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Phytochemical and Antimicrobial Studies of the Crude Extracts of the Leaves of *Carica papaya* Linn (Pawpaw) and *Psidium guajava* Linn (Guava)

Priscilla Alexander[1*], Ismaila Yada Sudi[2] and Martin Tizhe[1]

[1]Department of Pure and Applied Chemistry, Adamawa State University, P.M.B. 25, Mubi, Nigeria.
[2]Department of Biochemistry, Adamawa State University, P.M.B. 25, Mubi, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author PA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors PA and IYS managed the analyses of the study. Author MT managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Psidium Guajava (Guava) and *Carica Papaya* leaves which have some ethnomedicinal applications were investigated. Phytochemical screening of their leaves revealed the presence of flavonoids, saponins, terpenoids, steroids, tannins and glycosides. Antimicrobial screening of the crude ethanolic extracts showed activity against *Staphylococcus aureus, Streptococcus faecalis, and Escherichia coli*. The minimum inhibitory concentration (MIC) for *P. gujava* on the organism was found to be 5.00 mg/ml against *S. aureus*, *E. coli and S. faecalis,* while that of *C. papaya* leaves is 10.00 mg/ml against *S. aureus*, *E. coli* and 8.00 mg/ml against *S. faecalis* respectively. *C. papaya* ethanolic extract showed more active inhibition against *S. aureus* with mean zone inhibition of 9.54 ± 0.03. *P. gujava* ethanolic extract has more active inhibition against *E. coli* with antibacterial activity with mean zone of inhibition of 10.44±0.02 and *S. faecalis* with mean zone of inhibition of 6.72 ± 0.01 respectively. This study showed that the leaves extract of these plants are good sources of bioactive compounds. Demonstration of antibacterial activity against the test isolates is

**Corresponding author: E-mail: priscillaalexander21@yahoo.com;*

an indication that there is possibility of sourcing alternative antibiotic substances in these plants for the production of newer antibacterial agents. These plants therefore, could be an important source of medicine for the treatment of various diseases.

Keywords: C. papaya; P. guajava; secondary metabolites; crude extracts; S. faecalis; S. aureus and E. coil.

1. INTRODUCTION

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 the treatment of different human ailments [1]. According to (Danjuma and Osuagbalende [2], plants are being used as important sources of food and medicine for the prevention of illness and maintenance of human health. Medicinal plants are cheap and renewable sources of pharmacologically-active substances and are known to produce certain chemicals that are naturally toxic to bacteria. Report according to Alorkpa et al. [1] stressed that these plants are composed of good sources of cheap, and affordable drugs and medicinal plants which possess therapeutic effectiveness like other conventional drugs, however; these drugs have little or less side effects [3]. Many of plants parts such as roots, stems, barks, leaves, flowers, fruits, seeds and exudates constitute important components of drugs which are used in traditional herbal medicinal systems. However, the curative efficiency of the drugs used in these systems depends most importantly on the use of suitable and original raw materials [4]. However, the studying of medicinal plant extracts and plant products constitute the phytochemicals, antimicrobial and antioxidant properties which indicate that many of such plants are elementary sources of antibiotics [5]. For ages, native people have used the healing power of plants as their personal phytomedical cure [1,6].

The plant yields a natural compound known as (Annonaceous acetogenins) in its parts such as leaf, bark and twig tissues which have high anti-tumor and pesticide properties [7]. The plant extract of the leaf have also showed the presence of Anti-malarial and anti-plasmodial properties [8]. The leaf of the *C. papaya* plants contains karpain, this is an important part of the leaf that kills microorganisms which causes disturbance of the digestive functions [1,9]. Antioxidant are substances that can prevent or slow damage to cell caused by free radicals, unstable molecules that the body produces as a reaction to environmental and other pressures [10]. They are sometimes called “free-radical scavengers”. Free radicals are waste substances produced by cell as the body processes food and reacts to the environment. If the body cannot process and remove free radicals efficiently, oxidative stress can result [11]. This can harm cells and body function. Free radicals are also known as reactive oxygen species (ROS) [11]. Nutrient antioxidants such as vitamins C and E within the flavonoids are naturally occurring phenolic compounds in the body. An antimicrobial is a substance that kills or prevents the growth of microbes [12].

Psidium guajava L. is a native plant of tropical America but now cultivated throughout the tropics especially were the climate is suitable for the growth of the plant. *Psidium guajava i*s a medium sized tree with evergreen, opposite, aromatic short-petioled leaves [13]. *Psidium guajava* (Guava) is a phytotherapic plant used in traditional medicine that is believed by the indigenous group to posses’ active components that help to treat and manage several diseases [14]. Many parts of the plant like the leaves, sterms, and barks have traditionally been used in native medicine to manage conditions like malaria, gastroenteritis, vomiting, diarrhea, dysentery, wounds, ulcers, toothache, coughs, sore throat, inflamed gums, and a number of other conditions [15]. The phenolic compounds in the leaves of the plant have also been used for the controlling of life-changing conditions such as diabetes, hypertension, and obesity [16].

Being a rich source of secondary metabolites such as phenolic acids, flavonoids, tannins, alkaloids, and other small compounds, plants can be of interest in therapeutics. Various plants extracts and phytochemicals offer considerable potential for the development of new agents, effective against infections and could help curb the problem of multidrug-resistant organism [17].

Certain diseases related to lack of proper human diet, increases as population increased, also some pharmaceuticals causes side effects. Therefore it is pertinent to understand the benefits of medicinal plants because of their chemo therapeutic activities which could be due to the phytochemical content of these plants [18].

The aim of this study is to evaluate the phytochemical and antimicrobial activities of two selected medicinal plants grown in Bazza, Michika Local Government Area of Adamawa State, Nigeria.

2. MATERIALS AND METHODS

2.1 Sample Collection

The leaves of *C. papaya lnn* and *P. guajava* were collected from different trees, growing at Sylvester Wada Farm in Ngrippa, Bazza, Michika Local Government Area of Adamawa State, Nigeria. The Identification and Authentification of the plant leaves was carried out in the Department of Botany, Adamawa State University, Mubi, Nigeria.

2.2 Sample Preparation

The collected leaves samples of *C. papaya* and *P. guajava* were taken to the laboratory and washed thoroughly with ordinary water to remove dirt, dust, and other contaminants, then furthermore, washed with distilled water and allowed to drip. The plants leaves samples were air-dried at room temperature. The dried plant leaves were crush, ground into fine powder using mortar and pestle in the laboratory and then homogenize using laboratory blender. The powdered samples were sieved using 90 micron sieve and stored in polyethylene air- tight containers for further processing [19].

About 20 g of each powdered samples were weighed into soxhlet extractor and extracted for 4 hrs with 250 ml of ethyl acetate at 60-80ºC for defatting. The extract was filtered and then evaporated under reduced pressure using a rotary vacuum evaporator at 65ºC. These purified extracts were stored at 4ºC for further analysis [20].

2.3 Phytochemical Analysis of the Leaves Extracts

Phytochemical analysis for the screening and identification of bioactive chemical constituents such as flavonoids, terpenoids, alkaloids, glycosides, steroids, saponins, osozone, and tannins of the leaves extracts were determined qualitatively and quantitatively using standard procedures as described by Alorkpa et al. [1]; Edeoga et al. [21] and Sofowora [22] with slight modification.

2.4 Antimicrobial Analysis

The ethanolic leaves extracts were tested against some common organisms such as *S. aureus, S. faecalis, and E. coli* to determine their zone of inhibition and minimum inhibitory concentration. Agar well method of the agar diffusion technique was used to determine the antibacterial activity of the plant extracts [1].

Fresh and pure clinical isolates of *S. aureus, E. coli*, and *S. faecalis* were obtained from Department of Medical Microbiology, General Hospital Mubi in Adamawa State,Nigeria and were used for the experiment. The identification and characterization of the isolates was carried out by using three methods, namely gram staining, cultural characterization using selective or indicative media and biochemical characterization with reference to Cheesbrough [13]. The pure isolates of each of the test organisms were inoculated in sterile slants containing Nutrient agar and transported to the Department of Chemistry, ADSU Mubi and refrigerated at 4ºC before use. The bacteria isolates were first sub cultured in a broth and incubated at 37ºC for 24 hrs [13].

2.5 Antibacterial Activity

Whatman No.1 filter paper was used to filter the mixtures and the extracts were then evaporated to dryness using rotary evaporator and water bath. The One gram of the solid residues obtained was reconstituted in 5 ml of 5% DMSO to form stock concentration of 200 mg/ml, this was stored in the refrigerator at 4°C for further analysis. The agar well method was used to determine the antibacterial activity of the plant extracts. 0.1 ml of the different standardized organisms were introduced separately and thoroughly mixed with Mueller Hilton Agar in a sterile Petri dish and allowed to set then labeled. A sterile cork borer 6mm was then used to punch holes (i.e. 5 wells) in the inoculated agar and the agar was then removed. Four wells that were formed were filled with different concentrations of the extract which were labeled accordingly; 200 mg/ml, 150 mg/ml, 100 mg/ml and 50 mg/ml while the 5th well contained the solution used for the research to serve as control, tetracycline (Chi pharmaceutical limited, Lagos Nigeria) 125 mg/ml, was used as control in this research.

These were then left on the bench for 1 hour for adequate diffusion of the extracts and incubated at 37ºC for 24 hours. After incubation, the diameter of the zones of inhibition around each well were measured to the nearest millimeters along straight line and the mean of the readings were then calculated [13,14].

2.6 Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) was determined using the tube dilution method.

The Minimum Inhibitory Concentration (MIC) helps to measure more accurately the concentration of an antibiotic necessary to inhibit growth of standardized inoculum under defined conditions. In this method different concentrations of the extracts were placed on the plates containing nutrient broth. The plates were streaked with the test organisms and incubated at 37^{o} C for 24 hrs, after which they were examined for the presence or absence of growth. The MIC was taken as the lowest concentration that prevented the growth of the test microorganism [1].

2.7 Zone of Inhibition

The zone of inhibition was determined using the nutrient agar method Alorkpa et al. [1]. Ten (10) petri-dishes were used and each of the petri-dish corresponds to one test organism for each extract, which were well labelled and used. About 20 ml nutrient agar was transferred into each petri dish of the organism. The nutrient agar was then allowed to solidify and wells were created in them using the cork borer (6 mm). Each of the well was filled with its respective concentration of the plant extract and left for about 1 hour for complete diffusion with the extract within the nutrient agar. The petri-dishes containing the nutrient agar were then incubated between 37ºC and 42ºC for a period of 18 hours after which the zone of inhibition was determined [1].

3. RESULTS AND DISCUSSIONS

3.1 Phytochemical screening

The phytochemical screening of the plant extracts revealed the presence of tannins, saponins, alkaloids flavonoids, glycosides, terpenoids and steroids in *P. guajava* and *C. papaya* leaves (Table 1 and Table 2). Alkaloids and flavonoids are slightly high in *P. guajava* leaves extract while tannin is slightly high in *C. papaya* leaves extract. These metabolites are known to have varied pharmacological actions in man and animals [23].

3.2 Antibacterial Activity

From the result of the antimicrobial screening (Table 3), the ethanolic extract of both *P. guajava and C. papaya* are active on *S. aureus, E. coli* and *S. faecalis*. The mean zone of inhibition (mm) for the different plant extracts (Table 3) shows that the ethanol extracts of *P. guajava* and *C. papaya* has good antibacterial activity against *S. aureus* and *E. coli*, this is attributed to better solubility of the active component by the ethanol [19]. This shows similarities to the findings of Nwanneka et al.[24] and Ali et al [13] which investigated the antibacterial activity of *P. guajava* leaf extract, the results of their research showed that both aqueous and ethanolic extracts *of P*. guava leaf inhibited the growth of the bacteria and fungi tested, but the ethanolic extract showed stronger inhibition than the aqueous extract against the organisms [13]. In this study the *C. papaya* ethanolic extract showed more active inhibition against *S. aureus* with mean zone inhibition of 9.54 ± 0.03. *P. gujava* ethanolic extract has more active inhibition against *E. coli* with antibacterial activity with mean zone of inhibition of 10.44 ± 0.02 and *S. faecalis* with mean zone of inhibition of 6.72 ± 0.01 respectively, this report is in agreement with the work of Danjuma & Osuagbalende [2]. Therefore, the antibacterial activity of ethanolic leaf extract of *P. guajava* and *C. papaya* (Table 3) revealed that the mean diameter of zone of inhibition of extract on the test isolate was most susceptible isolate on *E.coli* with concentration (10.44 mm). *P. guajava* ethanolic extract showed higher antibacterial activity this might be attributed to better solubility of the active component by the ethanol [14].

3.3 Minimum Inhibitory Concentration (MIC)

Table 4 shows that the minimum inhibitory concentration (MIC) for *P. gujava* on the organism was found to be 5.00 mg/ml against *S. aureus*, *E. coli* and *S. faecalis*, while that of *C. papaya* leaves is 10.00 mg/ml against *S. aureus*, *E. coli* and 8.00 mg/ml against *S. faecalis* respectively. This study shows that the ethanol extracts of *C. papaya* have high antibacterial activity against *E. coli* (the frequently implicated organism in gastroenteritis and pelvic inflammation), and *S. aureus* (that brings about many skin diseases such as atopic eczema)[19], this is in agreement with the work of Adebayo-Tayo et al. [25].

Table 1. Phytochemical constituents of the ethanolic leaves extracts of *P. guajava* and *C. papaya*

Extracts	Phytochemicals						
	Tannins	Saponins	Alkaloids	Flavanoids	Glycoside	Terpenoids	Steroids
P. gujava	+	+	++	++	+	+	+
C. papaya	++	+	+	+	+	+	+

+ Present, ++ highly present

Table 2. Quantitative composition of the phytochemical constituents in the ethanolic leaves extracts of *P. guajava* and *C. papaya*

Extracts	Phytochemicals (mg/100 g)						
	Tannins	Saponins	Alkaloids	Flavanoids	Glycoside	Terpenoids	Steroids
P. guajava	0.43±0.01	0.39±0.02	0.95±0.10	2.43±0.02	0.54±0.02	0.26±0.01	0.19±0.01
C. papaya	1.05±0.00	0.43±0.01	0.16±0.01	0.87±0.01	0.48±0.01	0.23±0.01	0.38±0.01

Table 3. Antibacterial activity of the ethanolic leaves extracts of *P. guajava* and *C. papaya* on different test organisms

Extracts	Zone of inhibition (mm)/Test organisms		
	S. aureus	***E. coli***	***S. faecalis***
P. guajava	8.27±0.02	10.44±0.02	6.72±0.01
C. papaya	9.54±0.03	6.43±0.01	5.15±0.00

Table 4. The Minimum Inhibitory Concentration (MIC) of the ethanolic leaves extracts of *P. guajava* and *C. papaya*

Extracts	MIC (mg/ml)/ Test organisms		
	S. aureus	***E. coli***	***S. faecalis***
P. guajava	5.00	5.00	5.00
C. papaya	10.00	10.00	8.00

The phytochemical screening shows that the plant contains bioactive compounds which have been reported to have antibacterial potency and perhaps may have contributed to its antibacterial activity [26]. Flavonoids are known to be synthesized by plants in response to microbial infection. They have effective antibacterial activities *in vitro* against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and also with bacterial cell [16].

Many human physiological activities, such as stimulation of phagocytic cells, host-mediated tumor activity, and a wide range of anti-infective actions, have been attributed to tannins [16]. Their mode of action is to complex with proteins through nonspecific forces, such as hydrogen bonding as well as by covalent bond formation. They also complex with polysaccharides which are components of bacterial cell wall, Studies show that tannins can be toxic to filamentous fungi, yeasts, and bacteria. Thus, the mode of antibacterial action of this plant may be related to the ability of these bioactive constituents to inactivate microbial adhesins, enzymes, and envelope transport proteins [16].

4. CONCLUSION

Phytochemical screening of *P. guajava* and *C. papaya* leaves extracts demonstrated the presence of common photochemical in the leaf extracts which includes alkaloids, flavonoids, saponins, terpenoids, steroids, tannins and glycosides as major active constituents and the quantitative analysis indicates that *P. guajava* has high concentration of flavonoids while *C. papaya* is rich in tannins. *C. papaya* ethanolic leaf extract has more active inhibition against *S. aureus* than *P. guajava* ethanolic leaf extract, *S. aureus and E. coli* are the most susceptible isolates while *S. faecalis* is the least susceptible isolate. The populace should be educated and encouraged on the use of *P. guajava* and *C. Papaya* leaves as a source of medicine or a substitute for synthetic drugs, because of their phytochemical content and also these plants have no or less side effects as compared to synthetic drugs produced by pharmaceutical industries.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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