Tropical Journal of Pharmaceutical Research January 2020; 19 (1): 137-145
**ISSN:** 1596-5996 (print); 1596-9827 (electronic)


Available online at http://www.tjpr.org
**http://dx.doi.org/10.4314/tjpr.v19i1.21**

**Original Research Article**

# *In vivo* antimalarial effect of *Ananas comosus* (L) Merr (Bromeliaceae) fruit peel, and gas chromatography-mass spectroscopy profiling: A possible role for polyunsaturated fatty acid

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*Sent for review: 5 October 2018* *Revised accepted: 18 December 2019*

## *Abstract*

***Purpose:*** *To evaluate the antimalarial effect of Ananas comosus extract and fractions and also to identify the likely bioactive compounds.*
***Method:*** *The fruit peel of the plant was extracted with methanol, and the extract successively fractionated with n-hexane, dichloromethane, ethyl acetate, methanol and water. The n-hexane fraction was further subjected to vacuum liquid chromatography to afford four sub-fractions, one of which was also analyzed using gas chromatography-mass spectroscopy (GC-MS). Plasmodium berghei-infected mice were treated orally with three doses (100, 200 and 400 mg/kg) of the plant extract and a single dose (200 mg/kg) of each of the fractions and sub-fractions in a curative antimalarial model using artemisinin combination therapy (ACT) as the reference drug.*
***Results:*** *The extract exhibited significant ($p < 0.001$) non-dose dependent parasitemia inhibitory activity in the range of 44.84 to 76.09 %. All fractions displayed inhibitory effect ($p < 0.001$) in the range of 46.44 to 87.58 % with the dichloromethane fraction displaying the highest effect (87.58 %). The sub-fractions exhibited significant inhibitory effect ($p < 0.001$) in the range of 84.14 to 92.54 %. The ACT produced significant ($p < 0.001$) inhibitory effect of 83.92 %. GC-MS analysis revealed the presence of 17 bioactive compounds, the most abundant of which were linoleic acid and palmitic acid.*
***Conclusion:*** *A. comosus displays strong antimalarial activity which supports the folkloric use of the plant for malarial treatment. A polyunsaturated fatty acid (linoleic acid) was the most abundant phyto-constituent identified.*

***Keywords:*** *Ananas comosus, Antimalarial, Malaria, Pineapple, Plasmodium beighei*



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## INTRODUCTION

Malaria is a parasitic disease caused by the Plasmodium species which is associated with significant mortality and morbidity especially in the tropical and subtropical regions of the world [1]. Presently, the first line of treatment of the disease is artemisinin-based combination therapy (ACT). Unfortunately, resistance to ACT has been reported [2]. Therefore, there is urgent quest for novel antimalarial agents particularly

from medicinal plants which are the sources of the two important antimalarial drugs, quinine and artemisinin.

*Ananas comosus* (L.) Merr (pineapple) is a perennial plant belonging to the family Bromeliaceae. The plant juice is quite nutritious and consumed globally. Pineapple fruits, peels and fruit infusions contain numerous phyto-constituents including bromelain, polyphenols and alkaloids [3-5]. Apart from its nutritional uses, the plant is used in traditional medicines for various indications, among which include anthelmintic, hiccough, hemorrhoids, constipation, diuretic, laxative, gastric irritability, jaundice, edema, venereal diseases, typhoid fever, strangury, sinusitis, throat problem, toothache, analgesics and diabetes [6]. In addition, *A. comosus* is used in the folkloric treatment of malaria in many countries [7-11]. The antiplasmodial activity of the plant has also been reported *in vitro* [12]. However, the traditional claim for the antimalarial activity requires further investigation in animal models.

Based on the foregoing, the present study was therefore designed to experimentally investigate the antimalarial effect of *A. comosus* fruit peel in mice. The bioactive constituents of the plant were also explored.

## EXPERIMENTAL

### Plant materials

Fresh fruits of *A. comosus* were collected in April, 2018 from Nsukka town, Enugu State, Nigeria. The specimen was identified by Mr. F. Nwafor, a botanist at the Department of Pharmacognosy and Environmental Medicines, University of Nigeria, Nsukka and deposited in the herbarium of the same department with voucher number PCG/UNN/0023. The plants materials were washed in running water and the peels were obtained by peeling the fruit skins with a knife. The peels were dried in the oven at a temperature of 40$^{o}$C. The dry plant material was ground into fine powder and kept in the refrigerator prior to use.

### Extraction and fractionation of plant material

The powdered plant material (500 g) was macerated in 2.5 L of 95 % methanol for 72 h at room temperature (28 ± 2 $^{o}$C) with intermittent shaking. The mixture was filtered first through a muslin then followed by filtration through a filter paper (Whatman® Grade 42). The filtrate was evaporated using a rotary evaporator at 40 °C under reduced pressure to afford the crude extract coded as ACE. The extract (ACE) (80 g) was further subjected to fractionation by dissolving it successively in *n*-hexane, ethyl acetate, methanol and water. For each solvent, the mixture was filtered and the filtrate concentrated while the residue was dissolved in the next solvent of higher polarity as listed above. This procedure afforded the respective fractions coded as ACH (for *n*-hexane), ACD (for dichloromethane), ACEA (for ethyl acetate), ACM (for methanol) and ACW (for water) fractions. Based on the extractive yield, results of bioactivity assay and trial TLC, the *n*-hexane fraction (ACH) was fractionated on a silica gel vacuum liquid chromatography (VLC) column (70-230 mesh, 500 g, 20 × 6 cm) using gradient elution of *n*-hexane and acetone (volume ratio of 3:2, 1:1, 7:3, 1:4, each 500 mL) to afford four (4) sub-fractions coded as ACH1, ACH2, ACH3 and ACH4 respectively.

### Animals

The animals used for the study were albino mice of either sexes which weigh between 20 to 25 g. The animals were obtained from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were allowed *ad libitum* access to water and feed. They received humane care throughout the experimental period in accordance with the approved protocols (FV-UNN.IACUC.2019.0816) of the University of Nigeria Ethical Committee on the Care and Use of Laboratory Animals and the guidelines of the National Research Council [13].

### Parasite inoculation

The donor mouse infected with *P. berghei* was obtained from the animal farm of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. Inoculum was prepared by drawing blood from the donor mouse and diluting it in Alsever's solution to make a suspension containing about 1 x $10^7$ infected red blood cells (RBC) in every 0.2 mL suspension.

### Curative antimalarial test (Rane's test)

Curative test model was used for the antimalarial study [14]. On Day 0, the standard inoculum (0.2 mL) of 1 × $10^7$ infected RBC was inoculated intraperitoneally in mice. Seventy-two hours (72 h) later (Day 3), 70 mice were randomly segregated into 14 groups of five (5) mice and dosed (p.o) once daily for five days (Day 3 to Day 7). Mice in groups 1-3 received 100, 200 and 400 mg/kg of the extract ACE, groups 4-12 received 200 mg/kg each of the fractions/sub-fractions, ACH, ACD, ACE, ACM, ACW, ACH1,

ACH2, ACH3 and ACH4 respectively, group 13 received the standard drug(ACT) while group 14 (Control group) was untreated (infected but received vehicle only). Thin blood film was prepared from the tail of each mouse on Day 3 (before treatment) and Day 8 (one day after the last dose of drug administration) and stained using Geimsa stain in order to monitor parasitemia level. Three stained slides were prepared for each mouse and examined under a microscope with an oil immersion nosepiece of 100 × magnification. The following formula was used to calculate the average parasitemia from three different fields on each slide [15].

Parasitemia (%) = (number of parasitized RBC)/ (total number of RBC) ×100

**Mean survival time (MST) determination**

Each mouse was observed daily for the determination of the survival time. For each group of mice, MST was determined by calculating the average survival time (days) of mice from Day 0 to Day 29.

**Determination of packed cell volume (PCV)**

On Day 8, the PCV of the mice that received the extract (groups 1-3), as well as ACT (group 13), and the vehicle (group 14) was determined using a Micro-Hematocrit Reader [15]. The animals were sacrificed by ether inhalation.

**GC-MS analysis**

The GC-MS analysis of one of the *n*-hexane sub-fractions (ACH3) was done as previously described [16] using a gas chromatograph equipment that was coupled to a mass selective detector (GCMS-QP2010SE Shimadzu, Japan). The *n*-hexane fraction was chosen for GC-MS analysis as it was the most volatile fraction (thus most suitable for GC-MS analysis). The sub-fraction of *n*-hexane (ACH3) was relatively the most abundant subfraction and also produced a significant ($p < 0.001$) parasitemia reduction. A solution of the sample (2 mg) was made in hexane/acetone mixture, centrifuged at 3000 rpm for 15 min and the supernatant concentrated to dryness using a rotary evaporator. This was reconstituted with methanol. A sample solution (1 µL) was injected for GC-MS analysis in pulsed split mode. The injector and interface were operated at 250 and 270 $^{o}$C respectively. The carrier gas was helium with a flow rate of 1.0 mL/min. Detection by the mass spectrometer was performed in the electron impact (EI) positive mode utilizing high energy electrons (70 eV). The analysis was repeated three times. Identification of peak was done by matching their mass spectra and retention indices with those on the NIST 11 and Wiley 8 GC-MS data bases.

**Statistical analysis**

The statistical package (SPSS version 21.0 USA) was used for data analysis. The results were expressed as mean ± standard error of mean (SEM). Difference between means was determined by One-Way Analysis of Variance (ANOVA) followed by Dunnet's post hoc test. Values of $p < 0.05$ were considered significant.

## RESULTS

**Antimalarial activity**

The data from the antimalarial study (Table 1) revealed that the extract (ACE) produced a significant ($p < 0.001$) inhibition of parasitemia in the infected mice in a non-dose dependent manner. The lowest dose of 100 mg/kg produced better effect (76.09 % inhibition) than the highest dose of 400 mg/kg (44.84 %). All the fractions produced significant ($p < 0.001$) inhibition of parasitemia. The ACD produced the highest inhibitory effect (87.58 %), followed by ACEA (85.26 %), ACM (81.26 %) and ACH (79.91 %) while ACW produced the least effect (46.44 %). All the sub-fractions (ACH1 to ACH4) also produced significant inhibition of parasitemia ($p < 0.001$) in the range of 84.14 to 92.54 %, with the ACH2 showing the highest effect (92.54 %). The results of the sub-fractions (84.14-92.54 %, $p < 0.001$) were better than that of the standard drug (ACT) which produced 83.42 % ($p < 0.001$) inhibitory effect.

**Mean survival time (MST)**

The data show that the ACH4 significantly ($p < 0.5$) prolonged the MST of the infected mice (Table 1). The extract, fractions and the other sub-fractions also exhibited moderate prolongation of the survival time.

**Results of packed cell volume (PCV)**

Results of PCV (Table 2) show that the PCV of the mice was lowered on Day 3 (relative to Day 0) due to infection by the malaria parasite. The plant extract, however, produced a significant ($p < 0.001$) improvement in the PCV of the mice in a non-dose dependent manner. The standard drug (ACT) also caused a significant increase in the PCV.

**Table 1:** Effect of *A. comosus* extract and fractions on parasitemia level and MST of the mice in the curative antimalarial model

| Group | Treatment | Parasitemia (%, Day 3) | Parasitemia (%, Day 8) | Inhibition (%) | Mean survival time (days) |
|---|---|---|---|---|---|
| 1 | (ACE) (100 mg/kg) | 46.0 ± 3.56 | 11.0 ± 3.49*** | 76.09 | 24.5 ± 3.50 |
| 2 | (ACE) (200 mg/kg) | 38.2 ± 4.43 | 9.6 ± 1.72*** | 74.87 | 14.6 ± 4.21 |
| 3 | (ACE) (400 mg/kg) | 36.8 ± 3.33 | 20.3 ± 4.87*** | 44.84 | 16.0 ± 2.24 |
| 4 | (ACH) (200mg) | 42.8 ± 5.34 | 8.6 ± 2.42 *** | 79.91 | 19.6 ± 5.18 |
| 5 | (ACD) (200 mg/kg) | 44.3 ± 3.79 | 5.5 ± 1.04 *** | 87.58 | 22.3 ± 5.75 |
| 6 | (ACEA) (200 mg/kg) | 50.2 ± 2.92 | 7.4 ± 1.12 *** | 85.26 | 18.0 ± 6.12 |
| 7 | (ACM) (200 mg/kg) | 52.3 ± 2.06 | 9.8 ± 1.84 *** | 81.26 | 21.0 ± 4.04 |
| 8 | (ACW) (200 mg/kg) | 32.3 ± 5.02 | 17.3 ± 1.31 *** | 46.44 | 11.3 ± 2.75 |
| 9 | (ACH1) (200 mg/kg) | 57.6 ± 3.08 | 7.2 ± 1.24 *** | 87.50 | 23.2 ± 4.80 |
| 10 | (ACH2) (200 mg/kg) | 53.6 ± 1.38 | 4.0 ± 0.84 *** | 92.54 | 15.6 ± 5.38 |
| 11 | (ACH3) (200 mg/kg) | 45.4 ± 4.28 | 7.2 ± 2.08 *** | 84.14 | 24.4 ± 3.60 |
| 12 | (ACH4 ) (200 mg/kg) | 47.6 ± 5.85 | 4.8 ± 1.24 *** | 89.92 | 28.1± 0.01* |
| 13 | (ACT ) (7 mg/kg) | 38.6 ± 2.54 | 6.4 ± 0.93 *** | 83.42 | 28.0± 0.00* |
| 14 | (Control) | 44.4 ± 3.97 | 69.0 ± 1.05 | -55.41 | 10.8 ± 1.17 |

Data are mean ± SEM (n = 5), *$p$ < 0.05,**$p$ < 0.01, ***$p$ < 0.001 compared with control group; MST = mean survival time; ACE = *A. comosus* peel extract; ACH = *n*-hexane fraction; ACD = dichloromethane fraction; ACEA = ethyl acetate fraction; ACM = methanol fraction; ACW = aqueous fraction; ACH1-ACH4 = various fractions of ACH; ACT = artemether and lumefantrine combination therapy

**Table 2:** The effect of *A. comosus* extract on PCV of mice

| Parameter | PCV (Day 0) | PCV (Day 3) | PCV (Day 8) | Change in PCV (%, Days 3 and 8) |
|---|---|---|---|---|
| ACE 100 mg/kg | 39.5 ± 0.65 | 28.0 ± 1.83 | 38.3 ± 0.85*** | 26.89 |
| ACE 200 mg/kg | 40.2 ± 1.20 | 27.8 ± 1.74 | 38.0 ± 1.05*** | 36.69 |
| ACE 400 mg/kg | 41.8 ± 0.85 | 29.3 ± 2.06 | 40.0 ± 0.41*** | 36.51 |
| (ACT) 7 mg/kg | 40.2 ± 0.49 | 31.2 ± 0.86 | 38.4 ± 0.68*** | 18.75 |
| Control | 41.0 ± 0.55 | 31.6 ± 0.81 | 25.6 ± 0.93 | -18.99 |

Data are mean ± SEM (n = 5); *$p$ < 0.05, **$p$ < 0.01, ***$p$ < 0.001 compared with Control group; ACE = *A. comosus* peel extract; ACT = artemether and lumefantrine combination therapy

## Phytochemical constituents

Results of GC-MS analysis showed that 17 compounds were detected (Table 3; Figure 1). Based on their peak area, linoleic acid (peak area of 59.68 %, retention time, RT, = 17.320 min) was the most abundant compound, followed by palmitic acid (*n*-hexadecanoic acid, peak area of 33.18 %, RT = 16.072 min). These two compounds altogether make up to 92.86 % of the sample (ACH3) composition. The constituents which make up the remaining 7.14 % of the sample include other fatty acids (such as oleic acid, stearic acid), esters, ketones, aldehydes, alcohols and hydrocarbons.

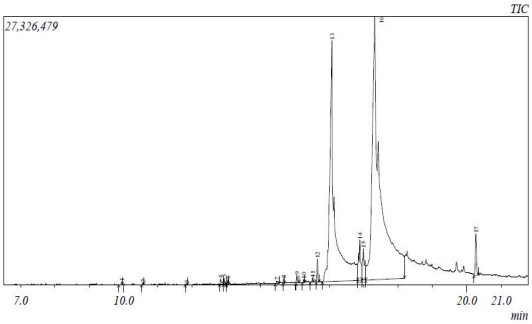



**Figure 1:** GC-MS chromatogram of the *n*-hexane sub-fraction of *A. comosus*

**Table 3:** Chemical composition of *n*-hexane subfraction of *A. comosus*

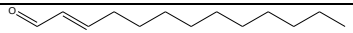
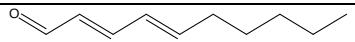
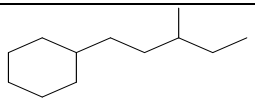
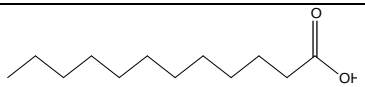
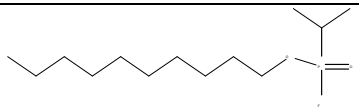
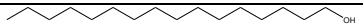
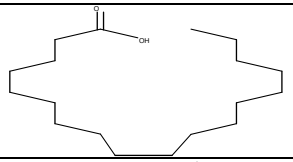
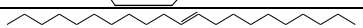
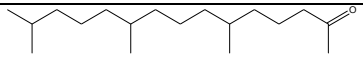
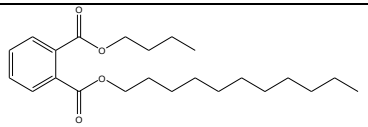
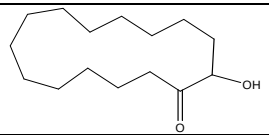
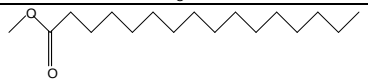
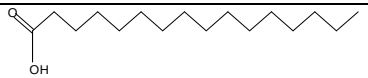
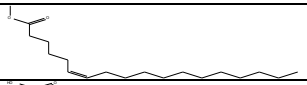
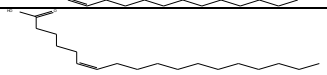
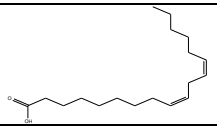
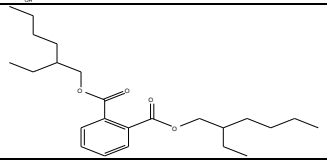
| Retention Time (RT) in minutes | Name of Compound | Molecular structure | Molecular formula | Molecular weight | Class of compound | Peak Area (%) |
|---|---|---|---|---|---|---|
| 9.949 | (2*E*)-2-Tridecenal |  | $C_{13}H_{24}O$ | 196.33 | Aldehyde | 0.05 |
| 10.562 | (E,E)-2,4-Decadienal |  | $C_{10}H_{16}O$ | 152.23 | Aldehyde | 0.03 |
| 11.836 | 3-Methylpentyl)cyclohexane |  | $C_{12}H_{24}$ | 168.32 | Aliphatic hydrocarbon | 0.02 |
| 12.824 | n-Dodecanoic acid (Laurostearic acid) |  | $C_{12}H_{24}O_2$ | 200.32 | Saturated fatty acid | 0.22 |
| 12.931 | Decyl-isopropylphosphonofluoridoate |  | $C_{13}H_{28}FO_2P$ | 266.33 | Fatty phosphoric ester | 0.16 |
| 13.040 | n-Cetyl alcohol |  | $C_{16}H_{34}O$ | 242.44 | Fatty alcohol | 0.11 |
| 14.462 | Stearic acid (octadecanoic acid) |  | $C_{18}H_{36}O_2$ | 284.48 | Saturated fatty acid | 0.12 |
| 14.666 | (10*E*)-10-Henicosene |  | $C_{21}H_{42}$ | 294.56 | Aliphatic hydrocarbon | 0.09 |
| 15.053 | 6,10,14-Trimethyl-2-pentadecanone |  | $C_{18}H_{36}O$ | 268.48 | Ketone | 0.16 |

**Table 3:** Chemical composition of *n*-hexane subfraction of *A. comosus* (*continued*)

| Retention Time (RT) in minutes | Name of Compound | Molecular structure | Molecular formula | Molecular weight | Class of compound | Peak Area (%) |
|---|---|---|---|---|---|---|
| 15.251 | Phthalic acid, butyl undecyl ester |  | $C_{23}H_{36}O_4$ | 276.53 | Fatty ester | 0.06 |
| 15.512 | 2-Hydroxycyclopentadecanone |  | $C_{15}H_{28}O_2$ | 240.21 | Ketone | 0.12 |
| 15.650 | Palmitic acid, methyl ester |  | $C_{17}H_{34}O_2$ | 270.45 | Fatty ester | 0.44 |
| 16.072 | *n*-Hexadecanoic acid (Palmitic acid) |  | $C_{16}H_{32}O_2$ | 256.00 | Saturated fatty acid | 33.18 |
| 16.887 | Methyl cis-6-octadecenoate (Methyl petroselinate) |  | $C_{19}H_{36}O_2$ | 296.49 | Fatty ester | 2.28 |
| 16.991 | *cis*-Oleic Acid |  | $C_{18}H_{34}O_2$ | 282.26 | Unsaturated fatty acid | 1.91 |
| 17.320 | (Z,Z)-9,12-Octadecadienoic acid (Linoleic acid) |  | $C_{18}H_{32}O_2$ | 280.45 | Polyunsaturated fatty acid (PUFA) | 59.68 |
| 20.275 | Bis(2-ethylhexyl) phthalate |  | $C_{24}H_{38}O_4$ | 390.28 | Fatty ester | 1.35 |

## DISCUSSION

The current work evaluated the malaria curative effect of *A. comosus* peel in mice. Curative test using *P. berghei* in mouse model is a widely accepted antimalarial evaluation model [17]. The extract was fractionated and one of the active fractions, the *n*-hexane fraction, further partially purified. The *n*-hexane fraction was chosen for further analysis as it is one of the fractions that gave high level of parasitemia inhibition and also the yield (data not shown) was higher than others.

The results showed that the extract and all its fractions and sub-fractions exhibited significant ($p < 0.05$) inhibition of parasitemia. It was also observed that as the extract was purified further, the level of parasitemia inhibition increased; such that the highest level of inhibition for the extract, fractions and subfractions were 76.09, 87.58 and 92.54 % respectively. The parasite inhibitory effect of the subfraction (92.54 %) was better than that of the reference drug (83.42 %). One of the subfractions also prolonged the survival time of the infected mice. It has been reported that antimalarial test samples that show over 30 % suppressive effect on parasitemia [18,19] or that can prolong the MST of the infected animals [19] are often considered effective antimalarial agent. Therefore, the results from the present study show strong indications that *A. comosus* possesses antimalarial activity which, interestingly, persisted as the extract was further purified.

These findings support the wide use of *A. comosus* fruit or peel in various communities for the treatment of malaria [7-11]. According to the classification of antimalarial activity by Deharo *et al* [20], the extract and fractions showed very good antiplasmodial activity. This antimalarial activity was further supported by the results of PCV which showed that the extract caused significant increase in this hematological parameter. This also suggests that the plant plays a role in reversing anemic signs induced by the parasite.

The GC-MS analysis was done to explore the likely bioactive principles of the plant. The results revealed the presence of 17 compounds. The fatty acids, linoleic acid and palmitic acid, were the most abundant phyto-constituents identified from the *n*-hexane subfraction. Linoleic acid was much more predominant than palmitic acid. It is suggested that linoleic acid, and to a lesser extent, palmitic acid, could have some roles to play in the antimalarial activity of the plant. Other lesser constituents such as oleic acid, stearic acid and the esters of fatty acid could also have some effect in the overall activity of the plant. Linoleic acid is an 18-carbon polyunsaturated omega-6 fatty acid, often denoted as 18:2 (n-6) or 18:2 cis-9, 12. It is one of the two essential fatty acids (the other being α-linolenic acid). Palmitic acid is a 16-carbon saturated fatty acid commonly found in living organisms. Fatty acids, particularly polyunsaturated fatty acids (PUFA), and diets rich in fats have been demonstrated to exhibit remarkable antimalarial property in a number of studies [21-23]. Thus, in the present study, it is possible that the linoleic acid acts in synergy with palmitic acid and the other identified constituents to exert the antimalarial effect.

Another interesting observation in the present study is that lower doses of the plant extract (100 and 200 mg/kg) produced better effect than the higher dose of 400 mg/kg. When the extract was purified and the fractions administered at a dose of 200 mg/kg, the effect became more pronounced. This suggests the presence of some metabolites that possibly antagonized the antimalarial activity of the plant at higher doses. One possible explanation is that the presence of antioxidants such as vitamin C, carotenoids and phenolic compounds in *A. comosus* [24] could have attenuated its antimalarial activity. Antioxidants were reported to have antagonistic action against parasite-killing by fatty acids when both are administered together [25]. Thus, the extract at higher dose of 400 mg/kg, could contain higher concentration of antioxidants that possibly antagonized the antimalarial action of the plant leading to lower activity.

In summary, fatty acids could play some role in the antimalarial activity of *A. comosus.* However, the actual roles of fatty acid in the activity of this plant and the mode of action need further investigation. Other fractions could also be investigated to determine the actual antimalarial constituents of the plant. It is possible that the antimalarial constituents of the plant are diverse in nature.

## CONCLUSION

The present study shows that *A. comosus* peel has antimalarial property. The finding supports the folkloric use of the plant for the treatment of malaria. The data from the present study also revealed the abundance of fatty acids, linoleic acid and palmitic acid, which could play some roles in the bioactivity of the plant. Further studies aimed at isolation the active principle of *A. comosus* and investigating the mode of action are recommended.

## DECLARATIONS

### *Acknowledgement*

The authors are grateful to Mr. Felix Nwafor for identifying the plant. They are also grateful to Zikora Uzor for providing some technical assistance.

### *Conflict of interest*

No conflict of interest is associated with this work.

### *Contribution of authors*

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. All authors contributed in the design, laboratory studies and analysis of the data. The first draft of the manuscript was written by PFU. All authors approved the final draft of the manuscript.



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