abundant. The plant extract increased the reaction time of the mice to radiant heat induced pain. All doses of the plant extract except the 800 mg/kg bwt dose showed the highest elevation of pain threshold at the 60 mins interval. The concentration of 1000 mg/kg body weight showed the highest elevation. Percentage inhibition was calculated as 27.5%, 20.6% and 44.4% for the three doses of the extract respectively. There was no significant difference ($p>0.05$) between the reaction time for the standard drug treated group and the extract treated

**Corresponding author: E-mail: petersidikioye@ymail.com, emmanuel_ezendiokwere@uniport.edu.ng;*

group. The increase in reaction time for the treated groups relative to the control was not significant at p≤0.05.
Conclusion: This study has revealed that *Maesobotrya barteri* has a mild analgesic property. Its use as a pain reliever in some local communities of Nigerian is hence not substantiated.

Keywords: Maesobotrya barteri; analgesic; reaction time; pain.

ABBREVIATIONS

M. barteri: Maesobotrya barteri; BWT: body weight; I.R: Infra-red.

1. INTRODUCTION

Phytochemicals are naturally occurring bioactive substances. They are found in different parts of plants in different concentrations. They are deemed valuable for their various functions [1] amongst which are: They play important protective and disease preventive role in plants and other organisms including humans, some of them attract pollinating agents, whereas others prevent predators. Due to the therapeutic properties of phytochemicals, most are now employed for culinary uses [2]. Recently, phytochemicals have attracted much interest from researchers in various fields because of their various applications [1]. They are valuable biosource of drugs used frequently in traditional systems of medicine, modern medicine, pharmaceutical intermediates and as leads in drug synthesis [3]. They have been reported to be responsible for the antimicrobial, antibiotic, anticancer, antihelminthic and antisickling properties of many products used in medical practice [4]. The important bioactive compounds found in plants include: alkaloids, flavonoids, tannins, phenolic compounds etc [5]. The extraction of these phytochemicals and their application has been the focus of advances in science of late [4]. The knowledge derived from their application is being harnessed daily in health care provision.

In pain, there is an uncomfortable sensitization due to actual or potential injury to tissues. There is usually an emotional experience which can be explained in terms of the damage done to tissues [6]. Depending on the duration, pain may be acute or chronic. Chronic pain always has a long lasting sensation and may be of no identifiable cause. In acute pain, the duration of the pain may be limited and it is usually attributed to an identifiable cause. Nociceptive pain is evoked by specialized afferent fibers in the absence of sensitization. They are otherwise called physiological pain. Damage to the nerves of the nervous system results to neuropathic pain [7]. When damage are done to tissues, stimuli are generated which are picked up and sent as signals via nociceptors to the dorsal aspect of the spinal cord. Inhibitory mechanisms to reduce the severity of perceived pain to tolerable levels is mediated via second order neurons of the spinothalamic tracts to higher brain centres. [8], [9-10]. Pain can be mediated via central or peripheral mechanisms. In central sensitization, there is a change in the functional state of neurons and nociceptive pathways throughout the neuraxis, caused by the increased membrane excitability and synaptic efficiency or by the decreased inhibition on the spinothalamic system. The series of processes that follow central sensitization include activation of wide range neurons, progressive increase of response provoked by a standard series of repeated stimuli, expansion of stimulus spatial extension and the triggering of changes lasting longer than the initial stimulus. Nociceptors play a vital role in peripheral sensitization. Action potentials are generated in the sensory endings of the healthy sensory nerve fibers once excited. Ectopic discharges occur when nerve fibers are impaired. Nociceptors utilize inflammatory mediators which are short-term evoked. Once nociceptive nerve fibers are sensitized, second messenger systems are activated which influence ion channels. Maintenance of pain however in spinal cord neurons that have undergone central sensitization can be intensified by the up-regulation of excitatory receptors [9]. Various classes of drugs can be used to achieve analgesia. These include: Non-steroidal anti-inflammatory drugs (NSAIDs), opioid drugs, and steroid based drugs [11]. Most NSAIDs, particularly aspirin act via the inhibition of prostaglandins, molecules which are important in mediating pain [12-13]. Some other analgesics mechanism of action involves the blockage of opioid receptors [14]. Plants have been reported to be potential sources of analgesics [15-17]. *Maesobotrya barteri* is a member of the family

Euphorbiaceae [18]. It is well distributed in Nigeria and known by various names such as mmiriogu, among the Igbos, Uvune in Etche land and Oruru in Benin. The report of a research finding states that bioactive compounds present in the plant include: tannins, saponins, cardiac glycosides, deoxy sugar and terpenes [19]. As of its pharmacological relevance, aside its many reported uses in the treatment of various health conditions like diarrhea, stomach ache and malaria, it's reportedly being used also as a pain reliever [20,21]. In view of its folkloric use in pain management, the present study has been designed to evaluate its analgesic property and possible mechanism of action. The doses of administration chosen were based on the result of the acute toxicity study.

2. MATERIALS AND METHODS

2.1 Sample Collection

Fresh leaves of *Maesobotrya barteri* were collected in Chokocho community, Etche Local Government Area, Rivers State, Nigeria, the plant sample was identified and authenticated at the Department of Plant Science and Biotechnology, University of Port Harcourt, Choba, Rivers State.

2.2 Sample Preparation

The leaves of the plant were obtained and dried under shade. They were ground into fine powder. The powder (2.4kg) was macerated in absolute ethanol (99.9%) at room temperature for 72 hours. It was then filtered using a filter paper and the filtrate was condensed in a rotary evaporator. The filtrate was further evaporated to dryness in a water bath at 50°C. A greenish residue weighing 36 g was obtained. The extract was kept in air tight sample bottles in a refrigerator until needed.

2.3 Phytochemical Screening

The crude leaf extract was phytochemically screened for the presence of alkaloids, tannins, glycosides, flavonoids, anthraquinones, saponins and cardenolides employing standard procedures and tests [21].

Quantification of the phytochemical constituents was done using a BUCK M910 Gas chromatography equipped with a flame ionization detector (FID).

2.4 Acute Toxicity Test

The safety of the leaf extract of *M. barteri* was determined using a standard method [22]. Rats were administered orally with doses of the leaf extract ranging from 1000 mg/kg to 5000 mg/kg body weight, the animals were all kept under the same conditions and observed for toxicity signs and mortality for 24 hours for a seven (7) days period. LD_{50} value was calculated as geometric mean of the dose that resulted in 0 and 100% lethality.

2.5 Experimental Design

2.5.1 Determination of analgesic activity of *M. barteri* using tail flick method

Twenty (20) albino mice weighing 20-25 g were selected and randomly placed in 5 groups of 4 animals each. Pain was induced by radiant heat (infra-red, I.R, intensity set at 20). [23]. The animals received oral treatments as shown below:

Group 1: Normal control mice treated with 10 ml/kg body weight distilled water.
Group 2: Received 100 mg/kg bwt aspirin.
Group 3: Received 500 mg/kg bwt *M. barteri* extract
Group 4: Received 800 mg/kg bwt *M. barteri* extract.
Group 5: Received 1000 mg/kg bwt *M. barteri* extract.

The animals were pre-treated orally before subjecting them to tail flick test. The animals were restrained on the apparatus panel and about 3 cm of their tails put over the I.R window. The pedal swing was depressed to activate the I.R source and the digital counter. The time in seconds taken for each mouse to flick its tail off the I.R window was noted as the reaction time. The animals were screened before drugs were given and those which flicked their tail before 30 secs were used in the experiment. Measurements were taken for one and a half hours at 30 minutes interval. The percentage pain inhibition was calculated using the formula:

Percentage pain inhibition =(Reaction time of treated group - Reaction time of the control/Reaction time of the Control)

2.6 Statistical Analysis

The data obtained from the experiment were expressed as mean ± SEM. One way analysis of variance (ANOVA) was used to analyze the data. Multiple comparison was done using post-Hoc. The values were considered to be statistically significant at $p < 0.05$ [24].

3. RESULTS AND DISCUSSION

Table 1 shows the preliminary phytochemical screening of the ethanolic leaf extract of *Maesobotrya barteri*. The result reveals that the plant contained alkaloids, flavonoids, steroids, carbohydrates, cardenolides and saponins. Flavonoids are the most abundant with kaempferol having a concentration of 45.5 ug/g,rutin, a concentration of 27.9 ug/g, and epicatechin and catechin present in the concentrations of 7.4 ug/g and 25.2 ug/g respectively (Table 2). This finding is supported by the report of Ogwuche et al [25] which stated that the aerial parts of *M.barteri* had these secondary metabolites in addition to tannins. According to Ojewole [26], the pharmacological relevance of ethnomedicinal plants is due to their phytochemical composition. As such, the active ingredients present in the plant is highly implicated in its pharmacological property. For instance, hesperidin, a flavanone-glycoside which is abundant in citrus has anti-inflammatory and analgesic properties. They have been reportedly used in the treatment of arthritis. Flavonoids traverse the blood-brain barrier and through several mechanisms including their effect on GABA receptors, opioid receptors and alpha-adrenergic receptors can inhibit enzymes involved in inflammation and pain in different parts of the nervous system including the ventral medulla oblongata, thus mediating analgesia [27]. Salvigenin obtained from *Salivia officinalis* has been shown to have a significant analgesic effect like morphine; The plant has been used for the relief of unilateral headaches and headaches with neurological origin. Flavonoids in inflamed tissues inhibit cyclooxygenase, thereby preventing the formation of prostaglandins. Some evidence have also shown that flavonoids inhibit tumor necrosis factor (TNF) secretion, hence their supposed anti-inflammatory action. Flavonoids like apigenin reduce the accumulation of lipids which are necessary for pain signals. The inhibition of phospholipase A_2 prevents the formation of arachidonic acid from phosphatidic acid, stalling the signaling cascade leading to pain. Previous studies have revealed that quercetin can inhibit lipoxygenase and cyclooxygenase activity [28]. Wang et al [29] reported that saponins demonstrated analgesic activity by inhibiting the release of histamine.The result of the acute toxicity studies conducted is contained in Table 3. The result shows that the plant extract did not cause mortality when administered orally at concentrations of 5000 mg/kg, 3000 mg/kg, 2000 mg/kg, and 1000 mg/kg body weight with the estimated median lethal dose of about 3000 mg/kg. The plant could be deemed safe for administration without concern for hepatic or cellular damage. This is in keeping with the findings of Hodge and Sterner [30] and Ghosh [31] who reported that with such concentration as median lethal dose, a plant could be considered relatively safe.

Table 1. Qualitative phytochemical composition of *M. Barteri*

Phytochemical	Status
Alkaloids	+
Flavonoids	+
Tannins	-
Anthraquinones	-
Triterpenoid / Steroids	+
Fixed oils	-
Carbohydrates	+
Cardenolides	+
Saponins	+

Key:+ = Present, -= Absent

Table 2. Quantitative phytochemical composition of ethanolic leaf extract of *M. Barteri*

Component	Subclass	Concentration (ug/g)
Flavonoid	Kaemferol	45.49
Flavonoid	Rutin	27.85
Flavonoid	Catechin	25.18
Flavonoid	Epicatechin	2. 54
Saponin	-	21.32
Phenol	-	7.42
Alkaloid	Ribalidine	1.75
Oxalate	-	1.50
Phytate	-	0.57

The effect of the ethanolic leaf extract of *M. baerteri* on reaction time is presented in Table 4. The reaction time was increased for all doses of the plant extract. The peak increase in reaction time was observed at the 60 mins interval for all doses of the extract except for the 800 mg/kg concentration which had its peak at 30 mins interval. The concentration of 1000

mg/kg bwt of the extract showed an increase from 4.46±0.14 to 6.30±1.30 at the maximal peak. Comparing the reaction time for all time intervals of the treated groups to the control showed an increase which was not statistically significant ($p>0.05$). The percentage pain inhibition of the plant extract is shown in Table 5. The various concentrations of 500 mg/kg, 800 mg/kg, and 1000 mg/kg bwt showed percentage inhibition of 27.5%, 20.6% and 44.4% respectively. The dose of 1000 mg/kg bwt had the highest pain inhibition. The percentage inhibition of the extract treated groups were similar, particularly at the lower concentrations to that of the standard drug treated group. The analgesic property of the plant extract could be described as mild. Umar et al [32] explained that the ability of a plant extract to prolong the reaction time in thermally induced pain within intervals of 30 and 60 mins is indicative of a mild analgesic activity. As of the mechanism of action, aspirin the standard drug used is an inhibitor of COX enzymes, the enzymes that feature prominently in the release of secondary molecules, prostaglandins which are messenger molecules especially in peripheral sensitization. Their under-production can have a negative effect on the ion pumps necessary for the generation of action potentials, hence lowering sensitivity. The animal response in this particular test is usually integrated at the lower levels in the central nervous system, thus giving information about the pain threshold [33]. Reports have confirmed that COX inhibitors produce mild action (little or moderate effect on reaction time) in thermally iduced pain. It's suggested that the plant extract may have acted through the inhibition of prostaglandin synthesis although this is not fully understood. The presence of the bioactive compounds present in the plant may have been responsible for the observed analgesic activity. Alkaloids have been shown to have analgesic and anti-inflammatory actions in natural products [34]. Flavonoids are known to target prostaglandin synthesis [35,36]. A few reports have also been released on the anti-nociceptive property of saponins [37].

Table 3. Result of Acute Toxicity Test of Ethanolic Leaf Extract of *M. barteri*

Group	Dose (mg/kg BW)	No of deaths	Mortality %
1	1000	0/3	0
2	2000	0/3	0
3	3000	0/3	0
4	5000	0/3	0

(LD_{50}= 2250mg/kg)

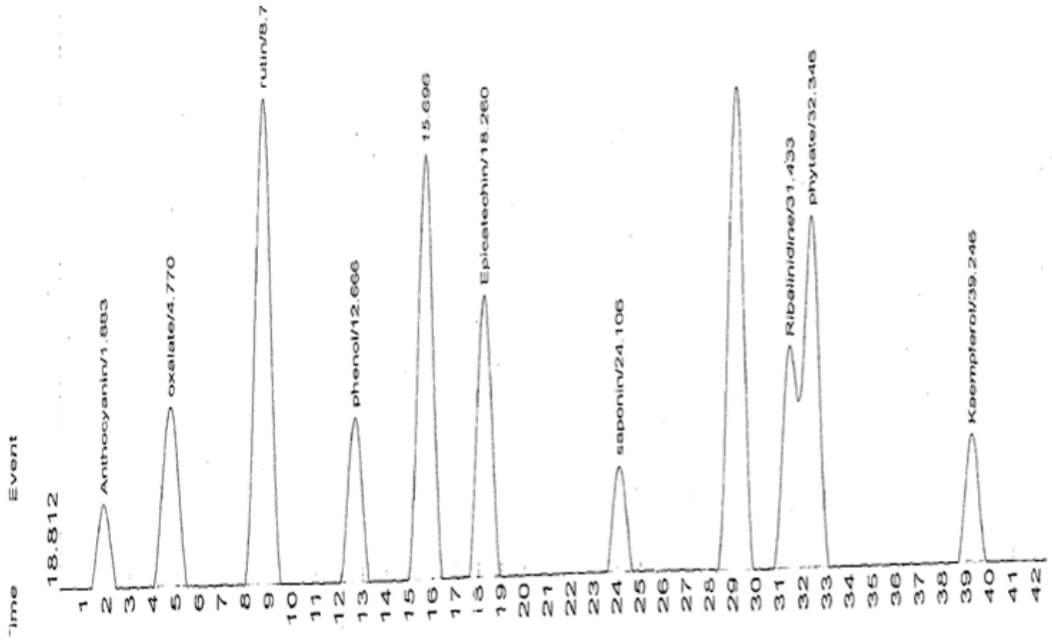

Fig. 1. Chromatogram of the phytochemical composition of *M. Barteri*

Table 4. Effect of ethanolic leaf extract of *M.barteri* on radiant heat induced pain in mice

Test groups	Zero mins	Thirty mins	Sixty mins	Ninety mins	One hundred and twenty mins
Group 1	3.63 ± 0.99	4.83 ± 0.86	4.36 ± 0.66	4.03 ±0.12	4.40 ± 0.43
Group 2	3.76 ± 0.73	5.06 ± 0.41	5.40 ± 0.41	4.86 ± 0.12	4.76 ± 0.74
Group 3	3.96 ± 0.42	5.50 ± 1.20	5.63± 1.38	5.63 ± 0.38	5.53 ± 0.92
Group 4	3.76 ± 0.52	5.33 ± 0.90	5.26 ± 1.31	4.73 ± 0.97	5.03 ± 0.84
Group 5	4.46 ± 0.14	6.03 ± 1.30	6.30 ± 1.30	5.90 ± 0.94	5.96 ± 1.04

Values are expressed as Mean ± SEM. N = 4. Values found in a column with common superscript letter a, are significantly different (p<0.05) when compared to the control while values without superscript a, are not significantly different (p>0.05) in comparison to the control.

Table 5. Percentage inhibition of pain of ethanolic leaf extract of *M. barteri* on tail flick test

Groups	Treatment	Dose (mg/kgBw)	Reaction time	% Inhibition
1	Distilled water	10ml/kg	4.36 ± 0.66	_
2	Aspirin	100	5.4 ± 0.41	25.2
3	*M. barteri* extract	500	5.63 ± 1.38	27.5
4	*M. barteri* extract	800	5.26 ± 1.31	20.6
5	*M. barteri* extract	1000	6.30 ± 1.30	44.4

4. CONCLUSION AND RECOMMENDATION

This research work has revealed that *Maesobotrya barteri* has mild analgesic property. It cannot be considered efficacious in the management of pain especially the chronic neuropathic kind. Its use as a painkiller is hence not substantiated. However, it is recommended that other models for evaluating analgesia should be adopted to further confirm the findings of this present study and possibly elucidate the mechanism of action of the plant extract.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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