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Toxicological and Antiplasmodial Suppressive Activities of Ethanolic Extracts of Orange (Citrus sinensis) peels, Grape (Citrus paradisi) andGuava (Psidium guajava)Leaves in Albino Rats.

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Abstract: *Malaria is one of the epidemic disease knownworldwide. Drug resistance have limited the choice of treatment of malaria. Hence, finding the new compounds to treat malaria is urgently needed. The present study aim at investigating the toxicological and anti-plasmodial effects of extracts ofOrange (Citrus sinensis) peels, Grape (Citrus paradisi) and Guava (Psidium guajava) Leaves on Albino rat infected with Plasmodium berghei. Leaves were extracted using soxhlet method and acute toxicity was evaluated. For efficacy test in vivo, standard 5-day suppressive test was carried out. Rats were inoculated with $1x10^7$/ml parasitized erythrocytes of Plasmodium berghei by intraperitoneal injection. The extracts (100,300, 500, 800, and 1000 mg/kg) of each plants were given separately to each group and orally once a day for 5 consecutive days. Average Percentage parasitemia and biochemical indices were estimated. Combisunate (10 mg/kg) was given to infected rats as reference control while untreated control was given only distilled water. It was found that ethanolic extracts of these leaves at different doses showed dose dependent parasitemia inhibition and have no physiological effects. Therefore, Orange (Citrus sinensis) peels, Grape (Citrus paradisi) and Guava (Psidium guajava) Leaves exact significant anti-plasmodial activity and prolonged survival time with no toxicity.*

Keywords:*Toxicological, antiplasmodial, Orange (Citrus sinensis), Grape (Citrus paradisi), Guava (Psidium guajava), Plasmodium berghei*

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I. Introdution

Malaria has been a devastating epidemic in especially sub-Saharan Africa countries. This, thus, makes it to be at the centre of several research studies. However, despite all the works on malaria, it has continued to have its strong toll on the entire populace and particularly pregnant women and children under age 5 [1]. The adverse effects of malaria infection on both pregnant women and children under age five, has continued to impact negatively on this target groups, of individuals. In fact, malaria infection in these groups of people has continued to be linked to increased mortality and morbidity in them [2]. Despite the increasing health devastation by malaria, its control is not impossible. Quite a number of ways of achieving this have been put forward, some of which include exploring evidence of immunity, revisiting abandoned vector control approach and investigating into traditionally used herbal medicines [3].

Rural and poor people are especially at risk because they are least likely to have the means to prevent and treat malaria. Children miss school because of the disease, suffer physically and intellectually, and often cannot contribute to their families' income through agricultural work. The WHO reports that many families spend up to a quarter of their annual income for malaria treatment [4], [5]. The publication of the genomes of Plasmodium falciparum and Anopheles gambiae in October 2002 has given new hope for the development of new anti-malarial drugs that may ultimately help to control the disease [6]. In the submission of Nzila et al., [7] new inhibitors are now needed to conquer the increasing difficulty of drug resistance in the treatment of Plasmodium falciparum infection. Aiming at the folate pathway has shown to be a strong approach for drug development against rapidly multiplying systems such as cancer cells and microorganisms. However, there are several other potential targets in the folate pathway that have not yet advanced to this stage, in addition to targets that are specific to parasite metabolism. Therefore, a successful treatment and prevention, demands continuous in-depth investigation of it [7].

The Orange (citrus sinensis) is a small evergreen tree in the Rutaceae (citrus family). The orange is known to be the most popular fruit, made up of peel, pulp, seeds and juice, it is eaten fresh too. The citrus sinensis fruit is used to produce 34% of juice, with 44% of peels as byproducts from which some phenolic

compounds could be obtained. Several of these phytochemicals had been reported to possess hapatoprotective activities, while there are some scavenge superoxides called pyroxynitrite, an action suspected be antiplasmodial.Phytochemical constituents of orange peels extract include tannin, saponin, steroids, reducing sugar, protein and carbohydrate, depending on the solvent used[8].

Next to oranges are the Citrus paradisi leaves are one of the most popular citrus leaves known for its combination of extreme bitterness and slight sweetness, this leaves is also versatile when added to salads, fruit medleys, and other recipes. Perhaps more important is its nutritional profile, which includes disease-fighting antioxidants. The grape leaves go by the scientific name of Citrus paradisi.Citrus paradisi is an important leaves and its production is increasing day by day due to its considerable medicinal importance [9].

Guava is a plant in the myrtle family (Myrtaceae) genus Psidium. It is a common shade tree or shrub in door-yard gardens in the tropics. Guava fruits are eaten fresh and made into drinks, ice cream, and preserves. Extracts from guava leaves or bark are implicated in therapeutic mechanisms against cancer, bacterial infections, inflammation and pain [10],[11],[12]. Essential oils from guava leaves display anti-cancer activity in vitro [13] guava leaves are used in folk medicine as a remedy for diarrhea and fever; the bark as antimicrobial and as an astringent [14]. Guava leaves or bark are used in traditional medicine as anti-diabetic [15].Phytochemical analyses of guava leaf reveal alkaloids, anthocyanins, carotenoids, essential oils, fatty acids, flavonoids (especially quercetin), lectins, phenols, saponins, tannins, triterpenes, and vitamin C (80 mg per 100 g of guava) [16]-[21]. The essential oil contains alpha pinene, caryophyllene, cineol, D-limonene, eugenol, and myrcene. The major constituents of the volatile acids include (E)-cinnamic acid and (Z)-3-hexenoic acid [22], while the seeds contain glycine-rich proteins, starch, and phenolic and flavonoid compounds.

1.1 Aim And Objectives Of The Study

This study is aimed at evaluating the toxicological and antiplasmodial effects of ethanolic extracts of peels of orange fruits (Citrus sinensis), leaves of grape (Citrus paradisi) and leaves of guava (**(Psidium guajava)**on Albino rats infected with Plasmodium berghei

Objective

In this study, the following objectives will be accomplished:

I To determine the median lethal dose (LD_{50}) of the extracts of peels of orange fruits (Citrus sinensis), leaves of grape (Citrus paradisi) and leaves of guava ((Psidium guajava).

II To determine the plasmodium suppressive effect of the extracts in albino rats infected with Plasmodium berghei.

III To determine the toxicological effect of the extracts on rats by assessment of biochemical parameters.

IV. To evaluate the effect of dose variation of each extract on the anti-plasmodial potency and their toxicological effects in albino rats.

II. Materials And Methods

2.1 Study Area

The study was carried out in animal House and the malaria research centre in the University of Port Harcourt, Rivers State. Port Harcourt is located in the tropical rain forest south/south Nigeria. It lies on latitude 50^0c 27^0c- 5^0,3/N and Longitude 6.55- 7.05E. The climate is tropical with the mean daily temperature of $29^0 \pm 5^0$c for the entire year.

2.2 Plant Materials And Sampling Collection And Identification.

The plant species of fresh peels of orange fruits, leaves of grape and guava were obtained from the Botanical Garden of the Department of Plant Science and Biotechnology, University of Port Harcourt. The plants parts were all identified by the Departmental Herbarium.

2.3 Determination Of Median Lethal Dose (Ld_{50})

The acute toxicity was determined by fixed dose procedure. A total of twelve rats were put into four groups labeled 1-4, with each group having three (3) rats each and these groups were replicated in three places each representing the extracts ofCitrus sinensis,Citrus paradisi Leavesand leaves of Psidium guajava respectively. The animals had an average weight of 124g. A single dose of 1000mg, 2000mg, 3000mg and 5000mg/kg body weight of the extract was administered to each rat in groups 1, 2, 3 and 4 respectively, using intubation canula. The animals were observed individually every 30 minutes after dosing during the first 24 hours. Special attention was given during the first 4 hours, and daily thereafter for a total of 7 days. Mortality and other physical factors like changes in skin and fur, eyes were monitored.

2.4 Experimental Animals

A total of seventy two (72) adult rats of both sexes weighing between 117 to 130g were used. They were acquired from Department of Physiology, University of Port Harcourt. They were acclimatized for several days before use. The animals were housed in a specially designed plastic/wire gauze animal cage and were placed on standard feed and given access to water ad libitum.

2.5 Experimental Design

The method used to evaluate the anti-malarial effect of orange fruits (Citrus sinensis), leaves of grape (Citrus paradisi) and leaves of guava (Psidium guajava)was that described by Ryley and Peters [23]. Seventy two (72) albino rats were selected and sixty eight (68) of these were injected intraperitoneally with 0.5ml of blood infected with 2×10^7/ml Plasmodium berghei (NK65 strain) on the first day. The inoculation of the parasites into the animals was done at the malaria research laboratory, centre for malaria research and phytomedicine University of Port Harcourt. The infection was confirmed by viewing the Giemsa – stain blood smear obtained from the tail of the infected rats and studied under the microscope. This was established on the fourth day. After the confirmation, the animals were put into eighteen groups of four rats per group. Group 1 was uninoculated and treated with distilled water. Group 2 was inoculated and treated with 1ml/kg distilled water daily. Groups 3 (which were inoculated) received daily dose of 10mg/kg body weight of combisurnate[@]. Five different concentrations of 100mg/kg, 300mg/kg, 500mg/kg, 800mg/kg and 1000mg/kg body weight of peels of Citrus sinensis, Citrus paradisi leavesand Psidium guajava leaves extracts were administered to groups 4-8,9-13 and 14-18 respectively for five days. All administration was by oral route. Suppressive effects were monitored. Biochemical (Plasma levels of ALT, AST, ALP T.Bil, Creatinin and the concentration of Urea) parameters were conducted at the end of the research to estimate the effects of the extracts on the animals.

III. Result

3.1 Acute Toxicity

The results of the acute toxicity evaluation of peels of orange fruits (Citrus sinensis), leaves of grape (Citrus paradisi) and leaves of guava (Psidium guajava) extracts showed no remarkable behavioral changes in the administered rats. No mortality occurred within the observation period of 7 days. However, behavioral signs of toxicity were observed in rats given 5000 mg/kg which include paw licking, salivation, stretching and reduce activity. There was however no mortality at all the doses used.

Table 1: results of the acute toxicity evaluation of peels of orange fruits (Citrus sinensis), leaves of grape (Citrus paradisi) and leaves of guava (Psidium guajava)extracts

PLANT EXTRACT	TREATMENT	NO. OF RAT	No. OF DEATH	% MORTALITY
peels of orange fruits (Citrus sinensis)	1000mg/kg	3	0/3	0
	2000mg/kg	3	0/3	0
	3000mg/kg	3	0/3	0
	5000mg/kg	3	0/3	0
grape (Citrus paradisi)	1000mg/kg	3	0/3	0
	2000mg/kg	3	0/3	0
	3000mg/kg	3	0/3	0
	5000mg/kg	3	0/3	0
leaves of guava (Psidium guajava)	1000mg/kg	3	0/3	0
	2000mg/kg	3	0/3	0
	3000mg/kg	3	0/3	0
	5000mg/kg	3	0/3	0

n=3 rats

Table 2.a: Percentage Suppression of the peel of Citrus sinensis

S/N	GROUP		Av % parasitemia	Av % Inhibition
1	NORMAL CONTROL	Food and water only (no inoculation)	0.00±0.00	0.00
2	NEGATIVE CONTROL	Inoculated but not treated	23.19±2.49	0.00
3	POSITIVE CONTROL	Inoculated + combisunate 10mg/kg	0.36±0.03	98.48
4	GC1	100mg/kg	3.75±0.16	80.06
5	GC2	300mg/kg	2.77±0.26	85.27
6	GC3	500mg/kg	2.01±0.11	89.31

7	GC4	800mg/kg	1.55±0.21	91.76
8	GC5	1000mg/kg	1.08±0.24	94.25

KEY: GC1-GC5 represents the administration of ethanolic extracts doses: 100, 300, 500, 800 and 1000 in mg/kg of Citrus x sinensis

Table 2b: Percentage suppression of Citrus paradisi leaves againt P. berghei

S/N	GROUP	Treatments	Av % parasitemia	Av % Inhibition
1	NORMAL CONTROL	Food and water only (no inoculation)	0.00±0.00	0.00
2	NEGATIVE CONTROL	Inoculated but not treated	23.19±2.49	0.00
3	POSITIVE CONTROL	Inoculated + combisunate 10mg/kg	0.36±0.03	98.48
9	GA1	100mg/kg	8.65±0.23	62.70
10	GA2	300mg/kg	6.25±0.79	73.05
11	GA3	500mg/kg	4.29±0.26	81.50
12	GA4	800mg/kg	1.10±0.10	95.26
13	GA5	1000mg/kg	1.02±0.10	95.60

Where: GA1-GA5 represent 100, 300, 500,800 and 1000mg/kg of Citrus paradisi leaf extracts respectively.

Table 2c: Average Percentage Suppression of Psidium guajava (leaf) ethanolic extracts against Plasmodium berghei

S/N	GROUP	Administration of ethanolic extracts in mg/kg of P.guajava	Av % parasitemia	Av % Inhibition
1	NORMAL CONTROL	Food and water only (no inoculation)	0.00±0.00	0.00
2	NEGATIVE CONTROL	Inoculated but not treated	23.19±2.49	0.00
3	POSITIVE CONTROL	Inoculated + combisunate 10mg/kg	0.36±0.03	98.48
14	**GB1**	100mg/kg	**0.93±0.10**	**94.88**
15	**GB2**	300mg/kg	**0.89±0.14**	**95.10**
16	**GB3**	500mg/kg	**0.71±0.09**	**96.09**
17	**GB4**	800mg/kg	**0.16±0.02**	**99.12**
18	**GB5**	1000mg/kg	**0.21±0.04**	**98.84**

Where: GB1-GB5 represent 100, 300, 500,800 and 1000mg/kg of Psidium guajava leaves extracts respectively.

Data are expressed as Mean ± SEM. n=4. Values found in a column with common superscript letter a, are significantly different ($p<0.05$) when compared to the normal control. Values with superscript b, are significantly different ($p<0.05$) relative to the negative control. While values with the superscript c, are significantly different ($p<0.05$) compared to the positive control.

3.2 Effect of Ethanolic Extracts of the peel of Citrus x sinensis on some Biochemical Parameters

From the table below, it can be infer that Citrus x sinensis also has hepato-protective effect as liver enzyme markers- AST, ALT, ALP are seen reducing progressively. Citrus x sinensis also showed an ameliorating effect on the kidney as values of urea and creatinin are observed to have reduced progressively as the concentration of the extracts increases. The total bilirubin also showed trend of reduction as the treatment progresses as all levels of administration indicating the healing effect of Citrus x sinensis.

Table 3a: Effect of Ethanolic Extracts of the peel of Citrus x sinensis on some biochemical Parameters

S/N	GROUP	AST	ALT	ALP	T.P	ALB	T.BIL	C.B	U	CRE
1	NORMAL CONTROL	9.00±0.70 ^{bc}	10.50±0.50 ^{bc}	118.75±1.49 ^{bc}	69.25±1.03 ^{bc}	37.75±0.85 ^{bc}	5.50±0.28 ^{bc}	2.50±0.28 ^{bc}	2.42±0.08 ^{bc}	61.25±1.75 ^{bc}
2	NEGATIVE CONTROL	40.75±1.88 ^{ac}	28.25±1.93 ^{c}	192.00±2.97 ^{ac}	53.00±1.87 ^{ac}	25.50±1.32 ^{ac}	18.75±1.25 ^{ac}	9.25±0.47 ^{ac}	4.90±0.18 ^{ac}	72.50±1.70 ^{ac}
3	POSITIVE CONTROL	25.75±1.65 ^{ab}	20.00±0.81 ^{ab}	146.00±3.58 ^{ab}	59.25±1.10 ^{ab}	33.00±1.29 ^{ab}	17.50±0.86 ^{ab}	7.25±0.62 ^{ab}	3.15±0.10 ^{ab}	67.50±1.04 ^{ab}
4	GC1	15.75±0.47 ^{abc}	20.75±0.62 ^{abc}	159.25±10.82 ^{abc}	65.75±0.85 ^{abc}	30.50±0.95 ^{abc}	21.00±0.70 ^{abc}	8.50±0.50 ^{abc}	4.32±0.10 ^{abc}	77.50±0.64 ^{abc}
5	GC2	12.75±0.47 ^{abc}	16.75±0.47 ^{abc}	165.75±3.52 ^{abc}	71.50±1.19 ^{abc}	37.00±0.91 ^{abc}	18.25±0.47 ^{abc}	8.25±0.62 ^{abc}	3.72±0.08 ^{abc}	68.75±1.10 ^{abc}
6	GC3	8.00±0.40 ^{abc}	11.25±0.85 ^{abc}	140.50±1.32 ^{abc}	79.25±0.85 ^{abc}	41.75±1.10 ^{abc}	11.00±0.57 ^{abc}	4.25±0.62 ^{abc}	2.65±0.10 ^{abc}	60.25±0.62 ^{abc}
7	GC4	6.50±0.6	10.50±0.	105.75±2.2	70.00±	39.50±	10.75±0	4.5±0.28	1.95±0.06	61.25±1.7

		4 abc	86 abc	8 abc	0.91 abc	0.64 abc	.85 abc	abc	abc	0 abc
8	GC5	5.00±0.70 abc	9.00±2.34 abc	97.00±1.95 abc	76.25±1.54 abc	45.25±1.75 abc	5.751.03 abc	2.00±0.40 abc	1.95±0.12 abc	52.50±0.86 abc

Data are expressed as Mean ± SEM. n=4. Values found in a column with common superscript letter a, are significantly different ($p<0.05$) when compared to the normal control. Values with superscript b, are significantly different ($p<0.05$) relative to the negative control. While values with the superscript c, are significantly different ($p<0.05$) compared to the positive control.**KEY:** GC1-GC5 represents the administration of ethanolic extracts doses: 100, 300, 500, 800 and 1000 in mg/kg of Citrus x sinensis

TABLE3b: EFFECT OF CITRUS PARADISI LEAVES EXTRACTS ON SOME BIOCHEMICAL PARAMETERS OF ALBINO RATS

S/N	GROUP	AST	ALT	ALP	T.P	ALB	T.BIL	CB	U	CRE
1	NORMAL CONTROL	9.00±0.70 bc	10.50±0.50 bc	118.75±1.49 bc	69.25±1.03 bc	37.75±0.85 bc	5.50±0.28 bc	2.50±0.28 bc	2.42±0.08 bc	61.25±1.75 bc
2	NEGATIVE CONTROL	40.75±1.88 ac	28.25±1.93 c	192.00±2.97 ac	53.00±1.87 ac	25.50±1.32 ac	18.75±1.25 ac	9.25±0.47 ac	4.90±0.18 ac	72.50±1.70 ac
3	POSITIVE CONTROL	25.75±1.65 ab	20.00±0.81 ab	146.00±3.58 ab	59.25±1.10 ab	33.00±1.29 ab	17.50±0.86 ab	7.25±0.62 ab	3.15±0.10 ab	67.50±1.04 ab
9	GA1	22.50±0.64 ab	24.50±0.64 bc	236.50±10.62 bc	71.75±1.65 abc	35.00±1.29 abc	21.50±1.19 abc	12.00±1.00 abc	3.60±0.12 ab	65.25±1.10 ab
10	GA2	20.00±0.40 ab	23.00±2.73 abc	217.00±23.44 abc	81.75±1.31 ab	40.75±0.85 bc	18.00±0.91 bc	11.00±0.81 abc	3.15±0.17 abc	54.25±1.84 abc
11	GA3	12.50±0.64 bc	12.50±1.44 abc	117.50±3.96 ac	83.00±1.29 ab	44.75±1.25 abc	10.75±0.62 abc	5.00±0.70 ab	2.85±0.35 bc	55.50±1.84 bc
12	GA4	11.00±0.70 bc	12.50±0.64 abc	109.25±4.028 abc	77.00±3.31 bc	43.75±1.93 abc	12.25±1.49 ac	6.25±1.10 ab	4.42±0.19 abc	74.00±2.91 abc
13	GA5	7.50±0.28 ac	10.00±0.40 ac	91.50±5.37 abc	84.50±1.19 bc	51.00±1.08 abc	8.75±1.10 abc	4.00±0.70 bc	5.17±0.23 abc	92.75±3.30 ac

KEY: GA1-GA5 represents the administration of ethanolic extracts doses: 100, 300, 500, 800 and 1000 in mg/kg of Citrus paradise. Data are expressed as Mean ± SEM. n=4. Values found in a column with common superscript letter a, are significantly different ($p<0.05$) when compared to the normal control. Values with superscript b, are significantly different ($p<0.05$) relative to the negative control. While values with the superscript c, are significantly different ($p<0.05$) compared to the positive control.

TABLE 3c: EFFECTS OF ETHANOLIC EXTRACTS OF Psidium guajava (leaf) ON SOME BIOCHEMICAL INDICES OF RATS

S/N	GROUP	AST	ALT	ALP	T.P	ALB	T.BIL	C.B	U	CRE
1	NORMAL CONTROL	9.00±0.70 bc	10.50±0.50 bc	118.75±1.49 bc	69.25±1.03 bc	37.75±0.85 bc	5.50±0.28 bc	2.50±0.28 bc	2.42±0.08 bc	61.25±1.75 bc
2	NEGATIVE CONTROL	40.75±1.88 ac	28.25±1.93 c	192.00±2.97 ac	53.00±1.87 ac	25.50±1.32 ac	18.75±1.25 ac	9.25±0.47 ac	4.90±0.18 ac	72.50±1.70 ac
3	POSITIVE CONTROL	25.75±1.65 ab	20.00±0.81 ab	146.00±3.58 ab	59.25±1.10 ab	33.00±1.29 ab	17.50±0.86 ab	7.25±0.62 ab	3.15±0.10 ab	67.50±1.04 ab
14	GB1	27.25±2.32 abc	21.25±2.05 abc	203.75±4.49 abc	51.50±0.64 abc	26.25±0.85 abc	14.25±0.62 abc	6.50±0.28 abc	4.77±0.17 abc	74.00±1.41 abc
15	GB2	23.25±1.25 abc	22.25±1.88 abc	183.75±3.56 abc	54.50±2.02 abc	27.50±0.95 abc	13.50±0.64 abc	5.50±0.28 abc	3.75±0.09 abc	69.00±1.68 abc
16	GB3	20.25±0.62 abc	19.00±0.70 abc	169.25±1.65 abc	55.00±1.47 abc	27.50±1.04 abc	10.75±0.47 abc	4.25±0.47 abc	3.30±0.14 abc	60.75±0.85 abc
17	GB4	14.50±0.64 abc	12.50±0.64 ab	155.75±1.65 abc	60.00±0.40 abc	31.50±0.64 abc	11.00±0.40 abc	4.00±0.40 abc	2.77±0.11 abc	59.00±1.08 abc
18	GB5	12.75±0.47 abc	11.75±0.85 abc	137.75±0.85 abc	59.50±0.86 abc	31.75±0.85 abc	9.50±0.28 abc	4.00±0.00 abc	2.45±0.02 abc	57.75±0.62 abc

KEY: GB1-GB5 represents the administration of ethanolic extracts doses: 100, 300, 500, 800 and 1000 in mg/kg of Psidium guajava.Data are expressed as Mean ± SEM. n=4. Values found in a column with common superscript letter a, are significantly different ($p<0.05$) when compared to the normal control. Values with superscript b, are significantly different ($p<0.05$) relative to the negative control. While values with the superscript c, are significantly different ($p<0.05$) compared to the positive control.

IV. Discussion

Plants are a major pool of potential antiparasitic and antimicrobial compounds of pharmaceutical needs, [24] The results of this study showed that the ethanolic extracts of Orange (Citrus sinensis) peels, Grape (Citrus paradise) and Guava (Psidium guajava) possess anti-malarial property. Flavonoids have been detected in the Artemisia species and have been reported to show significant anti-malarial activity against P.falciparium[25]. These set of compounds (alkaloids and flavonoids) were identified in these three species of plant extracts, hence the allusion that the presence of these secondary metabolites could be the reason for the plant's therapeutic actions. Meanwhile, the results partly corroborate claims made in traditional medicine of the anti-malarial efficacy of these plants [26],[27]. Data in this study indicated that combisunate$^{@}$ treatment during the infection almost completely abolished the parasites (percentage suppression of 99.45%). Physical signs of illness (diarrhea, lethargy, piloerection, reduced locomotor activity etc.) normally seen in malaria-infected rats were absent in combisunate-treated malarial rats and they appeared healthy after five days' post treatment. Results with combisunate indicate that the malarial model used in this study is sensitive to antimalarial agent (artemether and lumefantrine) and therefore justify its use in screening of antimalarial properties. Among the ethanolic extracts of Orange (Citrus sinensis) peels, Grape (Citrus paradisi) and Guave (Psidium guajava) leaves tested against malaria infection in this study, Guave (Psidium guajava) leaves exhibited the most potent antimalarial activity. Inhibition on parasitaemia reached almost **99.12**% even at the moderate dose of 800mg/kg. However, at the highest dose 98.84% (1000mg/kg) its potency tends to decline. Grape (Citrus paradisi) peel extracts also exhibited considerably significant antimalarial activity in this study but with inhibitory potency on parasitaemia (62.70% at 100mg/kg and 95.60% at 1000mg/kg) less than that observed with Guave (Psidium guajava) leaves. A previous study revealed that citric acid and flavonoid content of the grape were thought to be responsible for its antimalarial property [25],[28]. Orange is one of the most popular fruits in Nigeria but very few consume the peel, which is arguably the healthiest part of the whole fruit. Orange peels are rich in flavonoids, like hesperidin and polymethoxyflavones (PMFs), and other phytochemicals, which contribute many of their health benefits [29]. Flavonoids found in Citrus sinensis peel anantioxidant compounds are known for their role in helping to prevent chronic diseases like heart disease and cancer. Although, orange peel has the least antiplasmodial properties in this study, it also contains considerable amounts of calcium, copper, magnesium, vitamin A, folate and other B vitamins and dietary fiber [29]. They have an intense orange and bitter flavor, but the latter is often a clue that a food is healthy; the bitter taste is the result of the many flavonoids that orange peels contain. Furthermore, since oxidative stress is implicated in variety of degenerative conditions such as, malaria, cardiovascular diseases, atherosclerosis, diabetes, liver diseases, kidney diseases, cancer, cataract, Parkinson's disease, Alzheimer's disease, central nervous system disorders etc., consumption of orange peel have been reported to have health benefits via maintenance of blood sugar level [28]. From the acute toxicity carried out in this study via oral route administration of doses of the ethanolic extracts of Orange (Citrus sinensis) peels, Grape (Citrus paradisi) and Guava (Psidium guajava) ranging from 1000 up to 5000mg/kg showed that there are no toxic effects produced by a single exposure of these extracts as no sign of toxicity and death was recorded in the first four hours. Also, no toxic effects produced by multiple exposures of these extracts as no sign of toxicity and death was recorded in the subsequently daily administration of these extracts for 7 days. Acute toxicity of these ethanolic extracts were carried out using modified Lorke's method [30] This is an indication that even up to 5000mg/kg, the ethanolic extracts of this plants are still within physiologically tolerable range. To further ascertain the toxicological effects of these extracts on tissues and organs of the body, the liver and kidney integrity test (LIT and KIT) were carried out. It can be infer that the ethanolic extracts of Orange (Citrus sinensis) peels, Grape (Citrus paradisi) and Guava (Psidium guajava) leaves have hepato-protective effect as liver enzyme markers- AST, ALT, ALP are seen reducing progressively. The ethanolic extracts of these plants also showed an ameliorating effect on the kidney as values of urea and creatinin are observed to have reduced progressively as the concentration of the extracts increases. The total bilirubin also showed trend of reduction as the treatment progresses at all levels of administration indicating their healing effects. The physiological state of the rats treated with these plants were seen returning back to normal from the altered state it went after inoculation with the parasite.

V. Conclusions

Based on these findings, it is clear to us that the oral administration of ethanolic extracts of Orange (Citrus sinensis) peels, Grape (Citrus paradisi) and Guave (Psidium guajava) leaves of range within the dose

(100–1000 mg/kg) to rats for 4 days significantly suppressed parasitemia of P. berghei in experimental rats with nontoxicity. The implication of this finding is that the ethanolic extracts of Orange (Citrus sinensis) peels, Grape (Citrus paradisi) and Guave (Psidium guajava) leaves possess suppressive antimalarial effects and may therefore serve as potential sources of safe, effective, and affordable antimalarial drugs. They displayed high in vivo antimalarial properties and lack of toxic effect render Orange (Citrus sinensis) peels, Grape (Citrus paradisi) and Guave (Psidium guajava) leaves a candidate for future isolation of compounds which could develop into new lead structures and candidates for drug development programs against human malaria. Orange (Citrus sinensis) peels, Grape (Citrus paradisi) and Guave (Psidium guajava) leaves are generally non-toxic and have cardiac, hepato and renal protective abilities when consume within tolerable physiological range.

VI. Contribution To Knowledge

The outcome of the study is expected to contribute to the knowledge of pharmacology. The availability of natural products like medicinal plants and plant products will greatly help to solve the healthcare problems of rural communities.

- The plant extracts showed moderate antimalarial property.
- Looking at the dose administered and its effect on the enzyme markers, it can be deduced that Orange (Citrus sinensis) peels, Grape (Citrus paradisi) and Guave (Psidium guajava) leaveshave ameliorating effects based on the dose range administered.

Recommendations

On the basis of the findings of this research, the following are recommended:

1. Further work should be done to ascertain the use of the plant extracts in preventive malarial therapy.
2. The plants should be used in combination with other plant products with known antimalarial activity to understand how this synergy can boost the antimalarial property of such plants.
3. Attempts should be made to isolate the active substance responsible for specific therapeutic action.

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