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Phytochemicals and Antimicrobial Properties of the Root and Leaf Extract of Carica Papaya

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Abstract:
The use of Carica papaya parts in the treatment common illness have been going on for ages, but this claims have not been properly investigated, we therefore commenced on investigating most of this ethno medicinal claims
The phytochemical and antimicrobial screening of the ethanol leaf and root extracts of Carica papaya were determined by standard laboratory procedures and with the aid of Jenway 6303 Digital spectrophotometer. Results revealed that the leaf sample contained 0.488 mg/100100g alkaloid, 0.81 mg/100 g saponin, 0.30 mg/100 g flavonoid, 3.45 ppm Tannin and 40.4 ppm phenol. The root sample contained 2.22 mg/100g alkaloid, 1.32 mg/100 g saponin and 1.52 mg/100 g of flavonoid. Antimicrobial screening of the ethanolic extracts of the samples against five selected pathogens revealed that the microbes were resistant to the crude leaf extracts but were signicantly inhibited by the root extracts. The diameter of inhibition were as fellows; 18mm, 28mm, 30mm, 36mm and 30mm for Escherchia coli, Pseudomonas aureginosa, Klebsiella, Staphylococcus aureus and Streptococcus aureus respectively. These values were comparative with those obtained from 1mg standard antibiotics Oxacillin which gave values of 12 mm, 21 mm, 14 mm, 13 mm and 20 mm respectively for Escherichia coli, pseudomonas auregonosa, klebsiella, staphylococcus aureus and streptococcus aureus. The minimum inhibition concentrations (MIC) were found to be 6.5 mg/g, 12.2 mg/g, 6.5mg/g, 12.5 mg/g and 6.5 mg/g for E. coli, P. aureginosa, and Klebsiella. Staphylococcus aureus and streptococcus aureus respectively The root extracts of Carica papaya could be useful in treating infectious wound, pneumonia internal heat, stomach noise, and strange movement in the body, abdominal pains and a host of other diseases

Keywords: *Phytochemicals, carica papaya, ethanolic extracts, antimicrobial*

1. Introduction

Nigeria is a country blessed with abundant mineral and natural resources, with over 2000 species of tropical herbs and shrubs that are yet to be fully utilized for the benefit of its citizens. Some of these plants contain phytochemicals that can exhibit antibacterial and inhibitory functions (Okwu and Morah 2007). One of such plants is *Carica papaya*, believed to have been originated from southern Mexico in Central America and cultivated in most tropical climates. The ripped fruit contains high amount of pectin which can be used to make jellies, the green fruit and the trunk are rich in enzyme called papain. Papain helps to tender meat and is useful against stomach upset (Doughari *et al 2008).* Papain is also useful in treatment of cuts, rashes, and insect stings. *Carica papaya* seeds have been reported to have abortificant and contraceptive effects. The phytochemicals in this plant may suppress the effect of progesterone (Lohiya *et al* 2002). The matured and rip fruits are used to treats ringworm while the unripe fruits are used to treats high blood pressure and as aphrodisiac (Chinoy and Padman 1996). The seeds have anti-inflammatory, anthelmintic and analgesic properties. The roots also have analgesics properties, the leaves are used as a heart tonic and as an analgesic and to treat stomach ache. Two varieties of the genetically modified papaya include sunup and rainbow are been grown by several growers. The leaves contain carpaine an anthelmintic alkaloid which could be dangerous in high doses. This plant is a fast growing semi woody tree that grows to height 10 m

and belongs to the family of caricacea. Its hollow stem is gray or green in colour and the plant bears large leaves which are spirally arranged and evergreen. The trunk has scares where the leaves and fruits were borne, the flowers are similar in shape to the flowers of plueria but are much smaller and waxy like. The leaves contain alkaloids, dehydrocarpaines, psuedocarpine, flavonols, benzyl glycosinolates and tannins. The fruit contains butanoic acid, methyl butanoate, chymopapain (a and b), linalool, alpha linolenic acid, papain, alpha terpene, terpinolene, alpha phellandrene, 4- terpineol and gamma terpine. The leaves and fruit contain cyanogenic glycoside. (Flath and Forrey 1977)

2. Materials and Method

2.1. Plant Materials

The samples were obtained from a farm land in Ezinihitte Mbaise area Imo state, they were identified by Dr. Nmeregini of forestry department Michael Okpala University umudike, the voucher specimens were deposited in the. Forestry department Herbarium of Michael okpara University Umudike. They were washed with distilled water and room dried. The dried samples were milled with an electric milling machine and stored in air tight plastic bottles and kept for analys**is.**

2.2. Alkaloid Determination

5 g of the sample was weighed into a 250 cm^3 beaker and 200 cm^3 of 29 % acetic acid in ethanol was added and covered to stand for 6hrs. This was filtered and the extract was concentrated using a water bath to one quarter of the original volume. The Alkaloid was precipitated out using concentrated ammonium hydroxide which was added drop by drop until precipitation was complete. The solution was allowed to settle and the precipitation was collected by filtration using whatman filter paper, the precipitate was dried and weighed (Obadoni and Ochuko (2001).

2.3. Saponin Determination

20 g of the sample was weighed into a 250 cm^3 beaker and 200 cm^3 of 20 % ethanol was added and stirred using a glass rod. The mixture was heated over water bath for 4hrs with continuous stirring while the temperature was maintained at 55 °C. The mixture was extracted and the residue was extracted with 200 cm^3 of 20 % ethanol. The combined extract was reduced to 40 cm^3 over water bath at 90 °C. The concentrated extract was transferred into a 250 cm^3 separation funnel and 20 cm^3 of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. This process was repeated thrice. 60 cm^3 of n-butanol was added. The mixture was washed twice with a10 cm^3 of 5 % sodium chloride. The remaining solution was heated over water bath and the residue dried to constant weight. The saponin content was calculated in percentages (Obadoni and Ochuko 2001).

2.4. Flavonoid Determination

10 g of the plant sample were extracted repeatedly with 100 cm^3 of 80% of aqueous methanol at room temperature. The solution obtained was filtered with whatman filter paper no 45. The filtrates were later transferred into a crucible and evaporated to dryness over a water bath and weighed (Boham and Kocipai,1994)

2.5. Phenol Determination

2 g of the sample was defatted with 100 cm^3 of diethyl ether using a soxhlet apparatus for two hours. The defatted sample was boiled within 50 cm^3 of ether for 15 minutes, then 5 cm^3 of the extract was pipetted into a 50 cm^3 flask and 10 cm^3 of distilled water was added. 2cm^3 of ammonium hydroxide and 5 cm^3 of amyl alcohol were added. The samples were made up to the mark and left for colour development. The absorbance of the solution was measured using Jenway digital spectrophotometer model 6303 at 505 nm wavelength (Obandoni and Ochuko 2001, Harbone 1973)

2.6. Tannin Determination

0.5 g of the sample was weighed into 250 cm^3 beaker and 50 cm^3 of distilled water was added and stirred vigorously with a glass rod for one hour the solution was filtered into a 50 cm^3 volumetric flask and made up to the mark. 5 cm^3 of the filtrate was pipetted into a test tube and mixed with 3 cm^3 of O.I M $FeCl_3$ in O.IN HCI and 0.008M Potassium Ferro cyanide. The absorbance was measured with the Jenway digital spectrophotometer model 6303at 120 nm wave length. The absorbance was compared with those of standard made from tannic acid (Van-Burden and Robinson 1981)

2.7. Anti Microbial Analysis

The Micro Organisms*, Escherichia Coli, Pseudomonas Aureginosa, Klebsiella, Staphylococcus aureus and streptococcus aureus,* were used for the analysis. They were obtained from the stock cultures of the Federal Medical Centre Umuahia and were brought to the laboratory and were resuscitated in peptone water and there after subcultured into nutrient agar medium and incubated at 37 ^oC for 24 hrs (Okigbo and Omadamiro 2006)

2.8. Antibacterial Assay

The test solution of each extract was prepared by dissolving 0.1 g of the plant extract separately in 1.0 cm^3 of dimethyl sulphoxide (DMSO) to get a concentration of 100 mglcm^3. The antibacterial activity was performed by filter paper disc diffusion technique. Filter

paper disc (Watman No 1.6 mm diameter) were placed in glass petridishes and sterilized in hot air oven (Ekundayo and Ezeogu 2006). The media (10 g nutrient Agar in 200 cm^3 distilled water, auto-claved at 115 ^oC for 30 minutes) was cooled to 50 ^oC. The sterile nutrient Agar media were poured into the sterile petridish and allowed to solidify. The bacteria were swabbed with a sterile wire loop. Each disc was impregnated with 0.2 cm^3 of plant extract standard, Ciprofloxacin was used as a control on a disc with DMSO 100 $mg/cm^{3.}$ The discs were used after drying them in an incubator at 40 ^oC to remove any trace of solvent. Discs were introduced into the surface of the medium. The plates were microbated at 37 ^oC for 24 hrs to obtain zones of inhibition. The experiments were repeated three times for each extract and twice for reference antibiotics to minimize error and the average of these values were recorded.

2.9. Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of the extracts was determined by incorporating constant volume 0.2 cm^3 of each diluents of the extracts into the perforated disc on a seeded nutrient agar plate as described in the anti microbial susceptibility test section (Okigbo and Omodamiro 2006) 0.1 g of each extract was dissolved in $1cm^3$ of DMSO to obtain 100 mg/cm^3. This concentration of DMSO was then double to obtain 50 mg/ml, then double again to obtain 12.5 mg/cm^3 and again to obtain 6.25 mg/cm^3. Each concentration was then used in the method earlier described to obtain zone of inhibition. The least concentration that showed inhibitory zones was taken as the MIC.

3. Results and Discussion

The results obtained from the analysis of the root and leaf of *Carica papaya* are presented in tables 1-4 below

Phytochemical	Leaf	Root
Alkaloids	+	+
Flavonoid	+	+
Saponins	+	+
Tannins	+	-
Phenol	+	-

Table 1: Table of phytochemical screening of the leaves and root of Carica papaya

Phytochemical	Mg/100g root sample	Mg/100g leaf sample
Alkaloid	2.22	0.84
Saponins	1.32	0.81
Flavonoid	1.52	0.30
Tannin	-	3.45ppm
Phenol	-	40.4ppm

Table 2: Table of the phytochemical determination of the root and leaf of Carica papaya

Micro organism	Degree of inhibition (mm) Root	Leaf (mm)
E. coli	18	00
Pseudomonas aureginosa	28	00
Klebsiella	30	00
Staphylococcus aureus	36	00
Streptococcus aureus	24	00

Table 3: Anti microbial screening o f the root and leaf of Carica papaya

Microorganism	sample	MIC(mg/g)	1mg oxacillin
E.coli	18	6.5	12
Pseudomonas aureginosa	28	12.5	21
Klebsiella	30	0.5	14
Staphylococcus aureus	36	12.5	13
Streptococcus aureus	24	6.5	20

Table 4: Table of antimicrobial screening 1mg oxacillin as compared to root extract

4. Discussion

The result of the phytochemical screening the samples showed the presence of alkaloids, saponins and flavoniods in the samples. Tannins and phenols were found in the leaf sample and were absent in the root samples, (tablble1). The leaf and root of *Carica papaya* contains 0.84mg/100g and 2.22g/100g alkaloid respectively, (table 2) Alkaloid is ranked among the most efficient therapeutically significant plant substances. Pure isolated alkaloids and their synthetic derivatives are used by Ethinomedicinal practitioners for their analgesic, antispasmodic and bactericidal effects (Okwu and Okwu 2004). They exhibit marked physiological activity when

administered to animals, the high alkaloid content of these samples may be the reason for their use in the treatment of cough, wounds, rheumatism and skin infections. Most samples containing alkaloid are used in Nigeria for the treatment of malaria and fever. (Adesuga and Coker 20001)

Saponins were found to be available at 0.81mg/100g and1.32mg/100g in the leaf and root of *Carica papaya* respectively. The Saponin content justifies the use of the extract from these plants in the treatment of wounds. Some of the general characteristic of saponins includes formation of forms in aqueous solutions, hemolytic activity and cholesterol binding properties (Okwu 2005, Sodipo and Akuniyi 2010) Saponin has the natural tendency to ward off microbes and this makes them good candidates for treating fungal and yeast infections. These compounds served as natural antibiotic, helping the body to fight infections and microbial invasion.

The flavonoid content of *the leaf and root of Carica papaya were 0.30mg/100g and1.52mg/100g.* Flavonoids are distributed group of polycyclic compounds characterized by a common Benzo pyrone ring structure that has been reported to act as antioxidants in many biological systems. The family encompasses flavonoids, flavones, chalcones, catchins, anthocyanidins and isoflavonoids (Okwu and Aluwo 2008).In addition to their free radical scavenging activities, Flavonoids have multiple biological activities including ; vasodilatory, anti carcinogenic, anti allergic, antiviral, estrogenic effects as well as being inhibitors of phosphatase H_2, cycloxygenase, glutathione reductase and xanthine oxidase (Saleh *et al* 1995, Del-Rio *et al* 1997, Okwu 2004).They support lactogenecity (Asoegwu et al 2006). Flavonoids in intestinal tracks lower the risk of heart diseases. As anti-oxidant, favonoids provide anti inflammatory actions.

The phenolic content of the samples was 40.4ppm *for Carica papaya leaf.* There is a growing interest in polyphenolic compounds as therapeutic agents against many diseases such as cardiac and cerebral ischemic, arteriosclerosis and rheumatic or pulmonary diseases. (Saleh *et al* 1995, Middleton and Kandaswani 1992) The activated phagocytic cells are known to produce potentially destructive oxygen species like super oxide anion (O^{2-}), hydrogen peroxide (H_2O_2) and Hypochloric acid (HOCl) during chronic inflammatory disorder. Many polyphenolics are known to exhibit antioxidant properties; they are free radical's scavengers. Phenolic flavonoids are also excellent hydroxyl scavengers. These properties promote health, and prevents certain chronic disorders such as cancer, cardiovascular diseases, diabetics and arthritis. The presence of phenols means that the extract could act as anti inflammatory, anti clothing, anti oxidants, immune enhancers and hormone modulators. Phenols have been the subject of extensive research as disease preventives. (Saleh *et al* 1995, Duke 1992). The have the ability to block specific enzymes that causes inflammations. They modify the prostaglandin pathways and thereby protect platelets from clumping.

The Tannin content of *Carica papaya leaf was* 0.85 %. Tannins have astringent properties, hastening the healing of wounds and inflamed mucous membrane (okwu and Okwu 2004). The presence of Tannins in these samples supports their use in treating wounds, varicose ulcers, hemorrhoids, frost bites and burns in herbal medicine

The two extracts were tested against E. *coli, Pseudomonas aureginosa, Klebsiella, staphylococcus and Streptococcus*, table (.3). It was observed that whereas the pathogens were resistant to the crude ethanoic leaf extract, they were however signicantly inhibited by the crude root ethanolic extracts. Similar test was carried out with 1 mg Oxacillin, a standard antibiotic and the root extract compared favorably with it. (Table. 4) The major inhibiting component of the root may be due to the type of alkaloid they contain. Alkaloids have been shown to inhibit the growth of staphylococcus sp, Candida albicans, aspergillus Niger and staphylococcus aureus. The pathogens, P. aureginosa, Staphylococcus and streptococcus aureus have been implicated in infectious wounds, body heat, stomach noise, abdominal pains, pneumonia. The root extracts therefore could be useful in treating infectious wounds. The mechanism of the inhibitory action of these phytochemicals may be due to the impairment of variety of enzyme systems including those involved in energy production, interference with the replication of the cell membrane and structural component synthesis.

5. Conclusion

The root extracts of *Carica papaya* could be useful in treating infectious wound, pneumonia internal heat, stomach noise, and strange movement in the body, abdominal pains and a host of other diseases caused by *E. coli, Pseudomonas aureginosa, Staphylococcus aureus, Klebsiella. And Streptococcus aureginosa.*

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