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SHORT COMMUNICATION article

Evaluation of the antibacterial efficiency of *Carica papaya* leaves against multidrug-resistant *Escherichia coli*

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Abstract: Over the years, there has been an increase in multidrug-resistant bacteria such as *Escherichia coli,* which has become a serious public health concern globally. It has become obvious that there would possibly be no chemotherapeutic agent to which microorganisms will ultimately grow resistance. This study investigated the in vitro antibacterial activity and the antioxidant activity of the ethanolic extract of *Carica papaya* leaves against multidrug-resistant *Escherichia coli* isolates. The fresh *Carica Papaya* leaves were collected from the plant and air-dried, then the dried leaves were finely ground and soaked for 72 hrs in ethanol to obtain the crude extract. The extract obtained was tested for antibacterial activity against ten *Escherichia coli* isolates using the agar well diffusion method. The phytochemicals and antioxidant properties were tested as well. The isolates were subjected to antibiotic susceptibility testing, which confirmed them all as multidrug-resistant. The findings exhibited concentration-dependent antibacterial activity of the crude extract against the multidrug-resistant *Escherichia coli* isolates, with minimum inhibitory concentration ranging from 25 to 100 mg/mL for most of the isolates. The crude extract showed the presence of alkaloids, flavonoids, terpenoids, and cardiac glycosides. The antioxidant assay for the concentrations 20, 40, 60, 80, and 100 mg/mL indicated a strong radical scavenging ability with percentage values 59.8, 63.2, 69.2, 70.9, and 71.4%, respectively, which indicates that *Carica papaya* leaves possess significant antioxidant properties.

Introduction

Escherichia coli (*E. coli*), a Gram-negative normal intestinal flora of humans and animals, has arisen as one of the most reported pathogens displaying resistance to multiple antibiotics. Infections caused by multidrug-resistant (MDR) *E. coli* strains, such as urinary tract infections, lungs, wounds, septicemia, bone marrow, brain, blood system, and gastrointestinal diseases, have globally led to high morbidity and mortality rates [1]. This constant rise of resistance has pointedly reduced the efficacy of conventional antibiotics, requiring the quest for other healing agents from natural sources. Interest in plant-derived antimicrobial agents has recently intensified due to their availability, safety, and lower tendency to induce resistance compared to synthetic drugs [2]. *Carica papaya*, commonly known as pawpaw or papaya, is a widely cultivated tropical plant belonging to the family Caricaceae.

Various parts of the plant, such as leaves, seeds, fruits, and latex, have been used in old medicine to treat some sicknesses, including malaria, typhoid fever, hypertension, pyrexia, gonorrhea, diabetes, syphilis, inflammation, and bacterial infections [3, 4]. This study aims to estimate the *in vitro* antibacterial efficiency of *Carica papaya* leaf extracts against MDR *Escherichia coli*.

Materials and methods

Culture Media and Reagents: MacConkey agar, Nutrient agar, Mueller-Hinton agar (MHA), and Nutrient broth (Titan Biotech, India) were the culture media used. They were prepared according to the instructions of the manufacturers. Simmon's citrate (Oxoid, England), peptone water (Titan Biotech, India), hydrogen peroxide (SKG Pharma. Ltd., Nigeria), crystal violet, Lugol's iodine, safranin (May and Baker Ltd., England), Kovac's reagent (Liofilchems, Italy), ethanol, Acetic acid (BDH Chemical Ltd., England) and dimethylsufoxide (DMSO) were the reagents used.

Identification and standardization of test microorganisms: The test organism used in this work includes multidrug-resistant strains of *E. coli* collected from the Laboratory of Pharmaceutical Microbiology and Biotechnology, Nnamdi Azikiwe University, Awka. Each of the test organisms was streaked on a fresh MacConkey agar plate and incubated at 37 °C for 24 hrs. The isolates were identified using macroscopy, microscopy, and confirmatory biochemical tests (oxidase, catalase, indole, and citrate tests) [5, 6]. The bacterial isolates were sub-cultured onto a nutrient agar plate and incubated at 37 °C for 18 - 24 hrs. The inoculum was standardized by transferring a pure culture of the test organism into 3.0 mL of sterile nutrient broth using a sterile wire loop. The suspension was incubated in an oxygen-rich shaker water bath for 3 hrs at 37°C to allow for the growth of the test organism to a density equivalent to the turbidity of 0.5 McFarland [7].

Antibiotic susceptibility testing: The susceptibility tests were performed using the M2A6 disc diffusion method [7] as follows. The standardized inocula were swabbed onto a Mueller-Hinton agar plate, and the discs were placed on the inoculated plates and pressed firmly onto the agar plate for complete contact. The plates were left for 30 min to allow for pre-diffusion of antibiotics into the agar and then incubated at 37°C for 18 - 24 hrs. The susceptibility of each isolate to antibiotics was shown by a clear zone of growth inhibition, and the diameter of the zones of inhibition was then interpreted using a standard chart [8].

Plant material and processing: Fresh, mature, and fully expanded leaves of *Carica papaya* (**Figure 1**) were collected from the trees growing at the waterside of Agulu Lake, Anambra State, Nigeria. They were identified and authenticated by a plant taxonomist in the Department of Pharmacognosy and Traditional Medicine, Nnamdi Azikiwe University, Awka, Nigeria.

Figure 1: *Carica papaya* leaves

The leaves were air dried under shade for some days until properly dried. The dried leaves were milled, and the weight was checked. The grounded leaves were subjected to extraction using Ethyl acetate solvent according to [6] with slight modifications. 85 g of leaves were soaked in 850ml ethyl acetate in a 1000 m1 glass beaker, stirred thoroughly, and allowed to stand for 72 hrs to enable complete extraction. It was carefully decanted and placed in a water bath to evaporate the solvent at a reduced temperature of 40 ℃. Stock concentrations of 100 mg/ml of the extract were made by weighing 400 mg of the crude extract and reconstituting it in 4 ml DMSO.

Preliminary antimicrobial assay: The antimicrobial assay for the plant extract was carried out using an agar well diffusion assay as described. The standardized organisms were inoculated onto previously sterilized MHA plates. A sterile cork borer was used to make five wells (5 mm in diameter) on each of the plates. Two-fold serial dilution was made from the stock concentrations to get graded concentrations of 100, 50, 25, 12.5, and 6.25 mg/ml of the extract. Aliquots of 70 µl of each extract dilution were applied in each of the wells in the culture plates previously seeded with the test organisms. Ciprofloxacin (5.6 mg/ml) served as the positive control against the test organisms. The cultures were incubated at 37 °C for 24 hrs. The antimicrobial activity of each extract was determined by measuring the zone of inhibition around each well. The experiment was done in triplicate against each organism [9, 10].

Antioxidant assay of crude extract of Carica papaya: A 16 g of the dried leaf extract was first dissolved in 2.0 ml of DMSO to obtain a concentrated stock solution. From this stock, serial dilutions were prepared to give different concentrations of 100, 80, 60, 40, and 20 mg/ml using methanol as the diluting solvent. The volumes of methanol used for each dilution were 2.70, 2.76, 2.82, 2.88, and 2.94 ml, respectively. A 0.1 mM 2,2-Diphenyl-1-picrylhydrazyl (DPPH) solution was freshly prepared in methanol and protected from light throughout the procedure. The antioxidant reaction mixture for each concentration was prepared by mixing 0.25 ml of each extract dilution with 2.0 ml of the DPPH solution in a clean cuvette. The negative control consisted of methanol mixed with DPPH, while ascorbic acid was used as the positive control. All mixtures were incubated in the dark at room temperature for 30 min to allow complete reaction between the DPPH radicals and the antioxidant components of the extract. After incubation, the absorbance of each sample and control was measured at 517 nm using a spectrophotometer, with methanol serving as the blank. The percentage inhibition of DPPH radicals by the extract was calculated, and the results were noted [11].

Phytochemical analysis: The extract was tested for the presence of various plant constituents like alkaloids, flavonoids, reducing sugars, saponins, proteins, tannins, amino acids, steroids, triterpenoids, and glycosides [12-14].

Results

Identification of test organisms: The results of the colonial, microscopic, and biochemical characteristics confirmed them as *Escherichia coli* isolates (**Table 1**).

Antibiotic susceptibility profile of Escherichia coli isolates: The antibiotic susceptibility results, as presented in **Tables 2** and **3**, revealed that all isolates exhibited resistance to multiple classes of antibiotics, indicating multidrug resistance.

Antimicrobial activities of the Carica papaya crude extract against the test organisms: The result obtained from **Table 3** showed that the extracts have antibacterial activity against the tested *E. coli* isolates. The highest activity was recorded at 100 mg/ml. Isolates EC2, EC3, EC4, EC5, EC6, and EC9 showed inhibition zones ranging from 4 mm to 5 mm.

Table 1: Identification of test organisms

Isolate code	Colony morphology	Gram- character	Microscopy	CAT	IND	OXI	CIT
EC 1	Milky, opaque, convex	Gram negative	Rods	+	+	-	-
EC 2	Milky, opaque, convex	Gram negative	Rods	+	+	-	-
EC 3	Milky, opaque, convex	Gram negative	Rods	+	+	-	-
EC 4	Milky, opaque, convex	Gram negative	Rods	+	+	-	-
EC 5	Milky, opaque, convex	Gram negative	Rods	+	+	-	-
EC 3*	Milky, opaque, convex	Gram negative	Rods	+	+	-	-
EC 7	Milky, opaque, convex	Gram negative	Rods	+	+	-	-
EC 8	Milky, opaque, convex	Gram negative	Rods	+	+	-	-
EC 9	Milky, opaque, convex	Gram negative	Rods	+	+	-	-
EC 10	Milky, opaque, convex	Gram negative	Rods	+	+	-	-

Key: CAT: catalase, OXI: oxidase, IND: indole, CIT: citrate, EC: *Escherichia coli* tests: + (positive reaction); - (negative reaction)

Table 2: Antibiotic susceptibility profile of *Escherichia coli* isolates

Isolate code	Antibiotics/inhibition zone diameter (mm)											
	ACX	CTX	IMP	NF	AUG	OFX	GN	ZEM	NA	CRO	LBC	CXM
EC 1	0±0	0±0	0±0	0 ± 0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
EC 2	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
EC 3	0±0	0±0	0±0	12±0	0±0	0±0	9±0	0±0	0±0	14±0.7	15±0	0±0
EC 4	0±0	0±0	0±0	12±0.7	0±0	0±0	0±0	0±0	10±0	20±1.2	16±0	0±0
EC 5	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
EC 6	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
EC 7	0±0	0±0	0±0	14±0	0±0	17±0	15±0	0±0	0±0	14±0	16±0.7	0±0
EC 8	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
EC 9	0±0	0±0	0±0	0±0	0±0	9±1.2	11±0	0±0	0±0	19±0	20±0	0±0
EC 10	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0

Key: ACX: Ampiclox (10 µg), CTX: Ceftotaxime (25 µg), IMP: Imipenem/Cilastatin (10/10 µg), NF: Nitrofurantoin (300 µg), AUG: Amoxicillin Clavulanate (30 µg), OFX: Ofloxacin (5 µg), GN: Gentamycin (10 µg), ZEM: Cefexime (5 µg), NA: Nalidixic Acid (30 µg), CRO: Ceftriaxone Sulbactam (45 µg), LBC: Levofloxacin (5 µg); CXM: Cefuroxime (30 µg), EC (*Escherichia coli*); 0±0 no inhibition zone.

Table 3: Antimicrobial activities of the *Carica papaya* Crude extract against the test organisms

Conc. (mg/mL)	Test organisms/inhibition zone diameter (mm)									
	EC1	EC2	EC3	EC4	EC5	EC6	EC7	EC8	EC9	EC10
100	0±0	5±0	4±0	5±0	4±0	5±0	0±0	0±0	5±0	3±0
50	0±0	4±0.7	3±0.7	3±0.7	3±0	4±0	0±0	0±0	4±0	0±0
25	0±0	4±0	3±0	3±0	0±0	3±0	0±0	0±0	2.5±0	0±0
12.5	0±0	3±0	1.5±0	2.5±0	0±0	2±0	0±0	0±0	2±0	0±0
6.25	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0

Key: EC (*Escherichia coli),* 0±0 no inhibition zone.

Antioxidant assay of crude extract of Carica papaya: The crude extract of *Carica Papaya* showed a percentage inhibition which ranged from 59.8% at 20 mg/ml to 71.4% at 100 mg/ml, indicating that antioxidant activity increased with an increase in concentration (**Table 4**).

Phytochemical assay of Carica papaya extract: The result of the phytochemical screening of the *Carica papaya* leaf extract (**Table 5**) revealed the presence of Alkaloids, flavonoids, terpenoids, and cardiac glycosides. indicating the extract antimicrobial, antioxidant, and therapeutic activities [22]. However, reducing sugars, saponins, tannins, and proteins were not detected in the extract.

Table 4: Antioxidant assay of crude extract of *Carica papaya*

Concentration (mg/mL)	*Carica papaya*	Ascorbic acid
	Mean absorbance / % Inhibition	Mean absorbance / % Inhibition
100	0.230 (71.4)	0.200 (87.5)
80	0.233 (70.9)	0.205 (82.9)
60	0.248 (69.2)	0.211 (77.7)
40	0.296 (63.2)	0.216 (73.6)
20	0.323 (59.8)	0.220 (70.45)

Table 5: Phytochemical assay of *Carica papaya* extract

Plant	Alkaloid	Flavonoid	Terpenoid	Reducing sugars	Cardiac glycosides	Saponin	Tanin	Protein
Carica papaya	+	+	+	-	+	-	-	-

Discussion

The high resistance of the bacterial isolates to antibiotics agrees with reports that *Escherichia coli* rapidly acquires antibiotic resistance due to horizontal gene transfer and antibiotic misuse [15-17]. The antibacterial activity of the extract supports previous findings of [18], which revealed that *Carica papaya* is effective against resistant *E. coli* strains. The inhibition zone diameter decreased as the extract concentration decreased, which indicates a concentration-dependent antibacterial response. Conversely, EC1, EC7, EC8, and EC10 showed no inhibition zone diameter, which indicates resistance. The results confirm that *Carica papaya* possesses notable antioxidant potential and free radical scavenging ability. This can counter pro-oxidants through several signaling pathways that either help the expression of antioxidant enzymes or reduce ROS production. As such, it can be combined into supplements and medications to help handle health situations motivated by oxidative stress. Our work agrees with the studies of [19, 20] that reported the plant's antioxidant properties. The presence of these bioactive compounds gives Carica *papaya* leaves their medicinal value, as alkaloids and flavonoids are known for their strong antimicrobial and antioxidant properties [21], while terpenoids and cardiac glycosides contribute to antibacterial and anti-inflammatory activities [22].

Conclusion: This study demonstrates that the ethanolic extract of *Carica papaya* leaves possesses antibacterial and antioxidant effects against several multidrug-resistant *Escherichia coli* isolates within the environment, with activity increasing in a concentration-dependent manner. Their efficiency could be due to the presence of alkaloids, flavonoids, terpenoids, and cardiac glycosides found in the extract. We therefore recommend further studies on the extract to determine its safety and toxicity profile. Also, clinical studies should be conducted to assess its potential use for the management of resistant *Escherichia coli* infections.

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