Determination of Phytoconstituents and Antimicrobial Analysis of the Ethylacetate Extract of *Carica Papaya* Seed

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***Abstract*:-*Carica papaya* (pawpaw) belongs to the family of *Caricaceae* with over 22 species. It is a large perennial herb with a rapid growth rate. Different parts of the plants are used to treat several diseases such as ulcer, diabetes, malaria, hypertension and skin diseases. powdered papaya seeds (70 g) was macerated with 150 mL ethyl acetate to obtain 15 g extract. Phytochemical and antimicrobial studies of the extract were carried out based on standard procedures. Preliminary phytochemical screening of the extract revealed presence of saponins, alkaloids, tannins, Flavonoids, triterpenoids, reducing sugars, glycosides and steroids and absence of anthraquinone. Presence of these phytochemicals were further confirmed and quantified by Gas Chromatography Flame Ionzation Detector (GC-FID). GC-FID revealed presence of compounds such as catechin (36.8538 µg/g) quinine (16.6331 µg/g), kaempferol (1.5637 µg/g) and rutin (5.9840 µg/g) etc. The extract was active against disease-causing microbes such as *Vancomycinresistantenterococci (VRE), Proteusmirabilis, Coniophoraputeana, Fomitopsispinicola, Fusariumoxysporum, Scloratiumrolfsii Candidaalbicans,* and *Candidakrusei* with inhibition zones that ranged between 16 mm and 22 mm. Minimum inhibitory concentration MIC was 10 mg/mL against the sensitive pathogens except for *C. albicans* and *Scloratium rolfsii*, which was 5 mg/mL. Minimum bactericidal concentration (MBC) was 40 mg/mL and 20 mg/mL against *VRE* and *Proteusmirabilis*, respectively. Minimum fungicidal concentration (MFC) of extract against *Candida krusei, Coniophora puteana, Fomitopsispinicola* and *Fusarium oxysporum* was 40 mg/mL. The MFC of *C. albicans* and *Scloratium rolfsii* was 20 mg/mL. The present study showed that ethyl acetate extract of *C. papaya* is rich in phytochemicals and has potential antimicrobial activities.**

***Key words*: phytochemicals, antimicrobial, *Caricapapaya*, GC-FID.**

I. INTRODUCTION

Medicinal plants have been used in virtually all cultures as a source of medicine, food flavors and conservatives, to treat health disorders and to prevent diseases. Active components produced during secondary metabolism are usually responsible for the plants biological activities. Nowadays, antimicrobial activities of numerous plants have been carried out to confirm the use of these plants in treatment of several diseases traditionally (Singh, 2015).

Carica papaya Linnaeus, (pawpaw), belongs to the family of Caricaceae with over 22 species while only one member of the genus *carica* is cultivated as fruit tree. Papaya is not a tree but an herbaceous succulent plants that possess self-supporting stems. *Caricapapaya* is believed to have originated in the lowlands of Eastern Central America, from Mexico to Panama. Papaya plant is grown in tropical and subtropical countries, which includes 57 counties like India, Brazil, Indonesia, Mexico and Nigeria. Among these countries India is the largest producer of papaya. It is a large perennial herb with a rapid growth rate. The plants are usually short-lived, but can produce fruit for more than 20years. Reproduction of papaya is complicated as the plant can be male, female or hermaphroditic. The male is usually uncommon (Ayoola and Adeyeye, 2010; Oyeleke *et al*., 2013; Basalingappa *et al*., 2018).

The black seeds of the papaya are edible and have a sharp, spicy taste. They are sometimes ground and used as a substitute for black pepper. Dried papaya seeds actually look quite similar to peppercorns and can be used in just the same way. Grinding a couple over a meal, especially protein rich meals, is a simple way to add extra enzymes to your diet and improve your digestive health. The papaya seeds are very pungent and peppery, making them almost unpalatable. However, the seeds seem to have more potent medicinal values than the flesh (Jyotsna *et al*., 2014).

C. papaya seeds poses several pharmacological activities including anthelmintic, antifertility, contraceptive, anti-inflammatory, analgesic and antimicrobial property (Agarwal *et al*., 2016) The seed extract is used as vermifuge, thirst quencher, or pain alleviator. It is also used against internal worms when chewed (Bergonio and Perez, 2016). Other pharmacological uses of papaya seeds include carminative, emmenagogue, abortifacient, counterirritant, as paste in ringworm disease, psoriasis, anti-fertility agent in males. Seed juice also used to treat bleeding, piles and in large liver and spleen (Roshan *et al*., 2014). Papaya seeds protect fibroblasts from H_2O_2-induced stress due to the antioxidant activity of the water extract (Panzarini *et al*., 2014). *C. papaya* seeds are also used in the treatment of hypertension, diabetes mellitus and hypercholesterolemia.

Ocloo *et al*. (2012) carried out antibacterial activity of dried papaya seed against *S. aureus* (gram positive), *E.coli* (gram

positive) and Shigella flexneri (gram negative) using the disc diffusion method. Jyotsna *et al*. (2014) investigated the antibacterial activities of aqueous and methanolic extracts of papaya seeds using agar well diffusion method. The bacteria used included *Staphylococcus aureus, Pseudomonas aeruginosa, E. coli and Salmonella typhi.* It was revealed that the aqueous as well as the methanolic extract of seeds were effective to inhibit the pathogens.

Phytochemical characterization conducted by Ocloo *et al*. (2012) revealed that *C. papaya* seeds contained flavonoids, tannins, reducing sugars, alkaloids, phenols, saponins, and terpenoids in organic and aqueous extract of dried seeds of papaya.

The seeds of papaya are reported to contain crude proteins, crude fibre, fatty acids, papaya oil, carpaine, benzylisothiocynate, benzylthiourea, glucotropacolin, benzylglucosinolate, hentriacontane, β-sistosterol, caricin and an enzyme nyrosin (Natarjan*et al*., 2014; Nwofia *et al*., 2012)

Oyeleke *et al*. (2013) quantitatively determined the micronutrients; zinc, iron and manganese by spectrophotometric methods using Atomic Absorption Spectrophotometer. The study involved unripe, ripe and overripe papaya seeds. It was revealed that, all the samples contained almost similar amount of the micronutrients. This present study examined the phytochemicals present in the seed of *Carica papaya*. Specifically, qualitative and quantitative methods of determining the presence or absence of phytochemicals as well as the antimicrobial potency of the sample was investigated in this study.

II. MATERIALS AND METHODS

Plant Collection

The fresh seeds of *C. papaya* were collected from Bunu town in Tai L.G.A of Rivers State in the month of March, 2019. The seeds were thoroughly washed with water and left to dry under shed. The dried sample was ground to powder for extraction and further analyses.

Sample Extraction

The powdered sample, 70 g was macerated with 150 mL ethyl acetate for 48 hours. The mixture was then filtered and allowed to evaporate under room condition to obtain crude extract for the phytochemical screening and antimicrobial studies. About 3 g was put in glass vial and was later used for quantification of phytochemicals by GC-FID (Nna *et al*., 2018).

Qualitative Phytochemical Analysis

Preliminary phytochemical screening was carried out according to standard procedures described by (Sabri *et al.*, 2012; Satheesh *et al.*, 2012).

Test for steroids and triterpenoids (Liebermann-Burchard test)

About 3 mg of the extract was mixed with 3 drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was then added from the sides of the test tube and observed for the formation of a brown ring at the junction of two layers. Green coloration of the upper layer and the formation of deep red colour in the lower layer would indicate a positive test for steroids and triterpenoids, respectively.

Test for cardiac glycosides (Keller-Killiani Test)

About 3 mg of the extract was mixed with 3 drops of conc. glacial acetic acid and diluted ferric chloride solution and mixed. Concentrated sulphuric acid was added, and observed for the formation of two layers. Lower reddish brown layer and upper acetic acid layer which turn bluish green would indicate a positive test for glycosides.

Fehling's solution test for reducing sugars

About 1 mg of an extract was taken into a dry test tube and 5 cm^3 of ethyl acetate was added and shaken for 5 minutes. The extract was filtered. About 5 cm^3 of a mixture (1:1) of Fehling's solutions A and B was added to 2 cm^3 of the ethyl acetate extract in a test tube and boiled on water bath for 5 minutes. A brick-red precipitate would indicate presence of reducing sugar.

Test for phenolics and tannins (ferric chloride test)

About 2 mg each of the crude extract was dissolved in 2 mL of solvent of extraction and treated with 4 drops of ferric chloride solution. Formation of bluish black colour would indicate the presence of phenols. Generally, the formation of bluish-black colour would indicate the presence of gallic tannins and bluish-green would indicate the presence of cathechic tannins.

Test for flavonoids (alkaline test)

About 5 mg of the extract was added 5 mL of diluted sodium hydroxide solution. The appearance of yellow colour which would become colourless on addition of few drops of dilute hydrochloric acid would indicate the presence of flavonoids.

Test for saponins.

The ability of saponins to produce frothing in aqueous solution and to haemolyse red blood cells was used as screening test for these compounds (Deniz, 2009). Distilled water (5 mL) was added to the extract (5 mg) and strongly shaken in a test tube. Formation of a large amount of froths that would last for about 30 minutes indicated the presence of saponins.

Test for alkaloids.

About 3 mL of an extract was mixed with 1 mL of 10 % HCl in a test tube and heated for 20 minutes. This was allowed to cool and filtered; 1 mL of the filtrate was treated with few

drops of Mayer's reagent. Appearance of creamy precipitate would indicate the presence of alkaloids.

Test for anthraquinone.

To 1 mg of an extract in a test tube, 10 cm^3 of benzene was added and shaken for 5 minutes, filtered using filter paper and 5cm^3 of 10 % ammonia solution was added to the filtrate and shaken. The presence of a pink, red or violet colour in the ammoniacal (lower) phase would indicate presence of free anthraquinone.

Quantification of Phytochemicals by GC-FID

The quantitative analysis of phytochemicals was performed on a BUCK M910 Gas chromatography equipped with a flame ionization detector. A RESTEK 15 meter MXT-1 column (15 m x 250 µm x 0.15 µm) was used. The injector temperature was 280 ^{0}C with splitless injection of 2μl of sample and a linear velocity of 30 cmS^{-1}. Helium 5.0pa.s was the carrier gas with a flow rate of 40 m/min^{-1}. The oven operated initially at 200 ^{0}C, was heated to 330 ^{0}C at a rate of 3 $^0C\ min^{-1}$ and was kept at this temperature for 5 min. The detector was operated at a temperature of 320 ^{0}C.

Phytochemicals were determined by the ratio between the area and mass of internal standard and the area of the identified phytochemcals. The concentrations of the different phytochemicals were expressed in µg/g (Emejulu *et al*., 2017).

Antimicrobial Screening

The antimicrobial activity of the plant extracts was determined using some pathogenic microbes. The microbes were obtained from the Department of Medical Microbiology, University of Ibadan. The extract (0.1 g) was weighed and dissolved in 10 mL of DMSO to obtain a concentration of 10 mg/mL. This served as initial concentration for determination of the antimicrobial activity. Agar diffusion method was used for screening the extracts.

Mueller Hinton Agar (MHA) was medium used for growth of microbes. The media were prepared according to manufacturer instruction, sterilized at 121 °C for 15 minutes, poured into sterile Petri dishes and allowed to cool and solidify. The sterilized media were then seeded with 0.1 mL of the standard inocula of test microbes. Inocula were spread evenly over the media surfaces by the use of sterile swabs. Using a standard cork borer (6 mm), a well was cut at the center of each inoculated medium. Solution of the extract (0.1 ML) of concentrated 30 mg/mL was then introduced into each well on the medium. The inoculated medium was then incubated at 37 °C for 24 hours for bacteria and at 30 ^{0}C, for 7 days for fungi, after which each plate was observed for inhibition zone of growth. Zone of inhibition was measured with a transparent ruler and the result recorded in millimeters as described by Tor-Anyiin *et al*. (2015) and Linus *et al*., (2016).

III. RESULTS AND DISCUSION

Extraction and preliminary phytochemical screening

Extraction of powdered sample (70 g) of papaya seed with ethyl acetate yielded a smooth and brown extract that weighed 15 g (Table 1) with percentage yield of 21.4 %. Phytochemical screening of the stem of *C. papaya* seeds was conducted and the result is presented in Table 2. This study showed presence of saponins, alkaloids, tannins, Flavonoids, triterpenoids, reducing sugars, glycosides and steroids. Anthraquinones were however not detected. This result can be compared to that published by Tarik *etal*. (2015). The presence of these phytochemical could be responsible for activities of the plant extracts against some microorganism. The qualitative analysis of the extract was further confirmed by the quantitation of the metabolites by GC-FID (Table 3).

Table 1: Extraction Result of Papaya Seed

Extract	Weight (g)	Percentage yield	Texture	Colour
Ethyl acetate	15	21.4	smooth	Brown

Table 2: Preliminary Phytochemical Screening of Papaya Seed Extracts

Phytochemical Components	Remark
Steroids	+
Flavonoids	+
Alkaloids	+
Tannins	+
Saponins	+
Glycosides	+
Reducing sugars	+
Triterpenoids	+
Anthraquinones	-

Key: - = Absence; + = Presence

Quantitation of Phytochemicals

The quantitative phytochemical analysis of the powdered seed of *C. papaya* (Table 3) showed how much of the metabolites present in the plant. Flavonoids were the highest among the metabolites detected in the sample. Catechin was found to be the highest component of the seed with quantity of 36.8538 µg/g representing 23 % of the total phytochemicals quantified. Catechin is a flavanol polyphenol compound that is found in tea coffee and several fruits (Padam *etal*., 2013). Catechins regulate blood lipid metabolism. They play an important role in the improvement of the vascular endothelial environment and in the prevention of dysfunction. Catechins work by eliminating excessive active free radicals in order to prevent the oxidation of lipids in the human body (Chen *et al*., 2016). Another flavonoid detected in high quantity was flavan-3-ols (22.3255 µg/g), representing about 14 % of the total phytochemicals detected. Flavan-3-ols are widely distributed in foods and supplements such as cacao, beans, red wine,

beer, berries, apples, black soya bean and French maritime pine bark(Liu *et al*., 2010; Osakebe, 2013). It has been suggested that flavan 3-ols have a positive influence on human health, due to antioxidant, anti-inflammatory, and anti-thrombotic effects (Aron and Kennedy, 2008; Osakebe, 2013).

Other flavonoids quantified were protoanthocyanin (5.5448 µg/g), rutin, (5.9840 µg/g), resveratol (5.3311 µg/g), naringin (4.6117 µg/g), flavonones (3.5409 µg/g), Kaempferol (1.5637 µg/g), flavones (3.4903 µg/g) and naringenin (1.0672 µg/g). The meaningful quantities of these flavonoids especially epicatechin anthocyanin could be responsible for the reported antioxidant activities of this plant.

Alkaloids detected in the sample are quinine and ribatinidine. Quinine had a significant quantity (16.6331 µg/g) representing approximately 10.2 % of the total phytochemicals detected. The quantity of ribatidine was 6.7163 µg/g (4.2 %).

Sapogenin contributed to about 16.2 % (25.9386 µg/g) of the phytochemicals present. Saponins have several medicinal purposes such as antimicrobial, anti-tumor, hepatoprotective, haemolytic anti-insect and anti-inflammatory activities. They are also known to decrease blood cholesterol level and may be used as adjuvant in vaccines (Eskandar and Somayeh, 2015).

Other phytochemicals detected from papaya seed were tannins (6.0671 µg/g), steroids (9.9460 µg/g), phenol (3.5384 µg/g) and phytate (µg/g 0.8209) (Table 3).

Table 3: Quantitation of Phytochemicals by GC-FID

Phytochemical	**Class**	**Quantity (µg/g)**	**Percentage**
Protoanthocyanin	Flavonoid	5.5448	3.5
Rutin	Flavonoid	5.9840	3.7
Ribalidine	Alkaloid	6.7163	4.2
Quinine	Alkaloid	16.6331	10.2
Flavol-3-Ol	Flavonoid	22.3255	14.0
Sapogenin	Saponin	25.9386	16.2
Phenol	Phenol	3.5384	2.2
Flavonones	Flavonoid	3.5409	2.2
Steroids	Steroids	9.9460	6.2
Kaempferol	Flavonoid	1.5637	1.0
Phytate	Phenol	0.8209	0.5
Resveratol	Flavonoid	5.3311	3.3
Flavones	Flavonoid	3.4903	2.2
Naringenin	Flavonoid	1.0672	0.7
Tannin	Tannins	6.0671	3.8
Naringin	Flavonoid	4.6117	2.9
Catechin	Flavonoid	36.8538	23.0
Total		**160.0064**	

Table 4: Sensitivity and Inhibition Zones (mm)

Test organisms	**Extract**	**Spirofloxacin**	**ciprofloxacin**	**Ketoconazole**	**Fulcin**
MRSA	R	S(30)	R		
VRE	S(20)	S(29)	S(30)		
Staphylococcus aureus	R	S(32)	S(26)		
Escherichi coli	R	R	S(37)		
Proteus mirabilis	S(21)	S(32)	S(30)		
Psedomonas aeruginosa	R	R	S(25)		
Salmonella typhi	R	R	S(40)		
Candida albicans	S(22)			S(33)	S(32)
Candida krusei	S(20)			S(34)	R

Candida stellatoidea	R			S(35)	S(30)
Aspergillus fumigatus	R			R	S(28)
Aspergillus nigre	R			R	S(26)
Coniophora puteana	S(18)			S(23)	R
Estrophoria raclentii	R			R	S(28)
Fomitopsis pinicola	S(16)			S(27)	S(30)
Fusarium oxysporum	S(18)			R	S(27)
Fusarium proliferatum	R			S(28)	S(26)
Rhizopus sp.	R			R	S(32)
Scloratium rolfsii	S(20)			S(25)	R
Serpula lacrymans	R			S(26)	S(30)

Key: R= Risistant, S = Sensitive, *MRSA= Methicillin resistant staphylococcus aureus*, *VRE = Vancomycin resistant enterococci*

Table 5: Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) of Papaya Seeds

Test organisms	**MIC**						**MBC and MFC**					
	40 mg/mL	**20 mg/mL**	**10 mg/mL**	**5 mg/mL**	**2.5 mg/mL**	**1.25 mg/mL**	**40 mg/mL**	**20 mg/Ml**	**10 mg/mL**	**5 mg/mL**	**2.5 mg/mL**	**1.25 mg/Ml**
MRSA												
VRE	-	-	ox	+	++	+++	O*	+	++	++	+++	+++
Staphylococcus aureus												
Escherichi coli												
Proteus mirabilis	-	-	ox	+	++	+++	-	O*	+	+	++	+++
Psedomonas aeruginosa												
Salmonella typhi												
Candida albicans	-	-	-	Ox	++	++	-	O*	+	+	++	+++
Candida krusei	-	-	ox	+	++	+++	O*	+	++	++	+++	+++
Candida stellatoidea												
Aspergillus fumigatus												
Aspergillus nigre												
Coniophora puteana	-	-	ox	+	++	+++	O*	+	++	++	+++	+++
Estrophoria raclentii												
Fomitopsis pinicola	-	-	ox	+	++	+++	O*	+	++	++	+++	+++
Fusarium oxysporum	-	-	ox	+	++	+++	O*	+	++	++	+++	+++
Fusarium prokforatum												
Rhizopus sp.												
Scloratium rolfsii	-	-	-	Ox	+	++	-	O*	+	++	++	+++
Serpula lacrymans												

Key: - = No colony growth; ox = MIC; O* = MBC/MFC; + = scanty colony growth; ++ = moderate colony growth; +++ = heavy colony growth.

Antimicrobial activity of papaya seeds

Tables 4 and 5 showed the bioassay results of*C. papaya* seed extract. A total of twenty (20) pathogens consisting of seven (7) bacteria and thirteen (13) fungal strains were used for the experiment. Only two bacteria strains *Vancomycinresistantenterococci* and *Proteusmirabilis* were sensitive to extract of papaya seed with inhibition zones of 20 mm and 21 mm, respectively. The fungi strains that were sensitive to extracts are *Coniophoraputeana*, *Fomitopsispinicola*, *Fusariumoxysporum*, *Scloratiumrolfsii Candidaalbicans*, and *Candidakrusei* with inhibition zones that ranged between 16 mm and 22 mm. The most sensitive was *Candidaalbicans* (22 mm) and the least sensitive was *Fomitopsispinicola* (16 mm).

The papaya seed extract exhibited minimum inhibitory concentration MIC of 10 mg/mL against the sensitive pathogens except for *C. albicans* and *Scloratium rolfsii*, which was 5 mg/mL.

Similarly, minimum bactericidal concentration (MBC) of extract against Vancomycin resistant enterococci was 40 mg/mL and that of *Proteusmirabilis* was 20 mg/mL. Minimum fungicidal concentration (MFC) of extract against *Candida krusei*, *Coniophora puteana*, *Fomitopsispinicola* and *Fusarium oxysporum* was 40 mg/mL. The MFC of *C. albicans* and *Scloratium rolfsii* was 20 mg/mL.

Evaluation of antimicrobial activities of seed extract of papaya showed that *Proteusmirabilis*, *C. albicans* and *Scloratium rolfsii* were most susceptible. *Scloratium rolfsii* is a parasite of stem bases, roots, leaves and fruits. It is commonly parasitic on plants such as pepper, sweet potato, tomatoes, carrots, watermelon and eggplants etc. lesions of circular spots on sweet potato roots are usually caused by *Scloratium rolfsii.* Thus it can be suggested that a formulation of papaya seed can be used to treat diseases caused by these pathogens.

IV. CONCLUSION

Ethyl acetate extract of *C. papaya* seeds exhibited promising antimicrobial activities which is attributable to secondary metabolites present in the seeds. Some fungal and bacteria strains were susceptible while others were resistant to extract. *Proteusmirabilis* and *Vancomycinresistantenterococci* were highly inhibited. Seeds of the plant can be used to treat health complications such as urinary tract infection and sepsis caused by *Proteusmirabilis* and *Vancomycinresistantenterococci*, respectively. Candidiasis could be treated using extracts of papaya seeds.

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