PHYTOCHEMICAL AND ANTIMICROBIAL STUDIES OF THE CRUDE EXTRACTS OF THE LEAVES OF *CARICA PAPAYA LINN* (PAWPAW) AND *PSIDIUM GUAJAVA LINN* (GUAVA)

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 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*

Introduction

The basis for traditional medicine is biological activity, which uses the pharmacological efficacy of natural compounds which are present in herbal preparations for the treatment of human diseases [1]. Plants constitute a good source of cheap and affordable drugs and medicinal plants also possess therapeutic efficacy like their orthodox drugs counterpart, yet they have little or less side effects [2]. Report according to Alorkpa et al. [3] emphasized that these plants and their parts such as roots, stems, barks, leaves, flowers, fruits, seeds and exudates form an important major constituent of drugs used in traditional herbal medicinal systems. However, the therapeutic efficiency of the drugs used in these systems depends greatly on the use of proper and genuine raw materials [4]. However, the screening of medicinal plant extracts and plant

products for antimicrobial and antioxidant properties show that many of such plants are primary sources of antibiotics. For years, indigenous groups have used curing plants as their personal phytomedical remedy [5].

Carica Papaya linn (Pawpaw) plant produces a natural compound known as Annonaceousaceto genins in its leaves, bark and twig tissues that possess both highly anti-tumor and pesticide properties [6]. Anti-malarial and anti-plasmodia activities have also been demonstrated by the leaf extract of the plant [7]. The leaves of the *C. Papaya* plants contain karpain, a substance that kills microorganisms which often interfere with the digestive functions. Antioxidants are a special group of nutrients produced by the cell, which removes supplements that scavenge for free radicals [8]. The free radicals impair the proper functioning of the glutathione peroxidase and regulate the action of immune system, leading to various disease conditions. 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 [9].

Psidium guajava (Guava) is a phytotherapic plant used in folk medicine that is believed to have active components that help to treat and manage various diseases. Many parts of the plant have been used in traditional medicine to manage conditions like malaria, gastroenteritis, vomiting, diarrhea, dysentery, wounds, ulcers, toothache, coughs, sore throat, inflamed gums, and a number of other conditions [1]. This plant has also been used for the controlling of life-changing conditions such as diabetes, hypertension, and obesity [10].

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 [11].

Certain diseases related to lack of proper human diet, increases as population increases, 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 [3].

This study is aimed at evaluating the phytochemicals of two selected medicinal plants in Bazza, Michika Local Government Area of Adamawa State, Nigeria.

Materials and Methods

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. Identification and Authentification of the plant materials has been done at Herbarium unit in the Department of Botany, Adamawa State University, Mubi, Nigeria.

Sample Preparation

The collected leaves samples of *C. Papaya* and *P. guajava* were taken to the laboratory and were thoroughly washed with ordinary water to remove dirt, dust, and other contaminated agents 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 [11] [2].

About 20g of each powdered samples were weighed into soxhlet extractor and extracted for 4 hrs with 250ml of ethyl acetate at 60-80^{o}C for defatting. The extract was filtered and then evaporated under reduced pressure using a rotary vacuum evaporator at 65^{o}C. These purified extracts were stored at 4^{o}C for further analysis [1]

Phytochemical Screening 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. [3]; Edeoga et al. [13] and Sofowora [14] with slight modification.

Antimicrobial Analysis

The ethanol 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 [13] [3].

Clinical isolates of S. aureus, E. coli, and S. faecalis were obtained from Department of Medical Microbiology, General Hospital Mubi, Adamawa State, Nigeria for further experiment. Identification and characterization of the isolates was conducted there by using three procedures namely Gram staining, cultural characterization using selective or indicative media and biochemical characterization with reference to Chees brough [15]. The pure isolates of each of the test organism were inoculated in sterile slants containing Nutrient agar and transported to the Department of Chemistry, ADSU Mubi and refrigerated at 4°C before use. The bacteria isolates were first sub cultured in a broth and incubated at 37^{o} C for 24hrs [16].

Antibacterial Activity

The mixture was filtered using Whatman No.1 filter paper and the extracts were evaporated to dryness using rotary evaporator and water bath. One gram solid residues obtained were reconstituted in 5 ml of 5% DMSO to form stock concentration of 200 mg/ml, stored in the refrigerator at 4°C until used. 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 37°C 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 [17].

Determination of Minimum Inhibitory Concentration (MIC)

The estimation of MIC of the crude extracts was carried out using the method of [4]. The MIC helps to measure more exactly 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.

Zone of inhibition

The zone of inhibition was determined using the nutrient agar method Alorkpa et al.[3]. Ten (10) petri-dishes with each petri-dish corresponding to one test organism for each extract were well labelled and used. 20 ml nutrient agar was put in each petri dish or the organism. The nutrient agar was allowed to solidify and wells created in them using the cork borer (6 mm) with each well filled with its respective concentration of the plant extract and left for about 1 hour for complete diffusion of the extract within the nutrient agar. The petri-dishes containing the nutrient agar were then incubated between 37 °C and 42 °C for a period of 18 hours after which the zone of inhibition was determined

Results and Discussion

Results

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 (g/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 s	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

Discussion

The result of the phytochemical screening (Tables 1&2) showed that *P. guajava* and *C. papaya* plant leaves contains tannins, saponins, alkaloids flavonoids, glycosides, terpenoids and steroids. Alkaloids and flavonoids are of high concentrations in *P. guajava* leaves extract while tannin is of high concentration in *C. Papaya* leaves extract. These metabolites are known to have varied pharmacological actions in man and animals [3].From the result of the antimicrobial screening

(Table 3), the ethanolic extract of both *P. guava 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 [18]. This shows similarities to the findings of Nwanneka et al.[19] and Ali et al [15] which investigated the antibacterial activity of *P. guajava* leaf extract, the results showed that both aqueous and ethanolic extracts of 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. *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 [20]. 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.coil* 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.

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), which is in agreement with the work of Adebayo-Tayo et al. [21].

The phytochemical analysis shows that the plant contains bioactive compounds which have been reported to have antibacterial potency and perhaps may have contributed to its antibacterial activity [22]. 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 [15]. Their activity is probably due to their ability to complex with extracellular and soluble proteins and also with bacterial cell [23].

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 [20]. 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 [24].

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.

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