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Article

Qualitative Analysis of Phytochemicals and Antibacterial Screening of Extracts of *Carica papaya* Fruits and Seeds

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Abstract: The study evaluated the qualitative analysis of phytochemical and antimicrobial activity of various solvent extracts from *Carica papaya* fruits and seeds. Phytochemical screening indicated the presence of saponnins, alkaloids, cardiacglycoside, tannins and anthraquinnon in the extracts. The analysis shows that the unripe fruit of *Carica papaya* can be ranked as carbohydrate rich fruit due to its high carbohydrate and starch contents. The chemical solvents used were ethanol, chloroform and benzene. Different solvent extracts of *Carica papaya* were tested against Gram positive and Gram negative bacterial strains by observing the zone of inhibition. The Gram positive bacterial used in the test were *staphylococcus aureus* and *Bacillus cereus* and the Gram negative bacterial were *Escherichia coli, Pseudomonas aeruginosa* and *Shield flexner*. It was observed that ethanol, chloroform and benzene extracts of *Carica papaya* showed activity against bacteria, the chloroform extract of *Carica papaya* showed stronger activity against *Escherichia coli* and *Pseudomonas earuginosa*. The ethanol extract showed stronger activity on *Bacillus aurous* and *Escherichia coli*. Chloroform extract in bacteria showed a varying degree of inhibition to the growth of tested organism. The fruit and seed of *Carica papaya* of the same plant contained the same constituent and could be used for the same purpose. The result confirmed the presence of antibacterial and antifungal activity of *Carica papaya* extract against various human pathogenic bacteria.

Keywords: *Carica papaya*; phytochemical; antibacterial activity; qualitative screening.

1. Introduction

Nigeria is a home of variety of fruit crops among which is *Carica papaya*. This popular and economically important fruits tree grows in tropical and subtropical countries of the world [1], and the fruits are consumed world wide as fruit and as vegetable or used as processed products. It is becoming an important fruit internationally both as a fresh fruit and as processed products. The papaya industry in Brazil is one of the largest industries, which continues to show rapid growth [2]. Papaya fruits are covered with a smooth thin skin that turns to yellow or red when ripe, the flesh is succulent, varying in texture and colour ranging from yellow to orange to red [3].

Carica papaya contains many biologically active compound, two important compounds chymopapain and papain which are widely useful for digestive disorder and disturbance of the gastrointestinal tract, papaya derived papain, caricain, chymopapain and glycine endopeptids can survive acidic pH conditions and pepsin degradation [4]. However, at low pH a conformational transition that instantaneously converts their native forms into molten globuces that are quite unstable and rapidly degrade by pepsin thus, they may need to be protected against both acid denaturation and proteolysis for them to be effective in the gut after oral administration for the control of gastrointestinal nematodes.

A part from papain and chymopapain, carica papain contains many biologically actives compounds. Carica papain lipase or caplahydroloses which is tightly bound to water insoluble fraction of crude papain and is thus considered as a "naturally immobilized" biocatalyst [4]. The papaya oil seed contains saturated fatty acid (plasmatic satiric and arachnidan) and unsaturated fatty acid oleic, linoleum, and the seed yields 660-760 carpasemine. The 106 volatile components were identified in papaya [5]. Fermentation with brew's yeast and distillation yielded alcohol (ethanol), which is externally applied to burns and scalds. The allergies papaya fruit and latex papain were reported [6]. The extract from fruit showed effective anti-microbial activity against *Staphylococcus aurous*, *Bacillus cereus*, *Escherichia coli* and *Pseudomonas shigella* [7]. The extracts of papaya seed could be used as contraceptive in rat, and papaya latex is very much useful for curing dyspepsia and is externally applied to burns and scalds [8]. Report also showed that phytochemical screening indicated the presence of alkaloid, cardiac glycosides anthroquinones, saponins, turning, flavorins and carbohydrates. It was discovered that *Carica papaya* is a natural product and belong to carica cease group [8]. Papaya fruit and seed have anthelmintic and anti-amoebic activities, and the dried leaf

infusion is taken for stomach trouble. The fruits are ingested or applied on the uterus to cause abortion [9]

Recently, a study with rat at different stages of gestation showed that consumption of unripe and semi-ripe papaya fruit could be unsafe during pregnancy given the high levels of latex in the fruit at these stages of maturity [10]. But consumption of ripe fruit during pregnancy causes no risk. Inner barks are used for sore teeth and latex is used for syphilis in psoriasis ringworm and prescribed for the removal of cancerous growth in Cuba, the flowers have been used for a hypoglycaemic [10]. The present study evaluated the qualitative analysis of phytochemical and antimicrobial activity of various solvent extracts from *Carica papaya* fruits and seeds.

2. Materials and Methods

2.1. Extraction of Papaya Fruit and Seeds

Fresh unripe fruit of *Carica papaya* was obtained from Akwanga market in Akwanga Local government Area of Nasarawa State, Nigeria.

The *Carica papaya* fruit collected were peeled, seed removed and pulp cut into pieces, sun dried for about two weeks and there after transferred to chemistry laboratory, and dried under oven at 55 ^{o}C for two days. The dried substance was pounded into powdered form and properly stored in sealed sterilized polythene for extraction and phytochemical analysis. The 50 mg of each sample was extracted in 200 mL, 250 mL and 300 mL respectively of ethanol, chloroform and benzene by maceration for 60 h. The crude extract was decanted, filtered and concentrated using rotary evaporator until all the solvent was completely evacuated. The solid extract was stored in glass vials in a refrigerator for next experiments.

The phytochemical screening was carried out to determine the biological active, non-nutritive compound that contributed to the flavour, colour and other characteristics of plants such as alkaloids, tannin cardiac glycoside, saponins, flavorins among others.

2.2. Determination of Alkaloids

Procedure: 0.5 g of the sample was accurately weighed and defatted with 5% ethyl ether for 15 min. The defatted sample was extracted for 20 min with 5.0 mL of aqueous HCl on a steam. The resulting mixture was centrifuged and treated with a few drops of Mayer's reagent and the second 1.0 mL portion was treated similarly with dragnet. The result was recorded.

2.3. Determination of Tannins

Procedure: 0.5 g of dried extract was stirred with 10.0 mL of distilled water. This was filtered and ferric chloride ($FeCl_3$) solution was added to the filtrate and the result was recorded.

2.4. Determination of Saponins

Procedure: 0.5 g of dried extract was shaken with water in a test tube and warmed. The result was recorded.

2.5. Determination of Anthraquinnone

Procedures: 0.5 g of dried extract was shaken with 10.0 mL of benzene. This was filtered and 5.0 mL of 10% ammonia solution was added to the filtrate and shaken. The result was recorded.

2.6. Determination of Cardiac Glycosides

Procedure: 0.5 g of dried extract was dissolved in 2.0 mL of glacial acetic acid containing one drop of ferric chloride solution. This was then under laid with 1.0 mL of concentrated H_2SO_4 acid and the result was recorded.

2.7. Determination of Flavorins

Procedure: 1.0 mL of 10% ethanolic extract of *Carica papaya* was mixed with 0.5 mL of hydrochloric acid and magnesium metal and the result recorded.

2.8. Determination of Carbohydrates

Procedure: 0.5 mL of the extracts was heated on warmed water, iodine solution was added and the result recorded.

3. Results and Discussion

Tables 1 and 2 represent the results of the phytochemical analysis of *Carica papaya* fruits and seeds while Tables 3 and 4 represent the results of phytomicrobial screening of seed and fruit of papaya.

Phytochemical screening indicated the presence of saponnins, alkaloids, cardiacglycoside, tannins and anthraquinnon in the extracts of *Carica papaya* fruits and seeds. The analysis shows that the unripe fruit of *Carica papaya* can be ranked as carbohydrate rich fruit due to its high carbohydrate and starch contents. Furthermore, the moisture contents are also low, showing that the unripe pulp can be stored for a period of time without spoilage and it will not be susceptible to microbial growth.

Table 1. Qualitative screening of *Carica papaya* fruits

S/N	Test	Observation	Inference
I	Sample + 10.0 mL of distilled water + few drop of ferric chloride ($FeCl_3$) aq	A blue-black precipitate	Tannins present
Ii	Sample + H_2O + heat	Persistence frothing was observed.	Saponins present
iii	Sample + 5.0 mL of HCl (aq) + few drops of Mayer's reagent	A turbid precipitation was formed.	Alkaloids present
Iv	Sample + glacial acetic acid + few drops of $FeCl_3$ (aq) + 1.0 mL of conc. H_2S0_4	A brown ring formed the interface of the test tube.	Cardiac glycoside present
V	Sample + 10.0 mL of benzene +10% of ammonia solution + shaken	A violet colour was formed.	Anthraquinnone present
Vi	Sample + warm water + iodine solution	A blue black colour was formed.	Carbohydrate present
Vii	Sample + 1.0 mL of 10% ethanol + 0.5 mL of HCL	A reddish colour was formed.	Carbohydrate present

Protein contents were not determined in this study to confirm whether the unripe pulp of *Carica papaya* fruits and seed are good sources of protein or not. The concentration of mineral elements would have an advantage, if determined to seek if they can play important roles in the maintenance of normal glucose tolerance and in the release of insulin from better cell or not. These mineral may not be present in a detectable amount in the pulp and could be of great advantage to the consumers. The secondary plant metabolites are bioactive compounds, for example saponins and cardiac glycoside are important medicines to health status.

Glycoside is known to be used in the treatment of congestive heart failure. Also saponins inhibits the blockage of the entrance of Na^+ concentration in cell activating, strengthens the contraction of heart muscle and thereby reducing congestive heart failure.

Pulp of *Carica papaya* contributed to the presence of bioactive compound like glycoside, mineral salts and polysaccharide. These compounds have been shown to be responsible for hypoglycemic activity in *Mormerdica charantia*. Bitter mellon is English name of *Mormerdica charantia* which promote health and wellness with the herb, bitter lemon by miller food/cooking.

Table 2. Qualitative screening of *Carica papaya* seed extracts

S/N	Test	Observation	Inference
I	Sample + 10.0 mL of distilled water + few drop of ferric chloride ($Fecl_3$) aq	A blue-black precipitate	Tannins present
Ii	Sample + H_2O + heat	Persistence frothing was observed.	Saponins present
iii	Sample + 5.0 mL of HCl (aq) + few drops of Mayer's reagent	A turbid precipitation was formed.	Alkaloids present
Iv	Sample + glacial acetic acid + few drops of $FeCl_3$ (aq) + 1.0 mL of cenc. H_2S0_4	A brown ring formed the interface of the test tube.	Cardiac glycoside present
V	Sample + 10.0 mL of benzene +10% of ammonia solution + shaken	A violet colour was formed.	Anthraquinone present
Vi	Sample + 1.0 mL of 10% ethanol +0.5 mL of HCl	A reddish colour was formed.	Flavorins present
Vii	Sample + iodine solution	A blue black colour was formed.	Carbohydrate present

Table 3. Phytomicrobial screening of the fruit extracts

	Test Performed	Ethanol Extract	Chloroform Extract	Benzene Extract
Saponins	Chloroform and H_2SO_4	+	-	-
Flavorins	Shinoda test	+	-	-
Alkaloids	Dragendoff's test	+	+	-
Carbohydrate	Molish test	+	-	-
Cardiac glycosides	Molish test	+	-	-
Tannins	Natural $FeCl_3$	-	+	-
Anthraquinnon	Ferric chloride	+	+	-

Note: (+) = positive result while (-) = negative result.

The result of the antibacterial activity shows that all the extracts have an antibacterial activity equivalent to that of standard against the entire tested phytochemical ethanol, chloroform and benzene extracts (Tables 5 & 6).

Table 4. Phytomicrobial screening of the seed extracts

	Test performed	Ethanol Extract	Chloroform Extract	Benzene Extract
Saponins	Chloroform and H_2SO_4	+	-	-
Flovorins	Shinoda test	+	-	-
Alkaloids	Dragendoff's	+	+	-
Carbohydrate	Molish test	+	-	-
Cardiac glycosides	Molish test	+	-	-
Tannis	Natural $FeCl_3$	-	+	-
Anthraquinnon	Ferric chloride	+	+	-

Note: (+) = positive (-) = negative results

Table 5. Phytomicrobial screening of antibacterial activity of *Carica papya* fruit extract against different organisms

Organism	Ethanol	Chloroform	Benzene
Escherichia coli	++	+++	-
Staphylococcus cereus	+	+	-
Bacillus cereus	+++	-	-
Pseudomonas aeruginosa	+	++	-
Shegella flexineri	+	+	-

Table 6. Phytomicrobial screening of antibacterial activity of *Carica papya* seed extract against different organisms

Organism	Ethanol	Chloroform	Benzene
Escherichia coli	++	+++	-
Staphylococcus cereus	+	+	-
Bacillus cereus	+++	-	-
Pseudomonas aeruginosa	+	++	-
Shegella flexineri	+	+	-

Note: (+++) = most reactive, (++) = more reactive and (+) = reactive

Chloroform extract have shown better activity against *Bacillus cereus* and benzene extract has no effective against the bacterial. The therapeutic value of medical plants lies in the various chemical

constituent in it. The bioactivity of a plant is attributed to phytochemical constituent for instance plant rich in tannin's have antibacterial potentials due to their character that allow them to react with proteins to form stable water soluble compounds, thereby killing the bacteria by directly damaging it's cell membrane [11]. Flavouriods are major group of phenolic compound reported for the antiviral, antibacterial and spasmolytic properties [12]. Alkaloids isolated from plant have antimicrobial properties [13]. The presence of saponins supports the fact that *Carica papaya* fruit extracts have cytotoxic effect such as permealization of the intestine as saponins are cytotoxic [14]. Saponins have relation with sex hormone involved in controlling the release of milk [15]. Another important action of saponins is their expectorant action through the stimulation of the upper digestive tract [16].

Alkaloids are the most efficient therapeutically significant plant substance. Pure isolated alkaloid and the synthetic derivative are used as basic medical agent because of their analgesic, and antispasmodic and bacteria properties [17], which show markedly psychological effect when were administered to animals. The presence of alkaloids in the fruit and seed show that this plants is effective to malaria, since alkaloid consists of quinine which is anti-malaria. The cardiac glycosides therapeutically have the ability to increase the force and power of hearth beat without increasing the amount of oxygen needed by the heart muscles.

4. Conclusions

The extracts of *Carica Papaya* fruits and seeds have great medicinal and nutrient values which could be used for treating many ailments and maintenance of the body.

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