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INVESTIGATION OF BIOACTIVE COMPOUNDS AND ANTIMICROBIAL SENSITIVITY OF PAWPAW (CARICA PAPAYA) LEAVE EXTRACTS AGAINST MORBIFIC MICRO-ORGANISMS

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ABSTRACT
Pawpaw (*Carica papaya)* is a notable plant due to its medicinal benefits used globally to treat diseases which include dengue, malaria, inflammation, and skin infections. This study was aimed at investigating the bioactive compounds and antimicrobial sensitivity of Pawpaw (*Carica papaya)* leave extracts against morbific micro-organisms. The bioactive compounds of *C. papaya* leaves were analyzed using Chemical Standard techniques as reported in literatures and antimicrobial assay was done using agar well diffusion techniques. Qualitative phytochemical screening results of pawpaw leaves shows the presence of glycosides, tannins, flavonoids, steroids, saponins and terpenoids. The antimicrobial sensitivity result reveals higher zones of inhibition of methanolic extract against *Staphylococcus sp* (11.4±0.3 mm), *Vibrio sp* (10.3±0.2 mm), *E. coli* (9.7±0.3 mm), *Shigella sp* (9.1±0.3 mm), Yeast (9.1±0.3 mm) and *Penicillium sp* (8.3±0.4 mm). While aqueous extract shows higher zone of inhibition against Mould (8.0±0.5 mm). This study revealed pawpaw leaves contains secondary metabolites (bioactive compounds) while the antimicrobial sensitivity against the microorganisms explains the possibility of using the leaves as antibiotic substances (antimicrobial agents). Therefore, pawpaw leaves could be a significant source of medicine for the treatment of various fungal and bacterial infections.

INTRODUCTION

Plant parts or materials trading have gone high due to the demand for traditional herbal products globally. Therefore, the

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historical evidence of the early man's adventure in using various herbs in their crude/raw and cooked forms for medicine and food to keep fit is logical. Since then the use of traditional herbs has been known, practiced and accepted worldwide and also termed as the first art of treatment available to mankind [1].

The fundamental of traditional medicine, is their biological activity, which make use of the pharmacological effectiveness of original compounds found in the herbal preparations for various human and animal disease treatments [2]. Traditional medicinal plants are readily available, cheap, ecofriendly and renewable sources of pharmacologically-active substances and are known to produce secondary metabolites that are toxic to microbial pollutions naturally [3]. The value of these plants lies in these bioactive chemical substances which produces definite physiological actions on the human system [4]. These very significant bioactive compounds in various plants are flavonoids, steroids, tannins and alkaloids [5].

Microorganisms are known to cause several infections and the uprising of multidrug resistant microbes has necessitated the use of plants as a remedy to several diseases caused by *Klebsiella sp*. *Staphylococcus sp, Escherichia coli,* amongst others causing the increase of morbidity and mortality rates. The operative components inherent in these plants are expected to be inimical to the proliferation of some pathogens [6].

Carica papaya also known as pawpaw is one of such plants. It belongs to the family *Caricaceae* that has been used to treat various ailments. Taking into consideration the potency of plants as vital sources or alternative for antimicrobial drugs, an investigation was carried out to analyze the traditional vegetation (*Carica papaya)* for antimicrobial activity. The entire pawpaw plant ranging from the fruits, leaves, seeds and juice is used as a medicine traditionally.

The significant medicinal properties of pawpaw are antimicrobial, antitumor, wound – healing, etc. This study was therefore aimed at investigating the bioactive compounds and antimicrobial sensitivity of Pawpaw (*Carica papaya)* leave extracts against morbific micro-organisms.

MATERIALS AND METHODS

Collection and Preparation of Plant Samples

Fresh pawpaw leaves were plucked at the garden in Yenagoa and the leaves were identified and authenticated at the School of Agricultural Technology, Federal Polytechnic, Ekowe, Bayelsa State, Nigeria. It was washed in tap water and allowed the water to drain. The leaves were then sundried for 3 days, pulverized and stored in airtight container prior to laboratory investigation.

Phytochemical Analysis of pawpaw Leaves

Phytochemical analysis for the screening and identification of secondary metabolites such as glycosides, flavonoids, terpenoids, alkaloids, steroids, saponins and tannins of the leaves were determined qualitatively and quantitatively using standard procedures as described by Ogidi *et al.* [7,8].

Qualitative phytochemical screening

Test for saponins

The pawpaw leaves (0.5 g) and 5 ml of distilled water was added in a test tube and the solution shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Test for tannins

Weighed 0.5 g of the leaves were boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1 % ferric chloride were added and the solution observed for brownish green or a blue-black colouration.

Test for glycosides (Keller-Killiani test)

The leaves (0.5 g) were dissolved in 5 ml of water and 2 ml of glacial acetic acid solution containing one drop of ferric chloride solution was added. This was underlayed with 1ml of concentrated H_2SO_4. A brown ring at the interface indicated the presence of deoxysugar characteristics of cardenolides. A violet ring appear below the brown ring while in the acetic acid layer a greenish ring was form just above the brown ring and gradually spread throughout this layer.

Test for flavonoids

Dilute ammonia (5 ml) was added to 0.5 g of the leaves. Then, concentrated sulphuric acid (1 ml) was added. A yellow colouration indicated the presence of flavonoids.

Test for alkaloids

Weighed quantity (0.5 g) of the pawpaw leaves were dissolved in dilute HCl and filtered. Filtrates were treated with Mayer`s reagent (potassium mercuric iodide). Formation of a yellow coloured precipitate indicated the presence of alkaloids.

Test for steroids

Acetic anhydride (2 ml) was added to 0.5 g of the sample and filtered. Sulphuric acid (2 ml) was added to the filtrate and observed for color change from violet to blue or green, which indicates the presence of steroid.

Test for terpenoids

The pawpaw leaf samples of 0.5 g were dissolved in 1 ml of chloroform and 1 ml of acetic anhydride added, with 2 ml of concentrated H_2SO_4. Formation of reddish violet colour was observed.

Quantitative phytochemical analysis

Total tannins content determination

The tannins were determined by slightly modified Folin and Ciocalteu method. Briefly, 0.5 g of the leaf samples were added with 3.75 ml of distilled water and 0.25 ml of Folin Phenol reagent, 0.5 ml of 35 % sodium carbonate solution. The absorbance was measured at 725 nm. Tannic acid dilutions (0 - 0.5 mg/ml) were used as standard solutions. The results of tannins were expressed in terms of tannic acid in mg/ml of the biomass.

Total alkaloid content determination

Measured 40 ml of 10 % acetic acid in ethanol was added to 1 g of powdered leaf samples, covered and allowed to stand for 4 hours. The filtrate was then concentrated on a water bath to get 1/4th of its original volume. Concentrated ammonium hydroxide was added drop wise to the sample until the precipitation was complete. The whole solution was allowed to settle and collected precipitate was washed with dilute ammonium hydroxide and then filtered. The residue was dried and weighed.

Total flavonoid content determination

The total flavonoids content of samples was determined by following the Aluminum chloride method. Pawpaw leaf concentrates were mixed with distilled H_2O and $NaNO_2$ solution. After 6 minutes, $AlCl_3$ solution was added and allowed to stand for 6 minutes. NaOH solution was added to the mixture. Immediately distilled H_2O was added to bring to the final volume and then the mixture was extensively mixed and allowed to stand for another 15 minutes. Optical density of the mixture was recorded at 510 nm. Rutin was used as a standard compound for the evaluation of total flavonoid. The total flavonoids were calculated using the standard curve, and expressed as rutin equivalent in mg/g of extracts.

Total saponin content determination

Weighed 0.5 g of the leaf samples were dissolved in 80 % methanol, 2 ml of Vanillin in ethanol was added, mixed well and 2 ml of 72 % sulphuric acid solution was added, mixed well and heated on a water bath at 100°C for 10 minutes, absorbance was measured at 544 nm against reagent blank. Diosgenin was used as a standard material and compared the assay with Diosgenin equivalents.

Total Terpenoid Content Determination

Pawpaw leaves (1 g) was marcarated with 50 ml of ethanol and filtered. To the filtrate (2.5 ml), 2.5 ml of 5 % aqueous phosphomolybdic acid solution was added and 2.5 ml of concentrated H_2SO_4 was gradually added and mixed. The mixture was left to stand for 30 min and then made up to 12.5 ml with ethanol. The absorbance was taken at 700 nm.

Total Steroid Content Determination

The steroid content of the leaf samples were determined using the method described by Ogidi *et al.* [8]. A portion of 2 ml was taken from a solution of 2.5 g of powdered plant material prepared in 50 ml of distilled water after vigorous shaking for 1 hour. The extract solution was washed with 3 ml of 0.1M NaOH (pH 9) and later mixed with 2 ml of chloroform and 3 ml of ice cold acetic anhydride followed by the cautious addition of two drops of concentrated H_2SO_4. The absorbance of both sample and blank were measured using a spectrophotometer at 420 nm.

Plant Sample Extraction

Methanol and aqueous were used as extraction solvents in this study. 10 g of pawpaw leave and methanol (25 ml) was added and mixed by shaking in a beaker. It was centrifuged (3000 rmp) at10 minutes and supernatant was collected. The supernatant was allowed to evaporate with a gentle stream of nitrogen and reconstituted in dimethyl sulphoxide (10 ml) and was mixed. The same procedure was repeated for aqueous.

Sources of test organisms

The test organisms were common pathogenic organisms in our environment. They were gotten from stock cultures obtained at the Microbiology Laboratory of the Department of Microbiology, Niger Delta University Wilberforce Island, Bayelsa State, Nigeria. They were sub cultured in nutrient broth and incubated 37°C for 24hr.

Antimicrobial assay of the plant extracts

The agar well method of the agar diffusion technique was used to assay the antimicrobial (antibacterial and antifungal) activity of the extracts. Preparation of filter paper disc impregnated with *Carioca papaya* extracts. Filter paper disc of 6 mm diameter

were cut using a punching machine in Whatmann No. 1 filter paper. The discs were sterilized by dry heat sterilization. 20µl of each leaf extracts were added to the separate discs. The dried extract impregnated discs were used for testing antimicrobial activity against clinical isolates by disc diffusion method as described by Saravanasingh *et al*. [9].

Disposal Procedure

The agar plates where the filter paper disc was impregnated was decontaminated by sterilization using autoclave before disposal.

RESULTS

Bioactive Compounds of *Carica papaya* leaves

Phytochemical properties present in *Carica papaya* leaves were: tannin (1.9± 0.02%), terpenoid (0.8±0.01%), (3.4 ± 0.01%) for saponin, (1.5± 0.02%) for flavonoid, (2.5±0.01%) for alkaloid, (1.3±0.03%) for steroids and glycoside for both qualitatively and quantitatively (Tables 1 and 2).

Antimicrobial sensitivity

Antibacterial activity of *Staphylococcus sp.* shows 11.4±0.3 mm in diameter of inhibition zone for Methanol extract and 7.0±1.0 mm in diameter of inhibition zone for aqueous extract. While *E. coli* shows 9.7±0.3mm and 8.5±1.3mm in diameters of inhibition zones for methanol and aqueous extracts respectively. *Shigella sp.* shows 9.1±0.3mm and 8.3±0.6mm in diameter of inhibition zones for methanol and aqueous extracts respectively. *Vibrio sp.* shows 10.3±0.2mm and 9.5±1.3mm in diameter of inhibition zones for methanol and aqueous extracts respectively. While antifungal activity of *Penicillium sp*, Yeast and Moulds shows 8.3±0.4, 9.1± 0.3, and 7.4±0.4 mm in diameter of inhibition zones for methanol extract and aqueous extract 8.0±1.0, 7.2±0.3 and 8.0±0.5 mm respectively as shown in Tables 4 and 6.

Table 1: Qualitative phytochemical result of pawpaw leaf

Plant Sample	**Phytochemical properties**						
	Alkaloid	**Tannin**	**Flavonoid**	**Saponin**	**Glycosides**	**Terpenoid**	**Steroid**
Pawpaw (*Carica papaya*) leaf	+	+	++	+	++	+	++

Note; + (presence), ++ (abundance)

Table 2: Quantitative phytochemical results of pawpaw leaf

Plant Sample	**Phytochemicals properties (%)**					
	Alkaloid	**Tannin**	**Flavonoid**	**Saponin**	**Terpenoid**	**Steroid**
Pawpaw (*Carica papaya*) leaf	2.5±0.01	1.9±0.02	1.5±0.02	3.4±0.01	0.8±0.01	1.3±0.03

Table 3: Biochemical characteristics of some bacteria

Bacterial Isolates		*Shigella sp*	*E. coli*	*Staphylococcus sp.*	*Vibrio sp*
Cell morphology (cell shape)		Rod	Rod	Coccus	Comma
Colony (cell shape)		Round	Spindle	Circular	Curved
Gram reaction		-	-	+	-
Biochemical test	Nitrate reductive	+	+	+	+
	Oxidase	-	-	-	+
	Catalase	+	+	+	-
	Methyl red	+	+	+	-
	Voges Proskauer	+	-	+	+
	Indole	-	+	-	+
	Citrate	-	-	+	+
	Hydrogen sulfide reduction	-	-	-	-

	Ureas activity	-	-	+	-

Table 4: Antibacterial activity of pawpaw leaf extracts (Diameter of inhibition zone in mm) (Means ± SD)

Bacterial Isolates	**Solvents for extraction**	
	Methanol	**Aqueous**
Shigella sp	9.1±0.3	8.3±0.6
E. coli	9.7±0.3	8.5±1.3
Staphylococcus sp	11.4±0.3	7.0±1.0
Vibrio sp	10.3±0.2	9.5±1.3

Table 5: Identification of fungi with cultural morphology

Fungal Isolates	**Microscopic observation (Medium)**	**Microscopic observation (gram reaction)**
Yeast *Sp*	White colour, creamy growth on the media surface	Pink colour large cells obtained by gram are staining, oval, budding cells obtained by LPCB staining.
Penicillium	Greyish-green colour colonies, smooth colonies.	Brush like conidiophores and branched mycelium spores arranged on conidiophores
Mould	Black huge colonial growth	Heavy mycelial growth arranged in filamentous form.

Table 6: Antifungal activity of Pawpaw leaf extracts (Diameter of inhibition zone in mm) (Means ± SD)

Fungal Isolates	**Solvents for extraction**	
	Methanol	**Aqueous**
Penicillium sp	8.3±0.4	8.0±1.0
Yeast	9.1±0.3	7.2±0.3
Mould	7.4±0.4	8.0±0.5

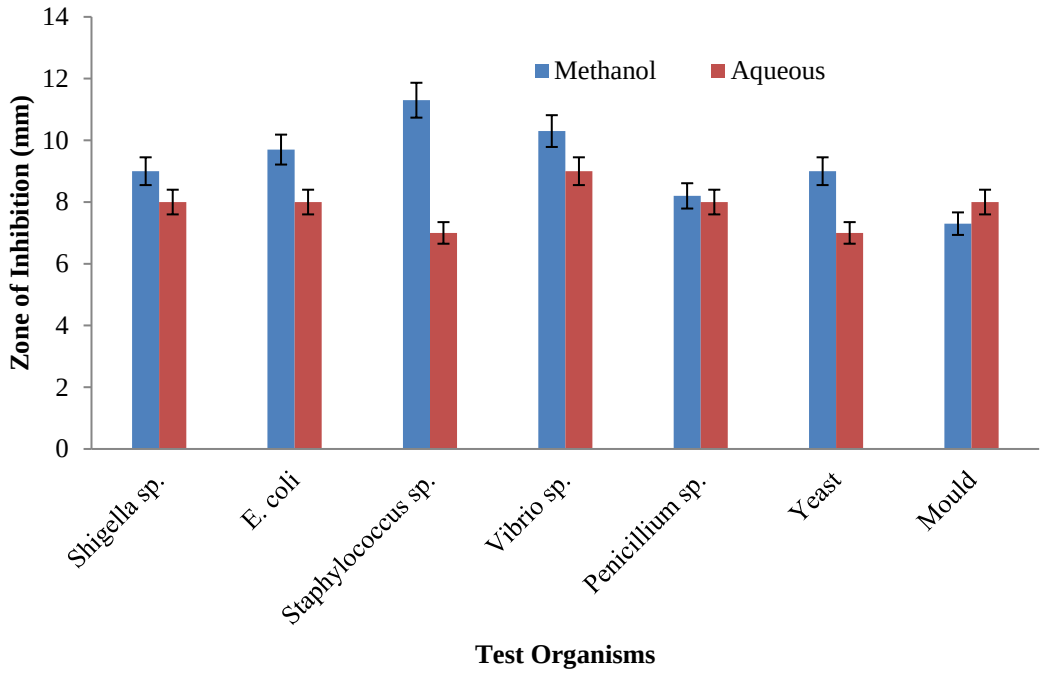

Fig. 1: Comparison of Methanol and Aqueous extracts of Test Organisms

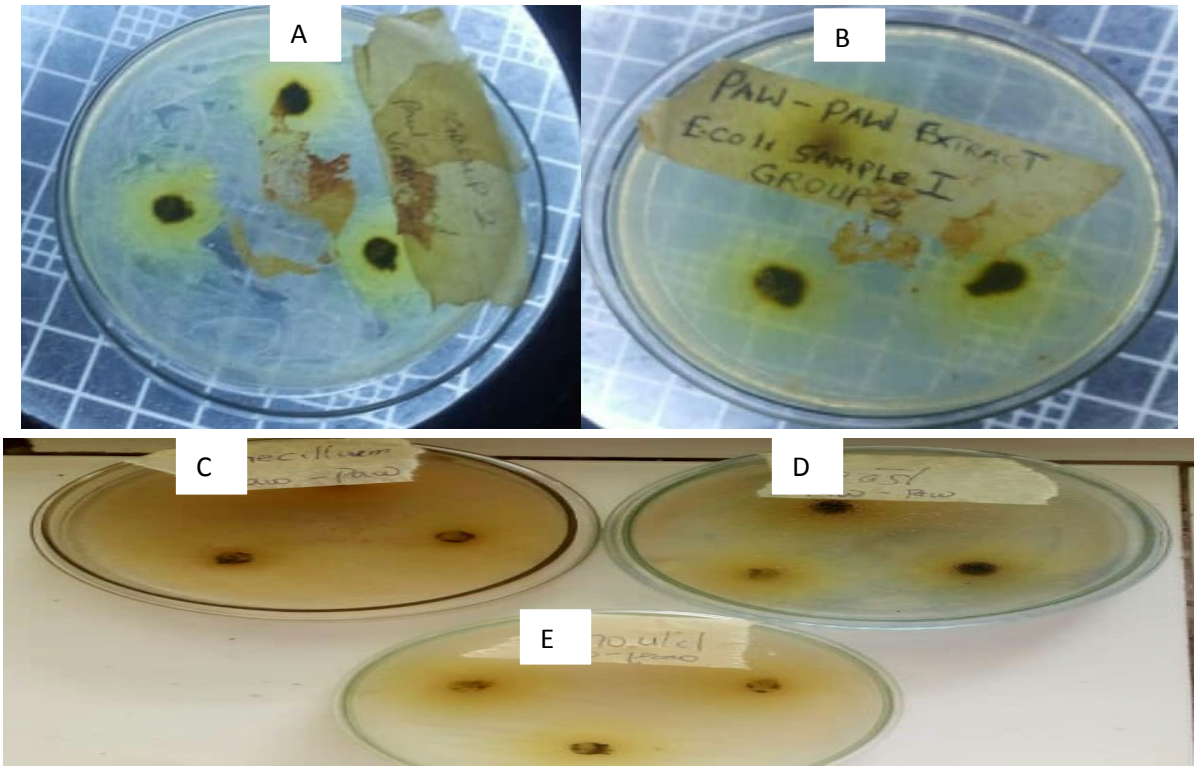

Fig. 2: Antimicrobial Activities of *Carica papaya* leaf Samples *Vibrio sp. (*B) *E. coli* (C) *Penicillium sp.* (D) Yeast (E) Mould

DISCUSSION

The global use of medicinal plants in human healthcare means that, the assessment of their chemical compounds has been evaluated due to the safety of the plants [10, 11]. Pawpaw (*Carica papaya)* is a notable plant due to its medicinal benefits used globally to treat diseases which include dengue, malaria, inflammation, and skin infections. On the report of World Health Organization, bioactive compounds can be naturally found in medicinal plants which are the best sources for novel drug discovery [12, 13].

The phytochemical screening (quantitatively) of Pawpaw leaves shows the presence of saponin, alkaloids, tannin, flavonoids, terpenoids, glycosides and steroids (Tables 1 and 2). This is in agreement with the research work by Omidiwura [14]. The above secondary metabolites are studied to be active biologically and thereby supporting the antibacterial and antifungal activities of *Carica papaya*. These secondary metabolites bring to bear antimicrobial activity through various mechanisms. For example, Shimada [15] reported that tannins formed irreversible complexes with proline rich protein which may cause the inhibition of the synthesis of cell proteins. Phytochemicals such as tannins involve in the reaction with protein in other to give the typical tanning effect which maybe used in the treatment of ulcerated tissues as reported by Parekh and Chanda [16]. Tannins are used for the treatment of diarrhea as reported by Dharmananda [17]. All these findings or reports support the application of the leaves of *Carica papaya* in curing some ailment caused by the test organisms. Alkaloids were also detected in the leaves of *Carica papaya*, alkaloids are toxic to the cells of some organisms. These activities have been widely investigated for their potency in the elimination and reduction of human cancer cell lines. The inhibitory effect of saponins on ulcerated cells and the usefulness of pawpaw plant in managing and treating inflammations as reported by Just *et al*. [18].

The antimicrobial sensitivity results shows methanol and aqueous extracts of *C. papaya* were active on both bacterial and fungal Isolates. The highest zone of inhibition (mm) for the methanol plant extracts for the test organisms were Staphylococcus sp., vibrio sp. and E. coli. While for aqueous extract were vibrio sp. as shown in Tables 4 and 6. This shows similarities to the findings of Ogidi *et al.* [8, 19] which investigated the antimicrobial activities of Mushroom and neem

leaf extracts, the results of their research showed that both aqueous and methanol extracts of mushroom and neem leaves were observed to have antimicrobial potencies [20].

Thus, the mode of antimicrobial actions of pawpaw (*Carica papaya*) leaves may be related to the ability of the bioactive compounds to inactivate microbial adhesins, enzymes, and envelope transport proteins [21]. In comparison with both extracts used in the antimicrobial activities, methanol extracts were observed to be more potent than aqueous extract as shown in Figure 1. This finding is synonymous with earlier reports by Ogidi *et al.* [22, 23, 24].

CONCLUSION

This study shows the presence of secondary metabolites such as alkaloids, flavonoids, glycosides, steroids, saponin, tannins and terpenoids. The effects of antimicrobial sensitivity against bacterial and fungal isolates in this study are a manifestation that the leaves have the potency of producing drugs with a wide-range of activity. However, the more effective extract observed was methanol extract. The findings of the study corroborate the local application of the plant leaves and shows that the plant contains secondary metabolites with antimicrobial (bacteria and fungi) effects which can be alternatively used as antimicrobial agents in novel drug production. And use for the treatment and management of diseases like otitis media, uretritis, gastroenteritis, wound infections caused by the test organisms.

FINANCIAL ASSISTANCE

Nil

CONFLICT OF INTEREST

The authors declare no conflict of interest

AUTHOR CONTRIBUTION

O. I. Ogidi and P. S. Tobia designed the study whereas A.R. Iyosayi and U. M. Akpan collected the data. O. I. Ogidi and D.N. Ijere analyzed the data while O. Omu, O. I. Ogidi and H. E. Carbom interpreted the data. All authors contributed to the preparation of the manuscript. All authors read and approved the final manuscript.

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