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ABSTRACT

Medicinal plants have extensively been used to cure and in some cases to manage diseases including infections by pathogenic organisms. The objective of the study was to ascertain quantitatively the phytochemical constituents, the antifungal effect and Minimum Inhibitory concentration (MIC) of carica papaya latex. The phytochemical screening for the chemical Composition was conducted with the unripe fruit, back and inside (latex) by making incisions and colleting the exuding liquid. The MIC and antifungal effect of the latex were studied at different concentrations of 1%, 5%, 10% and 20% using two test organisms namely *Candida albicans* and *Aspergillus niger* on Sabouraud Dextrose Agar. The constituents included saponin (3.8%), flavonoid (4.2%), glycoside (4.4%) for the back and alkaloid[3.8%], saponin (4.7%), tannin(1.2%), phenol(0.23%),essential oil(3.8%) for the inside. *Carica papaya* latex extract showed inhibitory effect against *Candida albicans* only. The zones of inhibition in millimeters were 8.15 ± 0.05 , 10.00±0.00, 12.88±0.15, 17.38±0.15 for the different concentrations respectively. The MIC was 0.25%. *Carica papaya* latex inhibited the growth of *Candida albicans*.

Keywords: paw-paw, extract, constituents, antifungal, effect, MIC.

INTRODUCTION

Plants are of numerous benefit to man. Apart from serving as source of food, shelter and raw materials they also play a big role in the prevention and treatment of diseases.

In most developing countries especially in the rural areas, people live in poor hygienic conditions with inadequate modern health care system. Therefore, there is possibility of contacting diseases and visit to

traditional healers is common practice. (Akerele, 2006; Heinrich et. al., 2008).

Carica papaya or paw-paw in English originated in Central America. It is commonly and erroneously referred to as a "tree" or shrub, reaching heights of 2-12 meters tall. The Northern common specie is deciduous while the Southern specie is evergreen. The plant is actually a large herb growing at the rate of 6-10 feet (1.8-3 meters) the first year and reaching 20-30 feet (6-9m) in height. The fruit of the common paw-paw is a large edible berry, 5-16 centimeters long, 3-7 centimeters broad and weighing 20-500 grams with numerous seeds. It is green when unripe, maturing to yellow or brown. The flowers are insect pollinated (Akah et al; 1997, Benneth et al., 2007).

Phytochemicals protect plants against micro-organisms such as viruses and bacteria. Fungi belong to a group of eukaryotic organisms such as yeast, molds and mushrooms (Akah et al; 1997; Ernest, 2009).

Medical claims of *Carica papaya* abound. Extracts from *Carica papaya* (Paw-paw) root, pulp and seed have been shown to have great anti bacterial effect. (Yates, 2010; Hazara et al; 2008). These claims suggest that the active properties of *Carica papaya* need to be investigated. Thus the aim of this study was to ascertain the constituents and determine the antifungal effect of *Carica papaya* on some fungi organisms namely *Candida albicans* and *Aspergillus niger*.

MATERIALS AND METHODS

Collection and Preparation of Plant Extract

The unripe fruit of *Carica papaya* was obtained from Enugu in Enugu State, Nigeria. Incisions were made on the fruit to collect the inside (pulp). The back and the inside were dried, grounded to powder and extracted into water and 70 % ethanol. The extracts were evaporated to dryness in a water bath at a temperature of 50 $^{\circ}$ c.

Phytochemical Screening

This was carried out with the extracts to find out the chemical constituents. The extracts were tested for tannins by lead acetate test, saponins by frothing test, Alkaloid with Wagner and Meyer's reagents, glycosides by hydrolysis tests, phenol and flavoniod by sodium hydroxide test and essential oil by osmic acid test. Quantitative analysis was by standard methods.

Antifungal Effect of Plants Extracts

This was carried out on Sabouraud Dextrose Agar (SDA) plates prepared by dissolving 6.5 grams Sabouraud Agar in 100 ml distilled water and autoclaved at 121° c for 15 minutes. Wells were bored on the SDA plates and filled with different concentrations of 1 %, 5 %, 10 % and 20 % of the plant extract. The plates were inoculated with peptone broth cultures of *Candida albicans* and *Aspergillus niger* and incubated for 24hrs at 37°c in an upright position. The diameter of the zones of lnhibition was measured in millimeter (mm) with a transparent meter rule.

Minimum Inhibitory Concentration (MIC)

Clean test tubes were sterilized, labeled (1-5) and 5ml of nutrient broth dispensed into each of the five test tubes. Serial two fold dilutions of the 1% *Carica papaya* latex extract were made in the nutrient broth tubes by adding 5ml of the 1% extract to tube 1. The mixture was properly mixed and 5ml was transferred to tube 2. This procedure was repeated up to tube 5 where 5ml was discarded. The five test tubes were inoculated with *Candida albicans*. This was done in triplicate and all tubes incubated for 24 hours at 37°c and scored for growth. Serial two-fold dilutions were also made and inoculated with *Aspergillus niger*. This was also done in triplicate, incubated and scored for growth.

The MIC was defined as the lowest concentration of the extract where fungal growth was not detected.

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Table 1: Qualitative analysis of <i>Carica papaya</i> Back and inside (latex)				
Sample	Parameter	With distilled	With Ethanol	
		Water		
<i>Carica papaya</i> (Paw-	Alkaloid	-	-	
paw) Back				
	Saponin	+++	++	
	Flavonoid	+++	-	
	Tannin	-	-	
	Phenol	-	-	
	Glycoside	+++	-	
	Essential Oil	-	-	
<i>Carica papaya</i> (Paw-				
paw)	Alkaloid	++	-	
Inside (latex)	Saponin	+++	+++	
	Flavoniod	-	-	
	Tannin	+	-	
	Phenol	+	+	
	Glycoside	-	-	
	Essential	-	+	

Result of Phytochemical Screening

Key: +++ = Present (abundant)

++= Present (significant amount)

+ = Present (traces)

- = Not Detected

Table 2: Quantitative Phytochemical Analysis on Paw-Paw Back and Inside

Sample	Alkaloid %	Saponin %	Flavonoid %	Tannin %	Phenol %	Glycoside %	Essential Oil %
Paw-Paw	ND ND	70 3.8		ND	ND		ND
Back		3.0	4.2	/ND		4.4	
Paw-Paw Inside	3.8	4.7	ND	I.2	0.23	ND	3.8

Result of Antifungal screening

Table 3: Effect of plant extract on test organisms

	Test Organisms		
Plant Extract	Candida albicans	Aspergillus Niger	
Carica Papaya latex	+	-	

Key: + = Inhibition

- = No inhibition

Table 4: Diameter of zones of inhibition (mm) for candida albicans $(mean \pm standard error of the mean)$

Plant Extract	1%	5%	10%	20%
Carica Papaya latex	8.15±0.05	10.00±0.00	12.88±0.15	17.38±0.15

Table 5: Minimum Inhibitory Concentration of plant extract on test organisms

Concentration of Plant	Candida albicans	Aspergillus niger
Extracts		
0.5% 1	-	Not Applicable
2	-	
3	-	
0.25% 1	-	Not Applicable
2	-	
3	-	
0.125 % I	+	Not Applicable
2	-	
3	+	
0.0625 % I	+	Not Applicable
2	+	
3	+	
0.0312% 1	+	Not Applicable
2	+	
3	+	

Key: - = Inhibition

+ = growth

DISCUSSION

The medicinal claims of *Carica papaya* fruit such as improving the digestion of protein, expelling worms, strengthening the immune system and fighting illnesses cannot be proven without the scientific discovery of the active natural chemical constituents. Our photochemical study of the *Carica papaya* fruit showed the presence of significant amounts of saponin, flavonoid, alkaloid, glucoside, tannins and trace amounts of phenol and essential oil. The back of paw-paw fruit has higher concentrations of flavonoid and glycoside while the inside contains more saponin, tannin and essential oil (table 2).

Flavonoids enhance health since they possess the ability to scavenge for superoxide anions and hydroxyl radicals (Hazara et al; 2008). Some alkaloids are liquids containing carbon, hydrogen and nitrogen while most alkaloids exist as solids such as atropine and are used as pharmaceuticals, narcotics, stimulants and poisons because of their potent biological activities (Cazanden et al; 2012).

Saponins have 'soap like' ability, producing foam and yield sapogenin on hydrolysis. Saponins haemolyse reds cells and emulsify olive oil. Phenols are colour pigments synthesized from phenylalanine by the action of phenylalanine ammonia lyase and applied in the control of human and plant pathogens (Ginseng, 2012). Glycosides are reducing sugars and characteristically susceptible to hydrolysis yielding sugar (glucose) and non-sugar (aglycone or genin). Tannins act as antiseptics due to the presence of a phenol group which may be responsible for the antifungal activity.

Essential oil is mainly an association of terpene and alcohol phenol, ketones and aldehyde esters found in plants. When taken internally it produces a gentle irritation. It also stimulates the secretion of gastric juice thus acting as appetizer (Coleby- Willan and Cai, 2008).

Fungi are non-photosynthetic microorganisms possessing relatively rigid cell walls and may be saprophytic, parasitic or pathogenic. From our study, *Carica papaya* (latex) showed no inhibitory effect on *Aspergillus niger* but inhibited *Candida albicans* (table 2). The genus *Aspergillus* is widespread, common in decaying vegetation and the commonest contaminant in the laboratory. It causes pulmonary infection in man and mycotic abortion in cattle (Vaibhar, 2010).

Candida albicans belong to the genus of yeast-like fungi and normally present in the mouth, intestine and vagina. It is responsible for infections in these sites and elsewhere when there is a disturbance of local conditions or impairment of the defense mechanisms. Infection is usually endogenous though cross-infection may occur between infants in a nursery. Opportunistic *Candida* infections may occur in pregnancy, neonatal debility, senility, minor trauma, continued exposure of the skin to moisture or when a patient is debilitated by diabetes or alcoholism. Addiction to drugs and the medical profession's increasingly active disturbance of the normal body flora with antibiotics and of the natural defense reactions of the body with immunosuppressive drugs and cytotoxic agents may lead to pulmonary and systemic infections (Beckstrom et al; 2011). The commonest *Candida* infection is vaginitis or vaginal thrush.

The minimum inhibitory concentration of *Carica papaya* latex on *Candida albicans* was 0.25%. At low concentration of 1%, the plant extract showed little inhibitory effect on *Candida albicans* while at 5% there was a significant inhibition. A further increase in concentration to 10% and 20% produced a greater inhibitory effect (table 3). This shows that the extent of antifungal effect of the extract is dependent on the concentration and can be used as herbal drug or medicine for the treatment of candidiasis.

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