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Determination of Antiplasmodial and Antityphoid Activity of Extract from Guava, Paw-Paw and Mango Leaves

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Abstract
One of the recently used strategies for the treatment of malaria and typhoid fever within northern Nigeria is the use of extract from mixture of guava, paw-paw and mango leaves. This study was aimed at screening antiplasmodial and antityphoid activity of methanol leaves extract from these plants by calculation of percentage parasitaemia and zone of inhibition respectively. Results showed that the extract possesses antiplasmodial and antityphoid properties that is dose and dosage dependent. However, the observed activity of the extract was lower than the standard antimalarial and antityphoid drugs. The present study demonstrated that combination of *Guava, Paw-Paw and Mango leaves* possess some antiplasmodial and antityphoid. Thus, supporting the traditional use of the plant for the treatment of malaria and typhoid fever.

Keywords: *Antiplasmodial, Antityphoid, Guava, Paw-Paw, Mango and Leaves*

Introduction

It is a common practice in Nigeria and other parts of the world to use plant in the form of crude extracts, decoction, infusion or tincture to treat common infection and chronic conditions. According to WHO, over 70% of the world populations rely on medicinal plants for primary health care (WHO, 2008) and there are reports from various researchers on natural substances of plant origin which are biologically active, with desirable antimicrobial and antioxidant properties (Hamid *et al.,* 2010). Despite tremendous progress in orthodox medicines, infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health. Their impact is particularly large in developing countries due to relative unavailability of medicines and the emergence of widespread drug resistance (Zampini *et al.,* 2009).

Due to their many uses, substances produced from plants have recently attracted a lot of attention. In terms of ancient systems of medicine, modern medications, nutraceuticals, dietary supplements, folk remedies, pharmaceutical intermediates, and chemical entities for synthesized compounds, medicinal plants constitute the richest bioresource (Ncube *et al*., 2008).

Malaria is a life-threatening disease spread to humans by some types of mosquitoes. It is mostly found in tropical countries. It is preventable and curable (WHO, 2023). According to the latest World malaria report, there were 247 million cases of malaria in 2021 compared to 245 million cases in 2020. The estimated number of malaria deaths stood at 619 000 in 2021 compared to 625 000 in 2020. Four African countries accounted for just over half of all malaria deaths worldwide: Nigeria (31.3%), the Democratic Republic of the Congo (12.6%), United Republic of Tanzania (4.1%) and Niger (3.9%) (WHO, 2023).

Malaria is a major public health concern in Nigeria, with an estimated 68 million cases and 194 000 deaths due to the disease in 2021 (WHO, 2022). Nigeria has the highest burden of malaria globally, accounting for nearly 27% of the global malaria burden. The risk of transmission exists throughout

the country, all year round. However, the incidence of malaria is highest in the northern and north-eastern parts of the country (WHO, 2022).

The Anopheles mosquito, which transmits the malaria parasite from one human being to another, thrives in warm, humid climates where pools of water provide perfect breeding grounds. It proliferates in conditions where awareness is low and where health care systems are weak (Fujioka and Aikawa, 2002). Five species of *Plasmodium* can infect and be spread by human species. The species *P. knowlesi* rarely causes disease in humans. It is typically transmitted through the bite of an infected a*nopheles'* mosquito which carries the *Plasmodium* parasite.

The parasite is released into the bloodstream. Once the parasites are inside the body, they travel to the liver, where they mature. After several days, the matured parasites enter the bloodstream and begin to infect red blood cells and within 48 to 72 hours, the parasites inside the red blood cells multiply, causing the infected cells to burst open. The parasites continue to infect red blood cells, resulting in symptoms that occur in cycles that last two to three days at a time.

Typhoid is disease characterized by spreading throughout the body and harms numerous organs. It can lead to major problems and even be fatal without early treatment. *Salmonella typhi*, a bacterium related to those that cause *salmonella* food poisoning, is the culprit behind it. The typhoid organism is very contagious. A person who is infected can expel the bacteria from their body through their faeces or, less frequently, through their urine. A person can contract the germs and develop typhoid fever if they consume food or drink water that has been tainted with a little amount of infected poop or pee (Ezeonu *et al*., 2016).

Materials and Methods

Collection and Processing of Leaves

Plant materials were collected in Damaturu town, Yobe state Nigeria. It was identified and authenticated at the herbarium section of Ahmadu Bello University Zaria. Their botanical name and specimen number were assigned as follows; *Carica papaya* (ABU01882), *Mangifera indica* (ABU01944), and *Psidium guajava* (ABU03253). The leaves were mixed in equal ratio and shade dried using a drying chamber and then crushed to coarse powder. Methanol was used to extract 100g of the powder using Soxhlet extractor. The extract was concentrated in a dryness rotary evaporator and then evaporated to dryness in a water bath (Babich *et al*., 2017). Qualitative and quantitative phytochemical screening was carried out as a preliminary investigation using standard methods.

Sourcing of Malaria Parasite

Blood samples infected with *Plasmodium falciparum* were collected from Haematology Department of Yobe State University Teaching Hospital, Damaturu.

Preparation of the working concentrations

A stock solution was prepared by dissolving 20mg the extract in 2ml dimethyl sulfoxide (DMSO). Concentrations of 500g/ml, 1000g/ml, 2000g/ml and 5000g/ml were made by serial dilution of the stock solution.

Preparation of Plasmodium falciparum Culture Medium

Roswell Park Memorial Institute (RPMI) 1640 was prepared by dissolving 1.04g of RPMI 1640 salt in 100 ml of distilled water, the mixture was stabilized by autoclaving at 121°C for 15 minutes. After cooling to room temperature, 40 µg/ml of Gentamycin Sulphate was added and supplemented with 5 ml of serum obtained from apparently healthy rabbit (Devo *et al*., 1985)

In-vitro Assay of the Activity of the Extracts on Plasmodium falciparum Culture

Eighteen test tubes were divided into six groups of three test tubes each, to each of the test tube 0.1 mL of erythrocytes containing 30% parasitemia and 0.2mL of prepared culture media were added and mixed thoroughly. For group one and two, 0.1ml normal saline and standard antimalarial drug (ACT) were added respectively. While group three, four, five and six received 0.1 ml of the extract containing 500g/ml, 1000g/ml, 2000g/ml and 5000g/ml respectively. The test tubes were incubated under a bell jar system with a lighted candle that ensured the condition was atmospherically inert (about 5% O_2, 2% CO_2, and 93% nitrogen gas).

After 24 hours of incubation, a thin smear from test tube in each was made on clean glass slides and fixed in absolute ethanol and then stained with Giemsa's stain. Each smear was observed under a microscope using oil immersion to count the number of infected erythrocytes. The same procedure was repeated after 48 and 72 hours of incubation to determine the activity. The activity of each of the tested samples was calculated as the percentage elimination of the parasites after incubation of 24, 48 and 72 hours using the formula below (Muktar *et al*., 2004)

$$\% = \frac{N}{Nx} \times 100$$

Where, % = Percentage activity of the extracts
N = Total number of cleared RBC
Nx = Total number of parasitized RBC

In Vitro Anti-Typhoid Activity

A gram-negative *Salmonella typhi* was obtained from Yobe State University Teaching Hospital, Damaturu. The test organisms were inoculated into the Mueller Hinton agar with a sterilized inoculating loop, and the plates were appropriately labelled with the name of the test organism as well as the plant extract for easy identification.

A stock solution of the plant extract was prepared by dissolving 100mg of the extract in 1ml of dimethyl sulfoxide (DMSO). Concentrations of 500g/ml, 1000g/ml, 2000g/ml and 5000g/ml were made by serial dilution of the stock solution. Freshly prepared medium was seeded along standard inoculum (0.1 ml) of the *S. typhi*. Thereafter, 0.1 ml of the extract solution concentration was added unto each plate. The MHA plates were incubated for 24 hours at 37°C. Zone of inhibition of the growth were then observed from the plate and calculated using a transparent ruler in millimetres (mm) and the value noted. MIC and MBC determination was also done according to Sallau *et al*. (2022).

Results

The results of qualitative and quantitative phytochemical screening of leaves mixture is shown in Table 1. The extract was found to be rich in flavonoids, followed by steroids, then phenol compounds, followed by alkaloids and tannin and finally saponin.

Table 1: Qualitative and Quantitative Phytochemical Screening of the Leaves Extract

S/N	Phytochemical	Inference	Quantity %
1	Alkaloid	+	3.73 ± 0.02
2	Flavonoids	+	14.55 ± 0.06
3	Phenols compounds	+	4.25 ± 0.05
4	Tannin	+	2.66 ± 0.04
5	Saponin	+	1.88 ± 0.02
6	Steroids	+	5.33 ± 0.02

Results are expressed in mean ± Standard deviation, n=3

Table 2 shows the anti-plasmodial activities of the extracts against *Plasmodium falciparum* parasite. ACT used as a standard drug showed the highest parasite clearance. Within the test groups, those administered with 5000g/ml showed the highest parasite clearance, followed by 2000g/ml, then 1000g/ml and finally 500g/ml with the least clearance.

Table 2: Antiplasmodial activities of Guava, Paw-Paw and Mango Leaves extract

Groups	Conc. (Ug/ml)	Average Initial count	Average Number during incubation			Average Number After Incubation	% elimination
			24 hrs	48 hrs	72 hrs		
Control Groups	Positive	46	07	01	0	2.66	94.21
	Negative	52	54	56	59	56.33	0.00
Test Groups	5000	54	39	36	31	35.33	34.57
	2000	55	40	39	37	38.66	29.71
	1000	56	43	40	41	41.33	26.20
	500	51	47	43	40	43.33	15.03

The results of the *in vitro* anti-typhoid activity of leaves mixture extract are expressed in terms of diameter of zone of inhibition, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Salmonella typhi* is shown in Table 3. The standard antibiotic has the lowest MIC which is similar to its MBC. The plant extract shows little anti-bacterial activity with the lowest MIC of 32mm at the highest dose of 5000g/ml.

Table 3: Anti typhoid Activities of Guava, Paw-Paw and Mango Leaves extract

Groups	Conc. (μg/ml)	Zone of Inhibition (mm)	MIC of the Leaves Extract (mg/ml)	MBC of the Leaves Extract (mg/ml)
Control Groups	Positive	45	1	1
	Negative	00	00	00
Test Groups	5000	32	5	15
	2000	29	10	20
	1000	25	15	30
	500	12	20	40

Discussion

Traditional medicine practice provides more than 40 percent of the medications in developing countries, however, there is little or no knowledge on the scientific basis for the use of most of these medications. One common practice of indigenous northern Nigerian cultures is the use of composite plants mixtures of Guava, Paw-paw and mango leaves in the treatment of malaria and typhoid fever. As a preliminary investigation, this research was carried out to ascertain their claim.

In vitro anti-malarial analysis of the extracts shows a dose and dosage dependent activity with the highest parasite elimination seen in group administered with 5000g/ml for 72 hours. Ogbuehi and Ebong (2015) reported *Carica papaya*, *Magnifera indica* and *Psidium guajava* among the 11 plants most widely used in Nigeria for the treatment of malaria fever. The percentage of used of these plants were 7.3, 4.2 and 3.1 respectively. The zone of inhibition of the extract under study against *Salmonella typhi* do not compare favorably with the control drug (ciprofloxacin) up to the maximum dosage of 5000g/ml.

The observed anti-malarial activity might be related to the phytochemicals constituents present in the extract. Preliminary phytochemical screening of the extract shows the presence of flavonoids, steroids, phenolics, alkaloids, tannin and saponin. Alkaloids have vast pharmacological effects and have recently been reviewed for their therapeutic importance

for treating COVID-19. Flavonoids are also an important group of polyphenols widely distributed among the plant flora and are active ingredients of many herbal medicines. Though Saponins are extremely poisonous but were also reported to have anti-inflammatory properties. Tannins have found fortuitous uses in medicine and have been used as anti-dysentery, antidiarrheal, anti-diabetes, anti-hypertension, analgesic, anti-inflammatory, antibacterial, antibiotic, antiseptic and antioxidant (Manisha, 2021; Mathew *et al*., 2019). The exact mechanism of action of this extract is still unclear. However, Liang and Fang (2006) reported some phytochemicals with antiplasmodial activity to attack the parasite at its intra-erythrocytic asexual stage there by preventing cell division and subsequent elimination of the parasite.

Conclusion

The study concludes that the extract has both antiplasmodial and anti-typhoid potential because of presence of various phytochemical constituents which can be isolated and purified through bio guided fractionation to be used as useful drugs for the treatment of these ailments.

Conflict of Interests

The authors declare no conflict of interest.

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