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# *Ananas comosus* (L) Merrill (pineapple) fruit peel extract demonstrates antimalarial, anti-nociceptive and anti-inflammatory activities in experimental models

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ABSTRACT

*Ethnopharmacology relevance:* Pineapple (*Ananas comosus)* peel is a major waste in pineapple canning industry and it is reported to be used in ethnomedicine as a component of herbal remedies for malarial management. This study aimed to evaluate the antimalarial, antinociceptive and anti-inflammatory properties of *Ananas comosus* peel extract (PEAC).

*Methods: Ananas comosus* peel was extracted with 80% methanol. PEAC (100, 200 and 400 mg/kg) was investigated for antimalarial effect using Peter's 4-day suppressive test (4-DST) model in mice. Antinociceptive activity of PEAC was investigated in hot plate, acetic acid-induced writhing and formalin tests in mice. The anti-inflammatory activity was evaluated using the lipopolysaccharides-induced sickness behavior in mice and carrageenan-induced air pouch in rats' models.

*Results:* PEAC could not significantly ($p > 0.05$) suppressed parasitemia level at 7-day post-infection in 4-DST. PEAC (400 mg/kg) mildly prolongs survival of infected mice up till day 21. PEAC demonstrated significant ($p < 0.05$) antinociceptive activity by increasing latency to jump on the hot plate, reduced number of writhings in acetic acid test and reduced paw licking time in $2^{nd}$ phase of formalin test. PEAC significantly reduced anxiogenic and depressive-like symptoms of sickness behavior in LPS-injected mice. PEAC demonstrated significant anti-inflammatory activity in carrageenan-induced air pouch experiment by reducing exudates formation, inflammatory cell counts, and nitrite, tumor necrosis factor-alpha and interleukin-6 levels.

*Conclusion: Ananas comosus* peel extract demonstrated mild antimalarial activity but significant anti-nociceptive and anti-inflammatory properties probably mediated via inhibition of pro-inflammatory mediators.

## 1. Introduction

Herbal medicine is considered an integral component of the traditional medicine which is gaining global attention as over three-quarter of the World's population now depends on it (Oreagba et al., 2011; James et al., 2018). Globally, more and more people are developing interest in advancing traditional and complementary alternative medicine due to age long belief in its safety and efficacy (Chung et al., 2020). An important component of traditional medicine is the use of medicinal plant parts such as seeds, roots, leaves, bark or flowers for the primary health care needs (Sofowora et al., 2013; Abd El-Ghani, 2016). Identification and characterization of these plants has fairly resulted in successful development of some conventional drugs. For example, anti-inflammatory drugs such as aspirin was developed from salicylic acid from white willow bark, antimalarial quinine and artemisinin from *Cinchona pubescens* bark and *Artemisia annua* leaves, respectively (Atanasov et al., 2015).

The World Health Organization recognized the place of traditional medicine for primary health care but also as source of international trade as annual revenue continues to rise in countries that have invested

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in developing medicinal products from plants (Tilburt and Kaptchuk, 2008; Bukar et al., 2016). China has continued to invest in herbal medicine following its success in the treatment of the severe acute respiratory syndrome (SARS) (World Health Organization (SARS), 2003). It is estimated that about 80% of Africa population use herbal medicine. The outbreak of the global pandemic of COVID-19 pushed millions to search and use different herbal medicines as chemo-preventives during the lockdown (ACEDHARS UNILAG COVID-19 Response Team, 2020; Benarba and Pandiella, 2020; Liu et al., 2020). Countries like Madagascar rolled out her herbal medicinal remedy against COVID-19, although lack of clinical trial data suppressed its global acceptance (Yang, 2020). This underscores the need to provide preclinical and clinical data on herbal remedies with anecdotal and folkloric use among the population.

There are threats to the advancement of herbal medicine which include lack of knowledge on how to sustain and preserve the plant populations (Oreagba et al., 2011); availability and sustainable cultivation of medicinal plants due to increased global demands (Gamaniel, 2005), and adulteration of herbal medicinal products due to lack of adequate national policies and regulations (Oreagba et al., 2011). To overcome the issues of sustainability, there is need to research more on renewable sources for herbal medicinal product. Therefore, researchers are turning to the largely unexploited fruit and vegetable by-products as a source for renewable herbal medicinal products. This is ideal for the economy of developing countries (Attanzio et al., 2018; Torres-León et al., 2018). Bioconversion of fruit by-products stands a chance of solving nutritional, economic and environmental problems associated with fruits bio-waste (Sagar et al., 2018). Potent antioxidant polyphenolic compounds have already been identified from skins, pulp and seeds of some fruits (Fierascu et al., 2020).

*Ananas comosus* (L) Merrill (Bromoliaceae) fruit is a tropical fruit that is widely cultivated with leading productions in countries like Thailand, Costa Rica, Phillipines, Brazil and India (Ali et al., 2020). The plant is known as Pineapple in English, “Ope oyinbo” in Yoruba (South-West, Nigeria) and “Nkw-aba” in Ibo (South-East, Nigeria) (Nwauzoma and Dappa, 2013). Nigeria ranked 7th on the list of pineapple producing countries with an estimated annual production of 1.4 million metric tonnes (Food and Agriculture Organization, 2013; Balogun et al., 2018). It is a tropical fruit with exceptional sweet sensory properties and health benefits whose processing into juice generates a lot of waste (Iwuchukwu et al., 2017; Torres-León et al., 2018). The peel, crown and core are major waste in pineapple canning industry (Ali et al., 2020). Azizan et al. (2020) identified several potentially bioactive metabolites in the peel, crown and core of pineapple and their antioxidants and alpha-glucosidase properties.

Pineapple peels are well known ingredients in ethnomedicine (Asase et al., 2010). There are anecdotal and folkloric reports of the use of pineapple peel alone or combination with other medicinal plants in the treatment of malaria (Odugbemi et al., 2007; Asase et al., 2010; Yetein et al., 2013; Iyamah and Idu, 2015), arthritis (Olatunji et al., 2014), typhoid infections (Fadimu et al., 2014), and gastrointestinal complaints (Kadir et al., 2012). Pineapple peel also serve the purpose of flavoring herbal decoctions in addition to its medicinal properties (Iyamah and Idu, 2015). Bromelain, a therapeutic enzyme is identified as the major bioactive ingredient in pineapple and is reportedly useful in the treatment of malignant cell growth, thrombus formation, inflammation and dermatological purposes (Sánchez-Burgos and de Lourdes García-Magaña, 2017). There are biological reports demonstrating anti-arthritic (Kargutkar and Brijesh, 2016), hypolipidemic (Emmanuel et al., 2016), antidiabetic (Das et al., 2019), and neuroprotective (Erukainure et al., 2011) effects of pineapple peel extract. Aqueous methanol has been used as a good extraction solvent due to its polarity (0.762) and ability to extract the phenolic content in pineapple peel (Lourenço et al., 2021; Jovanović et al., 2018). Aqueous methanol extract have been the most reported in several studies investigating antimalarial, antinociceptive and anti-inflammatory studies because of its ability to extract potent antioxidants phenolics (Ajayi et al., 2017a; Adeyemo-Salami et al., 2020). We have earlier reported that the aqueous methanol extract of pineapple peel possess atheroprotective and anti-inflammatory effect in High-fat diet fed rats (Ajayi et al., 2021). Therefore, considering the ethnopharmacological mention of the use of pineapple peel in malaria home remedy and its usefulness as a sustainable source of herbal medicinal product, we evaluated the antimalarial, anti-nociceptive and anti-inflammatory activities of peel extract of *Ananas comosus* (PEAC).

## 2. Materials and methods

### 2.1. Pineapple peel extraction

Pineapple was bought from the Oje fruit market in Ibadan, Oyo-State, Nigeria. The fruit with its crown was authenticated at the Botany Department Herbarium, University of Ibadan and a voucher specimen with herbarium number (UIH-22992) deposited. The plant name as checked on Plant List (http://www.theplantlist.org/) revealed the name was supplied on 2012-03-23 as *Ananas comosus* (L) Merr (Bromoliaceae). The fruit was thoroughly rinsed with tap water and peeled using a sterile knife. The wet peel was chopped into pieces and oven dried at 40$^{o}$C for 72 h. Dried peels were ground into powder using a mechanical grinder. Two hundred grams (200 g) of the powder was macerated in 1 L of 80% methanol for 48 h, filtered using a muslin clothe and evaporated to dryness in a rotary evaporator. The peel extract of *Ananas comosus* (PEAC) was brownish syrupy extract, stored at 4$^{o}$C and dissolved in distilled water for animal experiment.

### 2.2. Experimental animals

BALB/c mice (20–25 g) and Wistar rats (150–180 g) of either sex were obtained from the central animal house, University of Ibadan. The animals were housed in polypropylene plastic cages and maintained in standard environmental conditions of natural light and dark cycle (12:12 h), temperature not exceeding 28$^{o}$C and relative humidity. The animals were fed with laboratory rodent chow (Vital feeds Ltd, Ibadan, Nigeria) and allowed to drink water *ad libitum*. The animals were acclimatized for one week before subjecting them to any experiment. The animal housing, care and experimental ethics followed the guideline for experimental animals outlined in the “Principle of Laboratory Animal Care” (NIH Publication No. 85-23).

### 2.3. Oral acute toxicity test

The oral acute toxicity test followed the guideline as described by the Organization of Economic Cooperation and Development 423 (OECD) using female BALB/c mice. Mice ($n = 3$) fasted for 4 h were administered a single oral dose of PEAC (5000 mg/kg) at 0.1 mL/10 g. The control mice ($n = 3$) received 0.1 mL/10 g of distilled water. Observation for signs of toxicity was done immediately for 0–4 h. The animals were monitored daily for 14 days for any delayed toxicity effect. At the end of the 14 days, body weights and organ weights were measured, the organs were checked visually for any observable changes compared with control.

### 2.4. Evaluation of the antimalarial activity of PEAC using the suppressive test

The antimalarial activity of PEAC was tested in BALB/c mice using the modified Peter’s 4-day suppressive test (Peters et al., 1970). A total of thirty-five mice weighing between 18 and 25 g were inoculated with a standard inoculum of *Plasmodium beighei* NK 65 (Chloroquine sensitive strain donated by the Malaria Research and Reference Reagent Resource Centre, MR4, USA). Each mouse was intraperitoneally injected with $1 \times 10^7$ parasitized red blood cells from a donor mouse. The animals were

divided into negative control (infected untreated), PEAC (100, 200 and 400 mg/kg), Chloroquine (4 mg/kg) and Artemether-lumenfantrine (5 mg/kg: 30 mg/kg). Beginning from the 4th hour after inoculation, the animals were treated orally for four days. Blood was collected from the tail vein of mice for thin film smears from 24 h after last treatment and on days 5, 6, 7 and 14. Subsequently, the animals were monitored for survival till day 28. The thin film smears were fixed with methanol, stained with Giemsa stain. Parasitized red blood cells were counted under light microscope (100× magnification). The percentage parasitemia was calculated as follows

$$\%\ \text{Parasitemia} = \frac{\text{Number of parasitized RBC}}{\text{Total number of RBC counted}} \times 100$$

### 2.5. Evaluation of antinociceptive activity

#### 2.5.1. Hot plate test

Antinociceptive activity of PEAC was investigated using the thermal stimulus on the hot plate. Twenty-five mice were divided into five groups (n = 5) as negative control, PEAC (100, 200 and 400 mg/kg), and pethidine (5 mg/kg), respectively. The baseline latency was determined in mice by placing the animals singly on the hot plate set at 55 ± 1 °C. Mice showing initial nociceptive responses within 2 s were selected, while a post treatment cut-off time of 20 s was used to avoid paw tissue injury. Thereafter, animals were treated with vehicle (10 mL/kg), PEAC (100, 200 and 400 mg/kg) and pethidine (5 mg/kg). The latency to withdraw or jump from the hotplate was taken after treatment at 30, 60, 90 and 120 min. The mean percentage maximal possible effect (% MPE) was calculated as follows;

$$\%\ \text{MPE} = \frac{\text{Post} - \text{drug latency} - \text{Pre} - \text{drug latency}}{\text{Cut} - \text{off time} - \text{Post drug latency}} \times 100$$

#### 2.5.2. Acetic acid-induced writhing

Mice were divided into 5 groups (n = 5) and treated with vehicle (10 mL/kg), PEAC (100, 200 and 400 mg/kg) and indomethacin (10 mg/kg). 1 h after oral treatment, mice were injected intraperitoneally with 10 mL/kg of acetic acid (0.6%) to induce characteristic writhing as described by Koster et al. (1959). The number of writhing was counted from the 5th – 20th minutes after acetic acid injection. Furthermore, the plausible pathway involved in the antinociceptive activity of PEAC (400 mg/kg) was investigated. Animals were pretreated intraperitoneally for 15 min with L-NAME (10 mg/kg), atropine (10 mg/kg), glibenclamide (8 mg/kg), yohimbine (3 mg/kg), or cyproheptadine (8 mg/kg) before oral administration of PEAC (400 mg/kg).

#### 2.5.3. Formalin-induced paw licking test

Antinociceptive activity of PEAC was further investigated using the formalin-induced paw licking test as described by Hunskaar and Hole (1987). Mice were divided into 6 groups (n = 5) and treated with vehicle (10 mL/kg), PEAC (100, 200 and 400 mg/kg) and pethidine (5 mg/kg) and indomethacin (10 mg/kg). 1 h after pretreatment, each animal was injected with 20 μL formalin (2.5%) subcutaneously in the paw. Paw licking time was observed for the first 5 min representing the neurogenic pain phase and from 20th −30th min representing the inflammatory pain response.

### 2.6. Evaluation of anti-inflammatory activity of PEAC

#### 2.6.1. Lipopolysaccharides-induced acute sickness behavior in mice

Acute sickness behavior was induced in mice as an inflammatory response following injection of LPS (0.5 mg/kg) to mice pretreated with PEAC (200 and 400 mg/kg). Sickness behavior comprising of reduced locomotor activity, anxiety-like and depressive-like behaviors were assessed in mice (n = 5) using the open field test (OFT), elevated plus maze (EPM) and forced swim test (FST), respectively. The locomotor activity was determined by assessing the number of line crossing in the open field apparatus for each animal 2 h after LPS injection. The anxiety-like effect was determined in the EPM 24 h post LPS injection by placing each animal in the EPM platform. The number of entries and duration in each arm of EPM were recorded. Depressive-like behavior was assessed in mice 24 h post LPS injection using the FST paradigm. Mice were placed in a vertical glass cylinder (26 × 12 cm) with fresh water and allowed to swim. Time the animal stayed immobile or floating in an upright position within a 6 min duration was recorded by a trained observer (Soncini et al., 2012).

#### 2.6.2. Carrageenan-induced air pouch in rats

Air pouch was induced in male Wistar rats divided into 4 groups (n = 5) according to the method of Martin et al. (1994) as modified by Ajayi et al. (2017a). Animals were anaesthetized with diethyl ether and the dorsal back shaved. 20 mL sterile air was injected sub-cutaneously to form the dorsal air pouch. The pouch was re-inflated after 3 days with 10 mL sterile air. From day 4–6, rats in group 1 and 2 were treated with vehicle (10 mL/kg), group 3 treated with PEAC (400 mg/kg), and group 4 treated with indomethacin (5 mg/kg). 1 h after the last treatment on the 6th day, 2 mL carrageenan (1%) was injected into the pouch of animals in groups 2–4, while group 1 was injected with sterile normal saline. 24 h after carrageenan injection, rats were deeply anaesthetized with diethyl ether, each pouch was flushed with 2 mL sterile saline and exudates recovered with the aid of Pasteur pipette. The exudates were measured and aliquot for cell counts and biochemical assays.

*2.6.2.1. Total leucocytes and differential cell count.* The number of infiltrating leucocytes into the carrageenan-induced air pouch was assessed using the Turks stain. Aliquot of exudate were mixed with turk stain in an eppendorf tube, and leucocytes were counted in a Neubauer chamber under a light microscope. Exudates were fixed in a glass slide and stained with May Grunwald-Giemsa to count infiltrating neutrophils and monocytes under a light microscope (Nikkon Eclipse E200, USA).

*2.6.2.2. Assay for pro-inflammatory mediators.* Exudates were centrifuged at 10,000 rpm at 4 °C for 10 min. Cell free exudates were used for determination of protein, nitrites, tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6). Protein was measured using the Biuret reagent as described by Gornall et al. (1949) using bovine serum album as standard. Nitrite in exudates was reacted with Griess reagent and absorbance was measured at 540 nm using a spectrophotometer (Ajayi et al., 2017b). The levels of TNF-α and IL-6 were measured using ELISA MAX™ Deluxe kit (BioLegend, USA) according to the manufacturer's instruction.

### 2.7. Statistical analysis of data

Data obtained from animal studies were expressed as mean ± standard error of mean (SEM). Data were analysed for significant main effect using one-way analysis of variance (ANOVA) followed by Newman-Keuls post hoc multiple –comparison test. Values were considered statistically significant when $p < 0.05$.

## 3. Results

### 3.1. Acute toxicity

Administration of 5000 mg/kg of PEAC in mice showed no signs of morbidity or mortality immediately or during the observation period. No observable changes in behavioural, body weight and organ weights in both male and female mice.

### 3.2. Antimalarial activity

The course of infection in mice infected with $1 \times 10^6$ parasitized red

blood cells is as shown in Fig. 1. There was a steady increase in parasitemia levels in the animals throughout the 14 days. There was no significant difference in mean percent parasitemia between infected untreated control group and infected groups treated with PEAC (100, 200, 400 mg/kg). However, *P. beighei* infected mice treated with chloroquine and artemether-lumefantrine showed suppressed parasitemia in mice. In the survival measure, PEAC (400 mg/kg) prolong survival of mice up to 20% on day 21, while Chloroquine prolong survival to about 80% on day 28. Artemether-lumefantrine showed 100% survival on day 28 (Table 1).

### 3.3. Antinociceptive activity of PEAC in hot plate test

PEAC administered at 100, 200 and 400 mg/kg significantly increased the latency to thermal stimuli on the hot plate (Fig. 2A). The AUC for percentage maximal possible effect showed significant ($p < 0.05$) profile of the antinociceptive activity of PEAC (100, 200 and 400 mg/kg), with percentage of inhibition as 74.1%, 82.7% and 87.5%, respectively (Fig. 2B). Pethidine (5 mg/kg) showed significant inhibition of pain stimuli on the hot plate.

### 3.4. Acetic acid-induced writhing

The number of writhes was significantly ($p < 0.05$) decreased by PEAC at the doses of 100, 200 and 400 mg/kg (43.6 ± 1.77, 42.6 ± 3.99, and 31.2 ± 1.96) compared to vehicle (51.8 ± 1.77) corresponding to the inhibition by 15.8, 17.8 and 39.8%, respectively (Fig. 3A). Indomethacin (10 mg/kg) significantly reduced the number of writhes by 43.2%. The antinociceptive activity of PEAC was significantly ($p < 0.05$) reversed when animals were pre-treated with L-NAME (10 mg/kg) (Fig. 3B). No effect was observed when animals were pre-treated with atropine (10 mg/kg), glibenclamide (8 mg/kg) and yohimbine (3 mg/kg). An enhanced antinociceptive activity was recorded when mice were pretreated with cyproheptadine (8 mg/kg).

### 3.5. Formalin paw licking test

The PEAC at 400 mg/kg but not at 100 and 200 mg/kg dose significantly reduced the paw licking time in phase I of nociception when compared to the negative control group as shown in Fig. 4. PEAC at 100, 200 and 400 mg/kg showed a significant and dose dependent reduction in paw licking time even better than that of indomethacin in the inflammatory phase of the formalin paw licking test (Fig. 4). Pethidine (5 mg/kg) blocked formalin-induced nociception in both phases as demonstrated in reduced paw licking. Indomethacin (10 mg/kg) only significantly blocked the inflammatory pain of the formalin-induced pain in mice.

### 3.6. PEAC attenuates behavioral derangement in LPS-induced acute sickness behavior

There was a significant decrease in the locomotor activity after intraperitoneal injection of LPS (0.5 mg/kg) in the OFT ($F_{3, 23} = 57.78$; $p < 0.0001$; Fig. 5A) in mice pre-treated only with vehicle. Pre-treatment with PEAC (400 mg/kg) significantly reversed the LPS-mediated decrease in locomotor activity.

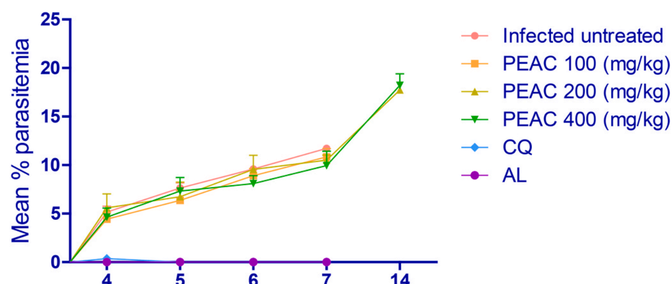


**Fig. 1.** Effect of PEAC on mean parasitemia in *P. beighei* infected mice. Values represents Mean ± SEM (n = 5).

**Table 1**
Percent survival of *P. beighei*-infected mice treated with PEAC.

| Treatment group | Survival (%)[a] | | | |
|---|---|---|---|---|
| | Day 7 | Day 14 | Day 21 | Day 28 |
| Infected untreated | 40 | 0 | 0 | 0 |
| PEAC 100 mg/kg | 20 | 0 | 0 | 0 |
| PEAC 200 mg/kg | 60 | 20 | 0 | 0 |
| PEAC 400 mg/kg | 80 | 60 | 20 | 0 |
| CQ | 80 | 80 | 80 | 80 |
| AL | 100 | 100 | 100 | 100 |

[a] Values represents Mean ± SEM (n = 5).

In addition, there was a significant reduction in the time spent in the open arm ($F_{3, 23} = 38.61$; $p < 0.0001$; Fig. 5B) and number of open arm entries ($F_{3, 23} = 10.85$; $p = 0.0002$; Fig. 5C) on the elevated plus maze 4 h after administration of LPS (0.5 mg/kg) in mice pre-treated only with vehicle. Pre-treatment with PEAC (200 and 400 mg/kg) significantly reversed the LPS-mediated decrease in the percentage open arm duration (Fig. 5B) and percentage open arm entries (Fig. 5C) on the EPM.

Furthermore, there was an increase in immobility time at 24 h after intraperitoneal injection of LPS (0.5 mg/kg) in the FST ($F_{3, 23} = 21.85$; $p < 0.0001$; Fig. 5D) in mice pre-treated only with vehicle. The 200 and 400 mg/kg of PEAC significantly ($p < 0.05$) reversed the LPS-induced increase in the immobility time.

### 3.7. Anti-inflammatory activity of PEAC in carrageenan-induced air pouch

#### 3.7.1. PEAC reduces carrageenan-induced exudate formation and vascular permeability

There was a significant increase in the exudate volume recovered 24 h after intra-pouch injection of carrageenan in rats treated with vehicle when compared with vehicle alone group (Fig. 6A). However, in animals that were pre-treated for 3 days with PEAC (400 mg/kg) before carrageenan injection, there was a significant inhibition of exudate formation by 22.1%. Indomethacin (5 mg/kg) significantly inhibited exudate formation by 31.8% (Fig. 6A). Exudate protein concentration was assessed as an index of increase vascular permeability. Carrageenan intra-pouch caused a significant 8-fold increase in protein concentration in vehicle-treated rats compared to vehicle group (Fig. 6B). Pre-treatment with PEAC (400 mg/kg) and indomethacin (5 mg/kg) significantly ($p < 0.05$) reduced exudate protein level by 55.2% and 38.9%, respectively (Fig. 6B).

#### 3.7.2. PEAC inhibits inflammatory cell migration into carrageenan-induced air pouch

As shown in Table 2, carrageenan injection into the pouch elicits a significant ($p < 0.05$) increase in the total leukocyte counts (7-fold), neutrophils (10-fold) and monocytes (14-fold). However, PEAC (400 mg/kg) pre-treatment significantly ($p < 0.05$) inhibited the total leucocytes (19.5%), neutrophils (58.2%) and monocytes (44.6%). Indomethacin (5 mg/kg) also significantly reduced total leucocytes count, neutrophils and monocytes in the pouch exudates.

#### 3.7.3. PEAC inhibits carrageenan-induced release of pro-inflammatory mediators

The results of inflammatory mediators' production in carrageenan-induced air pouch is as shown in Table 3. Carrageenan stimulates significant increase in exudates nitrites, TNF-α and IL-6 productions when compared with vehicle alone group. The exudates nitrite levels were significantly reduced in rats pre-treated with PEAC (400 mg/kg) and

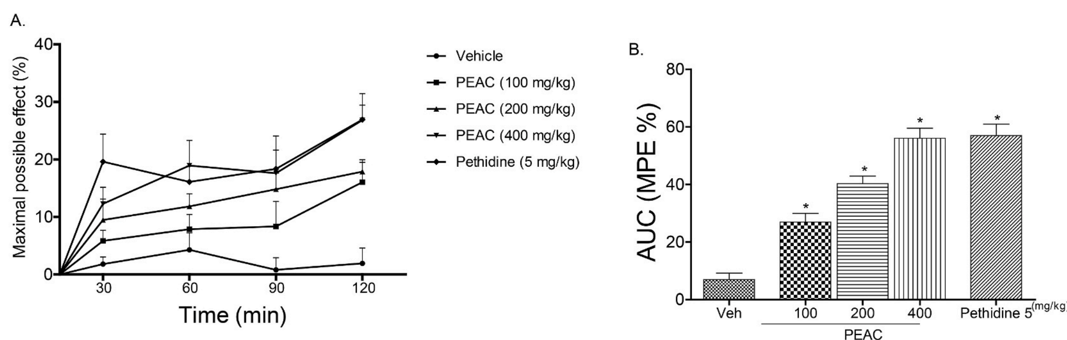


**Fig. 2.** Antinociceptive effect of peel extract of *Ananas comosus* (PEAC) on the hot plate test (A) Maximal possible effect (B) Area under the curve. Values represents mean ± SEM (n = 5). *$p < 0.05$ compared to control (vehicle) group using one-way ANOVA followed by NewMann Keuls *post hoc* test.

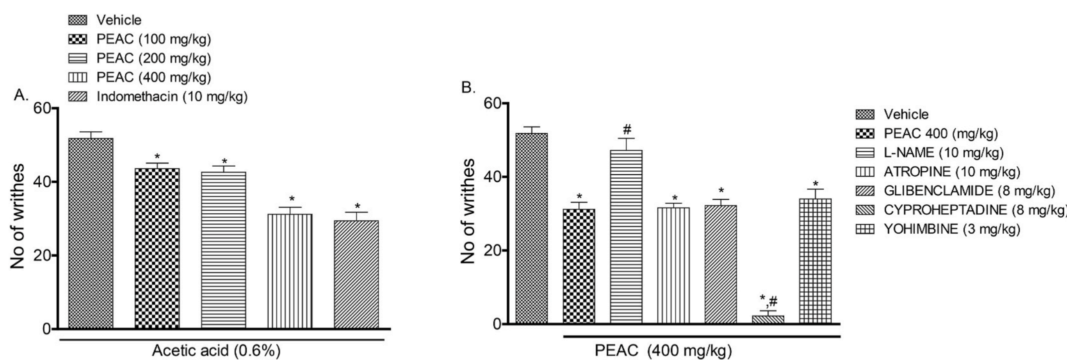


**Fig. 3.** Antinociceptive effect of peel extract of *Ananas comosus* (PEAC) in acetic-induced writhing test (A) Effect of PEAC (B) effect of blockers. Values represents mean ± SEM (n = 5). *$p < 0.05$ compared to control (vehicle), #$p < 0.05$ vs PEAC (400 mg/kg) using one-way ANOVA followed by NewMann Keuls *post hoc* test.

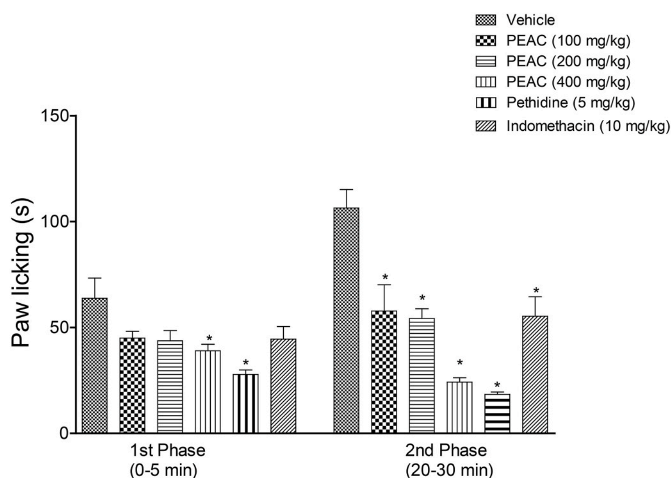


**Fig. 4.** Antinociceptive effect of peel extract of *Ananas comosus* (PEAC) in formalin-induced paw licking test. Values represents mean ± SEM (n = 5). *$p < 0.05$ compared to control (vehicle) group using one-way ANOVA followed by NewMann Keuls *post hoc* test.

indomethacin (5 mg/kg) by 39.4% and 47.6%, respectively. Furthermore, PEAC (400 mg/kg) significantly reduced TNF-α and IL-6 cytokines by 65.7% and 31.6%, respectively. Indomethacin (5 mg/kg) also significantly reduced TNF-α and IL-6 cytokines by 62.4% and 35.7%, respectively.

## 4. Discussion

The practice of using multicomponent plants parts as herbal remedy in home management of malaria is common place. However, pharmacological investigation is required to understand the contribution of each plant to the antimalarial activity demonstrated in such concoctions. In this study, we reported that *Ananas comosus* peel used as a component of malarial home herbal remedy possess mild antimalarial activity but significant anti-nociceptive and anti-inflammatory activities in rodents. Pain and inflammation often accompany *Plasmodium* infection in humans, many medicinal plants parts have been reported as a febrifuge and as such might actually be acting to relieve the symptoms without having direct effect on the *Plasmodium* development. Also, ethnomedicinal use of certain medicinal plants are to provide relief from pain and ache of acute malaria attack (Adzu et al., 2003).

Result of the oral acute toxicity test of PEAC showed no mortality or any significant change in the behavior of mice when PEAC was administered at 5 g/kg dose. Other studies have indicated no toxicity effect at 2000 mg/kg dose of pineapple peel extract (Kargutkar and Brijesh, 2016). The peel extract has also shown hepatoprotective effect (Dougnon et al., 2009).

*Ananas comosus* peel was reported in ethnomedicine as a component of antimalarial herbal mixtures. In our study, PEAC did not suppress parasite development in mice infected with *P. beighei* NK65 strain. PEAC at the tested doses showed increase parasite count post inoculation. Peter's 4-day suppressive test model is used as a first step in screening the *in vivo* antimalarial activity (Deharo et al., 2001). An extract or a compound is considered to possess active antimalarial activity when it shows at least a 30% suppression of parasitemia (Adugna et al., 2014). Survival time is an important parameter in evaluating antimalarial activity of extracts of compounds (Fidock et al., 2004). In this study, there was about 20% prolonging of survival in *P. beighei*-infected mice treated with the highest dose of PEAC. The prolonged survival might be due to increased immune boosting or possibly due to other anti-inflammatory effect of the extract. An extract that can prolong the survival time of *P. beighei* infected mice when compared with infected non-treated control could be considered active against malaria, particularly when

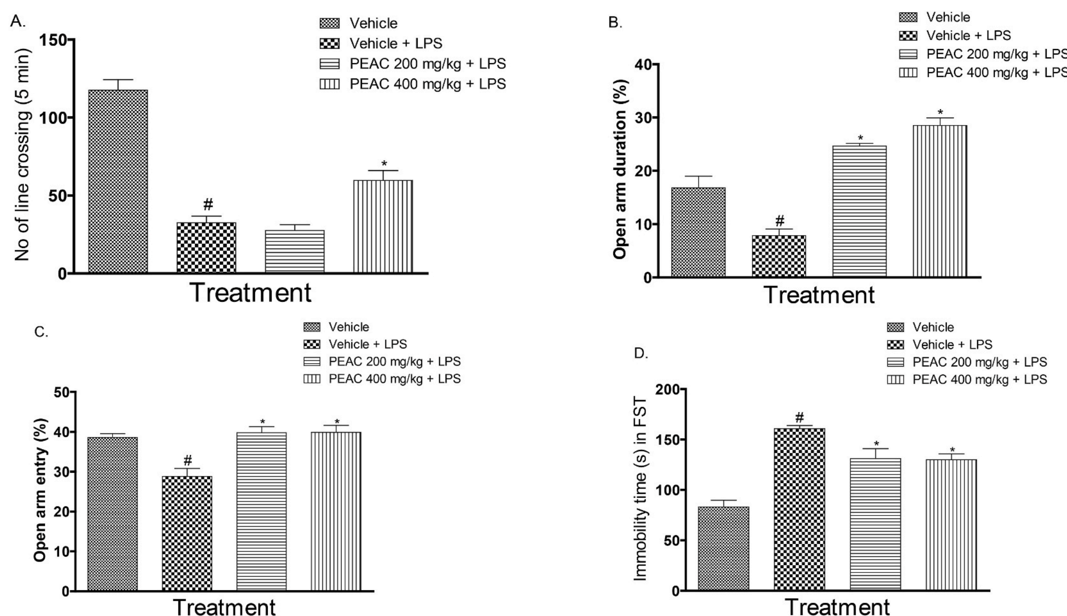


**Fig. 5.** Modulatory effect of peel extract of *Ananas comosus* (PEAC) in LPS-induced sickness behavior in mice (A) No of line crossing in OFT (B) Open arm duration in EPM, (C) Open arm entries in EPM, and (D) immobility time in FST. Values represents mean ± SEM (n = 5). #$p < 0.05$ compared to vehicle, *$p < 0.05$ compared to vehicle + LPS using one-way ANOVA followed by NewMann Keuls *post hoc* test.

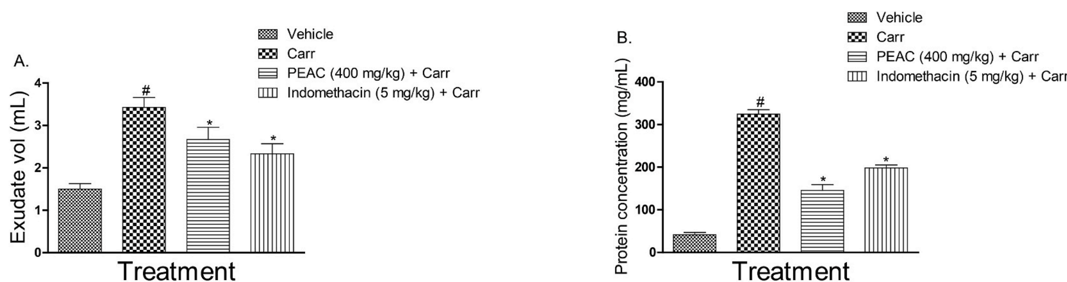


**Fig. 6.** Anti-inflammatory effect of peel extract of *Ananas comosus* (PEAC) in carrageenana-induced air pouch in rats (A) Exudates volume(B) protein concentration in exudate. Values represents mean ± SEM (n = 5). #$p < 0.05$ to compared vehicle, *$p < 0.05$ compared to carrageenan using one-way ANOVA followed by NewMann Keuls *post hoc* test.

**Table 2**
Effect of PEAC on carrageenan-induced inflammatory cell accumulation.

| Treatment | Inflammatory cells (x $10^6$)[a] | | |
|---|---|---|---|
| | Total leucocytes counts | Neutrophils | Monocytes |
| Vehicle | 6.72 ± 0.70 | 2.99 ± 0.24 | 0.12 ± 0.03 |
| Vehicle + Carrageenan | 47.43 ± 2.42# | 29.66 ± 2.46# | 1.66 ± 0.20# |
| PEAC (400 mg/kg) + Carrageenan | 38.16 ± 2.62* | 12.41 ± 1.89* | 0.92 ± 0.27* |
| Indomethacin (10 mg/kg) + Carrageenan | 31.27 ± 1.04* | 17.55 ± 2.61* | 0.79 ± 0.18* |

[a] Values represents Mean ± SEM (n = 5). #p < 0.05 vs vehicle, *p < 0.05 vs Carrageenan alone.

**Table 3**
Effect of PEAC on carrageenan-induced inflammatory mediators.

| Treatment | Inflammatory mediators [a] | | |
|---|---|---|---|
| | Nitrite (μmol/mg protein) | TNF-α (pg/mg protein) | IL-6 (pg/mg protein) |
| Vehicle | 7.32 ± 1.30 | 28.23 ± 3.48 | 18.79 ± 2.63 |
| Vehicle + Carrageenan | 35.74 ± 1.35# | 257.60 ± 43.76# | 46.04 ± 3.07# |
| PEAC (400 mg/kg) + Carrageenan | 21.67 ± 1.88* | 88.16 ± 9.44* | 31.48 ± 4.28* |
| Indomethacin (10 mg/kg) + Carrageenan | 18.72 ± 4.78* | 96.73 ± 11.20* | 29.61 ± 6.71* |

[a] Values represents Mean ± SEM (n = 5). #p < 0.05 vs vehicle, *p < 0.05 vs Carrageenan alone.

survival is beyond 12 days (Krettli et al., 2009; Ural et al., 2014). An earlier study had reported a weak antiplasmodial activity against *P. falciparum* in *in vitro* culture (Okokon et al., 2017). However, a more recent report showed that methanol extract of *A. comosus* peel and its hexane fraction demonstrated antimalarial activity against *P. beighei* infected mice using the Rane's test (Uzor et al., 2020).

The anti-nociceptive activity of PEAC was evaluated using the acetic acid-induced writhing test, hot plate test and the formalin paw licking test. Acetic acid writhing test is a standardized model for evaluating the analgesic properties of most compounds. This is due to the fact that acetic acid causes the release of various inflammatory mediators of the nociceptive neurons (Collier et al., 1968). These mediators include bradykinins, prostaglandins and pro-inflammatory cytokines which are released from macrophages and mast cells (Ikeda et al., 2001; Figueredo et al., 2011). From the results, it was observed that the ability of the oral administrations of PEAC in inhibiting the abdominal constrictions

evoked by acetic acid was dose-dependent. The formalin test is a biphasic test for nociception-neurogenic phase and inflammatory phase. The result demonstrated that PEAC attenuated the paw licking time dose-dependently at both phases. To further investigate the central acting effect of PEAC, the hot plate test was used. It measures responses to short-term harmful stimuli. From the results, PEAC increased the latency to the thermal stimuli dose-dependently. The percentage inhibition increased with increased doses as well. These tests help reveal the anti-nociceptive properties activity of PEAC both peripherally and centrally, either by interfering with the release of the inflammatory mediators or centrally by acting on neural structures. The demonstrated antinociceptive activity of *Ananas comosus* peel extract alludes scientific evidence behind the ethnomedicinal use of the pineapple peel in the management of acute malaria attack.

*Plasmodium*-induced inflammation has been found to cause sickness behavior (Clark et al., 2008). Sickness behavior are symptoms of loss of appetite, less activeness and changes in body temperature resulting from infection (Vale, 2018). Ameliorating anorexia during acute malaria attack is an important practice in folkloric management of the disease (Adegoke et al., 2011). In this study, PEAC attenuated LPS-induced sickness behavior in mice. LPS induction caused a decrease in novelty-induced exploration in the open field test, which is an important assessment of sickness behavior in mice (Desruisseaux et al., 2008). PEAC significantly attenuated this effect in LPS-induced mice. PEAC also attenuated the behavioral and emotional components in sickness behaviors as measured in the elevated plus maze and forced swim tests, respectively. Pro-inflammatory mediators such as TNF-α, IL-1β and IL-6 are the main drivers of these behavioral and emotional changes during pathogen infection (Clark et al., 2008; Lasselin et al., 2020). The ability of PEAC to attenuate these behavioral and emotional changes demonstrates its inherent anti-inflammatory properties.

To further demonstrate the anti-inflammatory activity of PEAC, a rat model of carrageenan-induced air pouch inflammation was used. In this model, inhibition of exudate volume, leucocytes migration and pro-inflammatory mediators is an indication of anti-inflammatory activity of drugs (Kim and KimJeong, 2006). Exudate volume, measured as an index of edema, and increased vascular permeability implicated due to the increased protein content were mitigated by pretreatment with PEAC (400 mg/kg) significantly. The rise in volume of exudates and increased vascular permeability are important markers of acute inflammation which can be caused by carrageenan injection. PEAC-treated rats reduced the infiltrating leukocytes, monocytes and neutrophils in the air pouch. Leukocyte migration is usually initiated as a response to inflammation usually associated with tissue injuries or inflammatory diseases (Ajayi et al., 2017a).

Inflammatory mediators including Nitrite, TNF-α and IL-6 were evaluated in the air pouch. TNF-α is a common pro-inflammatory cytokine implicated during acute inflammatory processes. It also plays a role in enhancing leukocyte migration during acute inflammation and is usually released from macrophages (Nourshargh and Alon, 2014; Kumar et al., 2018). From the results, pretreatment with PEAC reduced the activation of macrophages by suppressing the concentration of TNF-α. Carrageenan exposure also increased IL-6, which is an inflammatory mediator that plays a role in acute phase reactions and immune responses (Shin et al., 2009). Pre-treatment with PEAC also significantly inhibited IL-6 concentration that is measured in the exudate. Nitrite as well is seen as a major product of nitric oxide which is produced in inflammatory processes especially via the activation of iNOS activity. It increases vascular permeability and vasodilation (Moncada et al., 1991). Pre-treatment with PEAC significantly inhibited nitrite concentration in the exudates showing its potential in reducing vasodilation during inflammation. These findings show that *Ananas comosus* peel extract has capacity to block the release of pro-inflammatory mediators.

In conclusion, our investigation revealed that *Ananas comosus* peel possess mild antimalarial activity but significant anti-nociceptive and anti-inflammatory properties probably mediated via inhibition of pro-inflammatory mediators.

## Contributions

AMA and OGA designed the study. AIC with AMA performed the antinociceptive and antimalarial experiments. OTO and IMA and AMA carried out the anti-inflammatory experiments. AMA perform statistical analysis. AMA and OTO produced the draft manuscript. All authors contributed to and approved the final manuscript.

## Declaration of competing interest

Authors declares no conflict of interest.

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