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ANTIMICROBIAL AND PHYTOCHEMICAL STUDIES OF CARICA PAPAYA LEAVES AGAINST ESCHERICHIA COLI AND STAPHYLOCOCCUS AUREUS.

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ABSTRACT

The bioactive compound of Carica papaya leaves were extracted using methanol and hexane. They were investigated for their antibacterial activity against some selected organism, Escherichia coli and Staphylococcus aureus. Methanol extracts of Carica papaya showed higher zones of inhibition than hexane extract. Significant ($p < 0.05$) zones of inhibition were recorded as ranging from 20.0mm-28.00mm for E. coli at concentrations of 20-80mg/ml. They were higher than the zones of inhibition for S. aureus with zones of inhibition of 16.0mm-22.0mm at concentration of 20-80mg/ml. Preliminary phytochemical analysis showed that methanol extracts contained alkaloids, tannin, flavonoid, glycoside and saponin at a higher amount than hexane extracts. The minimum inhibitory concentration varied between 3.125 and 12.5mg/ml for methanol and hexane extracts respectively. From the study it was observed that antibacterial properties of Carica papaya could support its use as microbial agent.

KEYWORD: Carica papaya, methanol, extract, hexane, phytochemicals, microorganisms.

INTRODUCTION

The plant kingdom holds an inexhaustible source of active ingredients and the importance of herbs in the treatment of human aliments cannot be over emphasized. The use of herbs dates back to the time of the early man. It can be used in their raw and cooked forms and its use has been known and accepted by most nations and has been known as the first art of treatment available to men (Kafari, 1994). Active components exist in different structures of plants and

in varying concentrations. The therapeutic parts of plants are highly concentrated and they consist of woods, flowers, fruits, leaves, stem, bark, root, or the seed (Ifesan, 2013). *Carica* papaya belongs to the family Caricacea. The plant is a native of tropical America. The plant is known for their therapeutic purposes. They are antibacterial, cardiotonic, analgesic and amebicidal. *Carica papaya* contains biologically active compounds such as chymopapain and papain which aids in digestion (Barger *et al*., 2009). The leaf, bark and twig tissues posses annonaceous acetogenins which posses highly anti-tumor and pesticidal properties (Mc Langhlin, 1992). The unripe fruit are used as a remedy for ulcer and impotence (Elizabeth, 1994). The green unripe pawpaw are antiseptic, cleansing the intestines from bacteria and enabling the intestine to absorb vitamin and minerals especially vitamin B_{12} (Mantok, 2015). Brown, yellow and green leaves of Carica papaya at different stages of development are known to be rich in vitamins such as thiamine, riboflavin and ascorbic acid. Minerals such as calcium, magnesium, sodium, potassium and iron are present in considerable amounts. Studies on phytochemical analysis showed that leaves contained saponins, cardiac glycosides and alkaloids (Ayoola and Adeyeye, 2010). The presence of the secondary metabolites such as alkaloids, flavonoids, saponins and tannins are reported to be potent free radical scavengers and are antimicrobial in action (Ifesan, 2013). Pharmaceutical companies and research institutions are constantly in search for newer antibiotics to combat the emerging and re-emerging resistant infectious agents. The present work was carried out to determine the presence of phytochemicals in Carica papaya leaves and predict its antibacterial properties. This could support its use in place of synthetic drugs.

MATERIAL AND METHOD

2.1 Sample preparation

Fresh Carica papaya (pawpaw) leaves were obtained from Agbani farm in Enugu and authenticated in the department of crop science, Enugu State University of Science and Technology, Nigeria. They were properly washed in tap water and rinsed with distilled water. The leaves were dried at room temperature and pulverised with a blender under aspetic conditions and used for extraction. A total of 110gm of powdered pawpaw leaves were soaked separately in 250ml each of the n-hexane and methanol. The extracts were kept in orbital shaking incubator for 3 days and then centrifuged to remove debris. Clear solvent extracts were collected and the solvent evaporated using rotavapour (BUCHI, India) to get the concentrated residue of the solvent, and dissolved component of the plant material. The extracts were kept at 4^0C for further analysis.

2.2 Test Organisms

The test organisms used in this research consist of both Gram-positive and Gram-negative bacteria namely, *Escherichia coli* and *Staphylococcus aureus.* These organisms were obtained from the clinical laboratory, University of Nigeria Teaching hospital, Ituku- Ozalla, Enugu Nigeria.

2.2 Phyotchemical profiling

The extract of Carica papaya leaves were tested for the presence of alkaloid, flavonoid, saponin, tannin and glycoside using the standard procedure described by Tang (2005).

Phytochemical analysis of Carica papaya

Sample A- Methanol extract

Sample B - Hexane extract.

Qualitative test for tannin

2ml of the different extracts, sample A and B were measured into different test tubes, 0.5ml of Ferric chloride ($FeCl_3$) were added and shaken.

Qualitative test for flavonoid

1ml of the different extracts were measured in different test tubes, then 2 drops of each of NaOH solution, $AlCl_3$ and conc. H_2SO_4 were added and shaken vigorously.

Qualitative test for Saponin

2mls of the different extracts A and B were measured in different test tubes and 3mls of distilled water were added and shaken vigorously.

Qualitative test for Glycoside

5mls of distilled water were added to 2ml of the extract sample A and B in different test tube. Then 2mls of H_2SO_4 was also added to the mixture. The mixtures were brought to boil in a water bath for 15 mins and allowed to cool. Then they were neutralized with 20% KOH. Then 1ml of Fehling solution A and B were added and boiled for another 15 minutes.

Qualitative test for Alkaloid

2mls of the different extract A and B were measured in different test tubes and 2mls of 1% of HCL were added and filtered. Then 3 drops of Mayer's reagent were added and observed for change.

2.3 Antimicrobial sensitivity Test (Agar Diffusion method)

The standard dilution of 10^6 cfu/ml of the innoculum was seeded evenly onto the surface of Mueller-hinton agar plates (Oxoid, England) in triplicates with a sterile swab (Hugo and Russel, 1998). Using a sterile 6mm diameter cork borer, 3 wells were made in the agar onto which 0.1m1 of appropriate concentration (20, 40, 80 mg/ml) of methanol and hexane extracts were added as well as the standard drug (ampicillin) which served as the control. The plates were incubated at 37^0C for 24h and the zones of inhibition measured in millimetres to determine antimicrobial activity.

2.4 Determination of Minimum Inhibition Concentration (MIC) and Minimum Bacterical concentration (MBC)

The MIC of the extract was determined using broth dilution technique. The MIC determines the least concentration of extract and antibiotics necessary to inhibit growth of a standardized innoculum under defined conditions (Goe *et al*., 2001). Serial dilution of the extraction $1x10^1$–$1x10^8$ in liquid medium were prepared. 4ml of sterile nutrient broth and the dilution of the innoculum with a visual density equivalent to 10^6 cfu/ml, which corresponded to 0.5 Macfarland standard were employed in the study (Adebayo *et al*., 1989). 1ml of each extract was added to sterile tubes containing nutrient broth to give a final concentration of 0.5mg/ml to 12.5mg/ml. Using a volumetric pipette, 0.1ml of the test organisms were added to each of the tubes. The tubes containing bacterial cultures were incubated at 37^0C for 24h. The tubes were read macroscopically to determine the lowest concentration of CPY that did not permit any visible growth. Tetracycline served as positive control for the test organism.

Minimum bacterial concentration was determined for each set of test tubes used for the MIC test. A loopful of the broth culture was collected from those test tubes which showed no growth and was inoculated onto sterile nutrient agar by streaking. The test organisms were also streaked on the plates which contained only agar and was incubated at 37^0C for 24hrs. MBC was determined when no visible growth was seen.

RESULT

Qualitative test for phytochemicals from Carica papaya leaves

The standard qualitative test for phytochemicals from powdered *Carica papaya* leaves using their specific reagents were proven by their reactions and showed the presence of alkaloid, tannin, flavonoid, saponin and glycoside (Table 1).

Table 1: Qualitative test for phytochemicals from Carica papaya leaves.

Plant material	Test	Reagent Used	Colour change	Result
Carica papaya	Alkaloid	Mayer's reagent	Cream yello ppt	+
Carica papaya	Tannin	$FeCL_3$	Greenish	+
Carica papaya	Flavonoid	$NaOH_+AlCl_3+H_2SO_4$	Yellow ppt	+
Carica papaya	Saponin	Distilled water	Pesistant foam	+
Carica papaya	Glycoside	Distilled, H_20, H_2SO_4, Handfehling solution	Brick red ppt	+

Keynote + = positive confirmation.

Presence of phytochemicals in Carica papaya leaves extract using different solvents

The qualitative analysis of Carica papaya leaves of hexane extract showed the presence of alkaloid. Flavonoids, alkaloids, glycoside, tannin and saponin where present in methanol extract. The methanol extract provided a significant efficacy than the hexane preparation (Table 2).

Table 2: Presence of phytochemicals in *Carica papaya* leaves extract using different olvents.

Phytochemcials	Methanol	Hexane
Glycoside	-	-
Alkaloid	++	+
Tannin	+++	-
Flavoniod	-	-
Saponin	++	-

Keynote = absent, + = Intermediate, ++ = sensitive, +++ = highly sensitive.

Antibacterial activity of test agents

The results of the antimicrobial activity of the extracts against the extracts against the test organisms, namely, *E.* coli and *S.* aureus are shown in Table 3. The extracts showed varying degrees of growth inhibition against the isolates. The mean zones of inhibition of growth of isolates are functions of relative antimicrobial activity of the extracts. The methanol extracts of Carica papaya showed higher growth inhibition of 28.0mm and 22.0mm for *E. coli* and *S.* aureus respectively at 80mg/ml. Reduced sensitivity were observed with hexane. Methanol extract of Carica papaya produced significant ($p<0.05$) effect when compared with tetracycline.

Table 3: Antibacterial activity of test agents.

Microorganism/Drug Concentration (mg/ml)	Extracts/Mean zones of Inhibition(mm)		
	Methanol	Hexane	Ampicillin
Escherichia coli			
20	20.0 ± 2.68*	0.60 ± 1.77	24.0± 2.15*
40	26.0 ± 3.08	0.80 ± 2.11	26.0± 2.41
80	28.0 ± 1.81	2.0 ± 2.12	23.0 ± 2.68
Staphylococcus aureus			
20	16.0 ± 2.12*	__	18.7± 1.40*
40	18.0 ± 2.48	__	20.0 ± 3.35
80	22.0± 3.56	2.0 ± 1.50	26.0 ± 4.8

***p < 0.05 when compared with control.**

Minimum inhibitory concentration (MIC) of Carica papaya extract

The result of the MIC of the extracts against the test isolates are shown in Table 4. The MIC varied between 3.125 and 12.5mg/ml for methanol and hexane extracts respectively. Higher concentrations of the hexane extract were needed to inhibit the test organisms.

Table 4: Minimum inhibitory concentration (MIC) of *Carica papaya* extract.

Microorganism	*Carica papaya* concentration (mg/ml)					
	50MET HEX	25MET HEX	12.5MET HEX	6.25MET HEX	3.125MET HEX	1.56MET HEX
Escherichia coli	__ __	__ __	__ __	__ +	_ +	+ +
Staphylococcus aureus	__ __	__ __	__ __	__ +	_ +	+ +

MET, HEX = methanol, hexane extract respectively:-= no growth,+= growth.

DISCUSSION

The results of the study showed that qualitative analysis of *Carica papaya* extracts showed the presence of alkaloids, flavoniods, saponins, glycosides and tannins in the leaves of papaya (Table 1). This is in agreement with the study of Ayoola *et al.*, *(*2010) who found out that the leaves of papaya contained saponins, cardiac glycosides and alkaloids. This study is also in agreement with the study of Akhila *et al*., (2015) who found phyto compounds, alkaloids, phenolics, flavonoids and also amino acids in *Carica papaya* leaves. This study also matched the results obtained by (Ikeyi *et al*., 2013; Bhushan and Mrina, 2016 and Marshall *et al*., 2015). Secondary metabolites like alkaloids, flavonoids, phenolic compounds are responsible for various biological activities such as antioxidants and antimicrobials.

Bhadane et al., 2014 and Sheikh and Krishnamurthy, 2013 reported super oxide scavenging activity of Carica papaya leaf extract. The metabolites such as phenolics and flavonoids have been reported to be potent free radical scavengers. They have been reported in all parts of plant such as leaves, fruits, seeds, root and barks by Natrajan et al., 2000 and Ifesan, 2013. The study of Imaga et al., 2010 reports that flavonoids and glycosides are antioxidants and Bhadane et al., 2014 showed that the highest antioxidant activity through β-carotene bleaching assay was observed in unripe fruit followed by young leaves, ripe fruit and the seed of Carica papaya. The presence of glycosides and flavonoids in papaya leaves in the study could suggest its antioxidant activity. From this study the methanol extract revealed the presence of alkaloids, tannins, glycoside, flavonoids and saponin while hexane extract showed the presence of only alkaloids (Table 2). The study reveals that methanol is a better solvent for extraction of Carica papaya leaves. Topuriya et al., 1978 reported that the active ingredients vary from one extract to another, which could be due to differences in solubility of the active compounds. The results of this study showed that the extracts of Carica papaya inhibited the growth of the bacterial isolates tested (Table 3) and were found to possess anti bacterial activity which supports the work of Doughari et al (2007). The methanol extracts showed good antibacterial activity with zone of inhibition greater than or equal to 10mm indicating good antibacterial activity (Kujumgier et al., 1999). Significant ($p < 0.05$) zones of inhibition were recorded as ranging from 20.0mm-28.0mm for E.coli at concentrations of 20-80 mg/ml. They were higher than the zones of inhibition for Staphylococcus aureus with zones of inhibition from 16.0mm-22.0mm at concentrations of 20-80mg/ml. From the study, the antimicrobial activity of the methanol extract compared well with the standard drug ampicillin. This is in agreement with the study of Okunola et al., (2012) who revealed very significant antimicrobial activity with the extracts of Carica papaya demonstrating broad spectrum of activity against E. coli and S. aureus. This study is also in agreement with the study of Marshall et al., 2015 who found the plant as having broad spectrum activity against the test isolates with varying zones of inhibition ranging from 16.0mm and 18.4mm for S. aureus and E. coli respectively. Baskaran et al., 2012 also reported antimicrobial activity of C. papaya leaf extracts against various bacteria and fungi. MIC and MBC values (Table 4) were varied between 3.125 and 6.25mg/ml for methanol and hexane extracts; thus, indicating that evaluation of MIC is sufficient for measuring bactericidal activity (Natrajan et al., 2000). The results of MIC showed that ethanol extracts were more active against the test organisms even at low concentrations. Based on the limited spectrum of activity of hexane extracts

when compared with the methanol extracts, it suggests that the active component is more soluble in methanol than in hexane.

From the study the dried leaf extracts were active against both Gram-negative and Gram-positive bacteria which may suggest a broad spectrum of activity and is significant because of the possibility of incorporating its use against multidrug resistant organisms. This is in accordance with the reports of Doughari et al. (2007) who indicated the need of developing therapeutic substances that may be more active against multidrug-resistant organisms. The present work has shown phytochemical compositions and antimicrobial activity of Carica papaya leaf extracts confirming the great potential of bioactive compounds. This supports the use of this plant in herbal medicine.

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