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Evaluation of the Efficacy of *Carica papaya, Azadirachta indica* and *Psidium guajava* leave Extracts as a Combined Alternative Therapy for the Phyto-Therapy of Enteric Fever (Typhoid) and Malaria Fever (*Plasmodiasis*) among some Selected Communities in Northern Nigeria

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Abstract
Malaria and typhoid (enteric) fevers are the most serious and devastating diseases in Nigeria and probably in the whole world by extension. Nigeria is said to be among the most countries in the world with larger number of malaria and typhoid related cases yearly. This study evaluated the efficacy of Carica papaya, Azadirachta indica and Psidium guajava leaves extracts as a Combined Alternative Therapy for the Phyto-therapy of enteric fever (Typhoid) and Malaria Fever (Plasmodiasis) among some selected Communities in Northern Nigeria. The research therefore centered on evaluating Vis-à-vis the Folkloric Claims made on these plants, thereby unveiling the effect phytochemical constituents responsible for its medicinal value. The effects of all the leave extracts were determined by recording their zones of inhibitions against organisms under investigation.

Key Words*: Efficacy, therapy, Phyto-therapy, Phytochemical, enteric, extracts, Plasmodiasis, qualitative, bioactive, antimicrobial.*

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I. Introduction

Malaria is a mosquito-borne infectious disease of humans and other animals. It is caused by parasitic protozoans of the genus *Plasmodium*. Commonly, malarial fever is caused by *Plasmodium* parasite and is transmitted from an infected person to healthy person via a bite of an infected female *Anopheles* mosquito, which introduces the organisms from its saliva into a person's blood circulatory system.

Plasmodium is a parasite that infects about 154 to 289 million people per year, resulting in approximately 660,000 deaths worldwide.

Typhoid fever is caused by *Salmonella typhi* which is transmitted through the ingestion of contaminated food or water.

These diseases are widely spread in tropical and subtropical regions in a broad band around the equator, including much of Sub-Saharan Africa, Asia, and the America.

Geographical distribution

Malaria and typhoid fever generally occurs in areas where environmental conditions allow parasite multiplication in the vector in large number.

Malaria is an endemic disease and several cases are been reported around the globe yearly. In 2010 about 106 were affected by malaria.

Typhoid and malaria fever are highly prevalent in Africa, India and South America.

For the past decades, there has been an increasing interest in the investigation of the pharmacological effect of different extract obtained from plant as a source of drugs.

Plants are considered to be a treasure house of potential drugs and in recent years there has been an increasing awareness about the importance of medicinal plants.

The use of plants and plant products has been documented since centuries. Volumes of literature have been written describing the use of various shrubs, herbs and plants. Despite the presence of modern pharmaceutical drugs search still continues for medicinal plants of high therapeutic value to resolve both old and new problems.

Medicinal plants which are also known as medicinal herbs have been in use in the treatment of so many ailments since time immemorial. These plants are known to be rich in Nutraceuticals. Phyto-therapy and nutritional therapy are two newly emerging concepts in health sectors and has been receiving attentions from so many specialists towards its exploit in the field of modern medicine. Nutraceuticals are natural bioactive substances found in foods, dietary supplements and herbal products and exhibit health promoting beneficial effects.

Knowledge of traditional medical practice is a crucial and integral part of any culture and the interpretation of health by indigenous populations in many parts of the world (Anyinam C. 1995).For example, Indian Ayurveda and Chinese traditional medicine are among the most successful and acceptable medical practice still in existence worldwide. These systems try to promote health and improve the quality of life, with therapies based on the use of indigenous drugs of natural origin (Patwardhan B, et al. 2005). Given that plants have been widely used as herbal medicines, several approaches are now being carried out to discover new bioactive compounds.

STATEMENT OF THE RESEARCH PROBLEM

Carica papaya, Azadirachta indica and *Psidium guajava* are plants claimed to have a lot of values in field of medicines and pest control. With these claims, it has not been clearly justified. Therefore, this research is centered on investigating and analyzing the claims made on these plants thereby unveiling the chemical constituents responsible for its medicinal value. It has been observed that *Carica papaya, Azadirachta indica* and *Psidium guajava* are among the medically important plants. It checks disease problems such as anemia, blood pressure, piles and malaria. Herbalists use these plants infusion to treat these ailments locally.

AIM AND OBJECTIVES OF THE STUDY

- There are no available literatures on the use of leave extract of *Carica papaya, Azadirachta indica and Psidium guajava as* combined therapy for the treatment of *Enteric fever and malarial* fever in Nigeria. Hence, the aim and objectives of this work are;
- To study and evaluate the antimicrobial activity of the aforementioned plant leaves extracts against the selected pathogens.
- To investigate the bioactive component presence in crude extracts of *Carica papaya, Azadirachta indica* and *Psidium guajava* preparations

SCOPE AND LIMITATIONS OF THE STUDY

- This work is basically designed to study and evaluate the *in vitro* antimicrobial actions of *Azadirachta indica, Carica papaya* and *Psidium guajava* against pathogenic microbes causing typhoid and malarial fever in Northern Nigeria.
- Therefore, this work did not cover the etiology as well as the epidemiology of the diseases under investigation.
- The research was conducted in the laboratory of SLT department, Mai Idris Alooma Polytechnic, Geidam, Yobe State of Nigeria.

SIGNIFICANCE OF THE STUDY

Though, there were claims that these plants preparations plays role in reducing high blood pressure as well as blood sugar, the significance of this research was to justify the claims made about these plants for its medicinal value in curing malaria and typhoid fever.

The plant preparation also reduces abdominal discomfort as claim by some quotas.

MATERIALS AND METHODOLOGY

Materials and Equipment

Materials: Leaves of *Psidium guajava, Carica papaya, Azadirachta indica,* Peptone, glucose, beef extract, sodium hypochlorite, agar, nutrient agar, absolute methanol, spirit, McFarland reagent, Wagner's reagent, iodine solution, Ferric chloride, Potassium iodide, chloroform, Lactose broth, Potato dextrose agar (PDA), phosphate buffer saline (PBS), liquid nitrogen, cultures of *Plasmodium falcifarum,* and *Salmonella enterica serotype Thypi* bacteria.

Equipment: Lamina flow hood, incubator, autoclave, autoclave bags, brown papers, absorbent papers, roll of cotton wool, thread, pestle and mortar, L-shape glass spreader, inoculation loop, spirit burner, puncture, pipettes of varying measure, pipette tips, petri plates, conical flasks of different volume, surgical hand gloves, face masks, sieve, 10 specimen bottles (100ml), test tubes, eppendorf, spectrophotometer, filter papers, pH meter.

Preparation of leaf powders

Fresh leaves of *Carica papaya, Psidium guajava* and *Azadirachta indica* were collected and air dried under shed. The dried leaves were crushed into a fine powder. The powders were collected in air tight containers and stored in a cool dry place away from sunlight.

Preparation of methanol extract of guava leaf powder

The methanol extracts were prepared by mixing 20 grams of each plant leaf powder into 100 ml of methanol in separate specimen bottles and kept for 3 days in a cool, dark place along with occasional stirring. After 3 days, the extracts were filtered into sterilized specimen bottles.

Preparation of nutrient agar (500ml)

Appropriate amount of nutrient agar were prepared by dissolving 2.5g of peptone, 5g of glucose, 0.75g of beef extract and 10g of agar in 500ml distilled water in a conical flask. The mixture was sterilized in an autoclave.

An isolate of each test organisms were grown on nutrient agar plates by spread plate method and incubated at 37^oC for 24 hours.

Determination of antimicrobial activity of plant extracts

The antimicrobial activity of the methanol extracts of *Psidium guajava*, *C. papaya* and *A. indica* leaves were tested against the different microbial strains.

An inoculum was prepared by transferring few colonies from the cultured test organisms into different sterilized test tubes containing normal saline.

Examination of microbial growth in the presence and absence of each leaf extracts

For each microbial strain, four petri plates were prepared, of which one was served as control, while three were used as test plates.

A well was carefully created on the inoculated media plates using a sterilized puncture. Different amount of leave extracts 0.5ml, 1ml and 2ml respectively were placed in the created well and left to stand for 1 hour at room temperature for diffusion to take place. The petri plates were then incubated at 37^oC for 24 hours. Zone of inhibition were observed after 24 hours.

Estimation of zone of inhibition using Well Diffusion method

After 24 hours of incubation, the test plates were examined for zone of inhibition and measured using a ruler and recorded.

Examination of microbial growth in the presence and absence of mixed leaf extracts

Equal quantity (2ml) of each leave extracts were mixed to form a combined mixture.

Each test organism was inoculated on petri plates (in triplicate) using spread plate method and well created using a sterile puncture. The mixed extracts of the leaves were placed in the well and left to stand for one hour for diffusion to take place at room temperature and then incubated at 37^oC overnight.

After 24 hours of incubation, zones of inhibition were examined and measured using a ruler and recorded.

Antimicrobial Activity Assay Determination

Using ciprofloxacin disc, antimicrobial assay of each leave extracts as well as the combined mixture of the leave extracts were determined using well diffusion method.

Zone of inhibition observed, measured and recorded as well.

Qualitative analysis of the phytochemical content of each plant leaf extracts

Chemical tests for the screening and identification of bioactive chemical constituents of each plant leaf extract was determined using the standard procedure as described.

The following bioactive chemicals were tested for each leave extracts: alkaloids, anthraquinones, phenols, flavonoids, Saponins, tannins and triterpene.

II. Results And Discussion

Table 1: Positivity test of individual plant leave extract against each test organism

Pathogens	Conc.(ml)	*C. Papaya*	*P. Guajava*	*A. indica*
Plasmodium falcifarum	0.5	-	+	+
	1.0	+	+	+
	1.5	+	+	+
	2.0	+	+	+
Salmonella typhi enterica	0.5	+	+	-
	1.0	+	+	+
	1.5	+	+	+
	2.0	+	+	+

Keys: + indicate present of antimicrobial action, - indicate absent of antimicrobial action

Table 2: Positivity test of combined plant leave extracts against each test organism

Pathogens	Conc.(ml)	PG	PN	GN	PGN
Plasmodium falcifarum	0.5	+	+	+	+
	1.0	+	+	+	+
	1.5	+	+	+	+
	2.0	+	+	+	+
Salmonella typhi enterica	0.5	+	+	+	+
	1.0	+	+	+	+
	1.5	+	+	+	+
	2.0	+	+	+	+

Keys: + indicate present of antimicrobial action; - indicate absent of antimicrobial action; PG:*Papaya* and *Guava;* PN: *Papaya* and Neem; GN: *Guava* and Neem; PGN: *Papaya*, *Guava* and Neem

Table 1: Zones of inhibition of individual plant leaf extracts against *Plasmodium falcifarum*

Pathogens	**Conc. (ml)**	***C. papaya***	***P. guajava***	***A. indica***
P.falcifarum	0.50	0.00	8.33	9.21
	1.00	7.51	9.55	11.32
	1.50	9.00	9.87	20.02
	2.00	9.25	11.35	31.56

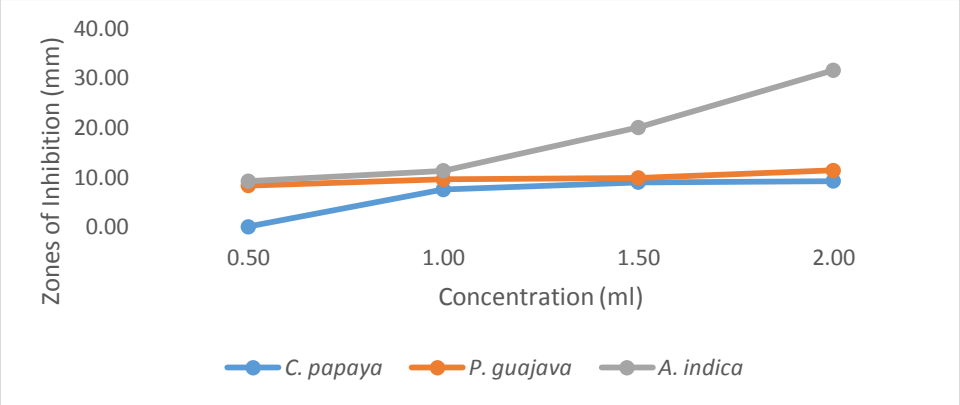

Figure 1: Line plot of zones of inhibition of individual plant leaf extracts against *Plasmodium falcifarum*

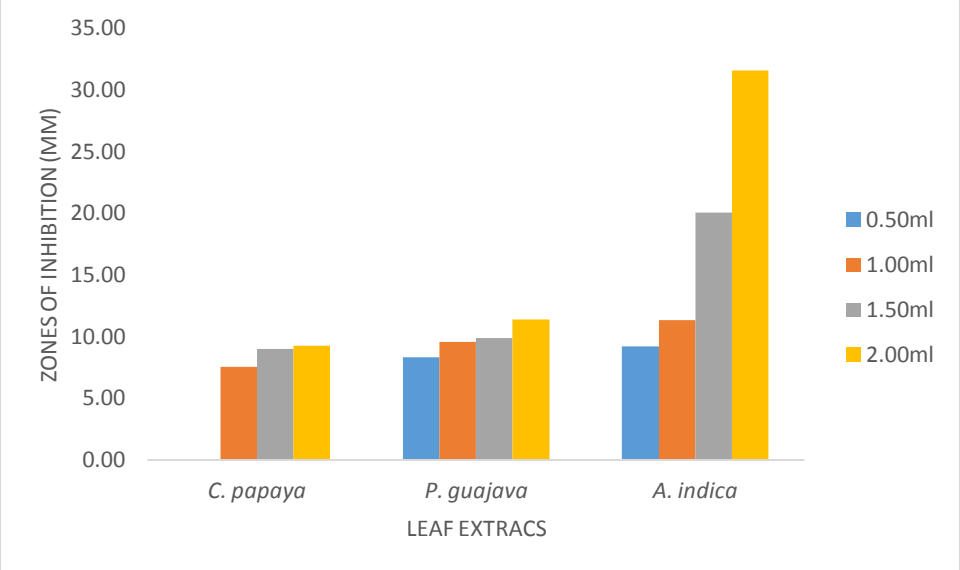

Figure 2: Graph Bar Chart of zones of inhibition of individual plant leaf extracts against *Plasmodium falcifarum*

Table 2: Zones of inhibition (in mm) of individual plant leaf extracts against *Salmonella typhi*

Pathogens	Conc. (ml)	*C. papaya*	*P. guajava*	*A. indica*
S. typhi	0.50	9.22	10.10	0.00
	1.00	9.89	15.26	8.00
	1.50	11.15	19.04	8.85
	2.00	26.00	25.62	9.15

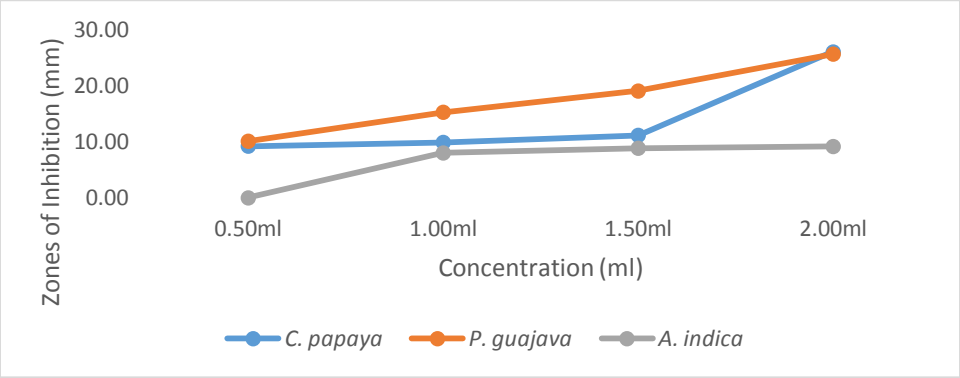

Figure 3: Line plot of zones of inhibition of individual plant leaf extracts against *Salmonella typhi*

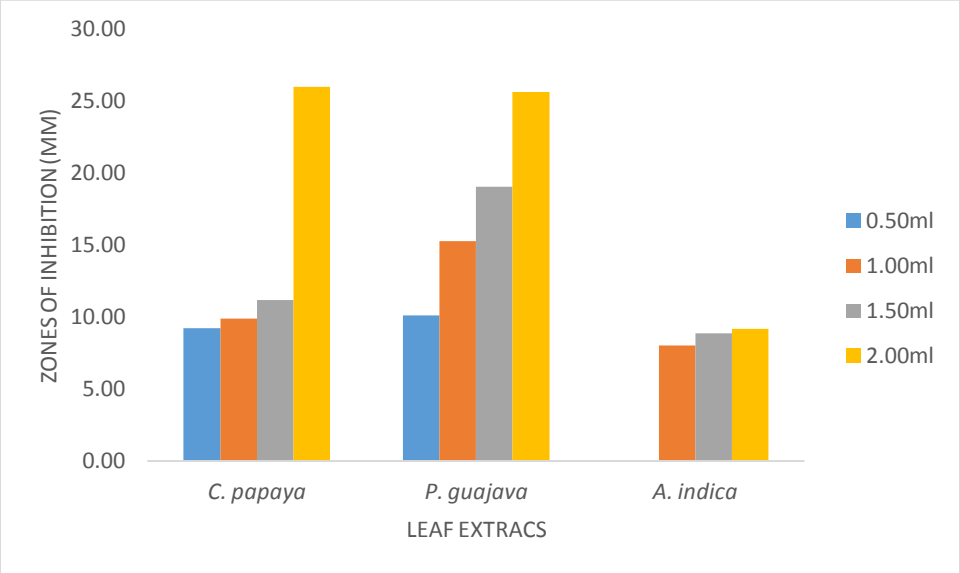

Figure 4: Graph chart of zones of inhibition of individual plant leaf extracts against *Salmonella typhi*

Table 3: Zones of inhibition (in mm) of combined plant leave extracts against *Plasmodium falcifarum*

Pathogens	Conc. (ml)	*PG*	*PN*	*GN*	*PGN*
P.falcifarum	0.50	9.55	10.54	10.00	12.19
	1.00	22.01	15.26	12.20	13.65
	1.50	26.65	16.78	13.00	20.42
	2.00	31.25	20.74	21.01	22.12

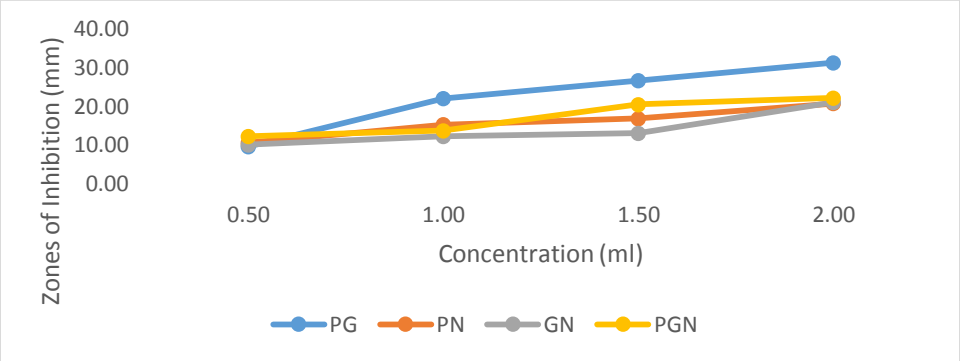

Figure 5: Line plot of zones of inhibition of combined plant leave extracts against *Plasmodium falcifarum*

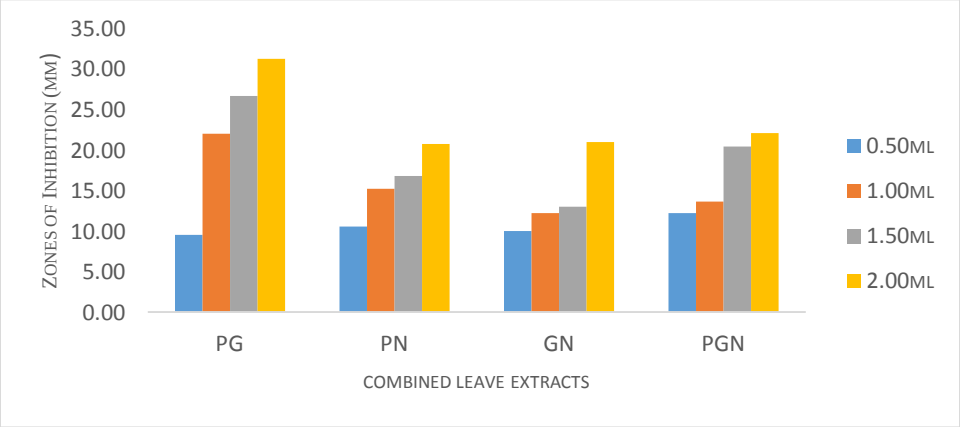

Figure 6: Graph of zones of inhibition of combined leave extracts against *Plasmodium falcifarum* concentration

Table 4: Zones of inhibition (in mm) of combined plant leave extracts against *Salmonella typhi*

Pathogens	**Conc. (ml)**	***PG***	***PN***	***GN***	***PGN***
S. typhi	0.50	10.11	9.58	11.23	15.00
	1.00	23.20	11.22	11.82	19.25
	1.50	26.32	22.19	19.00	23.34
	2.00	38.00	27.56	22.40	28.61

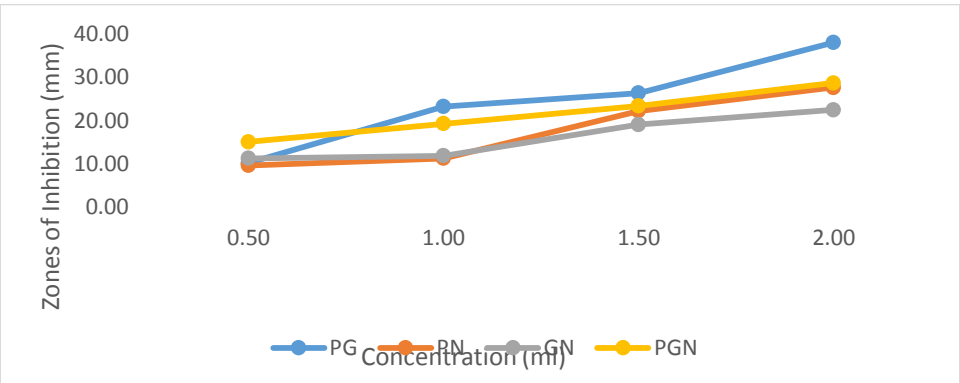

Figure 7: Line plot of zones of inhibition of combined plant leave extracts against *Salmonella typhi*

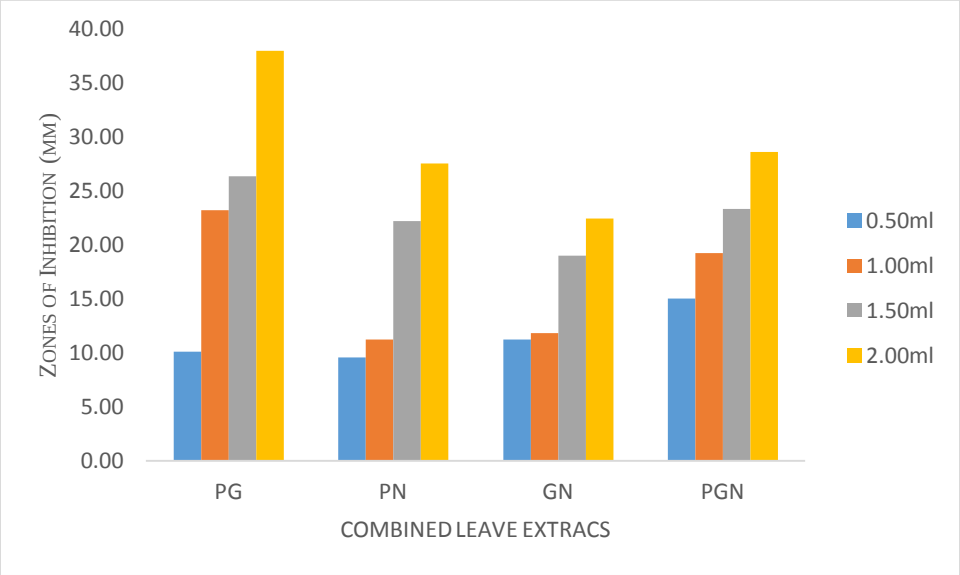

Figure 8: Graph of zones of inhibition of combined plant leave extracts against *Salmonella typhi*

Table 5: Phytochemical constituents of plants under investigation

Phytochemicals	Conc.(ml)	*C. Papaya*	*P. Guajava*	*A. indica*
Alkaloid	2.0	+	+	+
Flavonoids	2.0	+	+	+
Tannins	2.0	-	+	+
Terpenoids	2.0	-	-	-
Saponins	2.0	+	+	+
Phenols	2.0	-	+	-
Triterpene	2.0	-	+	-
Anthraquinones	2.0	-	+	-
Azadirachtin	2.0	-	-	+

Keys: + indicate present of chemical constituents, - indicate absent of chemical constituents

Antimicrobial activities of *Carica papaya, Azadirachta indica and Psidium guajava* leave extracts were determined against two pathogens; *Plasmodium falcifarum and Salmonella typhi*.

Extracts of *A. indica* shows strong antimicrobial effect against *P. falcifarum* with zone of inhibition of 31.56mm followed *P. guajava* with an inhibition zone of 11.35mm. *Carica papaya shows no effect at all* (table 1).

Carica papaya leaves extracts shows strong effect against *Salmonella typhi with inhibition zone of 26.00mm followed closely by Psidium guajava* with 25.62mm inhibition zone, while *Azadirachta indica* shows negative as shown in table 2.

Combination of extracts from *C. papaya* and *P. guajava* shows strong antimicrobial effect against *P. falcifarum* with 31.25mm inhibition zone followed combined effect of the three plants leave extracts (*A. indica, C. papaya* and *P. guajava)* with inhibition zone of 22.12mm when 2 ml was used (table 3). However, the least zone of inhibition (9.55mm) was shown by 0.5mm drop of combined *C. papaya* and *P. guajava* leave extract. Similarly 2ml of combined *P. guajava* and *C. papaya shows strong antimicrobial action against* Salmonella typhi with inhibition zone of 38mm followed by 2ml of combined extract of *P. guajava, C. papaya* and *A. indica* having an inhibition zone of 28.61mm. The least zone of inhibition was shown by 0.5ml of combined *C. papaya* and *P. guajava* leave extracts.

III. Conclusion

This study shows that leave extracts of *Carica papaya, Azadirachta indica* and *Psidium guajava* demonstrated strong antimicrobial activities against the selected pathogens with potentiality of curing both malarial fever and typhoid fever.

The antimicrobial actions of these plants may be attributed to their phytochemical constituents. Medicinal plants derive their therapeutic importance from these bioactive compounds. However, the mechanism of actions of these phytochemical compounds was not investigated in this work. Further research is required to investigate this. The commonest bioactive compounds of the plants under investigation were alkaloids, flavonoids and Saponins (table 5).

Combine effect of both plant extracts was less strong than combined effect of *C. papaya* and *P. guajava* against both *S. typhi* and *P. falcifarum* as shown in tables 3 and 4. Reason for this is not known to the researcher.

Data Analysis

Statistical package for social science (SPSS) was used in analyzing data in this research work. All data were subjected to analysis of variance and differences between means were determined using the least significance difference (LSD).

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