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Exploring the Antibacterial Properties of *Carica papaya* Unripe Fruit Extracts on Wound Isolates: Phytochemical Analysis and Susceptibility Testing

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ABSTRACT

The rise of multidrug-resistant bacterial pathogens poses a significant challenge in wound management. In this study, we investigated the phytochemical constituents and antibacterial properties of unripe fruit extracts of *Carica papaya* on bacterial isolates from wounds. Phytochemical analysis revealed the presence of phenols, tannins, saponins, alkaloids, terpenoids, reducing sugars, and cardiac glycosides in both aqueous and 50% ethanolic extracts. Antimicrobial susceptibility testing demonstrated significant activity against Staphylococcus aureus, Protues vulgaris, *Enterobacter aerogenes*, and Escherichia coli, with aqueous extract exhibiting greater inhibition than the ethanolic counterpart. However, no activity was observed against *Pseudomonas aeruginosa*. These findings underscore the potential of Carica papaya extracts in combating opportunistic infections in wound management.

Keywords: *Carica papaya*, wound, antibacterial, phytochemical, extracts, micro-organisms.

INTRODUCTION

Studies on ethnobotany have shown that a greater variety of East African plants are being utilised to cure wounds and other ailments [1]. Traditional treatments in East Africa are utilised to cure infections caused by bacterial pathogens, among other indications [2]. Human pathogenic bacterial resistance has been documented in recent years worldwide [3]. Antimicrobial agent-resistant bacteria are becoming more common as many bacteria have developed a variety of defensive mechanisms against antibiotics [4].

A wound is a breakdown in the protective function of the skin; the loss of continuity of epithelium, with or without loss of underlying connective tissue (i.e., muscle,bone, nerves), following injury to the skin or underlying tissues or organs caused by surgery, a cut, chemicals, heat or cold, friction or shear force, pressure, such as a leg, or as a result of disease such as leg ulcers or carcinomas[5, 6]In order to effectively heal wounds and combat the issue of these multidrug-resistant organisms, the application of plant extracts and phytochemicals may prove to be highly significant in therapeutic treatments [7, 8].

Carica papaya commonly known as "paw-paw" is a widely known plant for its nutritional, economical and medicinal benefits [9]. It is a member of a dicotyledonous family Caricaceae, polygamous plant and dioecious in nature [10, 11]. It is frequently grown in the Philippines, Sri Lanka, India, Bangladesh, Malaysia, and the West Indies, among other tropical American and African nations [12,13]. Numerous commercial goods, including fruit juice, sugar, and health supplements, are made from various portions of *C. papaya* trees [14]. It has long been believed that the various parts of the C. papaya plant—fruits, leaves, latex, and seeds can be consumed and utilised medicinally to treat a variety of illnesses [15,16]. *C. papaya* is utilised in candies, beverages, jams, dried fruit, and fruit crystals. It is a great source of vitamins A and C and calcium [14]. A number of phytochemical are found to be contained in extracts of *C. papaya* [17] and also possess antioxidant properties [18], anti-inflamatory properties [19], antimicrobial activities [20,21] antitumor properties [22] Cysteine endopeptidases, including glycol endopeptidases, cysteine proteinases, serine proteinase inhibitors, glutamine cyclase characin, class II chitinase, papain, and chymopapain, are abundant in papaya latex [23].

Antimicrobial activity of *C. papaya* include the activity of the proteolytic enzymes; chymopapain and papain mechanisms [24]. The medical benefits of papaya proteases are particularly noteworthy in the fields of gastrointestinal, wound healing, neurosurgery, ophthalmology, anthelmintic, and anticancer characteristics [25]. Because of its antibacterial properties, *Carica papaya* may be helpful in treating and promoting healing of persistent skin ulcers [26]. The objective of this study was to

identify and determine the antibacterial properties of *Carica papaya* on bacterial isolates from patients' in the Surgical Ward of Kampala International University Teaching Hospital (KIUTH).

METHODOLOGY

Study Design

The study design was experimental using *Carica papaya* unripe fruit extract and wound bacterial isolates from the surgical ward KIU-Teaching Hospital.

Setting of the Study

The study area was Ishaka-Bushenyi Municipality, and the experiments were conducted in the KIU Teaching Hospital Microbiology Laboratory in Ishaka, Uganda.

Selection of Study Population

The studies were conducted on wound bacterial isolates from patients' wounds in the Surgical Ward KIU-Teaching Hospital.

Determination of Sample Size

All patients in the surgical war were used to determine the sample size in study using Slovin's formula for sample size determination as follows:

$$n = \frac{N}{1 + N(e)2}$$

Where n = sample size = 32 patients
N = population size = 35patients
e = margin of error (usually 0.05 since the preferred confidence level in sampling is 95%).

$$n = \frac{35}{1+35(0.05)\,2} = 32 \text{ patients}$$

Exclusion and Inclusion Criteria

Only wounds caused by burns, surgical wounds and accidental wounds were selected in the surgical ward, while wounds caused by chronic diseases like diabetes, mellitus, and cancer were excluded from the study.

Sampling Techniques

All in-patients in the surgical ward at time of wound sample collection were used except those to be excluded.

Plant Material Collection and Identification

Fruit samples of *Carica papaya* plant were collected from Bwegirajje, Ishaka-Bushenyi district, Uganda, and identified by Dr. Okoruwa Godwin, Dean of the School of Pharmacy at KIU-Western Campus. Plant materials were transported to the laboratory in clean plastic bags.

Preparation of the Extract

The extract was prepared according to the method explained by Mahmood et al. [27].

The percentage yield of extraction from the ethanol or aqueous extract was calculated as follows:

Mean % yield = Mean weight of extract x 100.

Phytochemical Screening

The preliminary qualitative phytochemical analyses of the unripe fruit extracts of *Carica papaya* were performed to screen for the presence of phytochemicals in the unripe fruit extract using conventional procedures described by Trase and Evans, Sofowora, Harborne and Mace [28, 29, 30,31].

Microbial Culture and Identification

Different wound sample tissues were obtained from consented patients using sterile cotton swabs. The inoculation of each swab was carried out aseptically and bacteria were cultured using blood agar, MacConkey agar, and chocolate agar since they can act as both selective and enrichment media [32] and incubated at 37°C for 24 hours.The colonies obtained were identified using Gram staining, TSIA, citrate testing, and coagulate assay with the aid of a microbiologist.

Antimicrobial Susceptibility Test (AST)

The antimicrobial susceptibility testing was done by using the well diffusion technique according to Valgas *et al*., [33].

Outcome Measures

During the research, it was expected that extraction of unripe fruit of Carica papaya using both distilled water for aqueous extraction and 50% ethanol solvent would give different percentage yields of extract.

Ethical Considerations

Ethical approval was obtained from the Institutional Research and Ethics Committee (IREC) of Kampala International University Western Campus for collection of samples from wounds to culture and identify bacteria isolates from wounds in surgical ward KIUTH. A letter of permission and introduction was obtained from the Dean School of Pharmacy. Informed consent forms for sample collection were first explained to patients, and patient signatures were obtained from those who accepted to participate in the study. The patients were reassured of the confidentiality of their results by not disclosing the patients' names and personal information to unauthorized personnel or individuals.

Limitation of the Study.

Since the study involved only inpatients, the sample size was highly reduced due to the small number of patients in the surgical ward. Also, the number was highly limited because of the exclusion criteria in which patients in the surgical ward participated.

Statistical Analysis

The means of inhibition diameter zones of both aqueous extract and 50% ethanolic extract of unripe fruit of Carica papaya on various bacterial isolates from patients' wounds were measured and compared using one-way ANOVA, followed by Scheffe Post Hoc tests using SPSS (version 16.0).

RESULTS

Extract Percentage Yield

Extractive value of 50% ethanolic extract of unripe fruit of *Carica papaya* is indicated below.

Weight of fresh peeled pieces of unripe fruit of *Carica papaya* = 400g

Weight of yield extracted 50% Ethanol solvent = 22.9g

% yield due Ethanol extraction method is

$22.9g \times \frac{100}{400g} = 5.73\%$

While the extractive value of aqueous extract of unripe fruit of *Carica papaya* is indicated below

Weight of fresh peeled pieces of unripe fruit of *Carica papaya* — 400g

Weight of yield extracted aqueous solvent 30.7g

% yield due Ethanol extraction method is

$30.7\ g \times \frac{100}{400} = 7.73\%$

Table 1: Phytochemical screening test results of unripe fruit of *Carica papaya* extracts

Phytochemicals	Aqueous	ethanol
Saponin	++	+
Tannins	++	+
Phlobotannins	-	+
Alkaloids	++	+
Volatile oils	+	+
Reducing sugars	++	+
Cardiac glycosides	+	+
Steroids	-	+
Phenols	++	+

+ denotes present, + denotes abundant -denotes absent

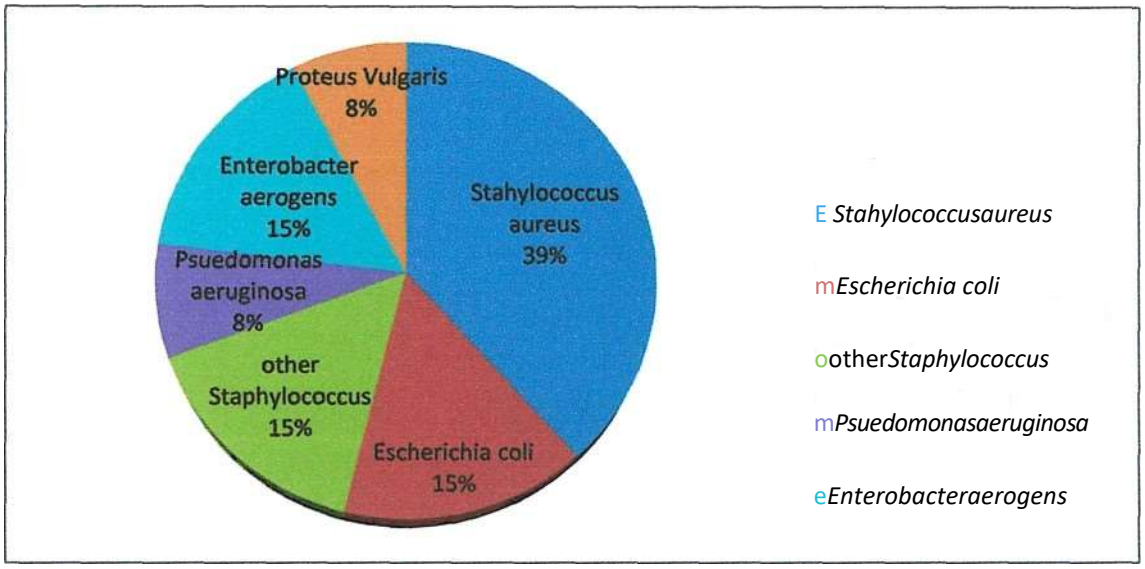

Figure 1: A pie-chart showing percentage of isolates

A total of 26 isolates were obtained and a pie chart above shows the percentage of different species of bacterial isolates that grew on the various agars. 39% for *Staphylococcus aureus* and 15% for other *Staphylococcus* species. This was followed by 15% respectively for *E. coli* and *Enterobacteraerogens* and

then by 8% respectively for *Proteus vulgaris* and *Pseudomonas aeruginosa.*

Table 2. The antimicrobial susceptibility testing using the well diffusion method

Organisms	Aqueous Extract Inhibition Zone Dameter(m'°)	Ethanol Extract inhibition zone diameter(mm)	AmpicillinDisc InhibitionZone Diameter (mm)	Solvent (Water) inhibition zonediameter	Solvent (50% Ethanol) inhibitionzone diameter
E. coli	7.75 ± 0.3[a]	6.50 ± 0.35°	0.00	0.00	0.00
Staphylococcus aureus	10.10 ± 0.74a	8.85 ± 0.75‘	0.00	0.00	0.00
Pseudomonas aeruginosa	0.00	0.00	0.00	0.00	0.00
Proteus vulgaris	7.00 ± 0.10[a]	4.50 0.05	0.00	0.00	0.00
Enterobacteraerogens	15.00 ± 0.58[a]	9.00 1.29°	4.75 ± 0.48	0.00	0.00
other *Staphylococcus*	15.50 ± 0.65[a]	13.25± 0.48'	5.25 ± 0.48‘	0.00	0.00

There was a significant increase in inhibition zone diameters against isolates like *E. coli*, *Staphylococcus aureus*, *Protues vulgaris*, *Enterobacteraerogens* and other *Staphylococcus* species for aqueous extract of *C. papaya* unripe fruit while only *Proteus vulgaris* and *Psuedomonas aeroginosa* did not exhibit any significant inhibition using both aqueous and ethanolic extract. The isolates of *Enterobacteraeruginosa* and other *staphylococcus* species apart from *Staphylococcus aureus* were inhibited by the control disc of Ampicillin10 mcg while no bacterial isolate was inhibited by the solvents used in extraction of unripe fruit of *Carica papaya.*

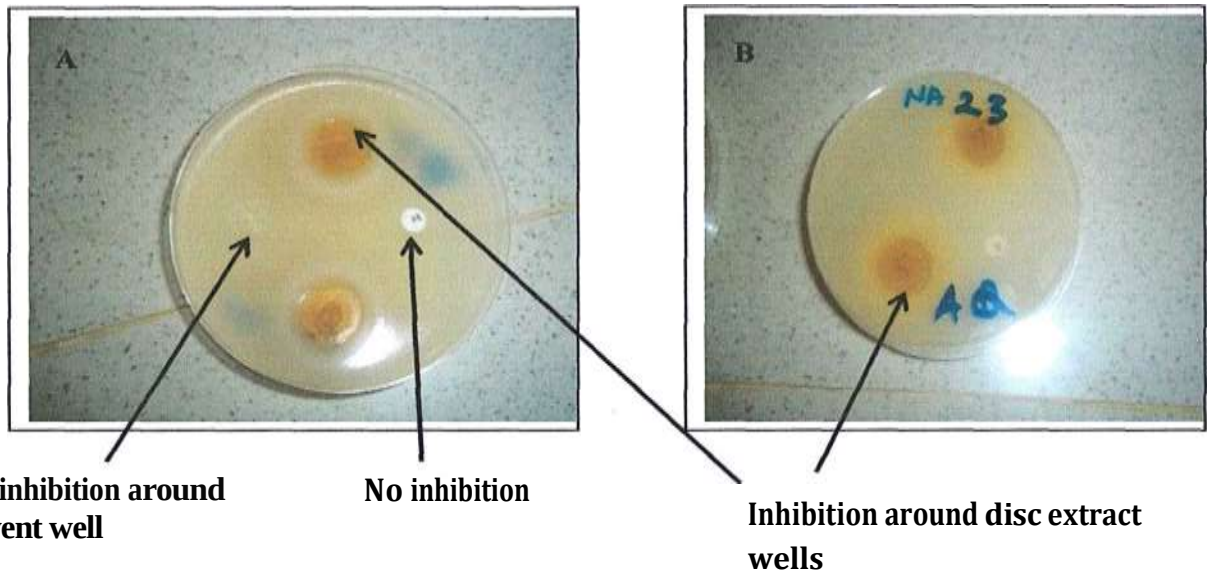

Figure 2: Zones of inhibition by aqueous extract on isolate 23 on same plate in a when open and in photo Bat the bottom In the figure above, no inhibition was recorded by each of the solvent(s) and Ampicillin while inhibition was recorded by the extracts.

DISCUSSION

Carica papaya is a member of the laticiferous plant family, which includes many specialised cells called laticifers scattered throughout most plant tissues. These lactifers secrete substance called "latex"-a milky-like fluid. Latex is an intricate blend of many chemical substances with a range of chemical properties [34]. Ripe papaya fruit contains low concentration latex, possibly because the latex-producing cells cease functioning or breakdown with age [35]. This explains why unripe fruit extract of *Carica papaya* contained phenols, tannins, saponins, alkaloids, terpenoids, reducing sugars and cardiac

glycosides as analyzed in the phytochemical analysis which is similar to findings of earlier researchers regarding phytochemical contents of some plants [17,36,37,38].

Wound infections especially surgical are caused by *Staphylococcus aureus*, *Escherichia coli*, *Proteus* species, *Klebsiella* species, *Enterococcus spp* [39]. *S. aureus* is carried in the nose and on the skin of many healthy people. It is easily spread in hospitals, particularly on surgical wards [40]. This may explain why 54% of bacterial isolates were of *Staphylococcus* species. The bacterial species identified from the wound samples match samples from patients with *Pseudomonas aeruginosa*, *Clostridium perfringens*, *Bacteroides fragilis*, and *Anaerobic cocci.*

Ampicillin control antibiotics shown considerable inhibition of *Enterobacteri aerogens* as well as other *Staphylococcus* species, such as *S. saprophyticus* and *S. epidermidis.* Since plasmid-coded beta-lactamases are produced by most *S. aureus* strains, especially hospital strains (like MRSA, or methicillin-resistant *S. aureus*), they are resistant to penicillin [41,42,43] which may explain why most of the isolates were resistant to ampicillin control disc.

Because of the selective character of the media or the rigorous growth needs of the bacteria (for example, strong anaerobic microorganisms cannot thrive under aerobic conditions), no growth of bacterial isolates may occur [44] thus the 19% no growth may have been due to strict conditions required for growth of microbes.

E. coli and Proteus vulgaris, *Enterobacteria aerogens* and *Staphylococcus* species were inhibited significantly by both aqueous and 50% ethanolic extracts of *Carica papaya* extracts similarly to Singh *et al.*,[45].

Carica papaya's antibacterial action has been linked to its ability to heal burn wounds [46]. *Carica papaya's* antibacterial properties could be attributed to the diverse phytochemicals-phenols, tannins, saponins, alkaloids, terpenoids, reducing sugars, and cardiac glycosides-that are found in the extract [38]. This study showed high antibacterial activity exhibited by both aqueous and 50% ethanolic extracts which could be attributed to the wound healing property of *C. papaya.*

CONCLUSION

Both aqueous and ethanolic extracts of *Carica papaya* unripe fruit possess diverse phytochemicals and exhibit considerable antibacterial activity against common wound pathogens. Aqueous extract showed superior efficacy, suggesting its potential in promoting wound healing by effectively combating bacterial infections. Further research to determine optimal concentrations for therapeutic use is recommended.

RECOMMENDATION

Further studies should be done to determine both minimum inhibitory concentration (MIC) and maximum bactericidal concentration so that required concentration for activity can be determined.

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