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Antipseudomonal Activities of Aqueous and Methanolic Peel Extracts of *Carica papaya* L. (Pawpaw) Unripe Fruits

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Abstract: *In response to the multi-drug resistance of Pseudomonas aeruginosa, the discovery of new effective agents is important in overcoming the infections resulting from the organisms. The present study assessed the antipseudomonal activities of aqueous and methanolic peel extracts of Carica papaya L. (pawpaw) unripe fruits against ten (10) multi-drug resistant (MDR) P. aeruginosa clinical isolates obtained from patients attending University of Maiduguri Teaching Hospital. The unripe fruit peels were extracted by maceration using distilled water and methanol (99%) and the antipseudomonal activities evaluated by modified diffusion method using concentrations of 20, 40, 80 and 160mg/ml. The activities of both extracts were compared by determining Extract Potency Index (EPI). The mean age ± standard deviation (range) of the patients from whom the isolates were obtained was 34.1 ± 14.3 (21.0 – 72.0) years. Significantly highest proportion (60.0%) of the isolates was obtained from wound swabs ($p < 0.05$). The aqueous peel extract had percentage yield of 24.6% (35g/145g) as against 21.1% (21.1g/100g) for methanolic peel extract ($\chi^2 = 1.2$, $df = 1$, $p > 0.05$). Both extracts demonstrated a concentration-dependent inhibition of all the 10 isolates with methanolic peel extract having larger zones of inhibition. The mean zone of inhibition produced by methanolic extract at 160mg/ml (17.9 ± 2.3mm) was similar to that of ciprofloxacin (30µg) with 19.7 ± 3.7mm ($p > 0.05$) but significantly higher than 14.5 ± 2.3mm produced by aqueous extract at 160mg/ml ($p < 0.05$). In addition, the overall mean ± standard deviation (range) of EPI was 0.77 ± 0.22 (0.25 – 1.00); this was significantly higher for methanolic peel extract with 0.93 ± 0.12 (0.75 – 1.00) than aqueous peel extract with 0.63 ± 0.32 (0.25 – 1.00) [$p < 0.05$]. These findings could contribute to effective use of C. papaya L. unripe fruits and may offer alternative treatment strategy for pseudomonal infections.*

Keywords: *Pseudomonas aeruginosa, Carica papaya L., Unripe fruit, Antibacterial activity*

I. Introduction

C. papaya L. (commonly called pawpaw or papaya) is a fast growing, usually unbranched tree or shrub with a single stem growing from 5 – 10m tall with spirally arranged leaves confined to the top of the trunk [1]. It belongs to the family Caricaceae. The fruit varies in shapes (oval, round, cylindrical or club-shaped) and sizes (0.5 – 2.25kg); the greenish unripe fruit contains latex which disappears as the fruit ripens to a light or dark yellow. The thick juicy flesh of the fruit may be yellow, orange or red in colour [2].

C. papaya L. is largely used in traditional medicine for treatment of various diseases, various parts are often used to treat different illnesses. For instance, the root of the male plant is chewed with seven seeds of Melegueta pepper during labour for prompt delivery. The leaves are used in many decoctions for bathing children [3]. The aqueous extract of the unripe fruits is used in southwest Nigeria to relief crises in patients with sickle cell anaemia [4]. The fresh and dried leaves are used in treatment of diarrhoea and typhoid. The roots and unripe fruits are made into concoctions to manage conditions such as asthma, stomach upset, diarrhoea and worm infestation [5,6].

In addition, previous studies have highlighted the pharmacological significance of the plant. For instance, the antibacterial activities of various parts of the plant have been described [7-10]. Other medicinal uses of the plant are treatment of malaria, typhoid fever, diarrhoea, dyspepsis, worm infestation and impotence among others [5,6]. These activities are attributed to presence of some phytoconstituents notably are chymopapain and papain [11].

Pseudomonas aeruginosa, a Gram negative, coccobacillus bacterium with unipolar motility belongs to the family Pseudomonadaceace. It is a major cause of nosocomial infections especially among immuno-compromised individuals [12,13]. It is implicated in several conditions such as cancer, nosocomial pneumonia, urinary tract infections, severe burns etc [14-16]. The multi-drug resistant nature of the bacteria markedly limits

therapeutic options, hence, the need to seek for alternative means for treatment of pseudomonal infections. Thus, this study assessed the antipseudomonal activities of aqueous and methanolic peel extracts of *C. papaya* L. unripe fruits.

II. Materials And Methods

2.1 Collection of bacterial isolates

Ten (10) clinical isolates of *P. aeruginosa* were obtained from the samples from patients seen at Medical Microbiology Unit, University of Maiduguri Teaching Hospital (UMTH), Maiduguri, Nigeria between February and May, 2015. The isolates were identified by morphological features on culture plate and biochemical analyses. The antibiogram for each isolate was determined as described by Cheesbrough [17] and the details of the samples were documented. The isolates were collected on sterile agar slants and kept as stock cultures in refrigerator at 4^oC until use.

2.2 Collection and authentication of the fruits

The unripe fruits of *C. papaya* L. were collected at University of Maiduguri Campus and the plant was authenticated by Prof. S. S. Sanusi of the Department of Biological Sciences, Faculty of Sciences, University of Maiduguri. A voucher specimen (Voucher Number 010) was deposited at Pharmacology Laboratory, Department of Clinical Pharmacology and Therapeutics, College of Medical Sciences, University of Maiduguri, Nigeria.

2.3 Preparation of the peel extracts

The fresh unripe fruits were thoroughly rinsed with clean water and the peels were carefully removed. The peels were spread on laboratory bench and air-dried within one week. The dried peels were ground into powder using electric grinder. Approximately, 145g of the powder was subjected to maceration in 4,400ml of distilled water and 100g in 800ml of methanol with intermittent shaking for 24 hours at room temperature to obtain aqueous and methanolic peel extracts, respectively. The solutions were filtered and the filtrates subjected to evaporation; the extract yield was determined [18].

2.4 Assessment of antipseudomonal activities of the peel extracts

The antipseudomonal activities of the peel extracts were conducted as previously described by Ifeoma *et al*. [18]. A fresh inoculum of each isolate was prepared in peptone water to match the turbidity of McFarland standard. The Mueller Hinton media was inoculated by spread plate method. A sterilized improvised cork borer of 6mm in diameter was then used to bore five (5) holes on each inoculated plate. Varying concentrations (20, 40, 80 and 160mg/ml) of the peel extracts were administered to the holes and properly labeled; distilled water was dispensed into the 5^{th} hole. Each of the isolate was done in triplicate. The plates were incubated at 37^oC for 24 hours, following which the zones of inhibition were measured to the nearest millimetres along two axes (90^o to each other) and the mean of the two readings recorded. In addition, the antipseudomonal activities of the two extracts were compared using Extract Potency Index defined as:

$$Extract\ Potency\ Index = \frac{Number\ of\ effective\ concentration}{Total\ number\ of\ concentration}$$

2.5 Data analysis

The data generated from the study were analyzed using statistical software, Statistical Package for Social Sciences version 21 [19]. Proportion was compared using Chi-square while means were compared using analysis of variance (ANOVA). Significance was inferred at $p \leq 0.05$.

III. Results

3.1 Profile of the *P. aeruginosa* clinical isolates

The mean age ± SD (range) of the patients from whom the 10 isolates were obtained was 34.1 ± 14.3 (21.0 – 72.0) years with 60% (6/10) of the patients being female. Significantly highest proportion (60.0%) of the isolates was obtained from wound swab ($p < 0.05$). The clinical isolates were sensitive to maximum of three antibacterial drugs and resistant to maximum of nine antibacterial drugs. Among the drugs tested, ciprofloxacin had the highest sensitivity of 70%, 7/10 ($p < 0.05$) [Table 1].

3.2 Nature of the peel extracts

The aqueous peel extract had percentage yield of 24.6% (35/145g) as against 21.1% (21.1/100g) for methanolic peel extract ($\chi^2 = 1.2$, df = 1, $p > 0.05$). The aqueous peel extract was brownish dry lumps while the methanolic peel extract was brownish oily paste.

3.3 Antipseudomonal activities of the peel extracts

Tables 2 and 3 show the zones of inhibition produced by the aqueous and methanolic peel extracts, respectively, against the ten (10) isolates. The extracts demonstrated a concentration-dependent inhibition of all the 10 isolates with methanolic peel extract having larger zones of inhibition. The mean zone of inhibition produced by methanolic extract at 160mg/ml (17.9 ± 2.3mm) was similar to that of ciprofloxacin (30µg) with 19.7 ± 3.7mm ($p > 0.05$) but significantly higher than 14.5 ± 2.3mm produced by aqueous extract at 160mg/ml ($p < 0.05$). In addition, the overall mean ± SD (range) EPI was 0.77 ± 0.22 (0.25 – 1.00); this was significantly higher for methanolic peel extract with 0.93 ± 0.12 (0.75 – 1.00) than aqueous peel extract with 0.63 ± 0.32 (0.25 – 1.00) [$p < 0.05$].

IV. Discussion

In the present study, the antipseudomonal activities of the aqueous and methanolic peel extracts of *C. papaya* L. unripe fruits were demonstrated against multi-drug resistant clinical isolates of *P. aeruginosa*.

The multi-drug resistance nature of *P. aeruginosa* has been previously described. The microbe is naturally resistant to commonly used antibacterial drugs and also often acquires resistance to initially sensitive antibacterial drugs [20]. Thus, the antibiogram displayed by the ten (10) isolates used in the present study is in accordance with previously reported antibiogram [20,21]. In addition, the fact that the majority of the isolates were obtained from wound swab is in agreement with the reports that the microbe frequently colonises wound and burns [22].

The assessment of the antipseudomonal activities revealed that both the aqueous and methanolic peel extracts of *C. papaya* L. unripe fruits demonstrated remarkable activities against all the ten (10) clinical isolates studied. This finding is in agreement with previous studies that have shown that some parts of *C. papaya* L. have pharmacological activities [5]. Similarly, Okunola *et al.* [10] had earlier reported the broad spectrum antibacterial activities of the fresh and dried leaves against Gram positive and negative clinical isolates including *P. aeruginosa*. The fact that the ten (10) isolates used in the present study were multi-drug resistance is an indication that the peel could be a potential source of drug against multi-drug resistant strains of bacteria. The authors opined that the higher EPI recorded for methanolic peel extract may be due to the ability of methanol to dissolve more biochemically active phytochemicals from the peel than water. *C. papaya* L. has been reported to contain some phytochemicals of antimicrobial importance, these include: alkaloids, tannins, saponins, phenolics and flavonoids [6]. Thus, the antipseudomonal activities observed in the present study may be attributed to the presence of certain phytochemicals present in the peel.

V. Conclusion

The aqueous and methanolic peel extracts of *C. papaya* L. unripe fruits demonstrated remarkable antipseudomonal activities with methanolic peel extract having higher activity. This finding provides evidence-based supports for some of the traditional uses of pawpaw.

Table 1: Profile of the *Pseudomonas aeruginosa* clinical isolates

Isolates	Source of Isolates				Antibiogram	
	Sample	Patient Age (years)	Patient Sex		Sensitive	Resistance
P1	Wound swab	30	Male		CPX, SXT, CN	S, R, AM, Z, PF, OFX,
P2	Wound swab	39	Female		CPX, SXT, OFX	CN, S, Z, AM, CH, SP,PF
P3	Wound swab	21	Female		R	CPX, SXT, S, OFX, CN, Z, CH, PF, SP
P4	Urine	35	Female		SP, CPX, CN	SXT, R, S, OFX, Z, PF, AM
P5	Urine	25	Female		CPX, S, OFX	CN, SXT, PF AM, SP, R, CH
P6	Wound swab	30	Male		CPX, SXT, CN	SP, AM, Z, S, PF, OFX, CH
P7	Ear swab	27	Male		R	CPX, SXT, S, OFX, CN, Z, CH, PF,SP
P8	Wound swab	34	Female		PF, LE	CPX, SXT, S, OFX, CN, CH, PF,SP
P9	Wound swab	28	Female		PF, CPS, LF	SP, SXT, S, OFX, CN, CH, SXT,
P10	Catheter tip	72	Male		CPX	SP, Z, PF, SXT, S, OFX, CN, CH, PF,

AM: Amoxacillin, CH: Chloramphenicol, CN: Gentamycin, CPX: Ciprofloxacin, LF: Levofloxacin, OFX: Ofloxacin, P1-P10: *Pseudomonas aeruginosa* clinical isolates, PF: Pefloxacin, R: Ceftriazone, S: Streptomycin, SP: Sparfloxacin, SXT: Cotrimoxazole, Z: Cefuroxime

Table 2: Zones of inhibition produced by aqueous peel extract

Extract Concentration (mg/ml)	Zones of Inhibition (mm)									
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
20	0.0±0.0	12.0±1.6	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	10.0±2.5	0.0±0.0	11.0±3.1
40	12.0±2.1	13.0±1.8	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	12.0±1.4	14.0±2.2	0.0±0.0	12.0±1.9
80	13.0±2.6	15.0±1.4	0.0±0.0	10.0±1.9	0.0±0.0	0.0±0.0	13.0±1.7	14.0±1.5	13.0±2.6	14.0±1.2
160	15.0±2.8	17.0±1.6	11.0±2.1	14.0±3.1	13.0±2.3	13.0±2.1	16.0±2.9	16.0±1.8	15.0±2.0	15.0±2.5

P1 – P10: *Pseudomonas aeruginosa* clinical isolates
Zone of inhibition for ciprofloxacin (30µg) = 19.7±3.7mm

Table 3: Zones of inhibition produced by methanolic peel extract

Extract Concentration (mg/ml)	Zones of Inhibition (mm)									
	P1	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**	**P9**	**P10**
20	14.0±2.1	13.0±1.7	13.0±1.3	14.0±1.3	0.0±0.0	0.0±0.0	0.0±0.0	13.0±2.9	12.0±2.2	11.0±1.6
40	19.0±2.7	14.0±2.3	15.0±2.5	16.0±1.8	13.0±2.9	11.0±1.1	14.0±2.3	15.0±2.7	13.0±1.9	12.0±2.1
80	21.0±3.1	19.0±1.5	17.0±3.0	17.0±3.1	14.0±1.6	12.0±2.9	15.0±1.5	19.0±1.4	15.0±2.4	14.0±1.8
160	23.0±1.2	20.0±2.6	20.0±1.9	18.0±2.2	16.0±1.7	13.0±2.9	16.0±2.3	20.0±3.1	19.0±1.7	14.0±2.9

P1 – P10: *Pseudomonas aeruginosa* clinical isolates
Zone of inhibition for ciprofloxacin (30µg) = 19.7±3.7mm

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