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ANTIPYRETIC, ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY OF POLYHERBAL FORMULATION (AGBO-IBA PMII) USED IN THE TREATMENT OF MALARIA IN SOUTHERN NIGERIA

Pass Chidiebere Chijindu
Department of Biological Sciences, University of Delta, Agbor, Delta State, Nigeria,
pass.chijindu@unidel.edu.ng

MacDonald Idu
Department of Plant Biology and Biotechnology, University of Benin, Benin City, Edo State, Nigeria

Benjamin Ogunma Gabriel
Department of Science Laboratory Technology, University of Benin, Benin City, Edo State, Nigeria

ARTICLE

Antipyretic, Anti-inflammatory and Analgesic Activity of Polyherbal Formulation (*Agbo-Iba PMII*) Used in the Treatment of Malaria in Southern Nigeria

Pass C. Chijindu [a,*], MacDonald Idu [b], Benjamin O. Gabriel [c]

[a] Department of Biological Sciences, University of Delta, Agbor, Delta State, Nigeria
[b] Department of Plant Biology and Biotechnology, University of Benin, Benin City, Edo State, Nigeria
[c] Department of Science Laboratory Technology, University of Benin, Benin City, Edo State, Nigeria

Abstract

The ideal antimalarial agent should not only possess antiplasmodial effects but also anti-inflammatory, antipyretic and analgesic activities. Hence, the aims of were to investigate the antipyretic, anti-inflammatory and analgesic activities of a traditional polyherbal formulation (*Agbo-Iba PMII*) used to treat malaria in Southern Nigeria. The antipyretic activity was determined by employing three models viz Yeast-induced hyperthermia, D-Amphetamine-induced hyperthermia and 2, 4- Dinitrophenol-induced hyperthermia. The anti-inflammatory activity was determined by employing the carrageenan induced rat paw oedema assay model. While the analgesic activity was determined by employing three models viz Acetic acid induced writhing, Hot Plate Method and Analgesy-meter Test (Randall−Selitto Test). The findings of this study revealed that '*Agbo-Iba PMII*' (Formulation 1:1:1:1) demonstrated significant ($p < 0.05$) dose-related antipyretic, anti-inflammatory and analgesic activities at dosages of 200, 400 and 800 mg/kg tested which may be as a result of synergistic interactions between the constituent plants and various phytochemicals present. The obtained results revealed that the polyherbal remedy (*Agbo-Iba PMII*) contains potent substances with antipyretic, anti-inflammatory and analgesic effects. Thereby, suggesting that these pharmacological effects are vital to the symptomatic management of malaria feverin Southern Nigeria. It is, therefore, recommended for subsequent development for clinical application in malaria therapy.

Keywords: Antipyretic, Anti-inflammatory, Analgesic, Polyherbal formulation, Southern Nigeria

1. Introduction

Malaria disease remains a major cause of death in Africa in general and Nigeria in particular. The female Anopheles mosquitoes serve as the vector for plasmodium parasite transmission in humans. Despite the many interventions including the provision of insecticide treated nets, household spraying and use of ACTs/prophylactics vis a vis continuous attempts to develop a vaccine for malaria, it remains a major cause of an estimated 214 million clinical episodes and 438,000 deaths globally. Of which 90% of the deaths occur in sub-Saharan Africa where Nigeria and the DR Congo accounted for 35% of these deaths [1].

Infections such as malaria also cause a lot of undesirable functional alterations in the host's body system including pyrexia, inflammation, pain and oxidative stress. Pyrexia emanates from infection [2]. Inflammation is also a usual occurrence in malaria infection arising with-in living tissues due to injury. During infection, malaria parasites produce diverse toxins, which stimulate the host immune cells to produce excessive cytokines, which drive the disease progression [3].

Though the pro-inflammatory response protects host from the parasites in the blood. It results in elevated temperature with attendant chills and body pains [3]. The inflammation in malaria occurs with elevated pro-inflammatory cytokines, including

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* Corresponding author.
E-mail addresses: alexthemain076@gmail.com, pass.chijindu@unidel.edu.ng (P.C. Chijindu), mcdonald@uniben.edu (M. Idu).

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interleukin Iβ (IL - 1β), IL − 6, IL − 8, IL − 23, gamma interferon (IFN − γ) and tumour necrosis factor alpha [3–8]. In contrast, decreased quantities of other pro-inflammatory cytokines like IL − 12 and IFN - α are related to intense malaria infection in humans [9].

Besides inflammation, increased pain is a frequently occurs in malaria infection [2]. It is proposed that inflammation heightens pain sensitivity, which is subject to the effects of analgesics. This principle therefore forms the basis on which Analgesic activity is usually measured [2,10].

From the high-points presented, it is therefore evident that malaria manifests symptoms of pyrexia, body pains, accompanied by inflammation. Therefore, the ideal antimalarial agent should not only possess antispasmodic effects but also anti-inflammatory, antipyretic and antinociceptive activities. Hence, this study investigated the antipyretic, anti-inflammatory and analgesic activities of a traditional polyherbal formulation (*Agbo-Iba PMII*) used to treat malaria in Southern Nigeria.

2. Methods

2.1. Selection, collection and processing of plant material

Sixteen (16) plants were selected based on the criterion of their frequency of usage identified from an ethnomedicinal survey by Iyamah and Idu [11] to constitute the polyherbal formulation (*Agbo-Iba PMII*) used in this study.

Fresh parts of the constituent plants of *Agbo-Iba PMII* (the leaves of *Azadirachtaindica, Cymbopogon, citratus, Mangiferaindica. Carica papaya, Psidiumguajava, Vernoniaamygdalina, Ocimum gratissimum, Chromolaena odorata, Anacardium occidentale* and *Persea americana*; stem barks of *Enantia chlorantha* and *Alstonia boonei; roots of Morinda lucida* and *Nauclea latifolia,* and the fruit skin of *Citrus aurantifolia* and *Ananas comosus*) were harvested from their natural habitat in the study area. The freshly harvested plant parts were air dried and pulverized separately. Powdered samples were then stored in airtight containers. Herbarium specimen were also prepared and deposited at the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Edo state, Nigeria with voucher numbers (UBH-O215; UBH-C311, UBH-A426, UBH-P154, UBH-E253, UBH-A105, UBH-M315, UBH-N115, UBH-C341, UBH-A131).

2.2. Preparation and extraction of AGBO-IBA PMII

One thousand grams (1000 g) each of the sixteen (16) powdered plant material were exhaustively extracted using a Soxhlet extractor in absolute ethanol. The extracts were evaporated in an air oven at 40 °C, weighed and kept in airtight containers at 4 °C prior to use [12].

An equal portion of each crude extract was weighed and dissolved in Dimethyl sulphate ($DMSO_4$) and subsequently diluted to lower concentration of $DMSO_4$ of <1% to prevent carry over (solvent) effect [13].

The sixteen (16) different plant extracts were grouped into four groups consisting of four plants each, based on their frequency of usage. The four plant extracts in each group were combined in a proportion of 1:1:1:1 (Table 1).

2.3. Phytochemical screening

The polyherbal formulation (*Agbo-Iba PMII*) was screened for phytochemical constituents using standard procedure as described by Evans [14].

2.4. Gas chromatography-mass spectrometry (Gc-Ms) analysis

The gas chromatography − mass spectrometry (GC−MS) analysis of the polyherbal formulation was performed using a GC−MS (Modal; QP2010 series, Shimadzu, Tokyo, Japan) equipped with a VF − 5 ms fused silica capillary column of 30m length, 0.25 mm diameter and 0.25 mm film thickness. For GCMS detection, an electron ionization system with ionization energy of 70eV was used. Helium gas (99.99 %) was used as a carrier gas at a constant flow rate of 1.51 N/min. Injection and mass transfer line temperature were set at 200 and 240 °C respectively. The oven temperature was programmed from 70 to 22 °C at 10 °C/min, held isothermal for 1min and finally raised to 300 °C at 10 °C/min 2 ml of water solution of the sample was manually injected in the split less mode, with a split ratio of 1:40 and with mass scan of 50–600 amu. Total running time of GC−MS is 35 min. The relative percentage of each extract constituents was expressed as a percentage with peak area normalization. Interpretation of mass spectrum of plant extracts was conducted using the data base of National Institute of Science and Technology (NIST) library having more than 62, 000 spectral patterns. The spectrum of the compounds was also compared with the spectrum of the National Institute of Standard and Technology (NIST) library database.

2.5. Animals used

Both sexes of albino Mice and Male Wistar rats were obtained from the Animal House unit of

Table 1. Grouping of the 16 plants from the highest frequency of citation to the least.

S/N	PLANTS	VOUCHER NUMBERS	FAMILY	LOCAL NAMES	COMMON NAME	PARTS USED	RFC	COMBINATION RATIO
GROUP I								
1	*Azadirachta indica*A. Juss	UBHdt/SN/131	Meliaceae	Dongoyaro (H)		Leaves	1.0	1
2	*Cymbopogon citratus*(D.C) Stapf.	UBHdt/SN/011	Poaceae	Ewe-tea, Kooko-oba (Y)	Lemon grass	Leaves	0.95	1
3	*Mangifera indica* L.	UBHdt/SN/023	Anacardiaceae	Mangoro(Y)	Mango	Leaves	0.95	1
4	*Carica papaya* L.	UBHdt/SN/086	Caricaceae	Eto-oyibo(U),Ibepe(Y)		Leaves	0.81	1
GROUP II								
5	*Psidium guajava* L.	UBHdt/SN/079	Myrtaceae	Gilofa (Y)	Guava	Leaves	0.70	1
6	*Citrus aurantifolia*(Chrism.). Swingle	UBHdt/SN/121	Rutaceae	Osan-wewe (Y), Oroma-nkirisi (I), Alimo-ebo(E)	Lime	Fruit skin	0.67	1
7	*Enantia chlorantha* Oliv.	UBHdt/SN/053	Annonaceae	Awopa (Y)	African yellow wood	Stembark	0.57	1
8	*Vernonia amygdalina* L.	UBHdt/SN/078	Asteraceae	Kiriologbo(Ij), Ewuro (Y).	Bitter Leaf	Leaves	0.53	1
9	*Morinda lucida* Benth	UBHdt/SN/072	Rubiaceae	Oruwo (Y), Njisi (I).	Brimstone tree	Roots	0.52	1
10	*Ocimum gratissimum* L	UBHdt/SN/047	Lamiaceae	Efinrin-ajase(Y), Ufuo-oyibo (U).	Tea bush, Scent Leaf	Leaves	0.51	1
11	*Chromolaena odorata*(L).R King&H. Robinson	UBHdt/SN/002	Asteraceae	Ewe-akintola, Ewe-awolowo (Y)	Siam weed	Leaves	0.49	1
12	*Anacardium occidentale* L.	UBHdt/SN/124	Anacardiaceae	Kasu(Y)	Cashew	Leaves	0.48	1
GROUP IV								
13	*Ananas comosus* (L). Merr.	UBHdt/SN/004	Bromeliaceae	Ope-Oyibo (U)	Pineapple	Fruit skin	0.47	1
14	*Persea americana*Mill	UBHdt/SN/057	Lauraceae	Pia(Y), Ube-oyibo(I), Uruvwon(U)	Avocadopear	Leaves	0.47	1
15	*Nauclea latifolia*(Smith) Bruce	UBHdt/SN/056	Rubiaceae	Egbesi (Y)	Africanpeach	Roots	0.46	1
16	*Alstoniaboonei* De Wild	UBHdt/SN/100	Apocynaceae	Ahun (Y)	Stool wood	Stem bark	0.46	1

Local names:(Y) − Yoruba,(I) − Igbo, (H) − Hausa, (B) − Benin, (E) − Efik, (Ij) − IJaw,(U) − Urhobo.

Emma Maria Scientific Research Laboratories and Consultancy, Abraka, weighing between 18-32 g and 100–150 g respectively. Both mice and rats were housed in well-ventilated animal unit with a temperature of 24 ± 2 °C, relative humidity 50–60 % and a 12 h light/dark cycle. The animals were supplied with standard grower mash diet and water *ad libitum* in a standard wire meshed wooden cages and allowed to acclimatize for 1 week before experiment. All the animal experimentation was carried out according to NIH Guide for Care and Use of Laboratory Animals (Pub. no, 85-23, revised 1985). Ethical approval for this study was obtained from the Nigerian Institute of Medical Research (NIMR) Institutional Review Board (IRB) (IRB/16/332).

2.6. Dosage preparation

The dose used for the experiment were prepared accordingly; 2, 4 and 8 g of the prepared polyherbal was weighed dissolved into 10 m/kgl of 5 % $DMSO_4$ to prepare 200, 400 and 800 mg/kg. The individual dosages were then calculated accordingly.

2.7. Antipyretic study

An antipyretic study was carried out using three (3) models described as follows:

2.8. Yeast-induced hyperthermia

This was carried out based on the method described by Mukherjee et al. [15]. Rats were randomly divided into five groups of six rats each. The basal rectal temperatures of the animals were recorded (T_0°C) over a period of one hour and the average basal rectal temperature of each animal was recorded. 10 ml/kg of yeast suspension (15 % in 0.5 % w/v methylcellulose) was injected subcutaneously into the rats to induce pyrexia. Nineteen hours after yeast injection, the rectal temperatures of animals were taken and animals showing rises in temperature of less than 0.6 °C were discarded [15]. After the establishment of pyrexia, 5 % $DMSO_4$ (10 ml/kg), (200, 400 and 800 mg/kg) of test compound (*Agbo-Iba PMII*) and Acetylsalicylic acid (100 mg/kg) were orally administered to qualified rats. The rectal temperatures of animals were then recorded at 1, 2, 3, and 4 h post-treatment (Tx°C).

2.9. D-amphetamine-induced hyperthermia

The basal rectal temperatures of rats fasted for 12 h were recorded (T_o°C) prior to the induction of pyrexia by intraperitoneal injection of D-amphetamine 10 mg/kg [16]. After confirmation of hyperthermia in the experimental animals 30 min after *D*-amphetamine administration, treatment was carried out in five groups of six animals each through the oral route; 5% $DMSO_4$ (10 ml/kg), (200, 400 and 800 mg/kg) of test compound (*Agbo-Iba PMII*) and Acetyl salicylic acid (100 mg/kg). The rectal temperatures of the animals were then recorded at 1, 2, 3, 4 h post-treatment (Tx °C).

2.10. 2, 4- Dinitrophenol-induced hyperthermia

The basal rectal temperature of rats fasted for 12 h was recorded (T_o°C). Pyrexia was then induced by intraperitoneal injection of 2, 4-DNP (prepared at a concentration of 1 mg/ml in 0.9 % Sodium Chloride solution) at a dose of 20 mg/kg using the method of Berkan et al. [16]. After the confirmation of hyperthermia 30 min after 2,4-DNP administration, treatment was then carried out orally in five groups of six animals each as outlined: 5% $DMSO_4$ (10 ml/kg), (200, 400 and 800 mg/kg) of test compound (*Agbo-Iba PMII*) and Acetylsalicylic acid (100 mg/kg). The rectal temperature of rats was then recorded at 1, 2, 3 and 4 h post-treatment (Tx°C).

2.11. Anti-inflammatory study

Anti-inflammatory activity of the polyherbal formulation (*Agbo-Iba PMII*) was evaluated using carrageenan induced rat paw oedema assay model.

2.12. Rat paw oedema assay

The animals were divided into five groups (5 rats per group) (pregnant females excluded) and were orally administered a dose (200, 400 and 800 mg/kg) of test compound (*Agbo-Iba PMII*), Indomethacin (10 mg/kg) and 5 % $DMSO_4$ (10 ml/kg) for control, After one hour, carrageenan suspension (0.1 ml, 1 %) in saline (0.9 % NaCl) solution was injected into the subplantar area of the right hind paw. The paw thickness was measured hourly over a period of 5 h

$$\text{Inhibition}\ (\%) = \frac{[\text{Tx}\,^{\circ}\text{C} - \text{To}\,^{\circ}\text{C Control}] - [\text{Tx}\,^{\circ}\text{C} - \text{To}\,^{\circ}\text{C Treatment}]}{[\text{Tx}\,^{0}\text{C} - \text{To}\,^{0}\text{C Control}]}$$

with the aid of veneer calliper. Anti-inflammatory activity was evaluated by the method of Duffy et al. [17] and the percentage inhibition of oedema level by drugs were compared to control. Mathematically, anti-inflammatory activity was evaluated using the formula below:

$$\% \text{ Inhibition} = 100 - \{100 \text{ x } (Dt / C)\}$$

Where Dt is the mean value for drug-treated animals and C is the mean value for animals treated without drug (control).

2.13. Evaluation of analgesic activity

Evaluation of Analgesic activity was carried out using three models described as follows:

2.14. Acetic acid induced writhing

The method of Koster et al. [36] were employed. The animals were divided into five groups with 5 mice in each group (pregnant females excluded). The animals were administered a dose (200, 400 and 800 mg/kg) of test compound (*Agbo-Iba PMII*) by oral cannula. After 1 h, the animals were injected intraperitoneally with 0.2 ml/mouse of 0.6 % v/v acetic acid solution. Acetic acid-induced writhing was counted and recorded within 30 min. 5 % $DMSO_4$ (10 ml/kg) was used as the negative control while acetylsalicylic acid (100 mg/kg) was used as reference drug. The mean of the abdominal constrictions for five mice in each group, which is an indication of analgesic activity, was recorded. Inhibition (%) of abdominal constrictions of the test compound was compared with the control group using the method of Duffy et al. [17]. Analgesic activity was computed in terms of inhibition calculated using the formula below:

$$\text{Inhibition } (\%) = 100 - \{100 \text{ x } (Dr / Cr)\}$$

Where Dr is the mean drug response and Cr is mean control response.

2.15. Hot plate method

The method described by Shetty and Anika [18] as modified by Franzotti et al. [19] was used for this study. Albino mice of both sexes were randomly grouped into five groups of five mice each, (pregnant females excluded), fasted for 12–18 h with adequate clean water provided *ad libitum*. Each of the mice was placed on a hot plate maintained at the temperature of 55 ± 1 °C and the pain reaction time (PRT) or latency period determined with a stop watch was recorded which represents the time taken for the mice to react to the pain stimulus. The response to pain stimulus considered included; jumping, raising and licking of the hind foot. The cut off time was fixed for 20 s. This served as control pain reaction time. The mice were then treated or administered orally, as follows: Group A received $DMSO_4$ solution (negative control) (10 ml/kg), Group B, C, D received the polyherbal formulation (200, 400 and 800 mg/kg respectively) and Group E received Morphine (10 mg/kg). After 1hr of treatment, the latency period observations were recorded at a time interval of 30, 60, 90, and 120 s.

2.16. Analgesy-meter test (Randall–Selitto Test)

The method of Randall and Selitto [20] and modified by Winter et al. [21] was used. Wistar rats (140–190 g) of either sex were randomly allocated into groups of at least five animals per group (pregnant females excluded). The animals were fasted over night with free access to water, which was only withdrawn during the experiment. The animals were administered orally 5% $DMSO_4$ (10 ml/kg), indomethacin (10 mg/kg), (200, 400 and 800 mg/kg) of test compound (*Agbo-Iba PMII*). One hour later, 0.1 ml of 1 % w/v carrageenan in normal saline was injected subcutaneously into the plantar surface of the right hind leg of the rat. Three hours later, the pressure was applied through a tip to the plantar surface of the rat's foot at a constant rate using the Analgesymeter (UgoBasil Apparatus for Biological Research, batch number: 37215). The pain threshold was considered reached when the animal struggles, squeals or attempts to bite. The weight at which this occurred was recorded. The percentage increase in pain threshold was obtained using the following formula:

2.17. Statistical analysis

The results were expressed as Mean ± SEM (standard error of the mean) and statistical significance of the treatment effect was analyzed using the student's t-test statistics (LSD t-Test), one way analysis of variance (ANOVA), followed by post Hoc LSD's test for multiple comparison, using software for social sciences (SPSS) version 20 windows software and significance at *P values < 0.05 while ^P value > 0.05 were considered to be statistically non-significant.

3. Results

The results obtained from this study are presented as follows:

3.1. Phytochemical analysis

The qualitative phytochemical analysis of the *Agbo-Iba PMII* formulation (1:1:1:1) having equal concentration of all plant extracts show the presence of various phytochemicals in different degrees (Table 2).

3.2. Gas chromatography mass spectrometry (GC–MS) analysis

In the present study, *Agbo-Iba PMII* (Formulation 1:1:1:1) was subjected to GC–MS analysis to identify the potential phytochemical constituents present (Fig. 1).

The GC–MS analysis show the presence of 42 compounds found in the polyherbal formulation (1:1:1:1) (Table 3).

4. Evaluation of antipyretic, anti-inflammatory and analgesic activities

4.1. Antipyretic study

4.1.1. Yeast-induced hyperthermia in rats

The polyherbal formulation (*Agbo-Iba PMII)* generated significant ($p < 0.05$) suppression of yeast-induced pyrexia across all dosages tested (200, 400 and 800 mg/kg) (Table 4). Both 400 and 800 mg/kg had the most significant effects from exactly 1hr, 2 h, 3 h and 4 h after induction compared to the negative control and 200 mg/kg, which only brought down the temperature 4 h later. *Agbo-Iba PMII* at 400 and 800 mg/kg also elicited better effects than the standard drug- Acetyl Salicylic acid that only produced significant effects 3–4 h later relative to control.

4.1.2. D-Amphetamine-induced hyperthermia in rats

The polyherbal formulation (200, 400 and 800 mg/kg) generated significant ($p < 0.05$) dose and time dependent reduction in hyperthermia induced by the administration of D-amphetamine 30 min after administration (Table 5). However, 800 mg/kg elicited significant effects 3–4 h after induction, relative to control. While the standard drug- Acetyl salicylic acid produced effects 1hr after induction relative to control.

4.1.3. 2, 4-Dinitrophenol-induced hyperthermia in rats

The polyherbal formulation (at doses 400 and 800 mg/kg) as well as the standard drug (Acetyl salicylic acid) caused significant ($p < 0.05$) dosage and time related inhibitory effects on temperature increase occurring at 1, 2, 3 and 4 h post treatment (Table 6). 200 mg/kg produced effects from 2 to 4 h post treatment relative to control.

4.2. Anti-inflammatory activity

4.2.1. Effect of Agbo-Iba PMII on carrageenan induced rat paw oedema

Agbo-Igba PMII exhibited effects on carrageenan-induced rat paw oedema as presented (Table 7). The

*Table 2. Qualitative phytochemical analysis of the polyherbal formulation (*Agbo-Iba PMII*).*

Chemicals	Test	Result
Proteins	Millon's test	++
Carbohydrates	Fehling's Test	++
	Iodine Test	++
Phenols		+++
Tannins	Ferric chloride	++−
Flavonoids	Shinoda	+++
	Alkaline Reagent	+++
	Sodium hydroxide	++
Phytosterol		++
Triterpenoids	Liebermann–Bucchard Test	+++
Phlobatannins		++
Saponins	Frothing	+++
Glycosides	Keller-kilanitest	++
	Liebermann's	+++
Steroid	Liebermann–Bucchard Test	++
Terpenoids		+++
Alkaloids	Dragendoff's test	+++
	Mayer's and Wagner's	+++
Anthraquinones	Modified Bontrager's	+++
	Bontrager's	++

Keys: + + + abundantly present; + + − Moderately present; + − − Present in trace amount.

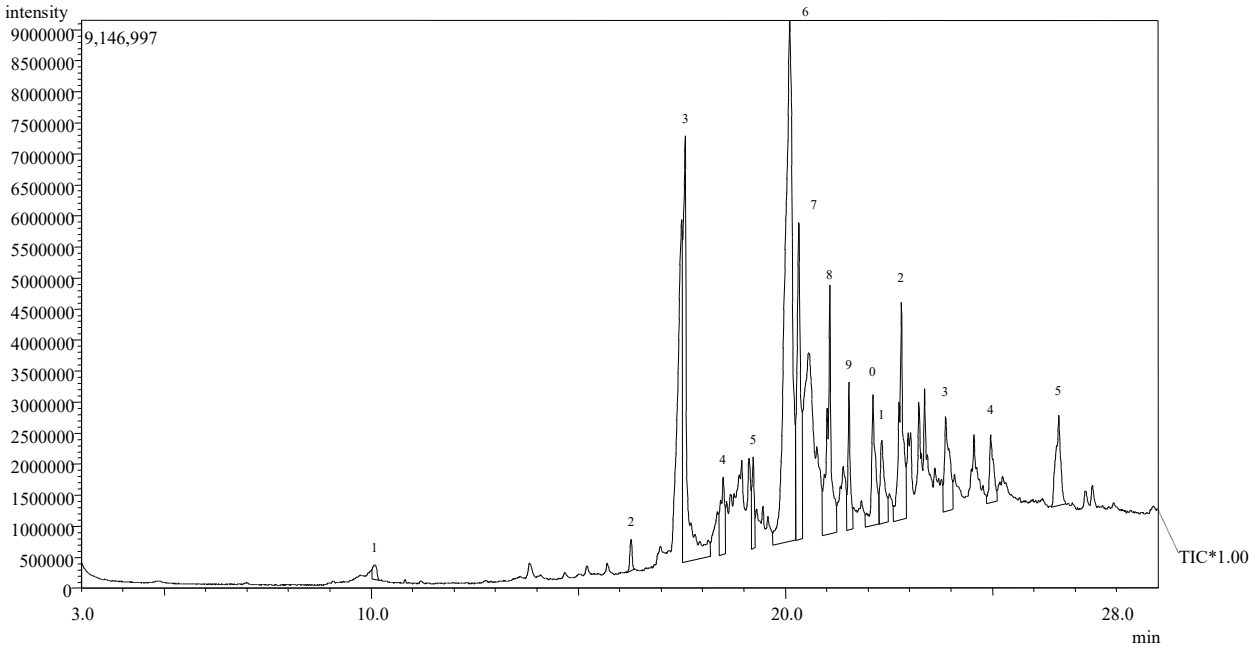
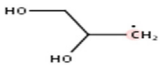
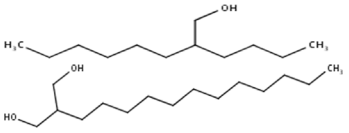
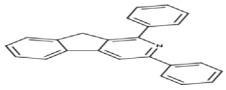
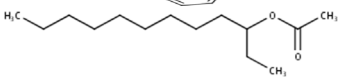
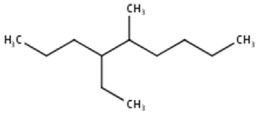
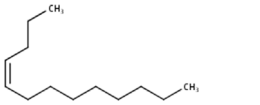
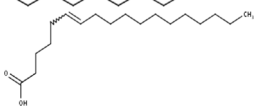
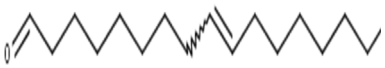
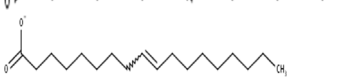
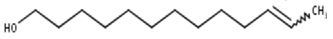


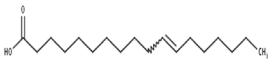
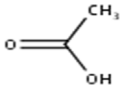
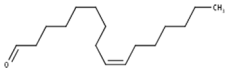
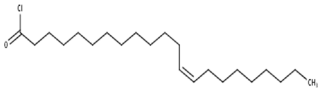
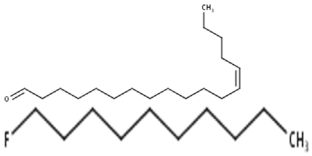
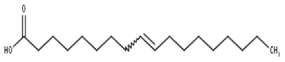
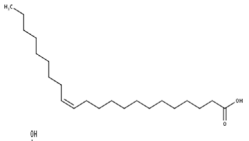
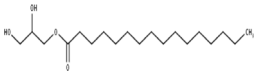
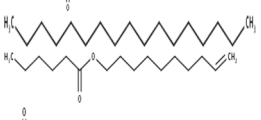
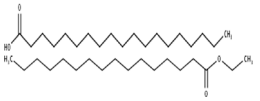
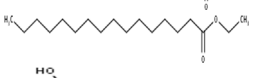
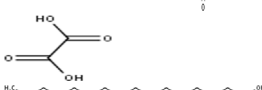
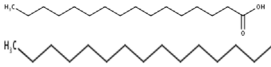
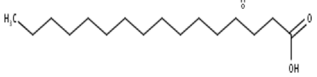
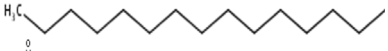
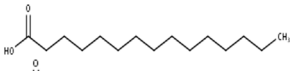
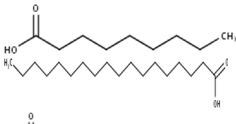
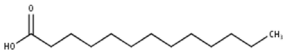
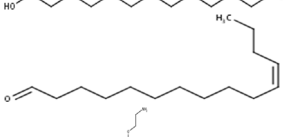
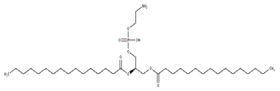
Fig. 1. Graph showing the various peaks from the GC–MS analysis.

*Table 3. Phytochemical constituents identified from GC–MS analysis of the polyherbal formulation (*Agbo-Iba PMII*).*

S/N	Compound	Molecular formula	Molecular weight g/mol	Structure
1.	2,3-Dihydroxypropyl	$C_{19}H_{38}O_4$	330	
2.	2-butyl-1-octanol	$C_{12}H_{26}O$	186	
3.	2-dodecyl-1,3-propanediol	$C_{15}H_{32}O_2$	244	
4.	1,3-Diphenyl-2-azafluorene	$C_{24}H_{17}N$	319.398	
5.	3-Acetoxydodecane	$C_{14}H_{28}O_2$	228	
6.	4-Ethyl-5-methylnonane	$C_{12}H_{26}$	170	
7.	4-Tridecene,(Z)	$C_{13}H_{26}$	182	
8.	6-octadecenoic acid	$C_{19}H_{36}O_2$	294	
9.	9-octadecenal	$C_{18}H_{34}O$	266	
10.	9-octadecenoate	$C_{18}H_{34}O_2$	282	
11.	11-Tridecen-1-ol	$C_{13}H_{26}O$	198	

(*continued on next page*)

Table 3. (continued)

S/N	Compound	Molecular formula	Molecular weight g/mol	Structure
12.	11-Octadecenoic acid	$C_{19}H_{36}O_2$	296	
13.	Acetic acid	$C_{10}H_{20}O_2$	172	
14.	Cis-9-Hexadecenal	$C_{16}H_{30}O$	238	
15.	Cis-13-Docosenoyl chloride	$C_{18}H_{34}O$	266	
16.	Cis-13-octadecenal	$C_{18}H_{34}O$	266	
17.	Decane 1-fluoro	$C_{10}H_{21}F$	160	
18.	9-octadecenoic acid	$C_{18}H_{34}O_2$	282.461	
19.	Delta 13-cis-Docosenoic acid	$C_{22}H_{42}O_2$	338	
20.	Glycerol 1-monopalmitate	$C_{19}H_{38}O_4$	330.509	
21.	Heptadecane	$C_{20}H_{42}$	282	
22.	Hexanoic acid 9-decen-l-yl ester	$C_{16}H_{30}O_2$	254	
23.	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256	
24.	Nonadecanoic acid	$C_{19}H_{38}O_2$	298	
25.	Ethyl hexadecanoate	$C_{18}H_{36}O_2$	284	
26.	Octadecanoic acid	$C_{22}H_{44}O_4$	372	
27.	Oxalic acid	$C_{20}H_{38}O_4$	342	
28.	Palmitate	$C_{36}H_{60}O_2$	524.8	
29.	Palmitic acid	$C_{17}H_{34}O_2$	270	
30.	Pentadecanecarboxylic acid	$C_{16}H_{32}O_2$	256	
31.	Pentadecanoic acid	$C_{17}H_{34}O_2$	270.457	
32.	Nonanoic acid	$C_{15}H_{30}O_2$	242	
33.	Stearic acid	$C_{18}H_{36}O_2$	284	
34.	Tridecanoic acid.	$C_{14}H_{28}O_2$	228	
35.	Z-11-pentadecenal	$C_{15}H_{28}O$	224.38	
36.	1,2-Dipalmitoyl-sn-glycero-3-phosphoethanolamine	$C_{49}H_{86}N_5O_{15}P$	1016.2	

(*continued on next page*)

Table 3. (continued)

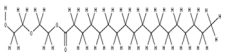
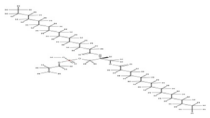
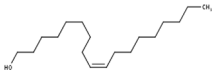
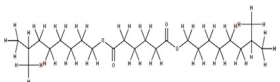
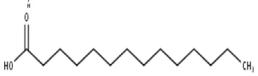
S/N	Compound	Molecular formula	Molecular weight g/mol	Structure
37.	Aqua cera	$C_{22}H_{44}O_4$	372	
38.	Dipalmitoylphosphoethanolamine	$C_{37}H_{74}NO_8P$	691.97	
39.	Cis-9-Octadecen-1-ol	$C_{18}H_{36}O$	268.4	
40.	Diisononyladipate	$C_{24}H_{46}O_4$	398.6	
41.	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.3	
42.	Eicosanoic acid	$C_{20}H_{40}O_2$	312.5	

Table 4. Effect of Agbo-Iba PMII *on Yeast-induced hyperthermia in rats.*

Groups	Doses (mg/kg)	Baseline	Rectal Temperature (°C) after 18 Hours Incubation	1 Hours	2 Hours	3 Hours	4 Hours
Control (DW)	0.5 ml	36.07 ± 0.25	38.49 ± 0.32	38.82 ± 0.20	38.49 ± 0.17	38.27 ± 0.19	38.00 ± 0.18
Polyherbal formulation	200	36.01 ± 0.19	38.56 ± 0.33	38.23 ± 0.32	37.93 ± 0.24	37.47 ± 0.15	37.02 ± 0.17*
Polyherbal formulation	400	36.31 ± 0.07	38.61 ± 0.22	37.95 ± 0.24*	37.57 ± 0.19*	37.25 ± 0.16*	36.57 ± 0.16*
Polyherbal formulation	800	36.12 ± 0.11	38.71 ± 0.25	37.92 ± 0.17*	37.28 ± 0.24*	36.64 ± 0.12*	36.03 ± 0.07*#
Acetylsalicylic acid	100	36.32 ± 0.27	38.54 ± 0.28	38.12 ± 0.23	37.67 ± 0.25	37.25 ± 0.29*	36.37 ± 0.29*

Data expressed as mean ± SEM, n = 6. *$p < 0.05$vs Control, #$p < 0.05$vs 200 mg/kg, $^{\alpha}p < 0.05$ vs 400 mg/kg, $^{\beta}p < 0.05$ vs 800 mg/kg.

result show increase in rat paw oedema in both the polyherbal formulation and Indomethacin at different time intervals especially in the first 4 h post treatment. However, significant reduction in rat paw oedema was observed with 400 and 800 mg/kg test drug as well as with the reference drug-Indomethacin between 5 and 6 h interval post treatments (Fig. 2).

4.3. Analgesic activity

4.3.1. Mouse writhing test

The polyherbal formulation (*Agbo-Iba PMII*) significantly ($p < 0.05$) reduced the acetic acid-induced writhes counts in mice in a dosage and time related manner (Table 8). The polyherbal formulation (*Agbo-Iba PMII*) across the various dosages employed (200, 400 and 800 mg/kg) provided pain relief above the 30 min observation period of the study (with 17.25%, 27.84% and 36.08% respectively) relative to control. However, these effects were less than that produced by the reference drug- Acetyl salicylic acid producing up to 65.49% suppression of acetic acid-induced writhes.

4.3.2. Hot plate test

The result of the hot plate test on the polyherbal formulation (*Agbo-Iba PMII*) are presented in Table 9. Elevation in latency time occurred in all treatment groups for 60 s. However, the test group (*Agbo-Iba PMII*) at 800 mg/kg exhibited significant ($p < 0.05$)

Table 5. Effect of Agbo-Iba PMII *on D-Amphetamine-induced hyperthermia in rats.*

Groups	Doses (mg/kg)	Baseline Temperature (°C)	1 Hour	2 Hours	3 Hours	4 Hours	5 Hours
Control (DW)	0.5 ml	36.81 ± 0.15	39.45 ± 0.23	38.18 ± 0.41	38.23 ± 0.30	37.99 ± 0.24	39.89 ± 0.35
Polyherbal formulation	200	36.74 ± 0.17	38.54 ± 0.43	38.19 ± 0.24	37.35 ± 0.09	37.07 ± 0.09	39.20 ± 0.50
Polyherbal formulation	400	36.97 ± 0.16	38.65 ± 0.23	37.97 ± 0.06	37.58 ± 0.07	37.25 ± 0.06	39.33 ± 0.40
Polyherbal formulation	800	36.60 ± 0.14	38.46 ± 0.37	37.36 ± 0.18	36.98 ± 0.25*	36.73 ± 0.24*	39.88 ± 0.46
Acetylsalicylic acid	100	36.90 ± 0.16	38.21 ± 0.33*	37.76 ± 0.19	37.53 ± 0.16	37.38 ± 0.14	39.12 ± 0.52

Data expressed as mean ± SEM, n = 6. *$p < 0.05$vs Control, #$p < 0.05$vs 200 mg/kg, $^{\alpha}p < 0.05$ vs 400 mg/kg, $^{\beta}p < 0.05$ vs 800 mg/kg.

Table 6. Effect of Agbo-Iba PMII *on 2,4-DNP-induced hyperthermia in rats.*

Groups	Doses (mg/kg)	Baseline Temperature (°C)	1 Hour	2 Hours	3 Hours	4 Hours	5 Hours
Control (DW)	0.5 ml	37.29 ± 0.17	39.70 ± 0.10	39.44 ± 0.07	39.17 ± 0.07	38.98 ± 0.07	40.67 ± 0.10
Polyherbal formulation	200	37.25 ± 0.06	39.69 ± 0.08	38.48 ± 0.08*	37.80 ± 0.06*	37.69 ± 0.05*	40.71 ± 0.09
Polyherbal formulation	400	37.44 ± 0.08	39.32 ± 0.04*$^{\#}$	38.20 ± 0.04*	37.29 ± 0.04*$^{\#}$	37.23 ± 0.05*$^{\#}$	40.70 ± 0.16
Polyherbal formulation	800	37.22 ± 0.06	38.74 ± 0.28*$^{\#}$	37.90 ± 0.11*$^{\#}$	37.14 ± 0.04*$^{\#}$	36.90 ± 0.06*$^{\#\alpha}$	40.94 ± 0.04
Acetylsalicylic acid	100	37.30 ± 0.05	39.28 ± 0.04*$^{\#\beta}$	38.13 ± 0.06*$^{\#}$	37.50 ± 0.07*$^{\beta}$	37.23 ± 0.03*$^{\#}$	40.83 ± 0.04

Data expressed as mean ± SEM, n = 6. *$p < 0.05$vs Control, $^{\#}p < 0.05$vs 200 mg/kg, $^{\alpha}p < 0.05$ vs 400 mg/kg, $^{\beta}p < 0.05$ vs 800 mg/kg.

Table 7. Effect of Agbo-Iba PMII *on carrageenan-induced paw oedema.*

Group	Doses (mg/kg)	1 Hour	2 Hours	3 Hours	4 Hours	5 Hours	6 Hours
Control (DW)	0.5 ml	3.48 ± 0.38	4.28 ± 0.23	4.92 ± 0.17	6.02 ± 0.17	7.28 ± 0.23	7.32 ± 0.17
Polyherbal formulation	200	3.88 ± 0.39	4.40 ± 0.53	4.80 ± 0.51	5.60 ± 0.51	6.70 ± 0.53	6.52 ± 0.37
Polyherbal formulation	400	3.20 ± 0.40	4.10 ± 0.10	4.42 ± 0.10	5.02 ± 0.10	6.10 ± 0.10*	6.00 ± 0.10*
Polyherbal formulation	800	3.36 ± 0.30	3.40 ± 0.10	3.84 ± 0.07	4.08 ± 0.22*$^{\#}$	4.16 ± 0.14*$^{\#\alpha}$	3.94 ± 0.21*$^{\#\alpha}$
Indomethacin	10	3.00 ± 0.27	3.92 ± 0.18	4.16 ± 0.14	4.46 ± 0.14*$^{\#}$	5.12 ± 0.18*$^{\#}$	4.64 ± 0.20*$^{\#\alpha}$

Data expressed as mean ± SEM, n = 5. *$p < 0.05$vs Control, $^{\#}p < 0.05$vs 200 mg/kg, $^{\alpha}p < 0.05$ vs 400 mg/kg, $p < 0.05$vs 800 mg/kg.

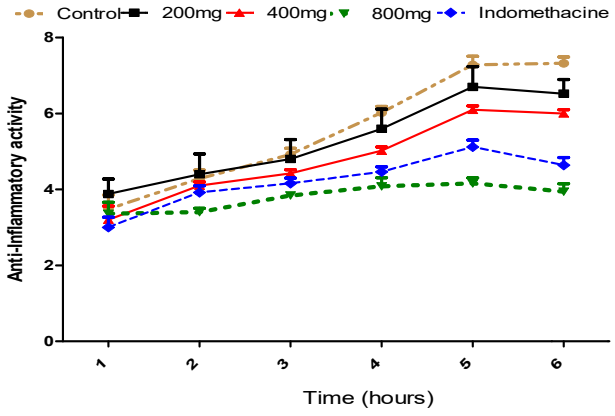


Fig. 2. Effect of polyherbal formulation (Agbo-Iba PMII) *on carrageenan-induced rat paw oedema.*

elevation in latency period up to 120 s relative to the reference drug.

4.3.3. Anagesy-meter test

The result obtained from this study show significant ($p < 0.05$) dosage and time related rise in pain threshold by the test drug (*Agbo-Iba PMII*) and reference drug (Indomethacine) compared to control in rats (Table 10). However, the polyherbal formulation (*Agbo-Iba PMII*) at 800 mg/kg caused a higher percentage inhibition (39.22%) relative to the reference drug (giving 39.00% inhibition).

5. Discussion

The result presented in this study reveals that the *Agbo-Iba PMII* which is a traditional polyherbal remedy used in the treatment of malaria in Southern Nigeria possess antipyretic, anti-inflammatory and analgesic activities.

Agbo-Iba PMII produced a significant reduction in yeast, D-amphetamine and 2,4 dinitrophenol (DNP)-induced pyrexia in rats in a dosage related pattern similar to the reference antipyretic drug (Acetyl Salicylic Acid) employed in this study. Pyrexia occurs due to infection, inflammation, malignancy as well as diseases states like malaria [22]. The infected tissue causes increased pro-inflammatory mediators production (cytokines including interleukin 1β, α, β and TNF-α) thereby elevating prostaglandins (PGE_2) production close to the preoptic hypothalamus area of the brain hence, stimulating the hypothalamus to raise the body

Table 8. Effect of Agbo-Iba PMII *on acetic acid induced writhing in mice.*

Groups	Doses (mg/kg)	No. of Writhes Pre-10 Min	No. of Writhes Pre-20 Min	No. of Writhes Pre-30 Min	Total	% Inhibition
Control (DW)	0.5 ml	16.67 ± 3.18	15.50 ± 3.17	19.40 ± 9.33	51.00 ± 9.49	0
Polyherbal Formulation	200	4.40 ± 0.81*	11.60 ± 1.57	26.20 ± 6.19	42.20 ± 6.12	17.25
Polyherbal Formulation	400	5.20 ± 0.66*	15.20 ± 2.78	16.40 ± 1.57	36.80 ± 3.85	27.84
Polyherbal Formulation	800	1.00 ± 1.30	15.20 ± 2.78	16.40 ± 1.29	32.60 ± 3.53	36.08
Acetylsalicylic acid	100	1.60 ± 0.51*$^{\beta}$	7.60 ± 1.21*$^{\alpha\beta}$	8.40 ± 0.81$^{\#}$	17.60 ± 1.86*$^{\#\alpha\beta}$	65.49

Data expressed as mean ± SEM, n = 5. *$p < 0.05$vs Control, $^{\#}p < 0.05$vs 200 mg/kg, $^{\alpha}p < 0.05$ vs 400 mg/kg, $p < 0.05$vs 200 mg/kg.

Table 9. Effect of polyherbal formulation (Agbo-Iba PMII)*on the latency time in the hot plate test in mice.*

Groups	Doses (mg/kg)	Baseline	30 s	60 s	90 s	120 s
Control	0.5 ml	15.06 ± 1.50	24.60 ± 2.16	27.00 ± 2.60	30.80 ± 2.03	23.20 ± 1.53
Polyherbal formulation	200	28.71 ± 5.02*	32.60 ± 1.94	38.00 ± 3.27*	38.40 ± 2.04	34.80 ± 2.48*
Polyherbal formulation	400	33.70 ± 1.63*	34.40 ± 2.11*	42.00 ± 1.52*	39.20 ± 2.80	34.80 ± 2.27*
Polyherbal formulation	800	42.24 ± 3.42*$^{\#\alpha}$	41.00 ± 3.22*	44.60 ± 2.69*	47.20 ± 2.66*	48.80 ± 3.67*$^{\#\alpha}$
Morphine	10	41.51 ± 3.68*$^{\#}$	41.60 ± 1.94*	49.40 ± 1.69*$^{\#}$	50.40 ± 0.24*$^{\#\alpha}$	48.40 ± 2.23*$^{\#\alpha}$

Data expressed as mean ± SEM, n = 5. *$p < 0.05$vs Control, $^{\#}p < 0.05$vs 200 mg/kg, $^{\alpha}p < 0.05$ vs 400 mg/kg, $^{\beta}p < 0.05$ vs 800 mg/kg.

Table 10. Effect of Agbo-Iba PMII *on Analgesy-meter test.*

Groups	Doses (mg/kg)	1 Hour	2 Hours	3 Hours	4 Hours	% Inhibition
Control	0.5 ml	3.72 ± 0.17	4.30 ± 0.16	4.88 ± 0.14	5.46 ± 0.16	0
Polyherbal formulation	200	2.88 ± 0.15*	3.20 ± 0.18*	3.50 ± 016*	3.74 ± 0.22*	27.45
Polyherbal formulation	400	2.94 ± 0.28	3.34 ± 0.32*	3.62 ± 0.20*	3.82 ± 0.32*	25.27
Polyherbal formulation	800	2.08 ± 0.15*$^{\#\alpha}$	2.80 ± 0.28*	3.00 ± 0.23*	3.28 ± 0.27*	39.22
Indomethacine	10	1.82 ± 0.06*$^{\#\alpha}$	2.42 ± 0.15*$^{\alpha}$	3.10 ± 0.25*	3.86 ± 0.12*	39.00

Data expressed as mean ± SEM, n = 5. *$p < 0.05$vs Control, $^{\#}p < 0.05$vs 200 mg, $^{\alpha}p < 0.05$ vs 400 mg, $^{\beta}p < 0.05$ vs 800 mg.

temperature [23]. Most known antipyretic medications suppresses COX − 2 expressions and the process hinders PGE_2 production to bring down the high body temperature. However, they are harmful to liver cells, cardiac muscle and glomeruli cortex of the kidney [22,24]. Natural antipyretic remedies such as *Agbo-Iba PMII* with minimal toxicity is therefore essential with its mechanism of action involving its effect on COX − 2 leading to decreased accumulation of prostaglandin in the brain [24], through the increase of innate production of hypothermic products including arginine and vasopressin [25] or by vasodilation of superficial blood vessels causing elevated rate of heat loss due to the hypothalamic temperature control centre [26].

The result from this study further revealed that *Agbo-Iba PMII* demonstrated anti-inflammatory activity with a significant dose and time dependent decrease in carrageenan − induced rat paw oedema. 800 mg/kg reduced the paw size down to 3.94 ± 0.21, six (6) hours later which was more effective than, that elicited by the reference drug (Indomethacin).

The carrageenan induced paw oedema models the active phase of severe inflammatory conditions. Development of oedema in the rat paw after inoculation with carrageenan is a two phased occurrence [27] with the first stage caused by the liberation of histamine and serotonin beginning instantly after injection and decreasing under one hour. While the next stage of swelling occurs due to the liberation of prostaglandin-like substances after one hour and remains for three hours [28].This stage of oedema is susceptible to therapeutic steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) as most NSAIDs hinder the second stage of carrageenan − induced oedema [24].The significant inhibition of rat paw oedema by *Agbo-Iba PMII* suggests that it contains active ingredients with anti-inflammatory effects. The significant anti-inflammatory activity displayed could be as a result of the suppression of any inflammatory intermediary and may also contribute immensely to the anti-malarial activity of *Agbo-Iba PMII*. In addition, the phytochemicals revealed from the qualitative phytochemical analysis could have contributed to the anti-inflammatory activity. Phenolic compounds like tannins found in the polyherbal have been known to be effective cyclooxygenase −1 suppressors as well as possessing antiphlogistic effects [29,30]. Glycosides, saponins and triterpenoids also present in this polyherbal are capable of hindering the process of inflammation by suppressing the actions of TNF-α interferon gamma, PGE, iNOS and NF-KB [3,31,32]. In addition, flavonoids equally present in *Agbo-Iba PMII* are also able to attack PGs present in late phase of acute inflammation and pain perception functioning by suppressing its biosynthesis as well as synthesis of other final products in the COX and LOX cycles of immune reactions [33,34].

The polyherbal remedy (*Agbo-Iba PMII*) at the doses tested also displayed analgesic activity as seen in all three models indicating a central and peripheral action. The writhing reaction caused by acetic ascertains peripherally active analgesics [22,35]. Generally, acetic acid generates pain by releasing endogenous substances such as prostaglandins (PGs), histamine, bradykinines, serotonin and substance P, which stimulates nerve endings [22]. The significant decrease in acetic acid-induced writhes by *Agbo-Iba PMII* show that it functions through suppression of PGs production and liberation [36] and more internally generated products.

Results obtained from the hot plate test also revealed that *Agbo-Iba PMII* produced a longer latency period than the negative control and was comparable to that elicited by the reference drug in a dosage related manner. The hot plate test is also regarded as being selective to centrally active drugs [37,38]. According to Biswas *et al.*it estimates the complex reaction to non-inflammatory acute pain input [39]. Therefore, the prolongation of the hot plate latency time by *Agbo-Iba PMII* may be centrally mediated. The analgesy-meter test determines centrally functioning analgesic activity which focuses mainly on alterations in the spinal cord [40]. The significant elevation in pain threshold caused by *Agbo-Iba PMII* in the analgesy-meter test even above the standard drug at a dosage of 800 mg/kg suggests involvement of central pain pathways and makes it a potential drug for the treatment of pain. These results indicate that *Agbo-Iba PMII* can significantly suppress reaction to mechanically and thermally induced pain dose dependently, exhibit strong analgesic activities at the doses administered and hence, contribute significantly to the antimalarial effect of *Agbo-Iba PMII*.

The simultaneous analgesic and anti-inflammatory activities displayed by *Agbo-Iba PMII* show similarities in action to that exhibited by most NSAIDS especially the salicylates and their derivatives thereby confirming its traditional application and existence of synergistic actions of its various constituents commonly associated with most traditional remedies. The result from the GC–MS analysis revealed forty-two (42) compounds which have been subjected to molecular docking studies where it was revealed that the various compounds especially 1, 3-Diphenyl-2-azafluorene had good binding affinities with *Plasmodium* receptor. Hence, it is capable of inhibiting the parasite and acts against malaria [41]. The result obtained in these studies are consistent with the works of Tarkang et al. [2] on a polyherbal remedy - Nefang which comprises of five (5) plants also present in *Agbo-Iba PMII*. It is also consistent with previously reported pharmacological activities of some of the *Agbo-Iba PMII* constituent plants including *O. gratissimum* [42], *Mangifera indica* bark and leaf [43,44], *C. papaya* [45] and *Psidiumguajava* [46]. Hence, confirming the pharmacological activity of *Agbo-Iba PMII* in the treatment of malaria.

6. Conclusions

The obtained results show that the polyherbal formulation (*Agbo-Iba PMII*) contains pharmacologically active ingredients with antipyretic, anti-inflammatory and analgesic effects. Thereby, suggesting that these effects are vital to the symptomatic management of malaria. These findings also gives empirical proof that the clinical effects of '*Agbo-Iba PMII*' are due to synergy between antiplasmodial and other biological activities of its constituent plants. Hence, justifying the traditional application of '*Agbo-Iba PMII*' in the treatment of malaria fever in SouthernNigeria. It is therefore, recommended for subsequent development for clinical application in malaria therapy.

Statement & declarations

We the authors intend to submit the manuscript to your reputable journal, a copy of this manuscript has not been under consideration or published in other journals. No issue concerning the Journal competing interest. All authors agreed to the publication of this manuscript.

Informed consent

Not applicable for this section.

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Data availability statement

Data obtained from this study were presented as Tables and Figures. The materials used for this study, such as; chemicals, medicine, kits, and experimental animals, were procured standard stores within and outside the country.

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