Supported by tetfund TERTIARY EDUCATION TRUST FUND

Phytochemical screening, Total flavonoid content assay and Evaluation of the Antimicrobial properties of Hydro alcoholic extract of the leaves of male *Carica papaya* Linn.

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Received: March 20, 2022 **Accepted:** June 18, 2022

Abstract: *Carica papaya* parts have been used traditionally to treat certain ailments. The aim of this study was to evaluate the antimicrobial potential of the total flavonoids of hydro alcoholic extract (TFHE) of the leaves of male *Carica papaya***.** Fresh healthy male leaves of *C. papaya* were thoroughly rinsed in running tap water and dried on a disinfected platform under shade for 10 days. The leaves were then pulverized, percolated in 1 L methanol and the extracts examined for the presence of phytochemicals using standard protocols. These extracts were further studied for their antimicrobial potential against some test isolates: *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*. Phytochemicals such as flavonoids, alkaloids, tannins, saponins, diterpines and cardiac glycosides were observed in the extracts. The extracts showed various degree of antimicrobial activities, with inhibitory zone diameters (IZDs) ranging from 13 mm – 24 mm (bacterial isolates) and 19 mm – 23 mm (fungal isolate). The extracts gave the highest IZD of 24 mm against *E. coli* at a concentration of 50 mg/ml and the least IZD of 13 mm against *S. aureus* at a concentration of 31.25 mg/ml. The extracts showed higher antifungal activity against the test fungal isolate compared to the standard fluconazole across the concentration gradient. These antimicrobial activities, especially antifungal activity greater than the standard drug observed in the methanolic leaves extracts could be due to the presence of potent secondary metabolites they harbour and these microbial activities decreases down the concentration gradient. The outcome of this study would make for further examination.

Keywords: Antimicrobial properties, *Carica papaya*, Hydro alcoholic extract, Phytochemical screening.

Introduction

Medicinal plants have been defined as one whose tissues or organs are said to contain substances with therapeutic importance or with the capacity to serve as a precursor for the synthesis of medications with great value in restoring health (Jahan *et al*., 2011). They have been used as a ready source of therapy for several centuries ago (Akpotu and Abdul, 2007; Abere *et al*., 2007). More than 35,000 plant species are said to have been used in folklore for medical purpose, where they are normally consumed as food (Kong *et al*., 2003). It has been reported that the bioactive compounds present in these medicinal plants are responsible for the healing abilities they elicit and notable among these compounds are the flavonoids, alkaloids, phenolic and tannins (Shihabudeen *et al*., 2010). The presence of these important bioactive compounds also known as secondary metabolites on the medicinal plants are dependent on their stage of growth as well as the season of year, with the younger plants producing richer secondary metabolites than the older ones and greater accumulation of total phenols, free amino acids and tannins higher during summer (May to June) and the phyic acid higher during rainy season (Tonar *et al*., 2015). In spite of the availability of different approaches for the discovery of new chemotherapeutics, natural products still prides itself as the best reservoir for novel structural types. Plants face many stresses such as drought, saline and rocky environment as well as been pitted by strong winds and ocean currents among others, making them to accumulate secondary metabolites and in the long run becoming largely immune to diseases and pests infestations during their life cycle (Akpotu *et al*., 2017; Farjana *et al*., 2014). In the last few decades, researches in medicinal plants had received great attention due to their antimicrobial and antioxidant properties as well as their relatively low tendencies to toxicity and their affordability compared to drugs used in main stream orthodox medicine (Chew *et al*., 2012). The antimicrobial property in medicinal plants has even elicited greater interest due to the global rise in antibiotic resistance to the existing medications used in therapy. This antimicrobial resistance poses great danger to public health, hence the need to develop new drugs with higher efficacies (Farjana *et al*., 2014).

Papaya, also called pawpaw, belongs to the family Caricaceae, known to possess four genera of which the most prominent genus, Carica, has twenty two known species and the notable, widely grown one among these species is the *Carica papaya* Linn (Krishna *et al*., 2008). This plant was said to be first domesticated in Mesoamerica, in the present day Southern Mexico and Costa Rica, which was later grown in plantations in India, South Africa, Hawaii, Philippines and most of the tropical and subtropical regions of the world (Krishna *et al*., 2008; Morton, 1987). The papaya tree is small, sparsely branched and usually growing up to heights ranging between 5 to 10m with leaves arranged helically and restricted at the uppermost part of the trunk. There is an obvious scarring on the lower trunk that once bears the leaves and fruits. The leaves are enormous, mostly growing up to 50 to 70 cm in diameter and deeply palmately lobed with seven lobes. The entire part of the plant usually contains latex (Heywood *et al*., 2007). The fruit, usually a large berry, grows between 15-45 cm long and 10- 30 cm in diameter (Heywood *et al*., 2007). The papaya tree is said to grow in three sexes: male, female and hermaphrodite. The male never fruits, but only produce pollen. The females are said to produce small, inedible fruits unless pollinated. The hermaphrodite on the other hand can self-pollinate, since its flowers contain both male stamens and female ovaries and this sex is common to commercial papaya orchard (Chia and Manshardt, 2001). Papaya leaves has been shown to harbour secondary metabolites like β –carotene, saponins, alkaloids, steroids, tannins, flavonoids and glycosides which have antimicrobial, immunomodulatory and antitumor activities (Anibijuwon and Udeze, 2009). It also contains chymopapain and papain which help in digestion and

greatly inhibit the progression of pathogens (Yismaw *et al*., 2008; Unaeze and Brikwa, 1986). Papaya leaves extract have been used in folklore to heal peptic ulcers as well as conferring protection against some dermatological issues such as pimples, acne and freckles (Udoh *et al*., 2005). The leaves of this plant has also found usefulness in folkloric medicine as a treatment for malaria (Titanji *et al*., 2008), an abortifacient, a purgative or smoked to provide relief for asthmatics (Morton, 1987).

This study was carried out to determine the antimicrobial properties of the hydro alcoholic extracts of the leaves of male *Carica papaya* Linn.

Methods

Sample collection and authentication

Healthy, fresh leaves of *Carica papaya* were collected at Ayepe Road in Sagamu, Ogun State in April 2018 and authenticated at the Forestry Research Institute of Nigeria (FRIN) in Ibadan, Oyo State where the voucher number: FH1111905 was assigned.

Sample preparation

The leaves were thoroughly rinsed under running water and subsequently dried at room temperature under shade for 10 days. The leaves were then pulverized and stored in clean, air tight containers away from light, heat and moisture until further analysis.

Total flavonoids extraction of the sample

The total flavonoids extraction of the sample was performed following the method of Shukla and co-workers with a little modification (Shukla *et al*., 2012). Briefly, 150g of the sample was weighed into a 2.5L capacity Winchester bottle and percolated with 1L of methanol with occasional agitation at room temperature for 72hours (3 days). The mixture was carefully filtered using Whatman filter paper and exact was concentrated to 50 mL under vacuum using a rotary evaporator to obtain the methanol extract (ME). The ME was further extracted twice with an equal volume of diethyl ether and the mixture separated into ethereal and methanol layers using a separating funnel. The ethereal layer was not treated further and the recovered methanol layer was extracted twice with an equal volume of chloroform. The resulting methanol extract (containing the precipitated total flavonoids) was kept in a refrigerator at -5°C and later concentrated to obtain a deep green paste labelled as the total flavonoids of the hydro alcoholic extract (TFHE).

Phytochemical screening

Part of the methanol extract was screened for the presence of alkaloids, flavonoids, tannins, saponins and cardiac glycosides in accordance with Poongothai and co-workers (Poogothai *et al*., 2011).

Antimicrobial assay of the extracts

This was carried out using a modification of the agar diffusion technique according to Akpotu and co-workers (Akpotu *et al*., 2017). Briefly, test isolates such as *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* obtained from the Pharmaceutical Microbiology Laboratory of Olabisi Onabanjo University, Sagamu Campus, Ogun state, Nigeria were standardized to 0.5 McFarland's standard and seeded unto the different freshly prepared Mueller Hinton agar (MHA) plates (for the bacterial test organisms) and Sabourauds dextrose agar (SDA) (for the fungal test organisms) prepared according to the manufacturer's directions and labelled accordingly in triplicates for each of the test isolates. The extracts were diluted by two-fold serial dilution into five different concentrations: 500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml and 31.25 mg/ml respectively using dimethyl sulphoxide (DMSO). Next, five holes were made equidistance apart my means of a sterile 6 mm cork borer into which 20 µL of the extracts were dispensed, with the first hole receiving 500 mg/ml concentration, the second hole 250 mg/ml, 125 mg/ml for the third hole and finally the fourth and fifth holes receiving 62.5 mg/ml and 31.25mg/ml respectively and the standard antimicrobial agent (Ciprofloxacin at 10 µg/ml) placed centrally to serve as positive control. The plates were incubated at 37°C for 24 hours. The above procedure was repeated using Sabouraud's Dextrose agar (SDA) for the fungal organism (*Candida albicans*) using fluconazole at 50 µg/ml as the positive control. The antifungal activity was observed after 72 hours, zones of inhibition was measured (in mm) in both categories and recorded. The DMSO was used as the negative control in both categories as well.

Results

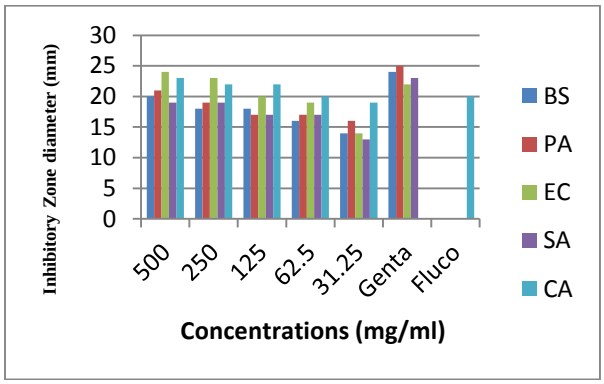
Plate 1: Male *C. papaya* plant

Plate 2: Female *C. papaya* plant

Table 1: Phytochemical constituents of the methanol extract (ME) of *Carica papaya* male leaves

Test	Inference
Flavanoids	+
Alkaloids	+
Tannins	+
Saponins	+
Diterpines	+
Cardiac glycosides	-

Key: + = present; - = absent



Key: BS: *Bacillus subtilis*; *PS*: *Pseudomonas aeruginosa*; EC: *Escherichia coli*; SA: *Staphylococcus aureus*; CA: *Candida albicans*

FIG 1: The inhibitory zone diameter of the extracts against the test organisms

Discussion

The methanolic extracts of the male *Carica papaya* used in this study (Plate 1) showed a number of phytochemical constituents such as flavonoids, alkaloids, tannins, saponins, diterpines and cardiac glycosides (Table 1). This is in consonance with earlier studies where *C. papaya* leaves showed several phytochemicals (Callixte *et al*., 2020; Arumugan *et al*., 2014). These phytochemical constituents otherwise known as secondary metabolites were produced in great numbers and quality. It has been reported that the age of the plant, especially the younger and tenderer leaves favours the production of quality secondary metabolites. In addition, it has been said that the weather, especially during summer to almost the end of rainy season favours quality production of secondary metabolites and this seems to be in agreement with our findings (Callixte *et al*., 2020; Tonar *et al*., 2015; Mayrhofer *et al*., 2005). The choice of extraction solvent, methanol may also be responsible for the impressive yield of secondary metabolites.

The extracts showed antimicrobial properties with the inhibitory zone diameters (IZDs) ranging between 13 mm – 24 mm for the bacteria test isolates and 19 mm – 23 mm for the fungal test isolate (*Candida albicans*). The highest IZD of 24 mm was given by *Escherichia coli* at a concentration of 500 mg/ml and the least IZD of 13 mm was given by *Staphylococcus aureus* at a concentration of 31.25 mg/ml. The extracts showed higher antimicrobial activity evidenced by their greater IZDs against the fungal test isolate (*C. albicans*) compared with the standard antifungal agent (fluconazole 50 µg/ml) (Figure 1). These extracts could be a promising source of a novel antifungal agent. It has been said that the biological activity elicited by medicinal plants are due largely to the secondary metabolites they harbor. These secondary metabolites harboured by the male leaf *C. papaya* could be responsible for the antimicrobial properties it elicits (Akpotu and Abdul, 2021; Akpotu *et al*., 2017; Shihabudeen *et al*., 2010; Abere *et al*., 2007). The bacterial isolates showed lower antimicrobial activities compared with the standard antibacterial agent, Gentamicin 10 µg/ml.

Conclusion

This study shows the presence of several useful phytochemicals in the methanolic extracts of the male leaves of *Carica papaya* L (Caricaceae). It also shows the capacity of these extracts to inhibit microbial growth, especially the *Candida albicans* test isolates, where it inhibits the isolate better than the standard fluconazole. This has arisen great interest for further investigations to determine the exact phytochemical that may have confer this important biological activity these extracts elicited and probably develop it into suitable drug moiety to enhance our armamentarium of antimicrobial therapy.

Conflict of interest

The authors declare that they do not have any conflict of interest.

Funding

The study was self-funded.

References

Abere TA, Onwukaeme DN and Eboka CJ (2007). Pharmacognostic evaluation of the leaves of Mitracarpus scaber Zucc (Rubiaceae). Tropical Journal of Pharmaceutical Research. 6(4): 849-853.

Akpotu MO and Abdul AO (2021). Preliminary investigation on the extracts of the fungal endophytes from *Mitracarpus scaber* for antimicrobial activity. Adamawa State University Journal of Scientific Research. 9 (1): 8-13.

Akpotu, MO., Eze, PM., Abba, CC., Umeokoli, BO., Nwachukwu, CU., Okoye, FBC., & Esimone, C.O. (2017). Antimicrobial activities of Secondary metabolites of endophytic fungi isolated from *Catharanthus roseus*. *Journal of Health Sciences*. *7*(1), 15-22. http://doi.org/10.17532/jhsci:2017.421 (1): 83-92.

Anibijuwon II and Udeze AO (2009). Antimicrobial activity of Carica papaya (Pawpaw leaf) on some pathogenic organisms of Clinical origin from South-Western Nigeria. Ethno botanical leaflets. 13 (7): 850-864.

Arumugan N, Boobalan T, Rajeswari PR and Duraimurugan MD (2014). Antimicrobial activity and phytochemical screening of *Cynodon dactylon* and *Carica papaya*. *Research in Biotechnology*. 5 (5): 21-31.

Callixte C, Baptiste NJ, Arwati H (2020). Phytochemical screening and Antimicrobial activities of methanolic and aqueous leaf extracts of *Carica papaya* grown in Rwanda. *Mol Cell Biomed Sci*. 4(1): 39-44.

Chew AL, Jessica JJ and Sasidharan S (2012). Antioxidant and antibacterial activity of different parts of *Leucas aspera*. Asian Pac J Trop Biomed. 2: 176-180.

Chia CL and Manshardt RM (2001). Why some papaya plants fail to fruit. College of Tropical Agriculture and Human Resources University of Hawaii at Manoa. Fruits and nuts (F&N-5) p1-2.

Farjana A, Zerin N and Kabir S (2014). Antimicrobial activity of medicinal plant leaf extracts against pathogenic bacteria. Asian Pacific Journal of Tropical disease. 4 (Suppl 2): S920-S923.

Heywood VH, Brummitt RK, Culham A, Seberg O (2007). Flowering plant families of the world. Firefly books. ISBN 9781554072064.

Jahan F, Lawrence R, Kumar V and Junaid M (2011). Evaluation of antimicrobial activity of plant extracts on antibiotic susceptibility and resistant Staphylococcus aureus strains. J. Chem. Pharm. Res. 3(4): 777-789.

Kong JM, Goh NK, Chia LS and Chia TF (2003). Recent advances in traditional plant drugs and orchids. Acta Pharmacol Sin. 24 (1): 7-21.

Krishna KL, Paridhavi M and Patel JA (2008). Review on nutritional, medical and Pharmacological properties of papaya (*Carica papaya* Linn). Natural Product Radiance. 7 (4): 364-373.

Mayrhofer S, Teuber M, Zimmer I, Louis S, Fischbach RJ, Schnitzler JP (2005). Diurnal and seasonal variation of isoprene biosynthesis-related genes in grey popular leaves. Plant Physiol. 139 (1): 474-484.

Morton JF (1987). Pawpaw. In: fruits of warm climates. Miami, Florida p336-346.

Poongothai A, Sreena KP, Sreejith K, Uthiralingam M, Annapoorani S (2011). Preliminary phytochemical screening of *Ficus racemosa* Linn bark. International Journal of Pharma and Bio-sciences. 2:431–434

Shihabudeen MH, Pricilla DH and Thirumurugan K (2010). Antimicrobial activity and Phytochemical analysis of selected Indian folk medicinal plants. Int J Pharma Sci Res. 1: 430-434.

Shukla P, Gopalkrishna B and Shukla P (2012). lsolation of rutin from *Phyllanthus amarus*. International Journal of Pharmaceutical Sciences and Research. 3 (4):1198-1201.

Titanji VPK, Zofou D and Ngemenya MN (2008). The antimalarial potential of medicinal plants used for the treatment of malaria in Cameroun folk medicine. Afr J Tradit Complement Altern Med. 5 (3): 302-321.

Tomar NS, Sharma M and Agarwa RM (2015). Phytochemical analysis of Jatropha curcas L during different seasons and developmental stages and seedling growth of wheat (Triticum aestivum L) as affected by extracts/leachates of Jatropha curcas L. Physiol Mol Biol Plants.

Udoh P, Essien I and Udoh F (2005). Effect of Carica papaya (paw paw) seeds extract on the morphology of pituitary-gonadal axis of male Wistar rats. Phytother Res. 19(12): 1065-8.

Unaeze MC and Brikwa PO (1986). Antimicrobial activity of certain medicinal plants used in traditional medicine in Nigeria: Preliminary study. Res J Microbiol. 6(2): 32-40.

Yismaw G, Tessema B, Mulu A and Tiruneh M (2008). The in vitro assessment of antibacterial effect of papaya seed extracts against bacterial pathogens isolated from urine, wound and stool. Ethiop Med J. 46(1): 71-77.