

IN-VITRO INVESTIGATION ON THE THERAPEUTIC POTENTIAL OF *CARICA PAPAYA* LEAF EXTRACT ON SOME PATHOGENIC BACTERIA

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Abstract

Carica papaya is one of the most valuable plants used for various purposes in medicinal field and ethnomedicine. The aim this study was to investigate the therapeutic potential of *Carica-C. papaya* leaf extract using cold maceration method with distilled water, methanol and petroleum-ether. Phytochemical screening of the extracts revealed the presence of the following: alkaloids, saponins flavonoids, steroids, cardiac glycosides and tannins. Antimicrobial activities of the extracts were determined by agar well diffusion method by measuring the diameters of zones of inhibition. In aqueous extract, *Klebsiella pneumonia* had the highest zone of inhibition (16 ± 0.00 mm) followed by *Escherichia coli* and *Staphylococcus aureus* at 14 ± 0.00 mm and 12 ± 0.5 mm respectively. In methanol extracts, *E. coli* had the highest zone of inhibition (15 ± 00 mm) followed by *Staphylococcus aureus* (12 ± 0.00 mm) while *K. pneumonia* had no inhibition. With Petroleum-ether, *Staphylococcus aureus* had the highest zone of inhibition (22 ± 0.00 mm) followed by *E. coli* (0.13 ± 0.00 mm) while *K. pneumonia* had no inhibition. It was cleared that *Carica-C. papaya* had antimicrobial activity but varied with organism and extraction solvent. Therefore, for development of drugs, different extraction solvent and bacteria must be used in order to develop effective and tailored made antimicrobials to curb the menace of antibiotics resistance.

Key words: *Carica papaya*, Bacteria, extract, Plant, and Phytochemical

1.0 Introduction

Carica papaya (pawpaw) have phenomenal restorative properties for treatment of various infirmities. The various parts of the *Carica-C. papaya* plant including leaves, seeds, latex and fruits showed to have restorative worth [1]. It is one of the most important plants utilized for different purposes in therapeutic field and ethnomedicine [2]. *Carica-C. papaya* has antimicrobial, antihelminthic and cancer prevention agent properties [3]. in leaf and bark, as well as twig tissues that have high anti-tumor and pesticide properties, as stated by Ali et al [4].

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Pawpaw is from *Caricaceae* family; its scientific name is *Carica papaya* L. It is a huge herbaceous plant on the grounds that the stem doesn't have a lot of wood, the stem develops from 5 to 10m tall with all the leaves on the top and are 50-70 cm wide [5]. It is broadly found in Indonesia and Nigeria, however the specific territory of origin is obscure, the papaya is accepted local to tropical America, maybe in southern Mexico and neighboring focal America [6].

Plants are significant natural source for items utilized in medication ~~in-for~~ numerous years [7]. Antimicrobial action in plant ~~extricates~~ is ascribed to the presence of phytochemicals, for example, phenolics, steroids, alkaloids, saponins and terpenoids [8]. The helpful properties of plants have been assessed by numerous examinations everywhere in the world and the vast majority of them have been uncovered to have antimicrobial action as ~~announced-reported~~ by

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Muhuha et al. [9]. It was also reported that alkaloid, reducing sugar, steroid, terpenoids, phenol, anthraquinone, tannin, saponin and flavonoids in the *Carica papaya* leaf extract [4].

Carica papaya L. contains numerous biochemically dynamic mixtures, two most significant mixtures are chymopapain and papain which should help in absorption [10]. Papain is utilized in the treatment of joint inflammation and the proteolytic catalyst, it has an abundance of industrial applications [11]. As of late, FDA has cleared chymopapain for intradiscal infusion in patients with reported lumbar intervertebral circles whose signs and indications have not reacted to conventional treatment throughout a sufficient timeframe [10].

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Papaya assumes a significant part in the customary treatment of dengue fever and malaria fever by heating up the leaves into tea which helps increment the white platelets and platelets, standardizes coagulating and fixes the liver [1]. The dark seeds of papaya are palatable and utilized as flavour for cooking and furthermore as a substitute for dark pepper [12]. Its underlying foundations can be utilized as medication for renal and urinary bladder issue [13]. The objective of this study was investigate the therapeutic potential of *Carica papaya* leaf extract on some pathogenic bacteria.

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2.0 Materials and methods

2.1 Sample collection and authentication

Pawpaw leaves (*Carica papaya* L.) was obtained from Shanu village, Minna, Niger State and transported ~~from there~~ in a clean polythene bag. They were identified by an ethnobotanist in the department of Plant Biology, Federal University of Technology, Minna, Niger State, Nigeria.

2.2 Preparation of the Plant Samples

The leaves were spread to dry for one week at room temperature. ~~After drying properly~~, the dried leaves were blended into powdery form. The powder was sieved so as to get fine powder of leaves.

2.3 Extraction of Plant Samples

The extraction of the leaves was carried out as described by Mbakwem-Aniebo *et al.* [14]. About 100 g of the powdered *Carica papaya* leaf was weighed in separate beakers and macerated with 500 ml of methanol, distilled water and petroleum-ether, each of these beakers ~~was~~ were properly sealed with aluminium foil and was thoroughly hand shaken after 30 minutes for 6 hours, then left for 72 hours. The mixture was filtered with a muslin cloth, the filtrate obtained was concentrated on a water bath. The concentrated extracts ~~was~~ were weighed and kept in a well labelled sterile specimen bottles and refrigerated at 4°C prior to use for antibacterial *in-vitro* investigations

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2.4 Phytochemical screening of *Carica papaya* extract

The phytochemical components of the plants were ~~determined~~ detected according to the method of Trease and Evans [15] and Sofowora [16]. The phytochemical screening was done to confirm the presence or absence of certain compounds that are responsible for the antimicrobial activities of plants extracts such as alkaloids, saponins, flavonoids, steroids, cardiac glycosides and tannins

2.5 Reconstitution of Extracts

The stock solution of the extract was prepared by dissolving 0.8g, 1.0g and 1.2g each in 2ml of DMSO and 3ml of sterile distilled water used in the extraction process to obtain 160mg/ml, 200mg/ml and 240mg/ml respectively. These were dispensed in sterile bottles; this procedure was repeated for each of the solvent used. These were stored at 15°C until further use.

2.6 Test Organisms and identity confirmation

The bacteria used for this study were *Staphylococcus aureus*, *Klebsiella pneumonia* and *Escherichia coli*. These organisms were obtained from Microbiology Department, Federal University of Technology, Minna, Niger State, Nigeria. Their identities were confirmed through morphological, biochemical and growth media.

2.7 Standardization of Organism

Approximately 0.1 ml of 1% barium chloride was applied to 9.9 ml of 1% sulphuric acid, which was then reconstituted into 10 ml of sterile distilled water to make a normal solution of 0.5 mL Mcfarland. The broth culture of the research organism was then contrasted to 0.5 percent Mcfarland in terms of turbidity. For the antibacterial assay of the plant extracts, a loop of the uniform culture was used [3].

2.8 Antibacterial Susceptibility Test

The agar well diffusion method was employed to test the antibacterial activities of the plant extracts according to Oyeleke *et al.* [17]. The standardized suspensions were used to inoculate the surfaces of sterile Mueller Hilton agar plates using sterile cotton swabs. Wells were punched (6mm in diameter) using sterile cork borer in duplicate agar plates each well was filled with 1mL of 240mg/ml, 200mg/ml and 160mg/ml of the plants respectively. The plates were allowed to stand for about 1-2 hours at room temperature for the extracts to diffuse into the agar, the agar plates were incubated at 37°C for 24 hours. The antibacterial activities of the plant extracts were assessed by measuring the diameter zone of inhibitions.

2.9 Data analysis.

The data obtained from diameter zones of inhibition produced by the isolates against the extracts used were analyzed using One-Way ANOVAs using statistical program SPSS 23.0 (Statistical Package for the Social Sciences). The values are expressed in mean \pm SEM,

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3.0 Results

Table 1: Qualitative Phytochemical screen of *Carica papaya* leaves extract

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Phytochemical	Aqueous	Methanol	Petroleum-ether
Alkaloids	+	+	+
Saponins	+	-	-
Flavonoids	-	+	+
Steroids	+	-	+
Cardiac glycosides	+	+	+
Tannins	+	+	-

Keys: '+' presence of compound, '-' absence of compound.

Table 2: Susceptibility of *Carica papaya* leave extract at 240mg/ml of all the solvents

Organisms	Aqueous 240 mg/ml	Methanol 240 mg/ml	Petroleum-ether 240 mg/ml	Standard drug (ciprofloxacin)
<i>Staph. aureus</i>	12±0.5 mm	12±0.00 mm	22±0.00 mm	36±0.00 mm
<i>Escherichia coli</i>	14±0.00 mm	15±00 mm	0.13±0.00 mm	36±0.00 mm
<i>K. pneumonia</i>	16±0.00 mm	NA	NA	35±0.00 mm
<i>Sal. enterica</i>		NA	NA	34±0.00 mm

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Keys: NA = No zone of inhibition.

Table 3: The result of confirmatory test of isolates

Gram Reaction	Shape	Catalase	Coagulase	Indole	Urease	TSIA			MSA	Hoekten agar	Citrate	Confirmed Bacteria
						Slope	H ₂ S	Butt				
-	Rods	+	-	-	+	R	+		-	+	+	<i>K. pneumonia</i>
+	Cocci	+	+	-	-	R	-	-	+	-	+	<i>S. aureus</i>
-	Rods	+	-	+	-	Y	-	+	-	-	-	<i>E. coli</i>

Keys: - = Negative, + = Positive, Y =Yellow, R = Red, TSIA = Triple Sugar Iron Agar and MSA = Mannitol Salt Agar.

UNDER PEER REVIEW

The qualitative phytochemical screening of ~~eariea~~ *Carica papaya* leaf extracts revealed the presence of the following: Alkaloids, Saponins, Flavonoids, Steroids, Cardiac glycosides and Tannins. However, different solvents revealed different bio constituents depending on their penetrating capacity (Table 1). All the phytochemicals screened were found in aqueous solvent except flavonoids. Saponins and steroids were absent in methanol. Petroleum-ether also lack saponin and tannins.

The results of antibacterial activity are given in the Table 2, which clearly show that all the extracts have shown antibacterial activity but not as high as that of standard drug against the entire tested organisms. Petroleum-ether extract was more effective against *Staphylococcus aureus* than both aqueous and methanol extracts. Methanol extract was more effective against *E. coli* than aqueous extract and petroleum-ether. Aqueous extract was the only one that was effective against *K. pneumonia* while methanol and petroleum ether extract were not effective against it.

The identity of the three isolates were confirmed to be: *Staphylococcus aureus*, *Escherichia coli* and *Klebsilla pneumonia* in Table 3

4.0 Discussion

In this research, the existence of alkaloids, saponins, flavonoids, steroids, cardiac glycosides and tannins in the *Carica papaya* leaf extract confirms the validity of the plant's long-standing usage for the treatment of various human ailments [18]. Muhuha et al. [8] reported that particular phytochemicals, concentration, bioactive concepts, antagonistic and synergistic behavior depend on the antimicrobial properties of medicinal plants. In related studies, Ali et al. [4] found alkaloids, saponins, flavonoids and steroids in *Carica C. papaya* leaf extract, with the exception of cardiac glycosides and tannins. Tannins in *Carica C. papaya* leaf extracts were discovered by Ajiboye and Olawoyin [3]. Due to the environmental condition, nature of solvents and method of extraction, plant phytoconstituents are not always the same as stated by Elijah et al. [19].

Ajiboye and Olawoyin [3] reported that tannin forms irreversible protein-rich proline complexes that result in cell protein synthesis inhibition. Tannins are known to react with protein to provide the typical tanning effect, which is important for the treatment of inflamed or ulcerated tissues, according to Parekh and Chanda [20]. As revealed by Ajiboye and Olawoyin [3], it is also used to treat conditions such as diarrhea.

Alkaloids, the most effective therapeutically important plant material, were another recognizable bioactive factor [21]. According to the report of Dwivedi et al. [22], alkaloids are a class of nitrogenous compounds known to be generated by plants. Due to their analgesic, antispasmodic and bacterial properties [21], pure alkaloids and synthetic derivatives are used as basic medicinal agents.

In several plants, the bioactive compound, flavonoids are known to protect plants from stress [22], as well as being anti-allergic, anticancer, hepatoprotective, anti-diabetic, antibacterial, anti-inflammatory and anti-viral in nature [23,22]. Ali et al. [4] reported that flavonoids inhibit the activity of enzymes by forming complexes with bacterial cell walls, extracellular and soluble proteins, more lipophilic flavonoids disrupt cell wall integrity or microbial membranes at low

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concentrations. Flavonoids are also reported for their antiviral, antimicrobial and spasmolytic properties [21].

Saponins are a wide group of foam-forming glycosides with detergent properties known in nature to be antimicrobial, anti-malarial, anti-allergic, anti-diabetic, insecticidal, and anti-inflammatory [9, 22]. Okigbo et al. [24] suggested that the existence of saponins supports the fact that *Carica-C. papaya* leaf has cytotoxic effects as saponins are cytotoxic, such as intestinal permeabilization.

Cardiac glycosides is effective in treatment of cancer [25] and congestive heart failure, inability to pump enough blood to take care of body needs [26]. In addition, Morsy [26] also reported that some cardiac glycosides display an inhibitory activity against rhinovirus

Steroids form a group of secondary metabolites whose structure and biological functions are diverse. In pursuit of these secondary metabolites as a possible lead in drug design/discovery, these natural products with many therapeutic uses and research is still ongoing [25].

The antimicrobial activities revealed from empirical measuring of diameter zone of inhibition varied. Petroleum ether had the highest zone of inhibition (22 ± 0.00 mm) with *Staphylococcus aureus* followed by *Klebsiella. Pneumonia* (16 ± 0.00 mm) *Escherichia coli* (15 ± 0.00 mm) in aqueous extracts and methanol extract respectively. The different might be as result of different phytoconstituents present in each of the extract even at same concentration [21]. Study was in agreement with Ajiboye and Olawoyin [3] who recorded different diameters zones of inhibition for different bacteria and extraction solvents at same concentration. petroleum ether extract and methanol extracts did not have activity on *Klebsiella. Pneumonia*. This might be as result key bioactive active components that were lacked in the extraction. However, Baskaran et al. [21] who conduct similar research like this recorded 0.00mm with *Klebsiella. Pneumonia*.

5.0 Conclusions

From this investigative study, it become crystal clear that all the reports of the medicinal values of *Carica papaya* for both traditional uses and scientific researches were true owing to the presence of some key bioactive components found and its antimicrobial effect on *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella Pneumonia*. It was also noted that different solvent had different behaviour in respect to the phytochemistry of the plant and antimicrobial activity.

Therefore, since there is no parallel antibacterial activity, further study is recommended for different extraction solvents and methods and different bacteria test in order to develop effective and tailored made antimicrobial agents to curb the menace of antibiotics resistance.

Reference

- 1 Sudhakar N and Vidhya RM. Potential Medicinal Properties of *Carica papaya* Linn. – A mini review. *Int. J.Pharm. Sci.* 2014;6(2): 1-4.
- 2 Attah F. Olatu O. Cletus AU. Otuoze VO. Garba DE. “Use of Medicinal Plants as Antimicrobial Agents: A Review of the Successes Story”. *International Journal of Medicinal Plants and Natural Products (IJMPNP)*. 2020: 6(3); 28-33.
- 3 Ajiboye AE. And olawoyin RA. Antibacterial activities and phytochemical screening of crude extract of carica papaya leaf against selected pathogens global journal of pure and applied sciences. 2020;26; 165-170
- 4 Ali M. Umar A. Nas FS. Antibacterial Efficacy of *Carica Papaya* Leaves and Stem Bark Extracts on Clinical Isolates of Methicillin Resistant *Staphylococcus Aureus* (MRSA). *Bioequivalence & Bioavailability International Journal*. 2018: 2(2); 1-8.
- 5 Nwinyi OC. Anthonia AB. Antifungal effects of pawpaw seed extracts and papain on post-harvest *Carica papaya* L. fruit rot. *African Journal of Agricultural Research*. 2010: 5(12): 1531-1535
- 6 Doughari JH, Elmahmood AM, Manzara S. Studies on the antibacterial activity of root extracts of *Carica papaya* L. *African Journal of Microbiology Research*. 2007;1(3); 37-41
- 7 Hassan RBA. (2012) Medicinal plants (Importance and uses). *Pharm Anal Acta* 3: e139.
- 8 Kasolo JN, Bimenya GS, Ojok L, Ochieng J, Ogwal-Okeng JW. Phytochemicals and uses of *Moringa oleifera* leaves in Ugandan rural communities. *J Med Plant Res*.2010: 4; 753-757.
- 9 Muhuha AW. Kang’ethe SK. Kirira PG. Antimicrobial Activity of *Moringa oleifera*, *Aloe vera* and *Warbugia ugandensis* on MultiDrug Resistant *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. *J Antimicrob Agents*. 2018;4(2): 162-168.
- 10 Anibijuwon II. Udeze AO. Antimicrobial Activity of Carica Papaya (Pawpaw Leaf) on Some Pathogenic Organisms of Clinical Origin from South-Western Nigeria. *Ethnobotanical Leaflets*. 2009;13: 850-64,
- 11 Tewari BB. Subramanian G. Gomathinayagam R. Antimicrobial Properties of *Carica papaya* (Papaya) Different Leaf Extract against *E. coli*, *S. aureus* and *C. albicans*. *American Journal of Pharmacology and Pharmacotherapeutics*. *AJPP*. 2014: 1(1); 025-039
- 12 Aravind G. Debjit B. Duraviel S. Harish G. Traditional and Medicinal uses of *Carica papaya*. *Journal of medicinal plants studies*. 2013;1(1): 7-15.
- 13 Romasi EF. Karina J. Parhusip, AJN. Antibacterial activity of papaya leaf extracts against pathogenic bacteria. *Makara teknologi*. 2011;15(2); 173-177.

- 14 Mbakwem-Aniebo C. Onianwa O. Okonko IO. Effects of *Ficus exasperata Vahl* on common dermatophytes and causative agent of Pityriasis Versicolor in rivers state, Nigeria. *American Journal of Dermatology and Venereology*. 2012;1(1):1-5.
- 15 Trease GE and Evans WC (1989) Pharmacognosy, 13th edition; ELBS Oxford University Press, London, UK: 245-263
16. Sofowora, A. Medicinal plants and Traditional Medicine in Africa, Spectrum Books Limited (Ibadan), Nigeria. 1993. p256.
- 17 Oyeleke SB. Dauda BEN. Boye OA. Antibacterial activity of *Ficus capensis*. *African Journal of Biotechnology*. 2008: 7(10); 1414-1417.
- 18 Vij T. Prashar Y. A review on medicinal properties of *Carica papaya Linn*. *Asian Pacific Journal of Tropical Disease*. 2015:5(1); 1-62
- 19 Elijah MI. Imohiosen O. Lamidi BT..Umar MS. Biochemical screening of pure honey and its antibacterial activity on some bacterial isolates compared with common antibiotics. *International Journal of Pharmaceutical Science Invention*. 2015: 4 (5); 15-20.
- 20 Parekh J. Chanda S. In vitro antibacterial activity of crude methanol extract of *Woodfordia fruticosa* Kurz flower (*Lythaceae*). *Brazilian Journal of Microbiology*. 2007: 38(3):2.
- 21 Balasankar D, Vanilarasu K, Preetha PS, Umadevi SR, Bhowmik D. Journal of Medicinal Plants Studies. *Journal of Medicinal Plants*. 2013:1(3);7-15.
- 22 Dwivedi, MK. Sonter, S. Mishra S. Patel DK. and Singh PK. Antioxidant, antibacterial activity, and phytochemical characterization of *Carica papaya* flowers. *Beni-Suef University Journal of Basic and Applied Sciences* (2020): 9(23); 1-11
- 23 Cushnie TPT. Andrew JL. Antimicrobial activity of flavonoids. *Int J Antimicrobial Agents*. 2005: 26(5); 343–356 33.
- 24 Okigbo RN. Anuagasi CL. Amadi JE. Advances in selected medicinal and aromatic plants indigenous to Africa. *J Medicinal Plants Research*. 2009:3(2); 086-095
- 25 Sultan A. Rauf RA. Steroids: A Diverse Class of Secondary Metabolites. *Med chem* 2015: 5; 310-317. doi: 10.4172/2161-0444.1000279
- 26 Morsy N. Cardiac glycosides in medicinal plants. *Aromatic and Medicinal Plants–Back to Nature*. London: *Intechopen*. 2017: 29-45.