INTRODUCTION

Addressing the increasing threat posed by chronic diseases to public health, it is essential to implement nutrition-based strategies for prevention. It may be difficult to address some issues medically, thus avoiding some illnesses requires ingesting basic functional foods. Eighty to nine percent of the factors affecting human health are social determinants, especially excellent eating habits, while ten to twenty percent of the factors are modifiable. A plant-based diet that is high in nutrients is linked to several health benefits, including a decreased risk of viral infections, obesity, type 2 diabetes, and cancer. It is also better for the environment. In terms of correcting nutritional deficiencies, functional agriculture—specifically, the production of functional food crops like papaya leaves (Carica papaya L.)—has emerged as a new field of study.

So, creating functional food crops with state-of-the-art technology and techniques from food science, agricultural science, and preventive medicine constitutes a significant field of research. In 2019 (Ugo et al. 2019)

Papaya, pawpaw, and kates are all common names for C. papaya L., a plant that belongs to the Caricaceae family. Native to southern Mexico, Central America, and the Mesoamerican Center, it is a perennial shrub used in gardening. The countries where it is grown most commonly are Brazil, Australia, Malaysia, China, Nigeria, India, Thailand, Myanmar, and other tropical and subtropical climates (Nandinhi et al. 2021). In addition to its luscious, juicy fruit, papayas are produced for their latex, seeds, leaves, roots, flowers, and barks, all of which have long been used in local medicine globally .

However, leaves have emerged as one of the most significant parts of plants due to their profusion of health-promoting compounds and activities.

Traditional medicine uses dried and cured papaya leaf cigars to treat respiratory ailments including asthma, and uses a fresh papaya leaf decoction to prepare tea to treat malaria. Some countries steam young papaya leaves and consume them as a leafy vegetable. Because papaya leaf extract is believed to aid in the recovery of patients from viral fevers by raising platelet count, red blood cell and white blood cell counts, boiling papaya leaves is an Ayurvedic treatment for malaria and dengue fevers in India (Singh et al. 2020)

Additionally, the extract has been shown to shield the patient from red blood cell sickling (Dharmarathna et al. 2013) Papaya leaves are utilized as a treatment for beriberi in many Asian countries. It has been determined that papaya leaves contain over fifty bioactive components, making them beneficial for treating a variety of human ailments. While Ayurvedic remedies employ papaya leaves, consumers nowadays are becoming more aware of the fruit's potential as a functional meal because of its strong antiviral and immunity-boosting qualities (Imaga et al. 2009)

Tea prepared from the juice extracted from papaya leaves is also used as a synergistic therapeutic dietary supplement for patients suffering from oxidative stress-related diseases because of its strong antioxidant potential (Ahmad et al. 2011). Few studies have shown that papaya leaves are antiseptic while they are fresh, while, dried papaya leaves can be used as a tonic to detoxify the blood and promote digestion. With regard to toxins in the human system, papaya leaf juice is now recognized for its powerful anticancer, anti - oxidative, anti-inflammatory, antimicrobial, and antisickling characteristics as well as nephron protecting, hepatoprotective, hypoglycaemic, and hypolipidemic benefits. (Sharma et al. 2022)

Because of its significant antioxidant properties, tea made from the juice of papaya leaves is also utilized as a synergistic therapeutic dietary supplement for patients with disorders connected to oxidative stress. While dried papaya leaves can be used as a tonic to purify the blood and aid in digestion, few studies have demonstrated that fresh papaya leaves are antimicrobial. Papaya leaf juice is currently known to have potent anticancer, anti-oxidative, anti-inflammatory, antibacterial, and antisickling properties in relation to toxins in the human body. It also has nephron-protective, hepatoprotective, hypoglycemic, and hypolipidemic effects (Tan et al. 2018). It has been demonstrated that papaya polar isolates have analgesic, wound-healing, and anti-HIV properties. An imbalance between the cellular antioxidant system and free radical activity is associated to several deadly diseases, including cardiovascular ailments and cancer. The aim of this research is to ascertain the antibacterial and phytochemical characteristics of Carica papaya leaf extract in light of recent studies that have concentrated on naturally occurring antimicrobial plant components.

MATERIALS AND METHODS

Sample collection

The papaya leaves were gathered at Ugbowo, Benin, Edo State, at BDPA. After gathering the sample of fresh leaves, it was properly cleaned in sterile distilled water. After being spread out on a mat and allowed to dry for four days in a cool atmosphere, it was ground into a fine powder with a dry blender, sealed in an airtight container, and preserved for examination. 500g of the ground leaves were steeped for 24 hours in 1000ml of distilled water. The combination was filtered through Whatman No. 1 filter paper at the conclusion of the 24-hour period, and the filtrates were concentrated to lower the volume. Muller Hinton agar media was made by following the manufacturer's instructions and dissolving 38g of the medium in 1000 ml of distilled water. The agar media was heated to 45–50 degrees Celsius with regular stirring, then boiled to fully dissolve the medium. It was then autoclaved at 121 degrees Celsius for 15 minutes to sanitize it. Fill a sterile petri dish with the agar. To keep the medium from becoming contaminated, it was poured within the laminar air flow chamber.

Using the Kirby Bauer disc diffusion method, the extract's sensitivity was tested. For this experiment, freshly generated bacterial culture was incubated for eighteen hours in nutritional broth.

For standardization, the broth culture was diluted until the bacterial suspension matched the turbidity of 0.5 McFarland turbidity standards. Exactly 0.1 ml of the standardized test isolates were evenly spread on an agar medium using a sterile glass rod. Four concentrations (12.5, 25, 50, and, 100) ug/ml of both methanolic and aqueous extract were prepared in a plain sterile sample bottle and a paper disc of 6mm in diameter was added to each of the bottles and allowed to diffuse for 1hr. Standard Streptomycin and Ampicillin antibiotics were tested alongside as controls.

The broth culture was diluted in order to standardize it, until the bacterial suspension had the same turbidity as the 0.5 McFarland standards. A sterile glass rod was used to evenly distribute exactly 0.1 ml of the standardized test isolates on an agar medium. Four methanolic and aqueous

extract concentrations (12.5, 25, 50, and 100 ug/ml) were made in a standard sterile sample container. A paper disc with a diameter of 6 mm was placed to each bottle, and the bottles were left to diffuse for an hour. The standard antibiotics ampicillin and streptomycin were also tested as controls.

Minimum inhibitory and bactericidal concentrations.

A loopful of the test organism that had been previously diluted to 0.5 MCFARLAND turbidity standard was added to the test tubes together with 1 ml of each sample at various concentrations. The tubes were then incubated, and the turbidity of the samples was checked after.

MIC (minimum inhibitory concentration) test

The microbial strains that were sensitive to the extracts in the disc diffusion procedure had their MIC values examined. The MIC is the lowest dose at which the test organisms are not killed. Each test organism was subjected to three tests of the extract used in this investigation.

Minimum bactericidal concentration

A 10 ml amount of the extract at four different concentrations (100 ug/ml, 50 ug/ml, 25 ug/ml, and 12.5 ug/ml) was filled with a loop full of the bacteria and incubated for 48 hours in McCartney bottles. It was then cultivated on a petri plate to determine which concentration was capable of totally destroying the organism. The MBC was determined to be the lowest concentration at which the organisms are fully killed. This concentration, when injected from McCartney bottles, did not show any growth on the media.

Test for antibiotic susceptibility

Test organisms underwent antibiotic sensitivity testing on prepared media using the Kirby Bauer disc diffusion method. There will be ten (10) distinct commercial antibiotic discs used. Using a sterile set of forceps, the antibiotic discs were gently and firmly placed on the inoculation plates. After being inverted, the plates were incubated for 24 hours at 37°C. Using a meter rule, the diameter of the zone of inhibition was calculated in millimeters (mm). To reduce the likelihood of error, the trials were run three times.

RESULTS

The cultural, morphological, and biochemical traits of the test organisms are displayed in Table 1. In this investigation, Pseudomonas, S. aureus, and E. coli were the bacteria that were evaluated

Table 1: Cultural, morphological, and biochemical characterization of test organisms

Parameters	**Organisms**		
	EC	**S**	**P**
Cultural characteristics			
Shape	Circular	Round	Round
Color	Cream	Milky	Milky
Size	Small	Small	Large
Elevation	Convex	Flat	Flat
Transparency	Opaque	Opaque	Opaque
Morphological			
Gram stain	_	+	_
	Rod	Cocci	Rod
Cell type			
Arrangement	Single		
Biochemical			
Catalase	+	+	+
Oxidase test	_	_	_
Indole test	+	+	_

Citrate test	-	+	+
Urease test	_	+	+
Bile test	_	_	+
Sugar fermentation			
Glucose	+	+	+
Sucrose	+	+	+
Lactose	+	+	+
Probable identity	E. *coli*	S. *aureus*	*Pseudomonas*

Key: + Means positive – means negative

The extract was able to stop the bacteria from growing at different concentrations, as shown in Table 2. At 1000 mg/ml, the zone of inhibition (ZOI) was 15.33±0.47 mm, and at 300 mg/ml, it was at least 8.00±0.00 mm.

Table 2a: Antibacterial activity of *Carica papaya* leaf extract against S. *aureus*

Concentration (mg/ml)	1000	500	450	400	350	300
Zone of inhibition (mm)	15.33±0.47	12.00±0.00	10.67±0.47	10.00±0.81	9.33±0.47	8.00±0.00

The antibacterial activity of Carica papaya leaf extract against Escherichia coli is displayed in Table 2b. The extract exhibited varying levels of activity, as indicated by the table, with the highest zone of inhibition (ZOI) at 1000 mg/ml being 14.00±0.81 mm and the lowest ZOI at 350 mg/ml being 8.00±0.00 mm.

Table 2b: Antibacterial activity of *Carica papaya* leaf extract against E. *coli*

Concentration (mg/ml)	1000	500	450	400	350	300	250
Zone of inhibition (mm)	14.00±0.81	11.33±0.47	10.00±0.81	9.33±0.47	8.00±0.00	0.00±0.00	0.00±0.00

The antibacterial activity of Carica papaya leaf extract against *Pseudomonas* is displayed in Table 2c. As the table illustrates, the extract revealed different activity with a zone of inhibition (ZOI) of 19.33±0.47mm at a concentration of 1000mg/ml and the least ZOI of 9.33±0.47mm at 62.5mg/ml.

Table 2c: Antibacterial activity of Carica papaya leaf extract against *Pseudomonas*

Concentration (mg/ml)	1000	500	250	125	62.5	31.25
Zone of inhibition (mm)	19.33±0.47	16.00±0.00	13.33±0.47	10.00±0.81	9.33±0.47	0.00±0.00

**values are mean ± standard deviation*

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Carica papaya leaf extract against test organisms reveals that the MIC of the extract against *S. aureus, E. coli*, and *Pseudomonas* are 300mg/ml, 350mg/ml and 62.5mg/ml respectively and the MBC 1000mg/ml for both S. aureus and E. coli as shown in table 3, meanwhile the extract was not bactericidal to Pseudomonas.

Table 3: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Carica papaya* leaf extract against test organisms

Test organisms	MIC (mg/ml)	MBC(mg/ml)
Staphylococcus	300	1000
E. *coli*	350	1000
Pseudomonas	62.5	-

Key: - means not

Table 4a and 4b displays the antibiotic susceptibility pattern of test organisms to Carica papaya leaf extract. It indicates that *E. coli* was found to be susceptible to Septrin, Chloramphenicol, and Streptomycin, but resistant to Ciprofloxacin, Amoxicillin, and Augmentin. *S. aureus* shown resistance to both Ampiclox and Amoxicillin.

Table 4a: Antibiotics susceptibility pattern of Gram-negative test organisms

Isolates	SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX	S
E. *coli*	S	S	I	R	R	R	I	I	I	S

Table 4b: Antibiotics susceptibility pattern of Gram-positive test organisms

Isolates	PEF	CN	APX	Z	AM	R	CPX	S	SXT	E
S. *aureus*	S	S	R	S	R	S	S	I	S	S

The multiple antibiotic resistance index of test organisms to common antibiotics is displayed in Figure 1. According to the data, S. aureus's MAR index is on the benchmark, but E. coli's resistance index of 0.3 is over the allowable limit of 0.2.

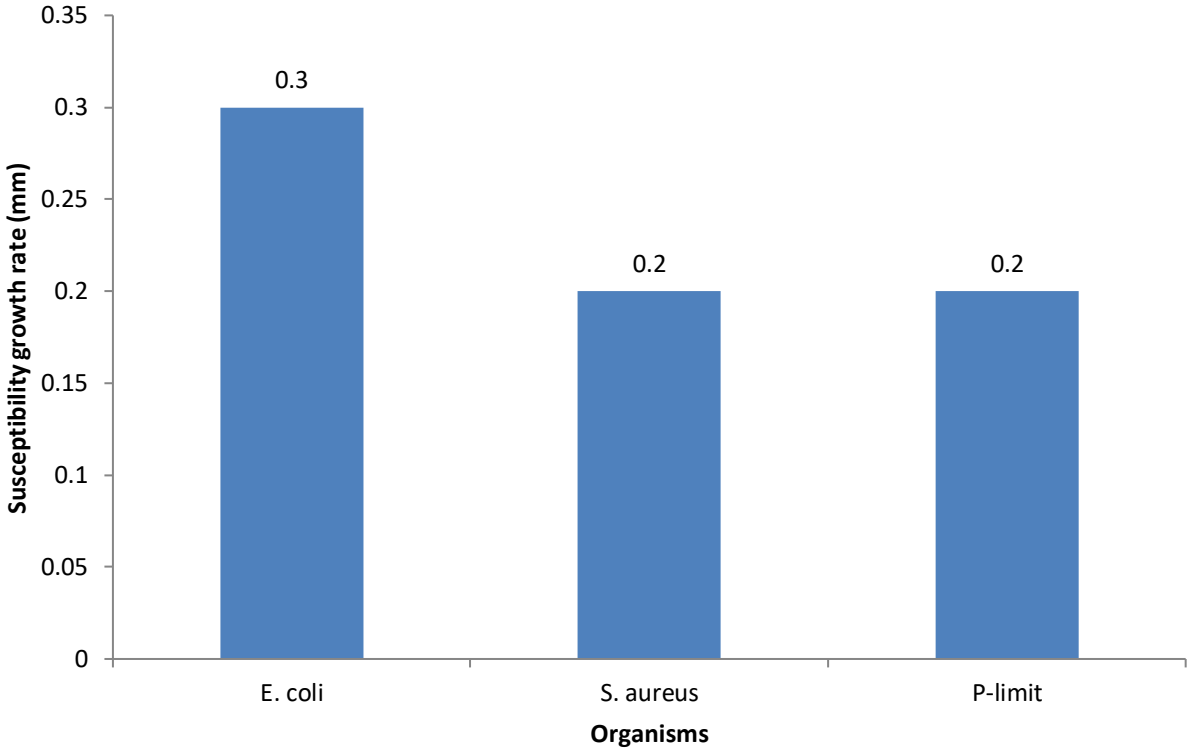

Figure 1: Multiple antibiotics resistant index of test organisms

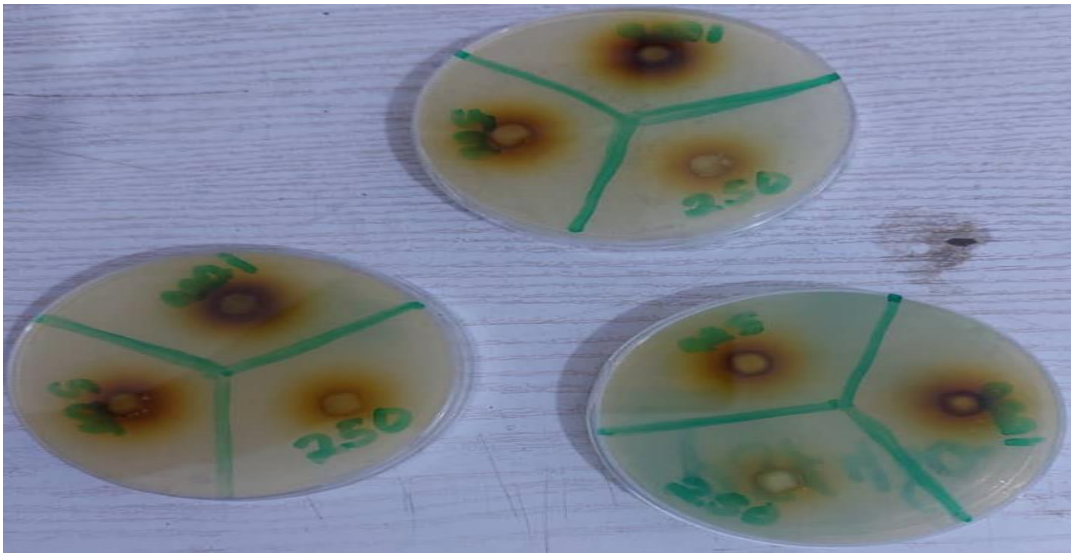

Plate 1: Plates from susceptibility testing using Kirby Bauer disc diffusion on prepared media

DISCUSSION

According to several studies, plants contain bioactive compounds. The evidence of antibacterial activity by the plant extracts employed in this investigation can be explained by the presence of bioactive compounds, which have been found to give resistance to plants against bacteria, fungus, and pests (Srinivasan et al. 2001).

According to the study's findings, Carica papaya leaf extract inhibited the growth of S. aureus at concentrations of 1000 mg/ml and 300 mg/ml, with a zone of inhibition (ZOI) of 15.33±0.47 mm and 8.00±0.00 mm, respectively. E. coli was also inhibited at a dose of 1000 mg/ml, with a ZOI of 14.00±0.81 mm and Pseudomonas demonstrated susceptibility, exhibiting a ZOI of 19.33±0.47mm at 1000 mg/ml and 9.33±0.47mm at 62.5 mg/ml. The findings of Anibijuwon and Udeze (2009) study on the antibacterial properties of Carica papaya, often known as pawpaw leaf, on a variety of pathogenic organisms with clinical origins in South-Western Nigeria are consistent with this outcome. Significant inhibition was seen in the aqueous leaf extract, which

was found to have higher activities against all tested gram-positive bacteria than gram-negative bacteria.

Pseudomonas aeruginosa showed the highest activity against this bacteria, with a 14 mm zone of inhibition. Additionally, they observed that root extracts had greater efficacy against Pseudomonas aeruginosa than against any of the gram-negative bacteria that were tested. Likewise, Dwivedi et al. (2020) noted action against E. coli (MTCC, 1687), with inhibition zones measured at 4.00 ± 0.08 mm, 0.30 ± 0.04 mm, and 0.50 ± 0.10 mm in methanol, chloroform, and aqueous extracts, respectively. According to Peter et al. (2014), a 70% methanolic extract of C. papaya seeds exhibited inhibitory action against E. coli, Pseudomonas aeruginosa, and Staphylococcus aureus. This result, however, is at odds with a prior study that discovered that plant extracts were more effective against Gram-positive bacteria than against Gram-negative bacteria, with C. papaya leaf extract being one of the most susceptible to Proteus mirabilis and other Gram-negative bacteria (Peter et al. 2014). Numerous factors, including past exposure to the agents or the properties of the medium being used, which may affect the agent's diffusibility, might make bacteria more susceptible to antibacterial agents. Different bioactive compounds inhibit the growth of microorganisms. According to Gyawali and Ibrahim (2014) a number of phenolic compounds particularly target the cytoplasmic membrane of bacterial cells in order to carry out their antibacterial function.

This has to do specifically with how many and where hydroxyl groups are found. According to Jigna and Sumitra (2006) potential membrane modification is a sign that additional antibacterial drugs have entered the bacterial membranes, disrupting their integrity. Nohynek et al. (2006) examined the potential of phenolic compounds found in cloudberry and raspberry extracts to damage Salmonella's internal membrane, as evidenced by increased release of [14° C] galactose-lipopolysaccharide and higher absorption of 1-N-phenylnaphthylamine. One of the phenolic group's possible antibacterial substances has also been reviewed: tannin. At a dosage of 25 mg/mL, Al-Maliki (2012) showed that tannin extracted from Ficus carica leaves was efficient in inhibiting S. aureus (11 mm of inhibition zone) and Proteus mirabilis at a dose of 90 mg/mL (8 mm of inhibitory zone). The extracts' activity was similar to that of antibiotics. The scientific basis for the local use of these herbs in the treatment of various ailments is provided by their action against the test microorganisms. The extracts' ability to combat both Gram-positive and Gram-negative bacteria under test may point to a wide range of activity. This finding holds great

significance as it may lead to the creation of medicinal compounds that effectively combat organisms resistant to multiple drugs.

Additionally, it is known that papaya leaves contain carpaine, which eliminates bacteria that frequently obstruct digestive processes. Phenolic substances found in papaya leaf extracts include quercetin, kaempferol, caffeic acid, p-coumaric acid, protocatechuic acid, and 5,7-dimethoxycoumarin. (Romasi et al. 2011). Additionally found are the alkaloids carpaine, pseudocarpaine, and dehydrocarpaine I and II, as well as choline, carposide, and vitamins C and E.

The extract's minimum inhibitory concentration (MIC) for S. aureus, E. coli, and Pseudomonas is 300 mg/ml, 350 mg/ml, and 62.5 mg/ml, respectively. For both S. aureus and E. coli, the MBC is 1000 mg/ml. However, the extract did not show bactericidal effects on Pseudomonas.

(Anibijuwon et al. 2009) conducted experiments that revealed the root extracts' Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) to be between 50 and 200 mg/ml. The observed high minimum inhibitory concentration (MIC) value suggests ineffectiveness against the bacterium or organisms that may become resistant to the bioactive chemicals.

E. Coli was susceptible to Septrin, Chloramphenicol, and Streptomycin but resistant to Ciprofloxacin, Amoxicillin, and Augmentin, according to the organisms' antibiotic susceptibility pattern to Carica papaya leaf extract. S. aureus shown resistance to both Ampiclox and Amoxicillin. E. Coli had a resistance index of 0.3, above the allowable limit of 0.2, according to the multiple antibiotics resistant index, a technique that indicates the importance of the organisms for public health, whereas S. aureus's MAR index was on the benchmark.

CONCLUSION

The current study's findings demonstrated that Carica papaya leaf extracts may have modest antibacterial action against a variety of harmful human microorganisms. Nonetheless, one could draw the conclusion that the plant's ability to exhibit antimicrobial action against both gram-positive and gram-negative bacteria suggests it has the potential to be a source for medications

with a wide range of therapeutic effects. The study's findings corroborate the plant's traditional use and imply that plant extracts contain antibacterially active compounds that could be incorporated into cutting-edge medications to treat otitis media, urethritis, gastroenteritis, and wound infections. Future research challenges include more pharmacological assessments, toxicological investigations, and potential isolation of the therapeutic antibiotic from this plant.

REFERENCES

Ahmad N, Fazal H, Ayaz M, Abbasi BH, Mohammad I , Fazal L. (2011). "Dengue fever treatment with *Carica papaya* leaves extracts," Asian Pacific Journal of Tropical Biomedicine **1**(4): 330–333.

Al-Maliki ADM. (2012). Isolation and identification of tannins from *Ficus carica* L. leaves and study of their medicinal activity against pathogenic bacteria. Journal of Thi-Qar Science **3**(3): 96-106.

Anibijuwon II, Udeze AO. (2009). Antimicrobial activity of *Carica papaya* (Pawpaw leaf) on some pathogenic organisms of clinical origin from South-Western Nigeria. Ethnobotanical Leaflets **13**: 850-864.

Dharmarathna SLCA., Wickramasinghe S, Waduge RN, Rajapakse RPVJ, Kularatne SAM. (2013). Does *Carica papaya* leaf extract increase the platelet count? An experimental study in a murine model. Asian Pacific Journal of Tropical Biomedicine **3**(9) 720–724.

Dwivedi MK, Sonter S, Mishra S, Patel DV, Singh PK 2020. Antioxidant, antibacterial activity, and phytochemical characterization of Carica papaya flowers. Beni-Suef University Journal of Basic and Applied Sciences **9**:23-44.

Gyawali R, Ibrahim SA. (2014). Natural products as antimicrobial agents. Food Control **46**: 412-429.

Imaga NOA, Gbenle GO and Okochi VI. (2009). Antisickling property of *Carica papaya* leaf extract. African Journal of Biochemistry Research **3**:102–106.

Jigna P, Sumitra C. (2006). *In-vitro* antimicrobial activities of extracts of *Launaea procumbns* Roxb. (Labiateae), *Vitis vinifera* L. (Vitaceae) and *Cyperus rotundus* L. (Cyperaceae) African Journal of Biomedical Research **9**(2): 89-93.

Nandini C, SubbaRao VM, Bovilla VR, Mohammad AK, Manjula NS, Jayashree K. (2021) "Platelet enhancement by *Carica papaya* L. leaf fractions in cyclophosphamide induced thrombocytopenic rats is due to elevated expression of CD110 receptor on megakaryocytes." Journal of Ethnopharmacology **275**: 114074.

Nohynek LJ, Alakomi HL, Kähkönen MP, Heinonen M, Helander IM, Oksman-Caldentey KM, Puupponen-pimiä RH. (2006). Berry phenolics: antimicrobial properties and mechanisms of action against severe human pathogens. Nutrition and Cancer **54**(1): 18-32.

Peter JK, Kumar Y, Pandey P, Masih H. (2014). Antibacterial activity of seed and leaf extract of *Carica papaya* var. *Pusa dwarf* Linn. International Organisation of Scientific Research Journal of Pharmacy and Biological Sciences **9**(2): 29-37

Romasi EF, Karina J, Parhusip AJN. (2011). Antibacterial activity of papaya leaf extracts against pathogenic bacteria. Makara Teknologi **15**(2): 173-177.

Sharma A, Sharma R, Sharma M, Kumar M, Barbhai MD, Lorenzo JM, Sharma S, Samota M K, Atanassova M, Caruso G, Naushad M, Chandran D, Prakash P, Hasan M, Rais N, Dey A, Mahato DP, Dhumal S, Singh S, Senapathy M, Rajalingam S, Visvanathan M, Saleena LAK**,** Mekhemar M. (2022). *Carica papaya* L. leaves: deciphering its antioxidant bioactives, biological activities, innovative products, and safety aspects. Hindawi Oxidative Medicine and Cellular Longevity **2022**: 245-265.

Singh SP, Kumar S, Mathan SV. (2020). Therapeutic application of *Carica papaya* leaf extract in the management of human diseases. DARU Journal of Pharmaceutical Sciences **28**(2) 735–744.

311 Srinivasan D, Perumalsamy L, Nathan P, Sures T. (2001). Antimicrobial activity of certain
312 Indian medicinal plants used in folkloric medicine. Journal of Ethnopharmacology **94**:
313 217-222.

314 Tan L, Norhaizan ME, Liew WPP, Sulaiman RH. (2018). Antioxidant and oxidative stress: a
315 mutual interplay in agerelated diseases. Frontiers in Pharmacology **9**:1162.

316 Ugo NJ, Ade AR, Joy AT. (2019). Nutrient composition of *Carica papaya* leaves extracts.
317 Journal of Food Science and Nutrition Research **2**: 3-5.