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Antimicrobial Potentials of *Carica Papaya* Latex and Seed Extract

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Abstract:
The increasing resistance of microorganism to antibiotics and toxicity of some antibiotics drugs has resulted in the search for more effective therapy from new organic molecules from plants with antibacterial and antifungal properties. This study is important to see the increasing resistance of micro-organisms to antibiotics. It is also carried out to see the effect of pawpaw seed extracts and latex on fungi and bacteria. The pawpaw latex and seed oil extract were analyzed for their antimicrobial activities. The pawpaw seed oil extract and latex had marked bactericidal effect against clinical isolates (B. subtilis, E. coli, S. aureus) and fungicidal effect against the isolates (Microsporumspp, Trichonphytonspp and Epidermophytonspp). The bactericidal and fungicidal effects of the extract were dose dependent. Minimum inhibitory concentration for the bacteria isolates (S. aureus and E. coli) were 2.5mg/ml and 1.0mg/ml while B. subilis showed no inhibition with latex but was inhibited by the seed oil extract at 1.50mg/ml. The minimum inhibitory concentration of the fungi was higher in the seed extract than the latex with the exception in C. albicans. The result obtained showed that pawpaw seed oil extract and latex have some antimicrobial properties. The scientific implications of these results are fully discussed.
Keywords: *Pawpaw, Pawpaw latex, Pawpaw seed oil, Antimicrobial Properties, Fungi, Bacteria,*

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I. Introduction

Antimicrobial agents are very important in reducing the global weight of infectious diseases[1]. Due to fewer, or even sometimes no effective antimicrobial agents available for the infection caused by pathogenic bacteria, emergence and dissemination of multidrug resistant (MDR) strain in pathogenic bacteria have become a known public health threat [2,3]. Many plants have been beneficial because of their antimicrobial traits, which are as a result of phytochemicals synthesized in the secondary metabolism of the plant[4,5].

Carica papaya belongs to family Caricaceaus widely known as papaya. It originated from tropical America and was introduce to India in 16th century. The plant is known by its weak and usually unbranched soft stem providing copious white latex and crowded by terminal cluster of large and long stalked leaves[6]. Papaya is a known tropical fruit rich in dietary antioxidants (vitamin C, tocopherols, total phenols, and β-carotene)[7] and bioactive phytochemicals with antioxidant activity (benzyl isothiocyanate)[8].

In folk medication, various parts of C. papaya (leaves, barks, roots, latex, fruit, flowers, and seeds) are used to treat a wide range of diseases[9]. In addition to all this, digestive enzyme papain can be found C. papaya which is used to treat causes of trauma, allergies and sports injuries. All the nutrients present in papaya as a whole improve cardiovascular system, protect against heart diseases, heart attacks, strokes and prevent colon cancer and the enzyme used to treat arthritis[10]. Many of these traditional uses have been validated by several scientific studies by proving that C. papaya displays a broad range of therapeutic activities (i.e., antiprotozoal, antifungal, antibacterial, antiviral, anti-inflammatory, antihypertensive, hypoglycemic and hypolipidemic, wound healing, antitumor, neuroprotective, diuretic, abortifacient, and antifertility). These properties mainly depend on the antioxidant activity of some secondary metabolites present in the C. papaya organ[9].

Traditional medicine or herbal medicine occupies a very important position in the communities care delivery of our traditional communities, whose population are mainly peasant and cannot afford the orthodox medicinal remedies. Knowing the unique effectiveness of herbal remedies in the treatment of diseases, scientists have carried out researches into the underlying principle responsibility for their efficacy. Promoting effect of these plants have been attributed bt various researchers traditionally and a lot of works have been carried out to find out specific basis involving the use of these plants as antimicrobial agents. Some specific parts of C. papaya have recorded antimicrobial activities or effect in some medicinal plants. Example is the antimicrobial properties of pawpaw seed and latex. It has been reported that latex extract prevents Candida albicans and seed extract against *Samonellatyphi*, *Escherichia coli* introvitro showing an antibacterial properly[10].

About 80% of four billion inhabitants of the earth which has been estimated rely on herbal medicine for their health care needs. Generally, plants have three main effects on man namely therapeutic, nourishing and psychological affects[11].

More effective therapy from new organic molecules from plants with antibacterial and antifungal properties has been discovered due to increasing resistance of microorganism to antibiotics and toxicity of some antibiotics drugs. Thus the objective of this present study is to verify the supported claim by various scientists who have work on the antimicrobial properties of pawpaw seed and latex. This study will also determine the chemical constituents that may be responsible for its other medical values. This study is important to see the increasing resistance of micro-organisms to antibiotics. It is also carried out to see the effect of pawpaw seed extracts and latex on fungi and bacteria.

II. Materials And Methods

Sample collection and preparation

The pawpaw seeds were collected from a fresh ripe pawpaw purchased at Uyo market, Akwa Ibom State. The sample at their point of collection were washed thoroughly under running tap water and air-dried at room temperature (28) before storing in polythene bags and labeled properly before being transported to the laboratory. The latex was collected from a matured pawpaw trunk in a sterilized bottle at room temperature (28 ℃) and was stored at 4 ℃. Two sets of microorganism were used for the assay. The first set was bacterial isolates comprising: *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus aureus*. Pure cultures of the isolates were derived from stock culture of the respective specie kept in the Department of Microbiology, University of Uyo, Akwa Ibom State. The second set comprised of pure cultures of dermotophytes namely *microsporumoudounii*, *Epidermophytonspp*, *Trichophyton mentagrophytes* and *Candida albicans* originating from clinical specimens were obtained from the medical laboratory unit of University of Uyo Health Centre. The identified fungal isolate were maintained on sabouraud dextrose broth (SOB) at 37 ℃ because they are diphosic and at room temperature (28 ℃) the organisms develop into flamentous forms. On the other hand the bacterial cultures were maintained in nutrient broth at 37 ℃.

Preparation of plant sample

After collection and identification of the sample (seed), it was dried in an oven at a temperature of 80 ℃. The cleaned dried samples where milled to obtain a uniform fine particle size powder with an electric blender (Model: BLG-595MK2 from China). The extraction of oil from the seed sample was done using soxhlet extraction method according to the method described by James *et al.*[12]

Preparation of Inocula

The dermatophytes (ringworm pathogen) were grown at 37 ℃ for 72 hours in sabouraud broth (SOB). The bacterial isolates were cultured on nutrient agar broth at 37 ℃ for 24 hours. The cultures were homogenized in a vorlex apparatus and served for antimicrobial assay.

Determination of Antimicrobial Activity of Pawpaw latex and Seed

The plant latex and seed extract were screened for antimicrobial activity using the cup-plate agar diffusion technique according to the method of Sarita *et al*.[13]

III. Results

Antifungal activity of pawpaw latex against clinical isolates of dermatophytes

Antifungal activities of pawpaw latex against clinical isolates of dermatophytes were shown in Table 1. The test organism *Trichophyton mentagrophytes* was sensitive to the latex at concentration of 1.00 mg/ml to 2.50 mg/ml and from 0.25 mg/m. to 0.50 mg/ml was resistance. *Microsporumoudouinii* was resistant at concentration of 0.25 mg/ml to 0.50 mg/ml and was sensitive at the concentration of 1.00 mg/ml to 2.5 mg/ml. *Microsporumoudouinii* showed the highest inhibitory activity against the latex at the concentration of 2.5mg/ml. *Epidermophytonspp* showed inhibition, at the 1.5mg/ml to 2.5 mg/ml and was resistant at 0.25 to 1.00 mg/ml *Candida albicans*was sensitive at the concentration of 2.0mg/ml and 2.5mg/ml was resistant at 0.25 mg/ml to 1.50 mg/ml.

Table 1: Antifungal activity of pawpaw latex against clinical isolates of dermatophytes

TEST ORGANISM	Concentration (mg/ml) zone of inhibition (mm)					
	0.25mg/ml (mm)	0.50mg/ml (mm)	1.00mg/ml (mm)	1.50mg/ml (mm)	2.00mg/ml (mm)	2.50mg/ml (mm)
Trichophyton mentagrophtes	3.8	9.21	14	19	23	26
Microsporum Oudouinii	4	8	10	16	18	19
Epidermophyton spp.	3.2	6.7	8.3	10.3	14	16
Candida albicans	2.2	4.5	8.1	9.3	11	13
Control	-	-	-	-	-	-

>10mm = sensitive <10mm = Resistant. The degree of sensitivity was expressed by a measure of the diameter of zone of inhibition. 10mm or higher was considered as an indication of the sensitivity of the isolates to the seed extract.

Antibacterial activity of pawpaw latex against clinical isolates of bacteria

Antibacterial activities of pawpaw latex against clinical isolates of bacteria were shown in Table 2. The result revealed that staphylococcus aureus showed inhibition at concentration of 2.50 mg/ml and resistant at 0.25 mg/ml to 2.00 mg/ml. *Esherichia coli* was inhibited at 1.00 mg/ml to 2.50 mg/ml and resistant at 0.25 mg/ml to 0.5 0mg/ml. *Bacillus subtilis* showed a complete resistance to the pawpaw latex in all the concentrations.

Table 2: Antibacterial activity of pawpaw latex against clinical isolates of bacteria

TEST ORGANISM	Concentration (mg/ml) zone of inhibition (mm)					
	0.25mg/ml (mm)	0.50mg/ml (mm)	1.00mg/ml (mm)	1.50mg/ml (mm)	2.00mg/ml (mm)	2.50mg/ml (mm)
Staphylococcus	4	5	5.8	7.3	9.1	13.1
Escherichia coli	6	9	13	14	16	17
Bacillus subtilis	4	6	7	7.5	8	9
Control	-	-	-	-	-	-

>10mm = sensitive <10mm = Resistant. The degree of sensitivity was expressed by a measure of the diameter of zone of inhibition. 10mm or higher was considered as an indication of the sensitivity of the isolates to the seed extract.

Antifungal activity of pawpaw seed oil extract against clinical isolates of dermatophytes

Antifungal activities of pawpaw seed oil extract against clinical isolates of dermatophytes were shown in Table 3. The result of the antifungal activity test of pawpaw seed oil extract on dematophytic fungi showed that the test organism *Trichonphytonmentagrphytes* was sensitive to the seed oil at the concentration of 1.50 mg/ml to 2.50 mg/ml and showed highest inhibitory activity at the concentration of 2.50 mg/ml and resistant at 0.25 mg/ml to 1.00 mg/ml. *Microsporumoudounii* showed inhibition at 1.50 mg/ml to 2.50 mg/ml and was resistant at 0.25 mg/ml to 1.00 mg/ml. *Epidermophytonspp* was resistant at 0.25 mg/ml to 1.50 mg/ml and was inhibited at 2.00 mg/ml to 2.50 mg/ml *Candida albicans* shared inhibition at 1.00 mg/ml to 2.50 mg/ml and resistant at 0.25 mg/ml to 1.00 mg/ml.

Table 3: Antifungal activity of pawpaw seed oil extract against clinical isolates of dermatophytes

TEST ORGANISM	Concentration (mg/ml) zone of inhibition (mm)					
	0.25mg/ml (mm)	0.50mg/ml (mm)	1.00mg/ml (mm)	1.50mg/ml (mm)	2.00mg/ml (mm)	2.50mg/ml (mm)
Trichophyton mentagrophtes	2.7	7.1	9.3	14	16	17

Microsporum Oudouinii	3	8	9	12	13	16
Epidermophyton spp.	2.9	7	8	9	10	12
Candida albicans	3	7.1	11	14	15	16
Control	-	-	-	-	-	-

>10mm = sensitive <10mm = Resistant. The degree of sensitivity was expressed by a measure of the diameter of zone of inhibition. 10mm or higher was considered as an indication of the sensitivity of the isolates to the seed extract.

Antibacterial activity of pawpaw seed oil extract against clinical isolates of bacteria

Antibacterial activities of pawpaw seed oil extract against clinical isolates of bacteria were shown in Table 4. The result showed that *Escherichia* coli showed inhibition at 1.00mg/ml to 2.50mg/ml and resistant at 0.25mg/ml to 0.50mg/ml. *E. coli* showed the highest inhibitory activity at 2.50mg/ml the test organism. *Staphylococcus aureus* showed inhibition at 2.50mg/ml and resistant at 0.25mg/ml. *Bacillus subtilis* was inhibited at 1.50mg/ml to 2.50mg/ml and resistant at 0.25mg/ml to 1.00mg/ml. These results show that the degree of inhibition or susceptibility of the organism to both extracts depend on the concentration of the latex and seed extract.

Table 4: Antibacterial activity of pawpaw seed oil extract against clinical isolates of bacteria

TEST ORGANISM	Concentration (mg/ml) zone of inhibition (mm)					
	0.25mg/ml (mm)	0.50mg/ml (mm)	1.00mg/ml (mm)	1.50mg/ml (mm)	2.00mg/ml (mm)	2.50mg/ml (mm)
Staphylococcus aureus	2	4	6	7	9	12
Escherichia coli	5	7	10	14	16	19
Bacillus subtilis	3	7	8	10	12	12
Control	-	-	-	-	-	-

>10mm = sensitive <10mm = Resistant. The degree of sensitivity was expressed by a measure of the diameter of zone of inhibition. 10mm or higher was considered as an indication of the sensitivity of the isolates to the seed extract.

IV. Discussion

Because of the presence of phytochemical which have long been known for their therapeutic functions, some plants are said to be medicinal. The antimicrobial properties of plants containing flavonoids, tannis, trapenoids, alkaloids, glycosides among other known phytochemical have been documented by many researchers[14]. The pharmacological properties of this class of plant make them beneficial as common primary health remedies in Africa, Asia, and Latin America[15]. In this research work, latex and seed oil extract of pawpaw has shown great antimicrobial properties, inhibiting the growth of wide spectrum of microorganism ranging from filamentous fungi (dermatophytes) to bacteria used in these experimental works. The research shows that the latex of papaya exhibit antifungal properties as well as antibacterial properties and inhibits the growth of *Trichonphytonsp* and *Candida albicans* (fungi). The experiment on *Staphylococcus aureus*, *Escherichia Coli* and *Bacillus subitilis* showed that the test organisms were sensitive to the antibacterial effect of the papaya latex except *B. subtilis*. This result agrees with the work of other researchers on the antimicrobial effect of papaya latex and other latex from medicinal plants. Osato *et al.*[16] reported that the latex of *carica papaya* shows bacteriostatic activities to *Entrobactacloaeae, E. coli, vulgaris*. Also, Lohiya *et al.*[17] also recorded antimicrobial activities of papaya latex, pinpointing its excellent growth inhibition on *Candida albicans* and other pathogenic fungi. He also reported bacteriostatic property of the latex of papaya against *E. coli, Salmonella typhi* and *Staphylococcus aureus.*

Papaya latex has been reported to be rich in various phytochemicals and proteolytic active components. Papaya latex is known to contain at least four proteolytically active components including papain, chymopapain A & B, and papaya peptidase A[18]. These proteolytic enzymes have been reported to inhibit the growth of Candida albicans by preventing it from attaching to the cell wall. The proteolytic activities of these enzymes can cause the description of bacteria cell wall or prevent the transpeptidation stage in the synthesis of bacteria cell wall. Pawpaw latex is known to contain flavonoids, alkaloids, terpenoids, tannin etc. which are known to have broad antimicrobial properties preventing the growth of various species of bacteria and fungi[19].

The result of antimicrobial effects of papaya seed oil compared with that of the latex showed the seed oil exhibits greater inhibitory effects on test bacteria than observed in latex. The seed oil exhibits detrimental effect on *B. subtilis* that showed resistance to latex in all concentrations. This is in accordance with the reported work of Lohiya, *et al.*[17] who reported that papaya possess bacteriostatic effect on *B. subtilis* and other microorganism. The resistance of *B. subtilis* to the latex of papaya may be attributed to the fact that it is a spore-forming bacteria but the sensitivity of seed oil of the same plant (papaya) to *B. subtilis* and bacteria and fungi with various zones of inhibition used in this experiment proves the variation of the same plants and this conforms to the reported work of Essien *et al.*[20]. In their work on "chemical analysis and antibacterial activity of seeds extract of ripe and unripe pawpaw fruits" showed that the sensitivity of *B. subtilis* to the seed oil extract was as a result of concentration of active constituents, the extracting solvent and their stability at room temperature.

The antimicrobial potential of papaya (seed oil and latex) is as a result of its high contents of phytochemicals such as flavonoids, tannis, alkaloids, terpenes etc. and many important proteolytic enzymes such as papain, chymopapain A & B, papaya peptidase A, Carpain etc. which are believed to interfere with the growth of microorganisms, exhibiting detrimental effect on them.

Flavonoid, one of the known phytochemicals with antimicrobial properties present in pawpaw latex and seed oil have been established to contain great antimicrobial potential as a result of its ability to complex with bacteria cell wall protein components and extracellular and soluble proteins. Lipophilic flavonoids are also reported to have damaging effect on microbial membranes[21]. Also predominant in the seed oil and latex are tannins. Studies show that tannins in plants have antimicrobial property and its mode of action has been reported to their ability to inactivate microbial adhesion enzymes, cell envelope transport protein or direct inactivation of microorganism[16].

The findings of this research work demonstrating the inhibitory effects of papaya seed oil and latex on Trichonphytonmentagrophytes, Microsporumoudouinii, Epidermophytonsp, Candkdaalbicans, E. coli, Staphylococcus aureus justify the reported use of papaya in the treatment of various skin infections, burns, wounds, gastrointestinal problems etc by the traditional medicine practitioners[22,23].

V. Conclusion

From the experiment, antimicrobial assay of pawpaw seed and latex were carried out. Pawpaw seed and latex showed an inhibitory effect on the growth of bacteria and fungi. Increase in concentration of seed and latex increases the effect of its inhibitory actions (i.e. the higher the concentration the more the zone of inhibition). One of the bacteria isolate was resistant to the latex e.g. *B. subtilis* even at the highest concentration but was inhibited by the seed oil extract. The activity of pawpaw seed and latex (i.e. antimicrobial activities) were due to the phytochemical constituents which includes terpenes, flavonoids, tannins etc. The result obtained in this work justifies the fact that pawpaw seed and latex is a medicinal plant, it was found to inhibit *S. aureus* and *E. coli* obtained from wound and stomach respectively and dematophytic fungi known to affect the skin, hair and nails of human. Thus it is effective in the treatment of a variety of ailments and also useful in industries and for dietetic purposes.

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