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Phytochemical, Antioxidant and Antimicrobial Potentials of Methanol Seed Extracts of Carica Papaya

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Abstract
The present study shows antioxidants and antimicrobial activity of methanol extract of Carica papaya against some pathogenic microorganism were carried out. The antibacterial activity was screened against Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus and E. coli using agar well diffusion method at various concentrations (160mg/ml, 120mg/ml and 80mg/ml). The minimum inhibitory concentration (MIC) was determined using serial dilution method while minimum bacterialcidal concentration (MBC) by plating various dilution of extraction. Results revealed the presence of total phenols and tannins, while steroids and phlobatannins were not detected. Quantification of the phytochemical also shows that seed extract contains 0.133 mg/ml (total flavonoids), 0.095 mg/ml (total phenol), 0.238 mg/g (alkaloids), 0.214µg/ml (saponins) and 3.196mg/ml (tannins). It is concluded that Carica papaya contains some useful phytochemicals with potential antioxidants and antibiotic reputations. Thus, it may be considered as a natural source of antimicrobials and antioxidants for therapeutic purposes.

Keywords Staphylococcus aureus antimicrobials and antioxidants activity.

1. Introduction

Pathogenic bacteria have always been considered as a leading cause of morbidity and mortality in humans. Even though a number of new anti-biotic have been developed by pharmaceutical organizations bacterial genetic ability to transmit and acquire resistance to these drug and therapeutic agents has generated a worldwide predicament of antimicrobial resistance (Nascimento *et al*., 2000). Antimicrobial resistance coupled with undesirable side effect associated with antibiotics has geared the scientist attention towards extracts and biologically active principles isolated from plant materials (Parekh *et al*., 2005). Antimicrobial agents from natural products represent an immense untapped source of medicines and further investigation of plant antimicrobials is highly welcome. Plant based antimicrobials are effective in the treatment of infectious diseases while concurrently extenuating the side effects that are often linked with syntheticanti-microbials (Essawi and Sour, 2000). Africa is blessed with enormous diversity of natural product with healing practice as revealed by several citations (Bashir *et al*., 2015)The medicinal virtue of natural products lays on some bioactive principles that produce a definite physiological action on the human body and that could serve as a candidate for the developments of new drugs. The most important of these bioactive compounds are classified as alkaloids, tannins flavonoids and phenolic compounds. Many plant leaves have antimicrobial principles such as tannins, essential oils and other aromatic compounds (Yusuf and Bewail, 2011). In addition, many biological activities and antibacterial effects have been reported for plant tannins and flavonoids (De Boer *et al*., 2005). *Carica* is a genus of about 20 species of evergreen trees native to tropical America. The papaya or pawpaw is the fruit of the plant carica papaya, it is the sole species in the genus caricaceae it is native to the tropics of the Americas, perhaps from southern Mexico and neighboring Central America it was first cultivated in Mexico

Several centuries before the emergence of the Mesoamerican Civilization (Korean *et al*., 2006). There are several scientific reports on the constituents and pharmacological activities of the *Carica papaya*. For instance, the stem bark has been reported to contain phenols, saponins, flavonoids, tannins, terpenoids, alkaloids and cardiac glycosides while saponins, terpenoids, cardiac glycosides and steroids are contained in the leaves (Colle *et al*., 2003). However, literature survey revealed very scanty information on the effectiveness of this plant against free radicals and some pathogenic organism. This study is therefore set out to evaluate the antioxidant and antimicrobial effects of methanol extract from the seed of this plant, with a view of bridging the gap in knowledge and providing scientific information regarding the clinical application

2 Material and Methods

Sample Collection

The Fresh seed of *Carica papaya* was collected from Minna Local, Niger State Nigeria. It was identified and authenticated by a Botanist in the Department of Biological Science, Federal, University, of Technology Minna. Niger State. The collected fresh seed of *Carica papaya* was destalked, washed with clean-water, dried at room temperature and finally grounded using electronic blending machine

Pure isolate of Salmonella typhii, Escherichia coli, Klebsiellapneumonea and Staphy-lococcus aureus were obtained from Microbiology Department, Federal, University, of Technology Minna. Niger State. The identity of the organism was confirmed by standard biochemical test and proper gram staining test.

3 Phytochemical Analysis

Test for alkaloids

Two (2) grams of the extract were mixed with 10ml of dilute Hcl and then filtered the filtrate was treated with Mayer's regent and dragendorifs reagent respectively the test was observed for colour changes (Harborne, 1973).

Test for Tannin

The methanol extract of *Carica papaya* (0.25g) was diluted with 10ml of distilled water, few drops of ferric chloride was added to the mixture. The reaction for the formation of blue-black precipitate (Sofowora, 1993).

Test for steroid

The methanol extract of *Carica papaya*(0.2g) was added to minimum quantity of chlorofoam, then 3-4 drops of acetic anhydride and 3 drops of concentrated suphuric acid were added and observed for redish brown colour formation (Harborne, 1973).

Test for Saponin

The methanol extract of *Carica papaya* (0.2g) was added to 10ml of water and shake well and observed for presence of a froth which does not break readily upon standing (Trease and Evans, 1989).

Test for Flavonoid

The methanol extract of *Carica papaya* (0.2g) was added to dilute sodium hydroxide. Then few drops of concentrated H_2S0_4 acid was added and boiled the result were observed for a yellow coloration.

Test for Anthraquinone

The methanol extract of *Carica papaya* (2g) was mixed with 2ml of chloroform and 10% ammonium solution the result was observed for the formation of brick red precipitate.

Test for Glycoside

The methanol extract of Carica papaya (0.5g) was mixed with 2m of glacial acetate and 1 drop of ferric chloride solution,and 1ml of concentrated sulphuric acid were added the reaction was observed for a brown ring formation(Trease and Evans, 1989).

Test forPhlobatannins

The methanol extract of *Carica papaya* (0.2g) were boiled with 5ml of 1%HCL.the reaction was observed for the formation of red precipitate (Sofowora, 1993).

4 Assay for Antibacterial Activity

Preparation of media

All the media used were prepared according to the manufacturer's instructions. Briefly, accurately weighed 2.8g of nutrient agar (Hi-media) was dissolved in the 100ml of distilled water. The medium was sterilized under 15Lb pressure for 15minutes in an autoclave. 30ml of this sterilized semisolid nutrient agar medium was poured in pre-sterilized 90mm glass petriplates under aseptic conditions in laminar flow. The plates were allowed to cool at room temperature to solidify the medium.

Preparation of inoculum

Active cultures for experiments were prepared by transferring a loopful of culture to 10 mL of nutrient broth and incubated at 37ºC for 24 hours in a rotary shaker for bacterial proliferation.

Agar-well diffusion method

Agar well bioassay was employed for testing antibacterial activity of the extracts. Each extracts were made to a final concentration of 50 mg/mL. 24 hour old cultures of test organisms (0.05 mL) were seeded onto agar (Media) plate and uniformly spread with a spreader. Wells (5mm) were made in the agar plate with a sterile corn borer. The plant extract was introduced into the well. The plates were then maintained at room temperature for 2 h allowing for diffusion of the solution. All plates were then incubated at 37 0C for 24 hours. Sensitivity was recorded by measuring the clean zone of growth inhibition on agar surface around the disc. The antibacterial assay for each of the extracts against all microorganisms tested was performed in triplicates (Farombi, 2004).

Determination of minimum inhibitory concentration (MIC).

MIC was determined by tube dilution method for each of the test organism in triplicates 20. To 0.5 ml of varying concentrations of the extracts (0 – 280 mg/ml), 2ml of nutrient broth was added and then a loopful of test organism was introduced to the tubes. A tube containing nutrient broth only seeded with the test organisms was served as control. Tubes containing bacterial cultures were

then incubated at 37 ^{0}C for 24 hours. After incubation the concentration at which no visible growth was seen was noted as MBC (Okwu and Josiah, 2004).

Antioxidant activity index determination

Antioxidants tricks of *Carica papaya* extract was assessed based on the 2, 2-diphenyl-2-piciylhydrazyl (DPPH) radical assay as described previously. Briefly, increasing (10 to 250 µg/mL) concentrations of *Carica papaya* extract were prepared and 100 µL of each dilution were mixed with 100 µL of DPPH (100 µg mL-1 in ethanol). Mixture was kept in dark at room temperature for 15 min, and then the absorbance was measured at 517 nm. Standard drug (Ascorbic acid) was used as references.

Percentage of free radical scavenging activity was calculated by the following equation:

$$RSA = \frac{\text{Abs (control) - Abs (sample)}}{\text{Abs (control)}} \times 100$$

The IC_{50} (concentration exerting 50 % inhibition) of extracts and standards was determinate using regression curves in the linear range of concentrations. The antioxidant activity index (AAI) was then calculated as follows:

$$\frac{[DPPH]\ (\mu g/mL)}{IC_{50}\ (\mu g/mL)}$$

[DPPH] = the final concentration of DPPH (Okwu and Josiah, 2004)

5 Statistical Analysis

Data were expressed as means ± standard error of mean (SEM). One-way analysis of variance (ANOVA) was performed to test for differences between the groups mean. Significant differences between the means were determined by Duncan's multiple range test and P values < 0.05 were regarded as significant.

6 Result and Discussion

Table: 1 Qualitative Phytochemical Composition of Aqueous Methanol Seed Extract of Carica papaya

Phytochemicals	Inferences
Alkaloids	+
Flavoinoids	+
Saponins	+
Steroids	-
Total phenols	+
Terpenoids	+
Tannins	+
Glycosides	+
Phlobatannins	-

KEY; **+ = presence. - = Absence**

Table: 2 Quantitative Phytochemical Composition of Methanol Seed Extract of *Carica papayas*

Phytochemicals	Inferences
Alkaloids	0.238±0.01 mg/ml
Flavoinoids	0.133±0.02 mg/ml
Saponins	0.214 ±0.01 mg/ml
Total phenols	0.095 ±0.03 mg/ml
Tannins	3.916±0.01 mg/ml

Table 3: Zone of Inhibition of Methanol Seed Extract of Carica papaya

Concentration (g/ml)	0.2	0.4	0.6	0.8	1.0	Ciprofloxacin (40 mg/ml)
S. typhi	10.00	18.00	23.00	23.00	24.50	26.00
S. aureus	6.00	10.00	10.00	20.00	20.00	24.00
E. coli	4.00	11.00	17.00	21.00	21.00	26.00

Table 4: minimum inhibitory concentration (MIC) of methanol seed extract of Carica papaya

Concentration (g/ml)	0.2	0.4	0.6	0.8	1.0
S. typhi	-	-	0.019	0.128	0.016
S. aureus	-	-	-	0.026	0.003
E. coli	-	-	-	0.026	0.003

Table 5: minimum bactericidal concentration (MBC) of methanol seed extract of *Carica papaya*

Concentration (g/ml)	0.2	0.4	0.6	0.8	1.0
S. typhi	-	-	0.48	2.4	0.4
S. aureus	-	-	-	0.64	0.05
E. coli	-	-	0.48	0.64	0.08

Table 6: DPPH radical scavenging activity of methanol leaf extract of *Carica papaya*

S/NO	Concentration mg/ml	*Carica papaya*	Vitamin E.
1	10	21.24±4.35	31.10±3.45
2	50	25.67±3.45	49.20±5.67
3	100	27.89±3.02	55.20±3.46
4	150	37.60±2.61	56.66±5.43
5	200	38.00±2.05	77.70±8.76
6	250	68.68±3.56	82.22±5.67

Discusion

Phytochemicals are secondary plant metabolites that occur in various parts of plants, they have diverse roles in plants which include provision of vigour to plant; attraction of insect for pollination and feeding defence against predators and provision of colour (Lawal *et al*., 2014). However this phytochemicals elicit varied biochemical and pharmacological actions when ingested by animals (Trease and Evans, 1989).This study revealed the presence of various medically important phytochemicals in *Carica papaya*. The results of this findings agrees with the study of Saha *et al*., (2008), who also reported the presence of these phytochemicals in aqueous extract of *Carica papaya*

Flavonoids are the most diversified groups of phenolic compound found in plant. It biological activity include, antibacterial, anti-inflammatory, anti-allergic, protect against ulcers, vineses and antitumor effect (Okwu and Josiah, 2004). Flavonoids are free radical scavengers, super antioxidant and potential water soluble which prevent oxidative cell, damage and have strong anti-cancer activity (Farombi, 2004). Alkaloid are the most efficient therapeutically significant plant substance pure isolated alkaloids and their synthetic derivatives are use as basic medicinal agent for their analgesis, antispasmodic and

antibacterial effect, Alkaloids has been found to have microbiocidal effect and the major anti-diarrheal effect is probably due to their effects on small intestine and antihypertensive antifungal, antiinflammatory, antifibrogenic effect (Satish *et al*., 2008).

Glycoside has been used for over two centuries as stimulant in cases of cardiac failure and diseases (Trease and Evans, 1978). The presenced of Glycoside also suggests the ability of *Carica papaya* in the treatment and management of hypertension (Taiwo *et al*., 2009). Tannin is non-toxic and can generate physiological responses in animals that consume them. The presence of tannin suggests the ability of *Carica papaya* to play major roles as antifungal, antidiarrheal, antioxidant and antihemorrhoidal agents (Naznin, 2009). The presence of important phytochemical is an indication that *Carica papaya* if properly screened could yield a drug of pharmaceutical significance. (Lawal *et al*., 2014).

The DPPH radicals were widely used to investigate the scavenging activity of some natural compounds. Table 4 shows the results of scavenging DPPH radical ability of methanol seed extract of *Carica papaya*at various concentrations in comparison with same doses of Vitamin E. *Carica papaya*was found to have a antioxidant property as confirmed by DPPH radical scavenging activities. The methanol seed extract of *Carica papaya* extract however, showed dose-dependent DPPH radicals scavenging activity than Vitamin E. The decrease in absorbance of DPPH caused by methanol seed extract of *Carica papaya* is due to the reaction between antioxidant molecules and radical, which results in the scavenging of the radical by hydrogen donation. This finding is in line with the study of Saha *et al*., (2008).The reducing power of a natural compound may serve as a significant indicator of its potential antioxidant activity (Lawal *et al*., 2015).

The antimicrobial activities of methanol seed extract of *Carica papaya* was investigated in this study, Increase in the concentration of the extracts yielded increase in the zones of inhibition. This linear relationship between the concentrations of extracts and zones of inhibition could be that the extracts were able to diffuse into the inoculated nutrient agar. The ability of methanol seed extract of *Carica papaya* to inhibit the growth of *E. coli*, a diarrhoea causing bacteria, explains why it is used in folk medicine to treat diarrhoea in Nigeria and other tropical countries (kumar *et al.*, 2008). The extracts were found highly active in a concentration of 360 mg/ml. this finding agrees with the report of Govindasamy and Kannan (2012) who also find out that anti-bacteria activities of leaf extract was higher at 360 mg/ml. Te antimicrobial activities demonstrated by this plant extract could be linked to its phytochemical constituents especially tannins which has been reported to excert antimicrobial activities (kumar *et al.*, 2008), However, the lack of activities at the dose of 40 and 80 mg/ml of the extract could indicate the low concentration of the antimicrobial phytochemicals at that dose. Therefore, methanol seed extract of *Carica papaya* has shown significant antimicrobial activities.

Conclusion

This study has shown that the methanol seed extract of *Carica papaya* from Nigeria contains some useful potential antibiotic principles that are inhibitory to a some pathogenic organism. Thus, it may be considered as a natural source of antimicrobials for therapeutic purposes.

Further studies to be carried out on determining mechanism of actions of these plant as well as on the isolation of bioactive principle that could be responsible for the observe antimicrobial effects. Also, pertinent scientist and stakeholders should look further into this plant for detailed authentification and subsequent commercialization

References

1. Bashir L, Shittu OK, Sani S, Busari MB, Adeniyi KA. (2015).African Natural Products with Potential Antitrypanosoma Properties: A Review. International Journal of Biochemistry Research & Review. 2015, 7(2): p45-79.
2. Colle CH, Lynes PM, Grange JM. Microbiological Methods (7thEdn.) Butterwort-Heinemann Ltd., Britain, 2003; 175-190.
3. Govindasamy C, Kannan R. Pharmacognosy of mangrove plants in the system of unani Medicine. Asian Pac J Trop Dis. 2012;2(1):S38–S41.
4. De Boer HJ, Kool A, Broberg A, Mziray WR, Hedberg I, LevenforsJJ. Antifungal & Antibacterial activity of some herbal remedies from Tanzania. J Ethnopharmacology, 2005, 96: p 461-469.
5. Essawi T, Srour M. Screening of some Palestinian medicinal plants for antibacterial activity. J Ethnopharmacol 2000; 70: 343-349.
6. Kurian A, Schweizer HP. Bacterial resistance to antibiotics: active efflux & reduced uptake, Advanced Drug Delivery Reviews, 2006; 57: 1486–1513.
7. Kumar M, Agarwala R, Deyb K, Raib V, Johnsonc B. Antimicrobial Activity of Aqueous Extract of Terminalia chebulaRetz. on Gram positive & Gram negative Microorganisms.International Journal of Current Pharmaceutical Research, 2008, 1(1): p 56-60.
8. Lawal B, Shittu OK, Ossai PC, Abubakar AN, Ibrahim AM. Antioxidant Activities of Giant African Snail (Achachatinamaginata)Haemolymph against CCl_4-Induced Hepatotoxicity in Albino Rats.British journal of pharmaceutical research. 2015; 6(3): 141-154.
9. Lawal B, Shittu OK, Kabiru AY, Jigam AA, Umar MB, Berinyuy EB, Alozieuw BU. Potential antimalarials from African natural products: A review. J. Intercultural Ethnopharm.,2014; 4(4): 318-343.
10. Nascimento GGF, Locatelli J, Freitas PC, Silva GL.. Antibacterial activity of plant extracts & Phytochemicals on antibiotic-resistant bacteria. Brazilian Journal of Microbiology, 2000; 31:247-256
11. Naznin AH. In vitro antioxidant activity of methanolic leaves & flowers extracts of Lippiaalba. Nur. Research Journal of Medical Science, 2009; 4(1): 107-11
12. Parekh J, Jadeja D, Cha S. Efficacy of Aqueous & Methanol Extracts of Some Medicinal Plants For Potential Antibacterial Activity.Turkish J Biology, 2005; 29, P 203-210.
13. Saha MR, Ashraful AR, Akter RJ. In-vitro free radical scavenging activity of IxoracoccineaL. Bangladesh Journal ofPharmacology, 2008; 3: P 90-96.
14. Taiwo O, Xu HX, Lee SF. Antibacterial Activities of Extracts from Nigerian Chewing Sticks. Phytotherapy Research 13: 2005; 675-679.

15. Yusuf OK, Bewaji CO. Evaluation of essential oils composition of methanolicAllium sativumextract on Trypanosomabruceiinfected Rats.Res. Pharm Biotechnology, 2011, 3(2): P 17 21.
16. Okwu D.E, C. Josiah. Evaluation of the chemical composition of two Nigerian medicinal plants. African Journal Biotechnol., 2006; 5: 357-361.
17. Trease MT., Evans SE. The Phytochemical Analysis and Anti-bacterial Screening of Extract of Some Common Vegetables. Chemical Science, Nigeria, 1978; 26: 57-58.
18. Farombi EO. African indigenous plants with chemotherapeutic properties and biotechnological approach to the production of bioactive prophylactic agents. Afr J Biotech. 2003; 2:662–671
19. Satish S, Raghavendra MP, Raveesha KA. Evaluation of antibacterial potential of some plants against human pathogenic bacteria. Adv Bio Res. 2008; 2(3–4):44–48. Sofowara AE..Medicinal plants and traditional medicine in Africa. Vol. 2. Spectrum Books Ltd, Ibadan, p. 1993; 288.