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# PROTECTIVE PROPERTIES OF PSIDIUM GUAJAVA AND ANNONA MURICATA LEAF EXTRACT ON P.BERGHEI INDUCED MALARIA IN MICE

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***Abstract:*** **Malaria is a major public health problem in developing countries. In this study, the leaves of Psidium guajava and Annona muricata are used for the management of malaria. Therefore, the current study aimed to evaluate antimalarial activity of ethanolic crude extract and solvent fractions of both extract in Plasmodium berghei infected mice. An 80 percent of ethanolic crude extract and solvent fractions of Psidium guajava and Annona muricata extract were tested for antimalarial activity at 100, 250 and 500 mg/kg doses. The parasitemia level, body weight, Relative Organ Weight, Liver enzymes and Kidney functional test were used to evaluate the antimalarial activity of the extracts. One-way ANOVA followed by post hoc Tukey's HSD multiple comparison test was employed and the result was expressed in mean + SEM (standard error of the mean). The experiment finding showed that the crude extract and solvent fractions of Psidium guajava and Annona muricata had significant curative and prophylaxis antiplasmodial activity. This result revealed that the Psidium guajava and Annona muricata leaf extract has promising antimalarial activity against Plasmodium berghei. However, further confirmatory studies, isolation and characterization of the active constituents are recommended.**

***Keywords:*** **Malaria, Psidium guajava and Annona muricata. major public health problem.**

## 1. INTRODUCTION

The history of malaria spans over 4,000 years, with the first documented case appearing in ancient Chinese medical writings, followed by Greek documentation in the fourth century BCE. Hippocrates recorded the symptoms, and the Susruta connected the disease to insect bites. In 1880, French surgeon Charles Louis Alphonse Laveran observed parasites in a patient's blood, identifying Oscillaria malariae as the cause of the disease. Giovanni Batissa Grassi and Raimondo Filetti named Plasmodium vivax and Plasmodium malariae in 1890, and William H. Welch renamed Oscillaria malariae to Plasmodium falciparium in 1897.

Plasmodium infections remain a significant public health threat, transmitted through the bite of an infected female Anopheles mosquito. The term "malaria" originates from the Italian "mala aria," meaning "bad air," reflecting its association with marshes. Plasmodium berghei, a single-celled parasite causing rodent malaria, is valuable in understanding the molecular and cellular biology of malaria parasites and is widely used as a model organism for human malaria studies.

Traditional medicine, including the use of medicinal plants, plays a crucial role in combating malaria. Ethnomedicine, practiced by indigenous populations worldwide, incorporates traditional medical practices such as Indian Ayurveda and Chinese medicine. These systems use natural remedies to promote health and improve quality of life. Ongoing research aims to discover new bioactive compounds from plants for the treatment of malaria.

## 2. MATERIAL AND METHOD

**Location and Duration of Study:** This study was carried in the Department of Human Physiology Faculty of Basic Medical Sciences Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria. The administration of the extract to the experimental animals lasted for 7 days, Acclimatization lasted for 3 weeks.

**MATERIALS:** 40 mice, Electronic Weighing Balance (M-Methlar Model M31L China), Centrifuge (Search Tech Instruments British Standard Model 80-2), Thermostat Oven (DHG-90 23A, PEC Medical USA), Rotary Evaporator (digital) TT-52 (Techmel and Techmel USA), Thermostatic water bath, Beakers, Measuring cylinders, 2ml hypodermic Syringe, Plasmodium berghei, Watt-man Number 1 filter paper, Normal saline, P.B (Light Microscope, A pyrochy), PB (Glass slides), cover slips, Leishman stain, Oil immersion, Refrigerator (Nexus), Latex gloves, Oral cannula, Dissecting kit, Cotton wool, EDTA bottles, Plain bottles, Chloroform (Guangdong Guandgua Chemical Factory Co. Ltd Shatou, Guondghuo, China), Feed and water for mice, Plastic plates, Standard cage, Guava leaves, Sour sop leaves.

## 3. METHODOLOGY

**Experimental Animals**: Experimental mice weighing between 21-31g were purchased from the Animal house, Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi campus. The animals were housed in standard cages kept at room temperature of 27±2°C and were provided with water and normal laboratory chow (Grower feed) ad libitium. The mice were acclimatized for 21 days before the start of the experiment (induction of Plasmodium berghei and treatment with ethanol leaf extract of Psidium guajava and Annona muricata.

**Experimental Design**: The experimental animals were divided and assigned into eight groups of five animals each as follows: Group A was the Positive control group, not induced and treated; Group B was the negative control group, induced with 0.2ml of Plasmodium berghei parasite without treatment; Group C was induced with 0.2ml of Plasmodium berghei and treated with 500mg/kg of standard drug (Artemether & Lumefantrine 80/480); Group D was induced with 0.2ml of Plasmodium berghei and treated with combination of the both extract (guava and sour sop leaves) at 250mg/kg each; Group E was induced with 0.2ml of Plasmodium berghei and treated with low dose of guava extract at 100mg/kg; Group F was induced with 0.2ml of Plasmodium berghei and treated with high dose of guava extract at 500mg/kg; Group G was induced with 0.2ml of Plasmodium berghei and treated with low dose of sour sop extract at 100mg/kg; Group H was induced with 0.2ml of Plasmodium berghei and treated with high dose of sour sop extract at 500mg/kg.

**Induction of Plasmodium berghei**: P. berghei ANKA strain parasitized erythrocytes were obtained from donor mice (Department of Zoology, University of Nigeria, Nsukka). 1ml of blood was dissolved in 19ml of normal saline in a ratio of 1:20. The mice were inoculated intraperitoneally with 0.2ml blood suspension (Basir et al., 2012). The animals were observed four days without treatment, after which parasite level was estimated quantitatively as described by the method of Fidock et al. (2004).

**Samples Collection and Parasitaemia measurement**: After the experiment was concluded following the administration of guava and sour sop leaves ethanol extract, the experimental animals were anesthetized using chloroform in an enclosed container for 2 minutes. Blood was collected from the retro-orbital sinus using heparinized capillary tube as described by Parasuraman et al., (2010) directly into an EDTA bottle, and centrifuged for 20minutes at 3000rpm, after which the serum was retrieved using a micropipette. The retrieved serum was used to assay kidney function test (urea and creatinine) and liver enzymes ( AST, ALT, and ALP). Both liver and kidneys were excise through intra-abdominal incision, weighed, and washed with normal saline; thereafter stored in 10% buffered formaline (Formosaline).

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**Statistical Analysis**: Data obtained from this study was analyzed using Statistical Package for Social Sciences (SPSS) version 25. Data obtained for plasmodium count, kidney enzymes, liver enzymes, body weight and histology of liver and kidney was analysed using ANOVA followed by post hoc Fisher's LSD.

## 4. RESULTS

**Table 4.1 effect of ethanolic leaf extract of Psidium guajava and Annona muricata on body weight following plasmodium toxicity**

| | Initial weight (g) MEAN±SEM | Final weight (g) MEAN±SEM | P-value | T-value |
|---|---|---|---|---|
| Group A (Positive control) | 25.54±1.34 | 45.21±1.30 | 0.017* | -7.493 |
| Group B (Malaria control) | 32.88±1.18 | 25.09±1.17 | 0.000* | 7.920 |
| Group C (Malaria + 500mg/kg of Standard drug) | 32.00±2.62 | 25.70±0.57 | 0.099a | 2.940 |
| Group D (Malaria + 250mg/kg of EPG + EAML) | 33.34±2.41 | 31.08±1.43 | 0.545a | 0.722 |
| Group E (Malaria + 100mg/kg of EPG) | 27.68±0.73 | 26.41±1.03 | 0.047* | 4.427 |
| Group F (Malaria + 500mg/kg of EPG) | 27.43±0.71 | 28.70±0.71 | 0.433a | -0.974 |
| Group G (Malaria + 100mg/kg of EMAL) | 27.00±0.58 | 31.18±0.67 | 0.073a | -3.494 |
| Group H (Malaria + 500mg/kg of EMAL) | 28.77±3.07 | 28.22±1.56 | 0.916a | 0.119 |

Data was analyzed using T-test, and values considered significant at $p < 0.05$. SEM: Standard error of mean. EPG: ethanolic leaf extract of Psidium guajava, EAML: ethanolic leaf extract of Annona muricata, significant (* ) and not significant (a ).

Table 4.1 resulted a showed a significant increase in-group A, groups B and E had a significant decrease, while C, D, and H had a non-significant decrease and groups F and G had a non-significant increase in the bodyweight when the initial weight was compared to the final weight.

**Table 4.2 effect of ethanolic leaf extract of Psidium guajava and Annona muricata on relative kidney and liver weight following plasmodium toxicity.**

| | Relative kidney weight (g) MEAN±SEM | Relative liver weight (g) MEAN±SEM |
|---|---|---|
| Group A (Positive control) | 0.014±0.001 | 0.030±0.004 |
| Group B (Malaria control) | 0.030±0.002* | 0.052±0.005a |
| Group C (Malaria + 500mg/kg of Standard drug) | 0.013±0.004* | 0.014±0.003* |
| Group D (Malaria + 250mg/kg of EPG + EAML) | 0.018±0.003a | 0.010±0.002* |
| Group E (Malaria + 100mg/kg of EPG) | 0.022±0.006a | 0.023±0.014a |
| Group F (Malaria + 500mg/kg of EPG) | 0.016±0.008a | 0.032±0.014a |
| Group G (Malaria + 100mg/kg of EMAL) | 0.021±0.005a | 0.034±0.018a |
| Group H (Malaria + 500mg/kg of EMAL) | 0.018±0.002a | 0.020±0.001* |
| F-value | **1.38** | **1.68** |

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Data was analyzed using ANOVA followed by post hoc Fisher's LSD and values were considered significant at p<0.005. SEM: Standard error of mean, significant (* ) and not significant (a ), EPG: ethanolic leaf extract of Psidium guajava, EAML: ethanolic leaf extract of Annona muricata.

**Table 4.2** result showed a significant increase in the relative kidney weight in group B compared to A (p=0.024), group C (p=0.018) had a significant decrease, while groups D, E, F, G, and H (p=0.089, p=0.214, p=0.055, p=0.166) had a non-significant decrease compared to group B. The relative liver weight result showed a non-significant increase in group B compared to A (p=0.141), while groups C, D, and H (p=0.018, p=0.011, p=0.040) had a significant decrease and groups.

**Table 4.3 effect of ethanolic leaf extract of Psidium guajava and Annona muricata on plasmodium count.**

| | Plasmodium count (Day 0) MEAN±SEM | Plasmodium count (Day 4) MEAN±SEM |
|---|---|---|
| Group A (Positive control) | 6.67±0.88 | 6.57±0.87 |
| Group B (Malaria control) | 25.33±0.33* | 24.67±0.55* |
| Group C (Malaria + 500mg/kg of Standard drug) | 16.67±4.48* | 14.27±0.03* |
| Group D (Malaria + 250mg/kg of EPG + EAML) | 14.00±1.73* | 12.52±0.02* |
| Group E (Malaria + 100mg/kg of EPG) | 12.00±1.52a | 14.89±0.14* |
| Group F (Malaria + 500mg/kg of EPG) | 16.33±2.02* | 12.25±0.14* |
| Group G (Malaria + 100mg/kg of EMAL) | 16.00±1.15* | 14.34±0.18* |
| Group H (Malaria + 500mg/kg of EMAL) | 13.33±0.88* | 11.45±0.01* |
| F-value | **6.82** | **8.68** |

Data was analyzed using ANOVA followed by post hoc Fisher's LSD and values were considered significant at p<0.005. SEM: Standard error of mean, significant (* ) and not significant (a ), EPG: ethanolic leaf extract of Psidium guajava, EAML: ethanolic leaf extract of Annona muricata.

**Table 4.3** result revealed a significant increase in the parasitaemia count in groups B, C, D, F, G, and H (p=0.001, p=0.003, p=0.021, p=0.004, p=0.005, p=0.033), while group E (p=0.080) had a non-significant increase compared to group A at day 0. At day 4, group B had a significant increase in the parasitemia count compared to group A (p=0.012), while groups C, D, E, F, G, and H (p=0.020, p=0.012, p=0.002, p=0.041, p=0.023, p=0.015) had a significant decrease compared to group B.

**Table 4.4 effect of ethanolic leaf extract of Psidium guajava and Annona muricata on urea and Creatinine level following P. berghei induced renal toxicity**

| | Urea level (mg/dl) MEAN±SEM | Creatinine level (mg/dl) MEAN±SEM |
|---|---|---|
| Group A (Positive control) | 35.45±5.63 | 1.39±0.28 |
| Group B (Malaria control) | 58.53±1.27* | 3.16±0.93* |
| Group C (Malaria + 500mg/kg of Standard drug) | 37.42±2.50* | 0.99±0.41* |
| Group D (Malaria + 250mg/kg of EPG + EAML) | 39.53±1.86* | 0.34±0.04* |
| Group E (Malaria + 100mg/kg of EPG) | 39.67±8.14* | 0.48±0.11* |
| Group F (Malaria + 500mg/kg of EPG) | 27.58±1.74* | 0.67±0.08* |
| Group G (Malaria + 100mg/kg of EMAL) | 32.66±5.68* | 0.59±0.03* |
| Group H (Malaria + 500mg/kg of EMAL) | 27.43±4.64* | 0.33±0.04* |
| F-value | **4.56** | **6.26** |

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Data was analyzed using ANOVA followed by post hoc Fisher's LSD and values were considered significant at p<0.005. SEM: Standard error of mean, significant (* ) and not significant (a ), EPG: ethanolic leaf extract of Psidium guajava, EAML: ethanolic leaf extract of Annona muricata.

**Table 4.5 effect of ethanolic leaf extract of Psidium guajava and Annona muricata on AST, ALT, and ALP level following P. berghei induced renal toxicity**

| | Aspartate Transaminase (IU/L) MEAN±SEM | Alanine Transaminase (IU/L) MEAN±SEM | Alkaline Phosphatase (IU/L) MEAN±SEM |
|---|---|---|---|
| Group A (Positive control) | 27.67±4.63 | 31.67±5.23 | 31.67±3.33 |
| Group B (Malaria control) | 41.33±3.33* | 49.67±7.79* | 65.10±4.77* |
| Group C (Malaria + 500mg/kg of Standard drug) | 27.66±1.76a | 26.00±2.51* | 16.57±2.23* |
| Group D (Malaria + 250mg/kg of EPG + EAML) | 22.67±5.82* | 37.00±4.16a | 27.00±3.55* |
| Group E (Malaria + 100mg/kg of EPG) | 26.67±5.78* | 32.67±8.29* | 16.86±2.66* |
| Group F (Malaria + 500mg/kg of EPG) | 27.00±4.58* | 30.33±4.37* | 20.47±4.20* |
| Group G (Malaria + 100mg/kg of EMAL) | 29.33±5.23a | 36.00±2.08a | 29.57±7.10* |
| Group H (Malaria + 500mg/kg of EMAL) | 31.00±5.13a | 31.33±3.38* | 14.63±0.97* |
| F-value | **1.34** | **1.85** | **16.86** |

Data was analyzed using ANOVA followed by post hoc Fisher's LSD and values were considered significant at p<0.05. SEM: Standard error of mean, significant (* ) and not significant (a ), EPG: ethanolic leaf extract of Psidium guajava, EAML: ethanolic leaf extract of Annona muricata.

Table 4.5 showed a non-significant increase in group A compared to B (p=0.057), groups C, G, and H had a non-significant decrease (p=0.057, p=0.091, p=0.141), while groups D, E, and F (p=0.013, p=0.043, p=0.047) had a significant decrease compared to group A as indicated in the AST result. The ALT result showed a significant increase in-group A compared to B (p=0.026), groups C, E, F, and H (p=0.005, p=0.034, p=0.018, p=0.024) while group D and G (p=0.104, p=0.081) had a non-significant decrease compared to group B. The ALP result showed a significant increase in-group B compared to A (p=0.012), groups C, D, E, F, G, and H (p=0.012, p=0.021, p=0.024, p=0.015, p=0.024, p=0.011) compared to group B.

## 5. CONCLUSION

The study demonstrated that ethanolic leaves extract of A. muricata and P. guajava possess antimalarial activities, however, both extracts improved liver and kidney enzymes as well as histoacrhitectural changes in both organs. This study demonstrated the antiplasmodial activity of Psidium guajava and Annona muricata through its reduction in Parasitaemia, and in increased Liver enzymes and Kidney Functional Test, at the given doses might contain potential lead molecule for the development of a new drug for malaria treatment. However, further confirmatory studies, isolation and characterization of the active constituents are recommended

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