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EVALUATION OF ANTIMALARIAL POTENTIAL OF A HERBAL PREPARATION CONTAINING THE FALLEN LEAVES OF FEMALE *Carica papaya* L (CARICACEAE) AND *Citrus aurantifolia* C (RUTACEAE) ON *PLASMODIUM BERGHEI* INFECTED MICE

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ABSTRACT
Background: Herbal preparations offers a complementary and alternative treatment of various diseases, including malaria. Aim: The aim of this study was to evaluate the antimalaria potential of a herbal preparation containing *Carica papaya* and *Citrus aurantifolia* on malaria infected mice. Methods: A 100g of the powdered female *Carica papaya* fallen leaves was decocted with 20%v/v *Citrus aurantifolia* juice. The extract was assessed for acute toxicity using Lorke's method. The antimalarial assay involving prophylaxis, suppressive and curative assessment of the extract was done *in vivo* using Chloroquine sensitive *Plasmodium berghei* infected mice. The extract was screened for phytochemical constituents. Fourier Transform Infra-red analysis was carried out. Results: The LD_{50} was estimated to be 5000mg because the animals showed no signs of toxicity. The prophylactic evaluation showed that 400 and 800mg/kg exhibited significant reduction of the parasitemia level against the control group. The suppressive study exhibited significant reduction of the parasitemia level against the control group while the curative study has no significant reduction. The phytochemical screening of the herbal combination showed the presence of tannins, flavonoids, phenols, deoxy sugars and triterpenes. The FTIR showed the presence of -NH, -OH, =C- and -CH. Conclusion: Herbal preparation of the 2 extracts is safe and has prophylaxis and suppressive effect but had no significant curative effect from the study. Therefore, the use of the herbal preparation in malaria prevention is evidently supported but not for malaria cure.

Keywords: *Carica papaya, Citrus aurantifolia, Plasmodium berghei,* Malaria

INTRODUCTION

Malaria is one of the most prevalent diseases afflicting humans. It is regarded as a tropical disease. Malaria is an endemic disease caused by the female anopheles' mosquito. It is a major public health issue as it results in 200 million cases and about 400,000 deaths worldwide each year (WHO, 2023). Malaria has been known to mankind for thousands of years. It is preventable and curable (Oyinloye *et al*., 2022). The 2023 World malaria report delves into the nexus between climate change and malaria. Changes in temperature, humidity and rainfall can influence the behavior and survival of the malaria -carrying anopheles' mosquito (WHO, 2023). The first evidence of malaria parasite was found in mosquitoes preserved in amber from the Paleogene period that are approximately 30 million years ago. *Plasmodium falciparum* is the most predominant species in the world. It is a type of malaria that most often causes severe and life-threatening malaria (CDC, 2024). People who are heavily exposed to the bites of mosquitoes infected with *P. falciparum* are most at risk of dying from malaria (Timperley *et al*., 2015. Malaria control efforts has been impaired by the emergence and spread of insecticide and drug resistant mosquitoes and parasites and as such, there is a need to develop new and potent antimalarial medications from natural sources (*Ranson et al.,* 2011). Herbal preparations are usually combination of plant material that are macerated, decocted or extracted with alcohol (Alamgir, 2017). Malaria is not also exempted from other diseases treated with herbal preparations.

Carica papaya has been used in traditional medicine to cure a number of ailments, including malaria (Suleiman & Nubani, 2025). *Carica papaya* Linn. (Caricaceae), a medicinally important plant species, is a member of the Carica genus, often known as papaya. The papaya tree is a widely distributed, naturally tropical, hollow, cylindrical, fast-growing herbaceous plant species (Badillo and Leal, 2020). *C. papaya* is used as a meal, culinary aid, and ethnomedicine to prevent and treat a variety of ailments and disorders. The papaya plant has a variety of pharmacological properties, including vermifuge, laxative, hypotensive, stomachic, febrifuge, amebicide, analgesic, digestive, cardiotonic, antibacterial, cholagogue, emmenagogue, wound healing, and many others (Aravind *et al*., 2013). A number of chemical compounds, which include dimehoxycoumarin, ferulic acid, rutin, carpain, nicotoflorin and so on have been reported from *C. papaya* (Kumarasinghe *et al.,* 2024). *Citrus aurantifolia* commonly called Lime belongs to the Rutaceae family. The fruit juice is acidic and fragrant, sour as lemon juice but more aromatic. It is usually valued for its unique flavour. The juice is used for the treatment of cough, sore throat, weight loss and malaria (Aibinu *et al.,* 2007; Suleiman *et al.,* 2020). The roots and leaves are reported to have antihypertensive, antibacterial and anticancer effect (Poulose *et al.,* 2005). D-limonene, D-dihydrocarbon, limonoids and flavonoids are among the major phytoconstituent in *C. aurantifolia* (Indriyani *et al*., 2023). This study aimed to investigate the phytochemical identity and evaluate the safely and anti-malaria potential of herbal extract that contain *C. papaya* and *C. aurantifolia*

MATERIALS AND METHODS

Plant Material

Fresh female *Carica papaya* fallen leaves were collected within the compound of University of Port Harcourt and fruits of Citrus aurantifolia were bought from Choba Market. The plants were

further identified and authenticated in the Herbarium of the Department of Pharmacognosy and Phytotherapy, Faculty of Pharmaceutical Sciences, University of Port Harcourt with the voucher number UPHC0611 and UPHR012. The leaves were thoroughly cleaned, air-dried, and powdered using a milling machine.

Preparation of the Herbal Extract
A 375ml of *Citrus aurantifolia* juice was mixed with 1500ml of distilled water to obtain 20%v/v *Citrus aurantifolia* juice. About 100g of the powdered leaves was boiled in 20%v/v *Citrus aurantifolia* for 10minutes.The plant mixture was allowed to cool for 6 hours. The mixture was filtered with filter paper and the filtrate concentrated on a water bath at a temperature of 50°C. A brown jelly extract was obtained. The extract was stored in an airtight container.

Phytochemical Screening
Phytochemicals was qualitatively assessed on the herbal extract to detect the presence of secondary metabolites using described standard methods (Sofowora, 1993; Harborne, 1998).

Fourier Transform Infrared Spectroscopy (FTIR) Assay
FTIR spectrum of the extract was obtained at frequency regions of 4,000 – 600 cm^{-1} and co-added at 32 scans in 4 cm^{-1} resolution on a Buck scientific M530 USA FTIR. The spectra were displayed as transmitter values.

Experimental Animals
Mice weighing 20-25g were obtained from Animal house, Faculty of Pharmaceutical Sciences, University of Port Harcourt. *Plasmodium berghei* infected mice (12-20g) were obtained from National Institute of Medical Research, Yaba Lagos. They were all and housed in a well-ventilated cage, fed and watered *ad libitum*, The animals were maintained at room temperature of 25ºC in the animal house.

Acute Toxicity Studies
A total of 18 mice of either sex weighing 20-25 g were used in the determination of the acute toxicity of the herbal extract. The mice were randomly divided into six groups of three (3) mice each and the first group was given 10mg/kg, the second group 100mg/kg and the third group 1000mg/kg of the herbal extract respectively via the oral route. The mice were observed for signs of toxicity, adverse effects or death. After 24 hours, the second three groups of mice were given 1600, 2900 and 5000 mg/kg of the herbal extract respectively and observations were noted as previously described (Lorke, 1983).

Plasmodium induction
A strain of Plasmodium specie was used for the induction of malaria in the experimental mice. The mice which was previously infected was used as donor and subsequently maintained in the laboratory by the serial passage of blood from the donor infected mice to the uninfected mice through the intra peritoneal route as described by Suleiman *et al.,* (2020).

Evaluation of the Prophylactic Anti-Malarial Activity of the herbal extract
The chemoprophylactic activity of the herbal extract was performed as described by (Suleiman *et al.,* 2021). There were five groups of *P. berghei* NK 65 infected mice in which each group contains five mice. The mice in groups 1 were administered distilled water, those in group 2 were administered 10 mg /kg of chloroquine orally while group 3, 4 and 5 were administered 200, 400 and 800mg/kg of the herbal extract orally respectively. The dosage administration continued once daily for the subsequent three days respectively. At 24 hours after dosage administration, the mice were injected with 32.3% parasitized erythrocytes by intraperitoneal injection. At 72 hours after the inoculation of the mice with the *P. berghei*, both thick and thin smears of the blood of each of the mice in all the groups were collected via the tail. The smears were fixed with ethanol and stained with Giemsa for identification and quantification of parasitized red blood cells microscopically.

Evaluation of the Suppressive Anti-Malarial Activity of the Herbal Preparation
Evaluation of the chemo-suppressive anti-malaria activity of the herbal using a modified standard four-day Suppressive test involves five groups of mice in which each group contains five mice each. Each of the groups was inoculated with 32.3% parasitized erythrocytes by intraperitoneal injection. Three hours after inoculation, the mice in groups 1 were administered distilled water, those in group 2 were administered 10 mg /kg of chloroquine orally while group 3, 4 and 5 were administered 200, 400 and 800mg/kg of the herbal extract orally respectively. The dosage administration continued once daily for the subsequent three days respectively. At 24 hours after day 3 of the dosage administration, both thick and thin smears of the blood of each of the mice in all the groups were obtained through the tail. The smears were fixed with ethanol and stained with Giemsa for identification and quantification of parasitized red blood cells microscopically.

Evaluation of the Curative Anti-Malarial Activity of the Herbal Preparation
The curative potential of the herbal preparation was evaluated as described by Suleiman *et al,* 2021). After three days post-induction of the *P. berghei* NK 65 parasite, the mice were grouped in five different cages containing three mice each. The mice in groups 1 were administered distilled water, those in group 2 were administered 10 mg /kg of chloroquine orally while group 3, 4 and 5 were administered 200, 400 and 800mg/kg of the herbal extract orally respectively. The smears were fixed with ethanol and stained with Giemsa for identification and quantification of parasitized red blood cells microscopically.

Determination of Parasitemia
The parasitemia level was determined by smearing on a microscope slide thin and tick blood smears that was obtained from the tail of each mouse and then counting the number of parasitized erythrocytes in random fields of the microscope. The smears were counted by a parasitologist from the Malaria Research and Reference Reagent Resource Center (M-RAB) of the University of Port Harcourt. The percentage parasitemia level was calculated by using the formula (Fidock *et al*., 2004).

$$\% \text{ Parasitemia} = \frac{\text{Number of infected RBC's X 100}}{\text{Total number of RBC}}$$

Determination of percentage survival
Mortality was monitored daily and throughout the period of study.

From the time of infection up to death for each mouse in both the treatment and control groups. The percentage survival time was calculated for each group by using the formula;

$$\% \text{ survival} = \frac{\text{number of animal (Day 0)} - \text{number of animal (Day 7)}}{\text{Number of animal (Day 0)}} \times 100$$

Statistical Analysis

Data were expressed as mean ± standard error and analyzed using the students t-test for significance.

RESULTS

Yield and Phytochemical Screening

The yield of 27.6% in Table 1 is quite significant. The secondary metabolites revealed in the herbal extract were flavonoids, tannins, phlobatannins, anthraquinones, alkaloids, triterpenoids, and cardiac glycosides as presented in Table 2.

FTIR Spectrum of the herbal extract

The infrared spectrum specifically shows the fingerprint pattern that identifies the herbal extract within the region of 500 – 1000 cm-1in Fig 1. The frequency region also showed the presence of OH (3106 cm-1) stretching, NH (3386-3514 cm-1) stretching, CH (2887-3106 cm-1) stretching and =C- stretching at 1615 cm-1.

Acute Toxicity Result

The animals survived the doses of the herbal extract administered to them at different phases of the study. They appeared active and healthy without sign of toxicity. The LD_{50} was therefore estimated to be 5000mg/kg.

Effect of Herbal Preparation on Prophylactic Evaluation

In Table 3, the treatments showed significant ($p>0.05$) percentage reduction of parasitaemia level in the *P. berghei* infected mice in a dose-dependent manner when compared to distilled water groups.

Effect of Herbal Preparation on Suppressive Evaluation

All the doses of the herbal extract showed no parasitemia in the infected mice as presented in Table 4. The standard drug (chloroquine) does not also have parasitemia.

Effect of Herbal Preparation on Curative Evaluation

The treatment doses of the herbal extract and chloroquine showed significant decrease when compared to the distilled water treated group on Table 5.

Percentage Survival in the Curative Study

Mortality of mice in this study, was monitored from the period of infection to day 4 in the treatment groups in the curative evaluation on Table 6. The group treated with 800mg/kg of the herbal extract had percentage survival of 80% while all other treatment groups had 100% survival.

Table 1. Percentage Yield

Herbal extract preparation	Percentage (%) Yield
Carica papaya (100g) + 20% *Citrus aurantifolia* juice (375 ml)	27.60g (27.6%)

Table 2. Phytochemical screening results of the herbal extract

Phytochemical constituents	Results
Tannins	+
Saponins	-
Cardiac glycosides	+
Anthraquinones	+
Triterpenoids & steroids	+
Alkaloids	+
Flavonoids	+
Phlobatannins	+

+ means Presence; - means Absence

Table 3. The effect of herbal preparation on the percentage parasitaemia level in *P. Berghei*-infected mice in the prophylactic evaluation

Treatment Groups	Mean parasitemia level ± Standard Deviation
Negative Control	2.45±0.698
Chloroquine 10mg/kg	0
Extract 200mg/kg	1.08 ± 0.295
Extract 400mg/kg	0
Extract 800mg/kg	0

Values represent Mean± Standard Deviation of 5 mice per group

Table 4. Suppressive effect of herbal preparation on *Plasmodium berghei*-infected mice

Treatment Groups	Mean parasitemia level ± Standard Deviation
Distilled water	13.18± 3.27
10mg/kg	0
200mg/kg	0
400mg/kg	0
800mg/kg	0

Values represent Mean± Standard Deviation of 5 mice per group

Table 5. Curative effect of herbal preparation on *Plasmodium berghei*-infected mice on day 4

Treatment Groups	Mean parasitemia level ± Standard Deviation
Distilled water	6.14± 1.03
10mg/kg	4.19± 0.66
200mg/kg	3.40 ± 0.58
400mg/kg	2.71± 0.26
800mg/kg	2.02 ± 0.04

Values represent Mean± Standard Deviation of 5 mice per group

Table 6. Percentage survival of infected treated mice in curative study after day 4

Days/treatment	200mg	400mg	800mg
Day 0	5/5	5/5	5/5
Day 4	5/5	5/5	4/5
Percentage survival	100	100	80

The numerator represents the number of mice that are active while the denominator represents the total number of mice.

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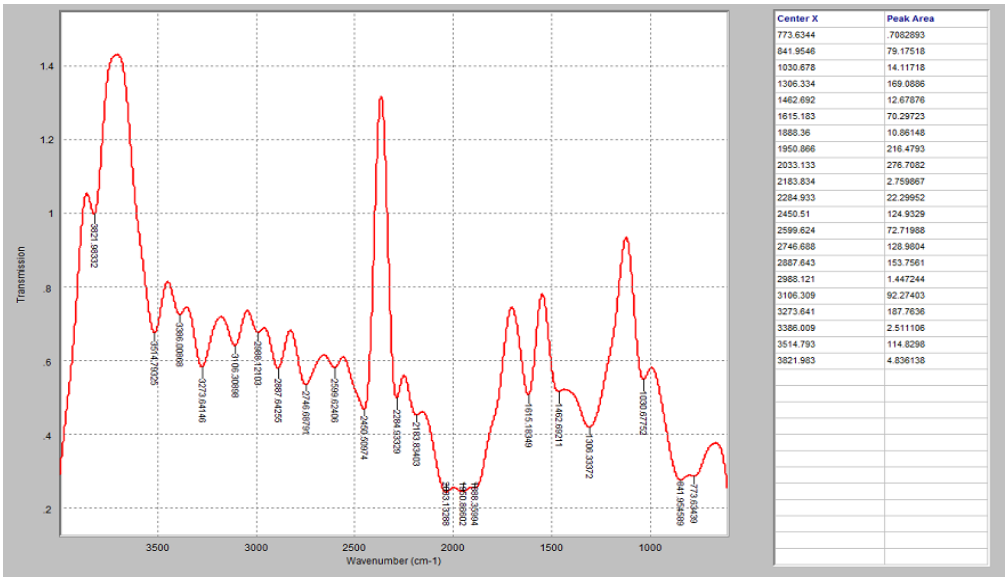

Center X	Peak Area
773.6344	.7082893
841.9546	79.17518
1030.678	14.11718
1306.334	169.0886
1462.692	12.67876
1615.183	70.29723
1888.36	10.86148
1950.866	216.4793
2033.133	276.7082
2183.834	2.759867
2284.933	22.29952
2450.51	124.9329
2599.624	72.71988
2746.688	128.9804
2887.643	153.7561
2988.121	1.447244
3106.309	92.27403
3273.641	187.7636
3386.009	2.511106
3514.793	114.8298
3821.983	4.836138

Figure 1: FTIR spectrum of the Herbal Extract

DISCUSSION

Plant to extract ratio usually varies due to the areal part extracted and the nature of solvent involved. The herbal extract exhibited a remarkable yield of 27.6% as shown in Table 1. This percentage exceeds the reported range by Monagas *et al.,* (2022). The presence of phenolics (tannins, flavonoids), cardiac glycosides, triterpenoids, alkaloids and anthraquinones as its phytoconstituents as presented in Table 2 is an indicative of a potent free radical scavenging mixture as earlier reported of the two plant materials (Boshtam *et al.,* 2011; Fatma *et al.,* 2019).

The herbal extract was analyzed using FTIR spectrophotometer at mid infrared region (4000-600 cm-1). FTIR spectroscopy is an ideal analytical technique in analyzing the functional groups in a mixture and a finger print the represent the identity of the plant extracts. The stretch at 3386 cm-1 as shown in Figure 1 is an N-H stretching vibrations (Howlett *et al*., 1997) indicating the presence of alkaloids. The stretching at 3273 cm-1 is a signature of -OH functional group (Howlett *et al*., 1997) that would be attributed to the polyphenols characteristic of the tannins as well as the flavonoids identified in the herbal extract. The region of 2887-3106 cm-1 is dominated by the C-H stretching of an aromatic ring with a complementary stretch at 1615cm-1 which could hastily be attributed to the presence of unsaturated =C- that characterized triterpenoids in Table 2. Evidently, the herbal preparation can be identified with its characteristic fingerprint region 500-1000 cm-1of the Infrared spectroscopy.

Interestingly, in this study, acute behavioral signs of toxicity such as paw licking, forced urination, forced defecation, salivation, stretching, and reduced activity, were not observed at dose 5000mg/kg, there was also no mortality at all the dose levels. The oral median lethal dose (LD_{50}) was established to be > 5000mg/kg body weight. According to the classification of LD_{50} by Loomis & Hayes, (I996), the herbal preparation falls under the range of 5000-15000mg/kg and is considered practically non-toxic thus the herbal preparation is safe.

In vivo evaluations of antimalarial activity begin with the use of the rodent malaria parasite. In addition, *in vivo* studies take into account any pro drug effect and the role of immune system in controlling malaria infection unlike in vitro ones (Fidock *et al*., 2004). The prophylactic antimalarial study in Table 3 showed that 400 and 800mg/kg exhibited significant reduction of the parasitemia level against the control group. Accordingly, this study evaluated the *in vivo* antimalarial activity using the 4-day suppressive test, which mainly evaluates the antimalarial activity of candidates on early infections, against *P. berghei* in mice. In the suppressive test of the herbal extract using five groups of mice with chloroquine as the positive control and distilled water as the negative control in Table 4, all the herbal extract doses showed maximum decrease in parasitemia level (total clearance of parasitized blood cells) which suggests that the extract was effective at preventing the infection. It could also mean that the extract is effective at eliminating the parasite at the earlier stage of malaria infection because the extract was administered three hours after inoculating the animals with the parasite, thus didn't give room for the parasite to mature in the blood since it takes 47-52 hours for *Plasmodium berghei* sporozoites to develop into mature schizonts.

In the curative test of the herbal preparation using five groups of mice with chloroquine as the positive control and distilled water as the negative control, it was observed that there was a significant decrease in the parasitemia level of the mice for all the herbal extract doses and the chloroquine positive group. The curative potential of the herbal preparation showed a dose dependent since increase in the dose corresponds with decrease in the parasitemia level (Ma *et al*., 2010). The test as well suggested that the doses of 400mg kg and 800mg/kg have a better curative potential than the chloroquine, which was used as the positive control (Karunajeewa *et al*., 2010), although there was no clearance of the parasite.

It was also observed that one of the mice in the highest dose group (800mg/kg) died before the end of the experiment in curative study. This therefore suggests that an increase in the dose of the herbal preparation may become toxic to the animals and does not hinder the progression of the parasitemia level in the mice (Karunajeewa *et al.,* 2010) thereby leading to the death of the animals treated with higher doses. It also suggests that lower doses below 400mg/kg could be better range to be administered to obtain a better

antimalarial suppressive effect as observed that the fixed smear of the blood of the animals treated with lower concentration of the herbal extract showed a lesser bleaching of the red blood cells of the animals.

Comparing the three studies it could be inferred that the herbal extract has a remarkable prophylactic and suppressive effect on malaria infection because it cleared the parasites in the blood. This helps to reduce the symptoms of malaria and prevents the disease from progressing. However, it does not eliminate the parasites when it has already grown as observed in the result of the curative study.

Conclusion

The study reveals that the Herbal extract of *C. papaya* and *C. aurantifolia* is safe at 5000mg/kg and has a significant prophylactic and suppressive effect at the doses of 400 and 800mg/kg. This study could also serve as a standard in the preparation of the herbal extract.

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Conflict of Interest

The authors declare no conflict of interest.

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