EVALUATION OF ANTI-INFLAMMATORY, ANALGESIC, ANTIPYRETIC EFFECT OF EICOSANE, PENTADECANE, OCTACOSANE, AND HENEICOSANE

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ABSTRACT

Objective: *Marantodes pumilum* (MP) is a plant widely used in traditional medicine in the Southeast Asian region and been reported to possess anti-inflammatory, analgesic, antipyretic, and antiulcer properties. The aim of the study is to identify the bioactive phytochemicals present in the purified leaf extract of MP using gas chromatography mass spectrometry (GC-MS), and to determine their anti-inflammatory, analgesic, and antipyretic effect in a rat model.

Methods: A dichloromethane crude extract of MP was partially purified using column chromatography, and the bioactive compounds were identified using GC-MS. The anti-inflammatory, analgesic, and antipyretic activities of the identified bioactive compounds were evaluated using formalin, carrageenan, acetic acid, and brewer's yeast to induce the rats.

Results: Eicosane, pentadecane, octacosane, and heneicosane were identified as bioactive compounds in purified leaf extract of MP. These bioactive compounds did not show any toxicity on the rats at 1000 mg/kg. There was no mortality, and no apparent behavioral, physiological, and morphological changes of the internal organs occurred. They exhibited a very significant ($p<0.05$) dose-dependent inhibition of acetic acid-induced writhing, formalin-induced paw licking, carrageenan-induced paw edema, and brewer's yeast-induced pyrexia.

Conclusions: Eicosane and pentadecane were able to show very strong anti-inflammatory, analgesic, and antipyretic effects. The observed effects of the bioactive compounds are similar to previous reports on crude and partially purified leaf extract of MP. The finding suggests that eicosane and pentadecane maybe the major bioactive compounds present in the purified leaf extract of MP.

Keywords: *Marantodes pumilum*, Anti-inflammatory, Analgesic, Antipyretic, Toxicity.

INTRODUCTION

Inflammation is a normal host defense mechanism that protects the host from infection and other insults; it initiates pathogen killing as well as tissue repair processes and helps to restore homeostasis at infected or damaged sites. It is characterized by redness, swelling, heat, pain, and loss of function, and involves interactions among many cell types and the production of, and responses to, a number of chemical mediators. Remedies of inflammation using medicinal plants are becoming popular, and it is used by a majority of the world's population. The efficacy of medicinal plants in the management of diseases is indubitable. The projection from the World Health Organization is that about 80% of the population of developing countries continues to use traditional medicine in primary medical problems. Several plants are typically used without considering the toxicity and pharmacological aspects of extract and phytochemicals present. The toxicity of herbal preparation and its bioactive constituents is usually unknown, and the population does not care, believing that if the material has been used so far, it should be devoid of toxicity [1]. It is very important to extract information on the toxicity of the plants and its bioactive compounds [2]. Since conventional anti-inflammatory drugs have not been successful in some inflammatory processes, there is an urgent need to have new and safe anti-inflammatory agents [3]. Attention is being focused on the investigation of the efficacy of plant-based drugs used in traditional medicine [4].

Marantodes pumilum (MP) is a plant commonly found in the Southeast Asian region. It is an herbaceous shrub with creeping rhizome that roots from the stem. MP has been reported to displace estradiol-binding antibodies [5] and modulates postmenopausal adiposity through the initiation of a lipolysis process in adipose tissues [6]. A crude and purified dichloromethane (DCM) leaf extract of MP has been reported to possess anti-inflammatory, analgesic, and antipyretic effects [7]. There has not been any pharmacological report on the identification of bioactive constituents in the crude and purified DCM leaf extract of MP. Gas chromatography/mass spectrometry (GC/MS) has become the choice method for identifying volatile compounds in complex mixtures [8]. The popularity of volatile compounds such as essential oils has gained interest with reported properties such as antimicrobial, antifungal, antioxidant, and free-radical scavenging activity [9-11]. Therefore, the present research aims to identify the bioactive phytochemicals present in the purified leaf extract of MP using GC-MS, and to determine the anti-inflammatory, analgesic, and antipyretic effects of the identified bioactive compounds in a rat model.

EXPERIMENTAL

Preparation of plant extract

The leaves of MP were purchased from the University Putra Malaysia, Serdang Selangor Darul Ehsan Malaysia. The plant was identified by Dr. Shamsul Khamis, a research officer (plant taxonomist) at the laboratory of Natural Products, Institute of Bioscience University Putra Malaysia. The leaves of MP (1 kg) were air-dried and powdered using a dry mill with 0.5 mm mesh size. 0.5 kg of the power was macerated for 48 h at room temperature in 5 L of DCM. The filtrate was concentrated using a rotary evaporator (BUCHI Rotavapor R-200, Switzerland) at a temperature of 35–40°C. After the evaporation process, a dark green residue was collected and left to dry in a fume cupboard [12].

Partial purification

A column (34 cm × 3 cm) was packed with silica gel (50 g) by pouring dry silica into a beaker of initial eluent of hexane (200 ml) to make slurry. The slurry was carefully poured into the column, previously for a quarter filled with hexane with the aid of a funnel. 20 g of DCM extract was loaded into the column and allowed to set before running the column. The partial purification of the DCM extract by column chromatography used a 42 cm × 2.5 cm vertical column equipped with a stop cock and glass frit to support the silica gel, pore size 60 Å, 200–400 mesh. 100% hexane was used to pre-elute the column. A decreasing polarity solvent ratio (100% hexane (Hx) – Hx: Ethyl acetate (EA) – EA: Methanol (MeOH) – 100% MeOH) was used to obtain Fraction E (60:40 Hx: EA to 100% MeOH). The fractions were collected based on their chromatogram profiles analyzed on TLC plates Silica gel 60 F254 and mobile phase 60:40 Hx: EA.[12]

GC-MS

10 mg of purified leaf extract of MP was dissolved in high-performance liquid chromatography grade methanol and was filtered through a 0.45 μm Millipore sterile syringe filter, before analysis. GC-MS was performed using the Agilent instrument model (5973). A HP-5MS 5% Phenyl Methyl Silox column with 30 m in length, 0.25 μm in diameter, and beta value of 250 μm was used.

Animal model

Male Sprague-Dawley rats weighing 200–220 g, obtained from the Institute of Medical Research, were used for the investigations. They were housed in groups of six in standard cages in animal holdings units at UCSI University, Kuala Lumpur, Malaysia. They were maintained under standard environmental conditions, fed with standard pellet diet and received water *ad libitum*. They were left to acclimatize for 2 weeks before the commencement of the experiment. All experiments were carried out in accordance with the UCSI University Ethical Guidelines on the usage of Laboratory Animals.

Acute toxicity test

The maximum of six Sprague-Dawley rats of three males and three females with an average weight of 220 g were fasted with only water provided *ad libitum* for 2 h before the administration of drugs. A single dose of 1000 mg/kg body weight of eicosane, pentadecane, octacosane, or heneicosane prepared in 10%v/v hexane and saline, was orally administered for 14 consecutive days. The animals were weighed before and after the administration of the compounds for the acute toxicity test. The animals were observed for any behavioral effects such as hyper excitability and depression or physiological effects such as diarrhea, salivation, motor impairment, as well as the incidence of mortality for 30 min, 4 h and daily for 14 days after the administration of the compounds [13]. After 14 days of treatment and observations, the animals were sacrificed, and the internal organs such as stomach, small intestine, kidneys, and liver were collected. The organs were anatomically examined for morphological changes and weight. The data were compared with the normal rat's organs.

Acetic acid-induced writhing

An acetic acid-induced writhing test was done using the method, according to Okechukwu and Ikujuni [14], for the analgesic actions of eicosane, pentadecane, octacosane, and heneicosane. Each rat in groups of six was injected intraperitoneally an 0.6% aqueous solution of acetic acid (10 ml/kg body weight), 1 h after receiving oral (p.o.) administered saline (1 ml) as the negative control, eicosane, pentadecane, octacosane, and heneicosane (5 and 10 mg/kg), or indomethacin (10 mg/kg), an anti-inflammatory drugs as positive control. Immediately after the acetic acid injection, each animal was placed in a transparent observation cage and the number of writhes per rat was counted for 30 min. The writhing activity comprised constriction of the abdominal muscles together with a stretching of the hind limbs. The percentage of inhibition of writhing was calculated using the equation below:

$$\% \text{Analgesic activity} = \frac{\text{(Control group} - \text{Treated group)}}{\text{Mean writhing count of control group}} \times 100$$

Formalin-induced paw licking

Based on the method described by Okechukwu *et al.*, Okechukwu and Ikujuni [7,14], a formalin-induced paw licking test was exploited to determine the analgesic actions of eicosane, pentadecane, octacosane, and heneicosane. Rat was injected with 50 μl of 2.5% formalin in 0.9% of saline solution into the subplantar surface of the left hind paw, 1 h after the administration of 0.9% saline, 100 mg/kg of aspirin and eicosane, pentadecane, octacosane, and heneicosane (5 and 10 mg/kg). Rats were then observed for 30 min, and the time spent licking the paw was recorded in two phases. The data were expressed as total licking time in the early phase (0–5 min) and the late phase (15–30 min) after formalin injection. Percentage of inhibition was calculated using the equation below:

$$\% \text{Anti} - \text{inflammatory effect} = \frac{\substack{\text{Mean paw licking, jecking, flexing} \\ \text{in (Control group} - \text{Treated group)}}}{\substack{\text{Mean paw licking, jecking,} \\ \text{flexing in control group}}} \times 100$$

D. Carrageenan-induced paw edema

Carrageenan-induced paw edema

The anti-inflammatory activity of eicosane, pentadecane, octacosane, and heneicosane was evaluated using carrageenan-induced paw edema, as described by Okechukwu and Ikujuni [14]. The rats were fasted overnight with water *ad libitum,* and the basal volume of right hind paw was measured before the administration of drugs by using a plethysmometer. Acute inflammation was induced by subplantar injection in the right hind paw of the rats, with 0.1 ml of (1.0%w/v) carrageenan suspension in 0.9% NaCl. The animals were orally administered with vehicle (saline), indomethacin (10 mg/kg), eicosane, pentadecane, octacosane, and heneicosane (5 and 10 mg/kg) 1 h before the carrageenan injection. The thickness of hind paw was monitored and measured at 1, 2, 3, 4, and 5 h after the carrageenan injection using the plethysmometer [14]. Percentage of inhibition was calculated using the equation below:

$$\% \text{Inhibition} = \frac{\substack{\text{Mean paw volume in} \\ \text{(control group} - \text{Treated group)}}}{\text{Mean paw volume in control group}} \times 100$$

Yeast-induced pyrexia

Hyperpyrexia was induced by subcutaneous administration of a (15%w/v) brewer's yeast suspension in 0.9% NaCl in a volume of 0.1 ml/10 g of bodyweight after the initial rectal temperature of each rat was measured and recorded using a digital clinical thermometer. Twenty-four hours after induction of hyperpyrexia, any animals that showed a minimal increase of 0.5°C in rectal temperature were selected for the experiment. The animals were orally administered with vehicle (saline), indomethacin (10 mg/kg), eicosane, pentadecane, octacosane, and heneicosane (5 and 10 mg/kg). The rectal temperature of each rat was measured at 1 h interval for 5 h [14]. The percentage of temperature reduction was calculated using the following equation:

$$\% \text{Antipyretic effect} = \frac{\text{(Yeast induced pyrexia} - \text{Post treatment)}}{\text{(Yeast induced pyrexia)}} \times 100$$

Statistical analysis

Data were expressed as mean ± standard deviations (SD). Statistically significant differences between groups were measured using a one-way analysis of variance followed by Dunnett's test. Statistical analysis

was performed using Minitab® Release 17 (Minitab Inc., US). Values of *p<0.05 were considered statistically significant.

RESULTS AND DISCUSION

Preparation of plant extract and partial purification

The crude extract yield from 1 kg leaf of MP was 50 g after the extraction process. DCM was the solvent used for the crude extraction. Partial purification yielded five different fractions A-E.

GC-MS

Fraction A was subjected to GC-MS because it is the fraction that retained bioactivity. The major bioactive compounds that were identified in this partially purified fraction A from the MP extract were: Phthalic esters; Bis(2-propylpentyl) phthalate and Bis(2-propylpentyl) phthalate (RT: 20.65, 17.8%), branched alkane hydrocarbons; Heneicosane, Hexadecane,1-iodo, Octacosane (RT:34.756, 11.72%), Pentadecane, Heptadecane and Heptacosane (RT: 23.142, 7.89%), tricosane and eicosane (RT: 26.902, 5.25%) and terpenes; β –amyrin and Urs-12-en-3-one (RT: 28.361, 6.84%). The molecular weight and nomenclature were taken from the National Institute of Standards and Technology, PubChem, ChemBook, and ChemSpider. The constituents with peak area >2% with their CAS number, molecular weight, molecular compound, retention time, and structure are reported in Table 1, Figs. 1a and 1b. The major constituents sum up to 49.5% of the total constituents.

Toxicity test

Oral administration of eicosane, pentadecane, octacosane, and heneicosane at 1000 mg/kg to rats did not cause any behavioral effects such as hyper excitability and depression or physiological effects such as diarrhea, salivation, and motor impairment. There was no mortality recorded throughout the 14 days treatment. The result shows that eicosane, pentadecane, octacosane, and heneicosane have no apparent toxicity to the rats at a concentration of 1000 mg/kg. Acute toxicity studies are widely used to ensure the safety of new drugs using animal models. They are performed to classify and label the potential hazard categories and estimate the concentration or dose required to cause toxicity [16]. Such assessment is important in the selection of a starting dose for Phase 1 human studies and provides the information relevant to avoid acute overdosing. Acute toxicity studies determine the LD50 values that provide indices of potential types of drug activities [17]. Lethal dosage (LD50) is the concentration of a substance or drug that will lead to the deaths of 50% of the population [18]. Eicosane, pentadecane, octacosane, and heneicosane at 1000 mg/kg did not show toxicity to the rats and are adjudicated to be safe to enter the next phase of the study as drug candidates.

Acetic acid-induced writhing

The oral administration of increasing concentration (5 and 10 mg/kg) of eicosane, pentadecane, octacosane, and heneicosane showed a significant reduction (p<0.05) in the number of writhing induced by acetic acid compared to the negative control. Eicosane showed the highest effect, followed by pentadecane. The results of acetic acid-induced writhing are shown in Fig. 2.

Acetic acid-induced writhing test is widely used to evaluate compounds with analgesic potential [19]. Intraperitoneal injection of acetic acid causes constriction of the abdominal muscle, which was accompanied by the twisting of the trunk followed by stretching of the hind limbs [20]. The acetic acid injection also results in the induction of inflammatory responses in subcutaneous and muscular layers of the abdominal wall [21]. These responses are the result of tissue damage

Table 1: Gas chromatography/mass spectrometry analysis of the fraction A of extract of *Marantodes pumilum*

S. No.	RT	Compounds	CAS registry	Molecular compound	MW	PA%
1	3.762	1-Butene, 2,3,3-trimethyl-	594-56-9[(NIST)]	C_7H_{14}	98.186	2.16
		2-Pentene, 4,4-dimethyl-, (Z)-	762-63-0[(NIST)]	C_7H_{14}	98.186	
		2-Pentene, 4,4-dimethyl-, (E)-	690-08-4[(NIST)]	C_7H_{14}	98.186	
2	14.302	2-Pentadecanone, 6,10,14-trimethyl	502-69-2[(NIST)]	$C_{18}H_{36}O$	268.478	4.46
3	15.097	1-Octacosanol	29739970[(CS)]	$C_{30}H_{57}F_3O_2$	506.768	2.61
		Docos-1-ene	1599-67-3[(NIST)]	$C_{22}H_{44}$	308.585	
		9-Nonadecene	31035-07-1[(NIST)]	$C_{19}H_{38}$	266.505	
4	15.372	Isophytol	505-32-8[(NIST)]	$C_{20}H_{40}O$	296.351	2.96
5	16.877	Tetracosyl heptafluorobutyrate	29739954[(CS)]	$C_{28}H_{49}F_7O_2$	550.676	2.38
6	17.661	Ethanol, 2-(octadecyloxy)-	2136-72-3[(NIST)]	$C_{20}H_{42}O_2$	314.546	2.03
		1-(4-Bromobutyl)-2-piperidinone	467195[(CS)]	$C_9H_{16}BrNO$	234.133	
		1-Decanol, 2-hexyl-	2425-77-6[(NIST)]	$C_{16}H_{34}O$	242.441	
7	18.530	Tricosane	638-67-5[(NIST)]	$C_{23}H_{48}$	324.627	2.56
9	20.659	Bis(2-ethylhexyl) phthalate	117-81-7[(NIST)]	$C_{24}H_{38}O_4$	390.556	17.80
		Bis(2-propylpentyl) phthalate	166657[(CS)]	$C_{24}H_{38}O_4$	390.556	
10	22.513	i-Propyl 9-octadecenoate	111-59-1[(PC)]	$C_{21}H_{40}O_2$	324.541	2.46
		Octadec-9-enoic acid	2027-47-6[(NIST)]	$C_{18}H_{34}O_2$	282.461	
11	23.142	Pentadecane	629-62-9[(NIST)]	$C_{15}H_{32}$	212.415	7.89
		Heptadecane, 9-octyl-	7225-64-1[(NIST)]	$C_{25}H_{52}$	352.680	
		Heptacosane, 1-chloro-	62016-79-9[(NIST)]	$C_{27}H_{55}Cl$	415.179	
12	24.756	Heneicosane	629-94-7[(NIST)]	$C_{21}H_{44}$	296.574	11.72
		Hexadecane, 1-iodo-	544-77-4[(NIST)]	$C_{16}H_{33}I$	352.338	
		Octacosane	630-02-4[(NIST)]	$C_{28}H_{58}$	394.760	
13	26.902	Eicosane	112-95-8[(NIST)]	$C_{20}H_{42}$	282.548	5.25
14	27.932	1,3-Dithiolane, 2-(28-norurs-12-en -17-yl)-	473220[(CS)]	$C_{32}H_{52}S_2$	500.885	2.65
		3,7,11-Trimethyl-dodeca-2,4,6,10-t etraenal	23255776[(CS)]	$C_{15}H_{22}O$	218.335	
		(22E)-3′,7β-Dihydrocycloprop[7,8]-5α-ergost-22-en-3-one	53755-18-3[(CB)]	NR	NR	
15	28.361	β–Amyrin	559-70-6[(NIST)]	$C_{30}H_{50}O$	426.717	6.84
		Urs-12-en-3-one	532729[(CS)]	$C_{30}H_{48}O$	424.702	

*RT: Retention time, #PA %: Peak area percentage, NR: Not reported, MW: Molecular weight (corrected to three decimal places), NIST: National Institute of Standards and Technology, PC: PubChem, CB: ChemBook, CS: ChemSpider
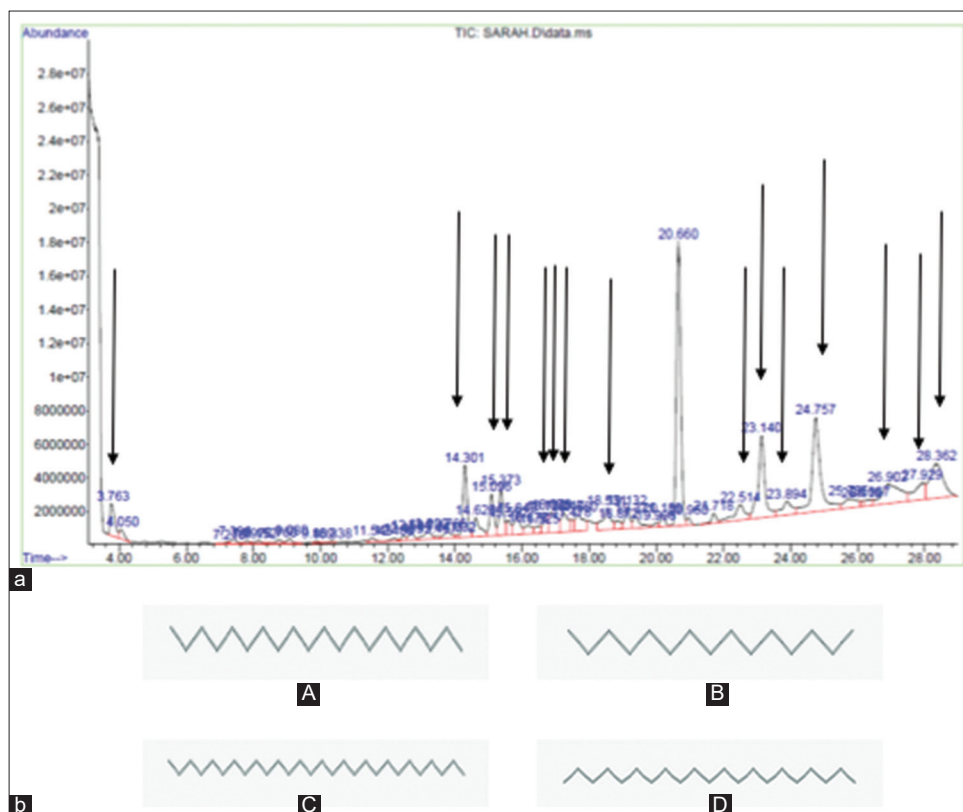
Fig. 1: (a) Gas chromatography/mass spectrometry analysis of the fraction A of extract of *Marantodes pumilum* (b) chemical structure of A=Eicosane, B=Pentadecane, C= Octacosane, and D=Heneicosane
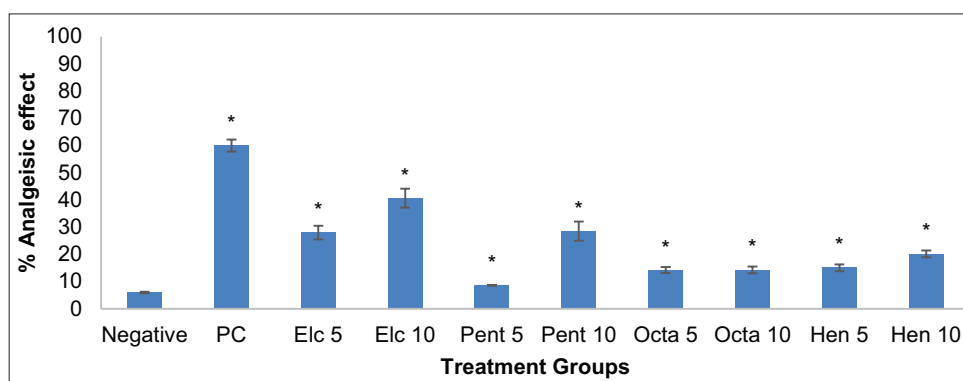

Fig. 2: Percentage analgesic effect on animals orally treated with saline solution (negative control), eicosane (Eic), pentadecane (Pent), octacosane (Octa), heneicosane (Hen) doses (5 and 10 mg/kg), and indomethacin (PC) (10 mg/kg) before the acetic acid (0.6%, i.p.). Values are expressed as mean±Standard deviation, n=6, the statistical significance by one-way analysis of variance followed by Dunnett's test. Values are significant at *p<0.05 compared to negative control when no drugs were given

and sensitization of nociceptive receptors to prostaglandins (PGs). They are also assumed to involve local peritoneal receptors and be mediated by peritoneal mast cells, acid-sensing ion channels, and the PG pathways. Prostaglandins have been widely reported to play an important role in the nociceptive mechanism [22]. Reports have shown that intraperitoneal injection of acetic acid triggers the release of several other mediators such as neurotransmitters, tumor necrosis factor-α (TNF-α), interleukin-1β (IL β) and IL-8, histamine, kinins, acetylcholine, and substance P [23]. The release of these mediators results in the increase of vascular permeability, reduction of the nociception threshold and stimulation of nociceptive neurons sensitive to nonsteroidal anti-inflammatory drugs (NSAIDs) and opioids [24,25]. Indomethacin is a NSAID, it is able to inhibit PG synthesis which leads to peripheral analgesic effect [26]. In this study, indomethacin may have reduced PG and thromboxane synthesis from arachidonic acid by inhibiting the activity of cyclooxygenase (COX) enzymes [27]. Indomethacin has also been previously reported to reduce leukocytes recruitment and inhibits neutrophil accumulation [28], by extension blocks the voltage-gated calcium channel resulting in the blockage of Ca^{2+} influx and intracellular Ca^{2+}-dependent mechanism [29]. Pre-treatment of the rats with eicosane, pentadecane, octacosane, and heneicosane reduced the acetic acid-induced writing response, and this analgesic role was similar to the effect observed with indomethacin (the reference drug). The result suggests that the compounds have the potential to inhibit the release and activation of mediators such as PG, TNF-α, IL-1β, IL-8, histamine, kinins, acetylcholine, and substance P. It may also have blocked cox enzymes or inhibit the leukocytes recruitment, inhibit neutrophil accumulation, as well as reduce the

influx of intracellular calcium. Eicosane and pentadecane showed the highest effect.

Formalin-induced paw licking

The subcutaneous injection of diluted (3.0%) formalin (0.1 ml) into the subplantar surface induced a nociceptive response, characterized by an increase in licking time. The oral administration of increasing concentrations (5 and 10 mg/kg) of eicosane, pentadecane, octacosane, and heneicosane did not show any significant reduction in the number of licking during the early phase. Eicosane, pentadecane, octacosane, and eicosane (at 5 and 10 mg/kg) and indomethacin (at 10 mg/kg) elicited significant reduction in the number of licking during the late phase. Eicosane and pentadecane showed the highest reduction and they are more potent than indomethacin. The results of formalin-induced paw licking are shown in Fig. 3. The injection of formalin in the hind paw produced nociceptive responses involving two different phases [30]. The first phase, known as neurogenic pain, starts immediately after formalin injection (0–5 min). This phase was due to the direct effect of formalin on sensory C fibers [31]. Earlier reports have suggested that substance P and bradykinin are involved in the first phase [32]. The first phase therefore reflects the centrally-mediated pain, which is usually suppressed by the μ-opioid receptor agonist, morphine [33]. The second phase starts approximately 20 min after the formalin injection. It reflects the development of an inflammatory response to tissue injury associated with the release of nociceptive mediators [32]. It was reported that histamine, serotonin, PG, and bradykinin were released during the second phase [34]. The reference drug, indomethacin, inhibits the late phase significantly, and this was expected since indomethacin is a peripheral acting drug that inhibits the enzymatic activity of COX from facilitating the release of inflammatory

mediators such as histamine, serotonin, PG, and bradykinin. Eicosane, pentadecane, octacosane, and heneicosane inhibit the late phase significantly, and the analgesic role was similar to the mechanism of action of the reference drug indomethacin. This finding suggests that they may be inhibiting the release of inflammatory mediators such as histamine, serotonin, PG, and bradykinin. They may also be interacting with cox enzymes. Eicosane and pentadecane at a dose of 5 mg were more potent than indomethacin at 10 mg.

Carrageenan-induced paw edema

Eicosane and pentadecane at 5 and 10 mg/kg and indomethacin (10 mg/kg) significantly inhibited carrageenan-induced rat paw edema ($p<0.05$). Octacosane and heneicosane (5 and 10 mg/kg) did not show any significant inhibition on carrageenan-induced paw edema. The inhibitory values of edema were based on 5 h post-carrageenan treatment, Fig. 4.

The carrageenan-induced paw edema test has been widely used as an investigational model of acute inflammation. It has been reported that carrageenan is a useful phlogistic tool for testing anti-inflammatory drugs, especially NSAIDs [35]. The injection of carrageenan results in the release of nitric oxide (NO), tumor necrosis factor-α (TNF-α), and IL-1β [36]. The release of these mediators causes vasodilatation, exudation of plasma and neutrophils migration to the site of injury [37]. The injection of carrageenan into the hind paw involves three phases. The first phase extends from 1 to 2 h, which is mediated through the release of histamine, serotonin, and kinins [38]. The second phase starts after 2–4 h predominantly due to the release of kinins, platelet-activating factors, and arachidonic acid metabolites such as PGs, thromboxanes (TXs), and leukotrienes (LTs).
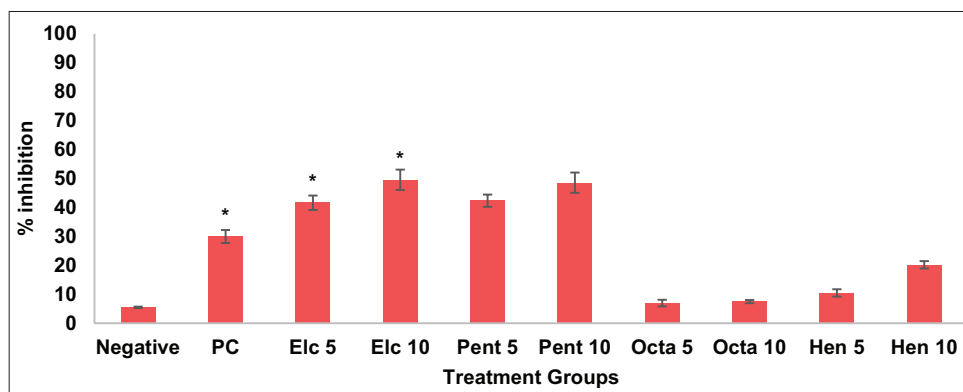

Fig. 3: Percentage inhibitions of formalin-induced paw licking, values are expressed as mean±Standard deviation, n=6, the statistical significance by one-way analysis of variance, followed by Dunnett's test. Values are significant at *p<0.05
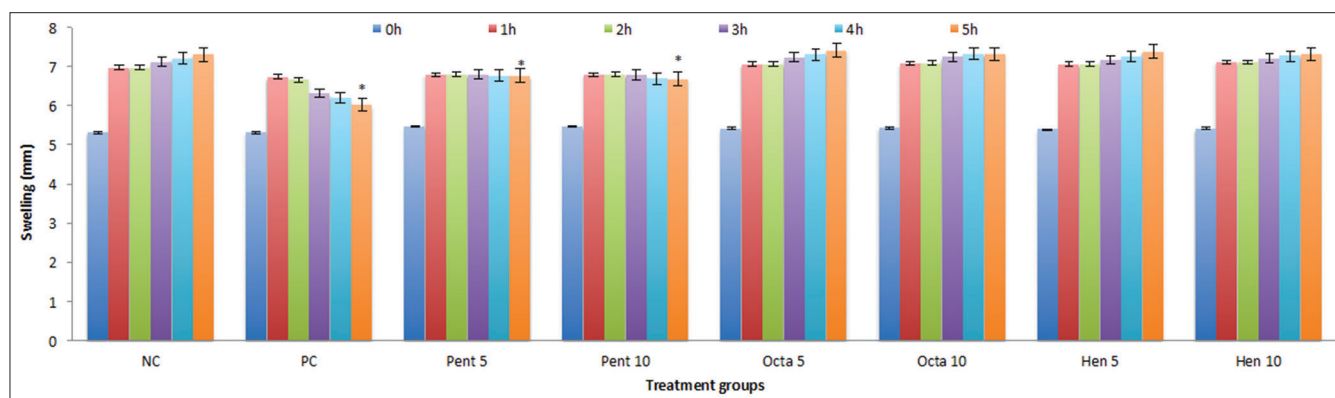

Fig. 4: Effects of the administration of eicosane, pentadecane, octacosane, heneicosane (5 and 10 mg/kg), and indomethacin (10 mg/kg) on rat paw edema induced by intraplantar injection of 0.1 ml of 1.0% carrageenan suspension. Values are expressed as mean±Standard deviation, n=6, the statistical significance by one-way analysis of variance, followed by Dunnett's test. Values are significant at *p<0.05 and nsP=Not significant compare to negative control when no drugs were given
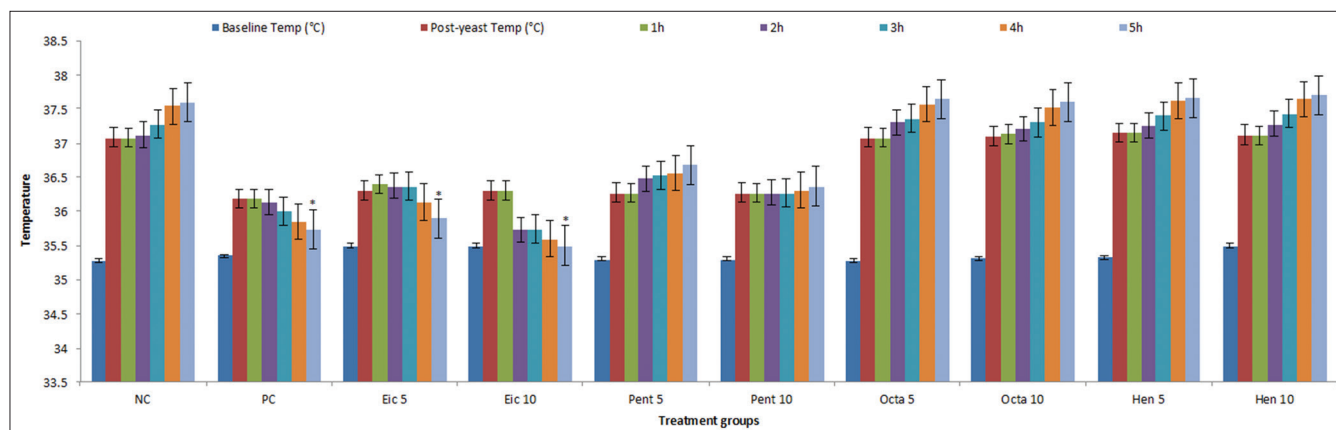
Fig. 5: Antipyretic effect of eicosane, pentadecane, octacosane, heneicosane (5 and 10 mg/kg), and indomethacin (10 mg/kg) on rats induced by yeast. Values are expressed as mean±Standard deviation, n=6, the statistical significance by one-way analysis of variance, followed by Dunnett's test. Values are significant at *p<0.05 and nsP=Not significant compare to negative control when no drugs were given

The release of these mediators causes TNF-α to release kinins and LTs and these results in the long-lasting nociceptive response [39]. The third phase starts at 4–5 h when the edema reaches its highest volume. The third phase is mediated by the release of bradykinin and overproduction of PGs, which lead to the formation of inflammatory exudates and hypersensitivity at the site of injury [40]. Oral administration of eicosane and pentadecane inhibited the three phases, suggesting that the inhibitory effect of eicosane and pentadecane on carrageenan-induced paw edema may be due to the inhibition of the release of an inflammatory mediator such as histamine, bradykinin, serotonin, kinins, and PGs. Octacosane and heneicosane did not show any effect. A previous report has shown that free fatty acids, including long-chain C16-C20 unsaturated fatty acids, have been suspected to be the bioactive components responsible for the anti-inflammatory activity reported in *Tinospora smilacina* Berth extract. Fatty acids can be of therapeutic value when infections are present in asthma patients (Desbois and Smith, 2010). This result is in line with the anti-asthmatic effect of purified leaf extract of MP reported by Okechukwu *et al.*, Okechukwu and Ikujuni, and Ekeuku and Okechukwu [7,14,15].

Yeast-induced pyrexia
Subcutaneous injection of a yeast suspension resulted in a markedly elevated rectal temperature as measured 24 h after administration. Treatment with increasing concentrations of eicosane (5 and 10 mg/kg) and indomethacin (10mg/kg) significantly (p<0.05) decreased the rectal temperature of the rats as compared to the control group. Pentadecane, octacosane, and heneicosane did not show any significant effect on decreasing the rectal temperature. The result of the antipyretic activity is shown in Fig. 5.

Subcutaneously injected yeast's cell wall products act as exogenous pyrogens and stimulate the immune cells, such as lymphocytes and macrophages [41]. The stimulation of immune cells results in the production of endogenous pyrogens in the form of cytokines such as TNF, IL-1b, IL-6, and interferon-α [42]. The cytokines enter the systemic circulation and increase the synthesis of PGE2 in the brain to elevate the hypothalamic set point for body temperature [43]. Reports show that COX-2 and iNOS are involved in the generation of a pyrexia response to brewer's yeast as the COX-2 isoform mediates pyrexia response through the production of E-type PG [44]. Therefore, a selective COX-2 antagonist is widely used due to its effectiveness in antipyretic activity [45]. The present study showed that the oral administration of indomethacin at 10 mg/kg exerted significant (p<0.05) antipyretic activity after the 2nd h of the treatment. Vane reported that indomethacin blocks the COX enzymes, therefore inhibiting the production of E-type PG. Indomethacin also reduces the elevation of the body temperature by inhibiting the inflammatory messages at the site of infection and within the thermoregulatory center [47]. It also reduces the production of

pyrogenic cytokines, block leukocytes interactions, and diminishes the thermoregulatory set point by blocking PG E2-production [48]. Oral administration of eicosane produced a significant (p<0.05) hypothermal activity against yeast-induced pyrexia on rats, suggesting that the anti-inflammatory role was similar to indomethacin (reference drug) by suppressing the production of PG E2. Pentadecane, octacosane, and heneicosane did not show any effect.

CONCLUSIONS

Eicosane and pentadecane mimic the effect of indomethacin, except in an antipyretic test where pentadecane did not show any effect. The compounds were more potent than indomethacin in the formalin-induced paw licking test. The compounds did not show any toxicity. Eicosane and pentadecane may be the major bioactive compound present in the fraction A extract of MP. The bioactive compounds may have inhibited the release of histamine, bradykinin, PGs, TXs, and LTs. They may have also reduced the stimulation of cytokines such as TNF, IL-1b, IL-6, and interferon-α and interacted with the cox enzyme. The effect is similar to the effect of the control drug indomethacin. The result is in consonance with an earlier report on purified leaf extract of MP.

AUTHORS' CONTRIBUTION

The project was done and authored by Dr. Patrick Nwabueze Okechukwu.

CONFLICTS OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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