

CHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF SEEDS EXTRACT OF RIPE AND UNRIPE PAWPAW (*Carica papaya*) FRUITS

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ABSTRACT

*Seeds from the ripe and unripe Carica papaya fruits were analysed for proximate composition, antinutritional factors, mineral and their antibacterial activity. The ripe seeds were richer in crude protein ($11.8 \pm 0.61\text{g}/100\text{g}$), crude fat ($29.0 \pm 0.74\text{g}/100\text{g}$) and calories (491 Kcal) than the unripe seeds. The unripe seeds however had higher levels of ash ($9.76 \pm 0.06\text{g}/100\text{g}$), carbohydrate ($43.9 \pm 0.83\text{g}/100\text{g}$) and tannins ($0.02 \pm 0.05\text{g}/100\text{g}$) than the ripe seeds. The oxalate, hydrocyanic acid and mineral levels were about the same in the ripe and unripe pawpaw seeds. The ethanol extract of the seeds had a high antibacterial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus faecalis* and *S. pyogenes*, with a more pronounced activity recorded for the unripe seed extract at 37°C . Pawpaw seeds, if thoroughly explored, could be of great nutritional, industrial and medical importance.*

INTRODUCTION

Carica papaya is dicotyledonous plant of the Caricaceae family. It is a plant that is indigenous to West Indies and tropical America but widely cultivated in many African countries (1). The berry fruits are green when unripe but turn yellowish green, yellow or orange when ripe. The ripe fruits have pink or

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red fleshy pulp and a central cavity containing a number of small dark-brown seeds supported by a translucent mucilaginous layer (2). The unripe fruits have white or milk pulp with white seeds. The pulp is eaten while the seeds are usually discarded.

Ripe fruit pulp is very refreshing, thirst quenching and appetising. The pulp has been reported to be highly nutritive (3). The seeds have been successfully incorporated in traditional concoctions for the treatment of ailments like pile and dermatitis. It has been reported that chewing the seeds could help to clear nasal congestion (4). Pawpaw seeds are therefore suspected to be rich in essential oil, minerals and alkaloids of nutritional and medicinal importance. This research is geared towards assessing the proximate composition, antinutritional factors and minerals; as well as testing the antibacterial activity of ethanol extract of ripe and unripe pawpaw seeds.

MATERIALS AND METHODS

Chemical Analysis

Pawpaw fruits (ripe and unripe) were plucked from pawpaw trees in the botanical garden of University of Uyo, Uyo, Akwa Ibom State, Nigeria. The fruits were washed and cut into halves to obtain the seeds.

The moisture content was determined by dry weight analysis in a vacuum oven at 80°C. The oven dried seeds were ground using a Moulinex blender and milled to pass through a 40-mesh sieve (aperture size of 0.42mm) to obtain a fine powder. This was stored in air tight bottles and used for further analyses.

The crude protein, fat, carbohydrate, fibre, ash contents and calorific value were determined using Standard procedures (5, 6 and 7).

The oxalate and hydrocyanic acid contents were estimated following the procedure of AOAC (5) while the method of Burns (8) was used in estimating the concentration of tannins. Samples were digested with mixture of perchloric acid and nitric acid and the cations analysed with the aid of a flame photometer (Jenway PF 7) and an atomic absorption spectrophotometer

Unicam, analytical system model 919.

Antibacterial Activity

The crude extract of the ripe and unripe seeds were separately analysed for their antibacterial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus faecalis* and *S. pyogenes* using the cup plate agar diffusion technique (9). Two grams of the dry powdery extract was dissolved in 10ml of 95% ethanol (extraction solvent) to give 200mg/ml standard solution for the antibacterial activity test.

The test was carried out on Tryptone soya peptone agar (TSA) plates. Using a sterile glass spreader, 0.2ml of a day old pure cultures of the test organisms were uniformly spread over the surface of a sterile TSA agar plates and allowed to dry. Three equally spaced wells (3mm in diameter) were cut in the agar and filled with about 5ml of the standard extract solution. TSA plates with agar wells filled with 96% ethanol only (without seed extract) were also made to serve as control. The inoculated plates were incubated at 37°C for 24 hours before examination.

The degree of sensitivity of test organism to the extract was expressed as a measure of zone of inhibition in millimeters. The inhibition zone was measured along two diameters at right angles after 24h incubation. An average diameter of 10mm or higher is considered positive and an indication of the antibacterial potential of the seeds.

RESULTS AND DISCUSSION

The data on proximate composition indicate that the ripe seeds had higher levels of crude fat, crude protein and calorific value than the unripe seeds (Table 1). The unripe seeds on the other hand had higher levels of ash, crude fibre and crude carbohydrate than the ripe seeds. The ash and crude fat levels from this investigation are appreciably higher than the levels (1.5g/100g and 11.7g/100g respectively) reported earlier for pawpaw seeds (3).

The data on antinutritional factors indicate that the seeds from the unripe fruit have a high level of tannins than the seeds from the ripe fruit (Table II). The levels of oxalate and hydrocyanic acid were about the same in both ripe and unripe seeds.

The unripe seeds had higher levels of iron (0.064%) and copper (0.00583%) but lower levels of manganese (0.015%) than the ripe seeds. The generally higher mineral element content of the unripe seeds should be expected because the unripe seeds also had a higher ash content.

The variation in composition between ripe and unripe seeds may be due to the biochemical events that occur during the ripening or maturation process.

The ethanol extract of the seeds had a high antibacterial effect on the test organisms at 37°C. The extract from the unripe seeds demonstrated a more pronounced antibacterial activity after 24 hours, although no significant difference ($p \geq 0.05$) in antibacterial activity was derived between the ripe and unripe seed extracts (Table III). The high antibacterial potential of the extract may be attributed to the presence of antinutrient or antioxidative constituents (Tannins, and Hydrocyanic acids). The slight variation in antibacterial effect observed might be partly due to the differences in the concentration of the active constituents and their stability at the incubation temperature (37°C).

The apparent minimal level of activity of the 95% ethanol control against the test organisms was anticipated (Table IV). Its antigrowth effect in the sensitivity agar would possibly be diluted by the presence of water used in the preparation of the medium (10). Therefore the level of inhibition exhibited by the control (alcohol) was very low and did not appreciably affect the inherent antimicrobial properties of the seeds extract and the interpretation of extracts activity against the test bacteria.

The antinutrient, mineral and proximate composition of the seeds and the high lipid content in particular are of great interest. The positive antibacterial activity of the extract has further confirmed the therapeutic efficiency of pawpaw seeds. The potentials of the seed oil of *Carica papaya* could be explored

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to determine its suitability for nutritional, medicinal and industrial purposes.

Table 1. Proximate Composition (g/100g) of Pawpaw Seeds

Parameters	Ripe	Unripe
Moisture	55.3 \pm 0.65	54.1 \pm 0.52
Ash	9.76 \pm 0.60	10.3 \pm 0.07
Crude Fat	29.8 \pm 0.74	23.4 \pm 0.72
Crude Protein	11.8 \pm 0.61	8.5 \pm 0.44
Carbohydrate	43.9 \pm 0.83	55.4 \pm 0.74
Calorific Value	491 Kcal	466 Kcal
Crude Fibre	3.53 \pm 0.02	3.75 \pm 0.04

All values are mean and standard deviation (\pm) of triplicate determinations.

Table II. Antinutrients Properties (g/100g) of Pawpaw Seeds

Parameters	Ripe	unripe
Total Oxalate	0.016 \pm 0.003	0.018 \pm 0.004
Sol. Oxalate	0.008 \pm 0.002	0.009 \pm 0.002
Hydrocyanic Acid	0.005 \pm 0.001	0.006 \pm 0.003
Tannins	0.006 \pm 0.002	0.021 \pm 0.005

All values are mean and standard deviation (\pm) of triplicate determinations

Table III. Mineral Elements Concentration (g/100g) in Pawpaw Seeds

Parameters	Ripe	Unripe
Iron	0.0518	0.0647
Copper	0.0052	0.0058
Manganese	0.0196	0.0151

All values are mean of triplicate determinations

Table IV. Antibacterial Activity of Ethanol Extract of Pawpaw Seeds at 37°C

Extract Source	Zone of Inhibition (mm)			
	<i>P. aeruginosa</i>	<i>Staph. aureus</i>	<i>S. faecalis</i>	<i>S. Pyogenes</i>
Ripe seeds				
Unripe seeds	18	21	20	22
Ethanol	22	23	22	23
(Control)	4	4	5	5

10mm + = Sensitive

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