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Preliminary Pharmaceutical Constituents of Crude Solvent Extracts of Flower and Stalk of Male *Carica papaya*

E.I. Okoye

Department of Pure and Industrial Chemistry, Chukwuemeka Odumegwu Ojukwu University, Uli, Anambra State, Nigeria

Abstract Qualitative and quantitative phytochemical screening of flower and stalk of male *Carica papaya* indicated the presence of these phytocompounds: alkaloids 0.53±0.01%flavonoids 0.86±0.02%, saponins 0.37±0.02%, tannins 2.06±0.01%, terpenoid 0.21±0.01%, steroid 0.08±0.01% and cardiac glycoside 1.87±0.02%. The ten fungi investigated in this research are: *Aspergillus niger, Microsporum gypseum, Candida albican, Aspergillus flavus, Fusarium verticilloids, Aspergillus parasiticus, Fusarium oxysporum, Candida glabrata, Candida parasilosis, Candida tropicalis*. Three solvents were used in the extraction of active ingredients in flower and stalk of make *Carica papaya* and they are: distil water, ethanolic and N-hexane. Aqueous extract of flower and stalk of male *Carica papaya* cannot inhibit two out of ten test fungi. These are: *Fusarium verticilloides* and *Fusarium oxysporum*. Ethanolic extract cannot inhibit three out of ten test fungi. These are: *Fusarium verticilloides, Aspergillus parasiticus* and *Fusarium oxysporum*. N-hexane extract cannot inhibit four out of ten test fungi. These are: *Microsporum gypseum, Aspergillus flavus, Fusarium verticilloides* and *Fusarium oxysporum*. The minimum inhibitory concentrations (MIC) of ten fungi investigated as mentioned above using three solvents are: aqueous extract, 0.05±0.01mg/ml, 9.35±0.02mg/ml, 0.05±0.02mg/ml, 0.05±0.02mg/ml, no inhibition, 0.05±0.01mg/ml no inhibition, and 0.05±0.01mg/ml, 0.05±0.01mg/ml and 0.05±0.01mg/ml respectively. The MIC for ethanolic extract are: 0.25±0.01mg/ml, 0.25±0.03mg/ml, 0.05±0.01mg/ml, 0.05±0.01mg/ml, no inhibition, no inhibition, no inhibition, 0.05±0.01mg/ml, 0.05±0.01mg/ml and 0.25±0.03mg/ml respectively. The MIC for n-hexane extract are: 0.45±0.03mg/ml, no inhibition, 0.05±0.01mg/ml, no inhibition, no inhibition, 0.05±0.01mg/ml, no inhibition 0.05±0.01mg/ml, 0.05±0.01mg/ml and 0.05±0.01mg/ml respectively. The minimum fungicidal concentration (MFC) for ten test fungi as mentioned above are: for aqueous extract 0.04±0.01mg/ml, 0.30±0.01mg/ml, 0.02±0.01mg/ml, 0.05±0.01mg/ml, no inhibition, 0.05±0.01mg/ml, no inhibition, 0.05±0.03mg/ml 0.5±0.01mg/ml and 0.03±0.01mg/ml respectively. The MFC for ethanolic extract are: 0.15±0.03mg/ml, 0.25±0.01mg/ml, 0.02±0.01mg/ml, 0.05±0.02mg/ml, no inhibition, no inhibition, no inhibition, 0.03±0.01mg/ml, 0.03±0.01mg/ml and 0.05±0.01mg/ml. The MFC for n-hexane extract are: 0.23±0.02mg/ml, no inhibition, 0.02±0.02mg/ml, no inhibition, no inhibition, 0.03±0.01mg/ml, no inhibition, 0.02±0.01, 0.05±0.02mg/ml and 0.05±0.01mg/ml respectively. Hence, crude solvent extracts of flower and stalk of male *Carica papaya* are antibiotic in nature.

Keywords Phytochemical screening, male *Carica papaya*, flower and stalk, fungi, zone of inhibition, minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC).

Introduction

Male pawpaw tree have flowers that grow on short stalks. *Carica papaya* is a native of the tropics of America, perhaps from Southern Mixico and neighouring central American [1]. According to Eno *et al*. (2000), pawpaw is the fruits, of the plant [2]. *Carica papaya* belong to the genus carica. Preliminary qualitative and quantitative phytochemical analysis of ethanol and aqueous extracts of *C. papaya* showed the presence of many phytocompounds. These extracts were found to inhibit these eight test micro-organisms: *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, Escherichia coli, Aspergillus niger, Penicillium notatum, Fusarium solani* and *Candida albican* [3]. Proximate analysis of leaves of *C. papaya* also showed appreciable quantity of ash content, crude protein, crude fat, crude fibre, carbohydrate and high calorific value [4]. In this present work, the author intends to study the preliminary pharmaceutical constituents of crude solvent extracts of flower and stalk of male *Carica papaya* since little or no work had been done in this area. The aim and objective of this work is to determined the phytocompounds present in flower and stalk of male *Carica papaya*; to extract the crude solvent extracts of the flower and stalk of male *C. papaya* and to find out whether the solvent extracts can inhibit the growth of ten pathogenic fungi. *Carica papaya* is composed of many biological active compounds, many of which are found concentrated in the latex, which is present in parts of the plant [5].

Within *Carica papaya* plants, the concentration of bio-actives will vary with position of plant, age of plant and cutivar. Also, concentration of bioactive differs between male, hermaphrodite and female plants. Female plants exude more latex than hermaphrodite and male plants. *Carica papaya* latex is rich in cystein proteinases which are proteolytic enzymes (caricain, chymopapain, papain and glucylendopeptidase) these constitute 80% of latex enxymes. Other enzymes present are glycosyl hydrolases (β-1, 3-glucanases, chitiriases and lyzozynus) protease inhibitors (cystertin and ghtaminylcyclotrunsferces and lipases [6]. It was reported that intake of two table spoons of pulverized papaya seeds mixed with hot water twice per day is used in the traditional management of diabetes and obesity [7]. *Carica papaya* (pawpaw) contains the enzyme papain, a protease used for tendering meat and other proteins [8]. The fruits are popularly used and processed into juice and wine, and also cooked as vegetable [9]. The seeds are medically important in the treatment of sickle cell disease and poisoning related disorder. The lead tea or extract had a reputation as a tumor destroyer agent. The flesh green tea is antiseptic while the brown dried leaves are best served as tonic and blood purifier [10]. Due to its antioxidant and fibre content, it is used in treatment of ailments such as chronic indigestion, overweighing, obesity, high blood pressure [11].

Sample Collection and Preparation

Flower stalk of male *Carica papaya* was collected from Adazi-enu in Anaochia Local Government Area of Anambra State, Nigeria. It was dried under air and mild sun-shine, for about three weeks and ground into powders. The ground sample was then kept in a clean polyethylene bottle until needed for analysis. Phytochemical and the extraction of the active components are determined by the methods outlined by Harbon [12]. The antifungal activity of flower and stalk of male *C. papaya* was determined by agar well diffusion method [13]. The zone of inhibition was recorded to the nearest size in mm [14]. After extraction of the active components using three different solvents separately (Ethanol, Water and N-hexane), the solvent extracts were evaporated to dryness at about 67, 98 and 66°C respectively in a water bath separately. 1, 2, 3, 4 and 5 mg of dry ethanolic, n-hexane and water extracts were weighed into five different labeled test tubes differently. Then 10 ml of the corresponding solvents used for extraction was added to the dried extracts to make 0.1, 0.2, 0.3, 0.4 and 0.5 mg/ml concentrations of the extracts.

The MIC of flower and stalk of male *Carica papaya* were found out by using 0.1, 0.2, 0.3, 0.4, and 0.5 mg/ml of each extract which were added to test tubes containing 1ml of sterile medium. The tubes were then inoculated with a drop of microbial suspension and incubated for 48 hours at 25°C. Then 0.1, 0.2, 0.3, 0.4, and 0.5 mg/ml of amphotericin B (for *A. flavus, F. verticilloides, A. parasiticus, F. oxysporum* and *Fluconazole*) for all candida was used for positive control and water for negative control respectively. The MIC value was determined, macroscopically after incubation in comparison with the growth and sterility control. MFC; the plates (petri-dishes) were divided into six different sections and labeled with the different concentration on the base of the plates, these were used to plate out the contents of each tube with the respective sections of the plate. The plates were incubated

for 18 – 24 hours at 37°C after which the MFC were recorded. Three replicates were done for each extract concentration and control against the fungi.

Results

Tables 1: Qualitative Phytochemical Analysis of Flower and Stalk of Male *Carica papaya*

Class of phytocompound	Inference
Alkaloid	+
Flavonoid	+
Saponin	+
Tannins	++
Terpenoids	+
Steroids	+
Phenol	-
Cardiac glycosides	++

Key**:** + = present, - = absent

Table 2: Quantitative Estimates of Phytochemical Constituents of Flower and Stalk of Male *Carica papaya*

Class of phytocompound	Inference
Alkaloid	0.53±0.01
Flavonoid	0.86±0.02
Saponin	0.37±0.02
Tannins	2.60±0.01
Terpenoids	0.21±0.01
Steroids	0.08±0.01
Phenol	Nil
Cardiac glycosides	1.87±0.02

Table 3: Antifungal activities of crude solvent extracts of flowers and stalk of male *Carica papaya*

Text organisms (fungi)	Conc. of extract (mg/ml)	Average Diameter (mm) of Inhibition Zone			+ve control for all Candida: Fluconazole others: Anaphotericin	-ve Control distil Water
		Distill H_2O	EtOH	N-Hexane		
Aspergillus niger	0.1	1.9 ± 0.20	NA	NA	16.26 ± 0.25	NA
	0.2	2.8 ± 0.10	NA	NA	19.00 ± 0.10	NA
	0.3	3.1 ± 0.02	2.33 ± 0.02	NA	21. 6 ± 0.21	NA
	0.4	4.04 ± 0.10	3.21 ± 0.01	NA	23.2 ± 0.28	NA
	0.5	5.00 ± 0.02	3.98 ± 0.10	2.41 ± 0.02	24.80 ± 0.01	NA
Microsporum gypseum	0.1	NA	NA	NA	8.00 ± 0.02	NA
	0.2	NA	NA	NA	8.60 ± 0.10	NA
	0.3	NA	2-33 ± 0.02.	NA	8.60 ± 0.06	NA
	0.4	2.61± 0.01	3.21 ± 0.01	NA	9.98 ± 0.22	NA
	0.5	3.22 ± 0.01	3.89 ± 0.10	NA	10.40 ± 0.01	NA
Candida albican	0.1	2.40± 0.01	7.5 ± 0.02	2.00 ± 0.01	30.08 ± 0.02	NA
	0.2	2.63± 0.01	8.2 ± 0.01	2.88 ± 0.02	33.20 ± 0.02	NA
	0.3	3.91± 0.02	9.00 ± 0.03	3.12± 0.01	35.80 ± 0.10	NA
	0.4	4.62± 0.02	9.97 ± 0.01	3. 92 ± 0.01	37.00 ± 0.03	NA

	0.5	4.88 ± 0.10	11. 00 ± 0.02	4.17 ± 0.02	30.28 ± 0.17	
Aspergillus flavus	0.1	1.21± 0.01	2.80 ± 0.20	NA	17.80 ± 0.10	NA
	0.2	1.90 ± 0.10	3.65 ±0.10	NA	21. 00 ± 0.20	NA
	0.3	2.40 ± 0.20	4.00 ± 0.02	NA	23.23 ± 0.10	NA
	0.4	2.86 ± 0.01	4.86 ± 0.01	NA	25.00 ± 0.05	NA
	0.5	3.16 ± 0.30	5.37 ± 0.20	NA	28.4 ± 0.10	NA
Fusarium Verticilloides	0.1	NA	NA	NA	7.00 ± 0.30	NA
	0.2	NA	NA	NA	7.90 ± 0.02	NA
	0.3	NA	NA	NA	9.00 ± 0.30	NA
	0.4	NA	NA	NA	9.58 ± 0.01	NA
	0.5	NA	NA	NA	10.22± 0.07	NA
Aspergillus parasiticus	0.1	1.38 ± 0.02	NA	3.73± 0.01	23. 80 ± 0.2	NA
	0.2	2.06 ± 0.01	NA	4.02 ± 0.30	24.00 ± 0.10	NA
	0.3	2.97 ± 0.02	NA	4.08 ± 0.20	25.61 ± 0.50	NA
	0.4	3.30 ± 0.02	NA	4.60 ± 0.01	26. 00 ± 0.20	NA
	0.5	3.88± 0.01	NA	4.88 ± 0.02	27.20 ± 0.10	NA
Fusarium oxysporum	0.1	NA	NA	NA	18.37 ± 0.40	NA
	0.2	NA	NA	NA	20.00 ± 0.20	NA
	0.3	NA	NA	NA	21.56 ± 0.10	NA
	0.4	NA	NA	NA	22.26 ± 0.10	NA
	0.5	NA	NA	NA	24.00 ± 0.01	NA
Candida glabrata	0.1	1. 35 ± 0.01	2. 60 ± 0.01	4. 30 ± 0.01	22.00 ± 0.01	N a
	0.2	2.29 ± 0.03	4.31 ± 0.03	2. 10 ± 0.02	24.1 8 ± 0.20	NA
	0.3	2 .96 ± 0.02	5.65 ± 0.01	2.80 ± 0.03	26.00 ± 0.02	NA
	0.4	3. 12 ± 0.03	7. 04 ± 0.01	3. 20 ± 0.02	27.86 ± 0.30	NA
	0.5	3. 84 ± 0.01	8. 52 ± 0.02	3. 90 ± 0.01	29.00 ± 0.10	NA
Candida parasilosis ticum	0.1	3.80 ± 0.02	4.20 ± 0.02	2.02 ± 0.01	23.00 ± 0.10	NA
	0.2	4.22 ± 0.02	4.86 ± 0.01	2.23 ± 0.02	23.86± 0.20	NA
	0.3	4.92 ± 0.01	5.12 ± 0.02	2.77 ± 0.01	24.12 ± 0.02	NA
	0.4	5.28 ± 0.02	5.66 ± 0.01	3. 12 ± 0.04	26.00 ± 0.10	NA
	0.5	5. 88 ± 0.01	5.91 ± 0.01	3.93 ± 0.01	27.88 ± 0.30	NA
Candida tropicalis	0.1	3.48 ± 0.20	NA	0.10 ± 0.02	2 1.00 ± 0.20	NA
	0.2	4.01 ± 0.01	NA	2.1 5 ± 0.02	2 1.95 ± 0.10	NA
	0.3	4.69 ± 0.02	3.66 ± 0.02	2.54 ± 0.01	23.00 ± 0.10	NA
	0.4	5.20 ± 0.01	5.00± 0.03	3.23 ± 0.20	23.86 ± 0.02	NA
	0.5	5.87 ± 0.01	6.91± 0.01	3. 87 ± 0.04	25.00 ± 0.03	NA

Tables 4: Minimum Inhibitory Concentration (MIC)

	Minimum inhibitory concentration (mg/ml)		
Test organisms (Fungi)	**Water extract (MIC)**	**Ethanol extract (MIC)**	**Hexane extract (MIC)**
Aspergillus niger	0.05± 0.01	0.25± 0.01	0.45 ± 0.01
Microsporum gypseum	0.35± 0.02	0.25± 0.03	NI
Candida albican	0.05± 0.02	0.05± 0.01	0.05 ± 0.01
Aspergillus flavus	0.05± 0.02	0.05± 0.01	NI
Fusarium verticilloides	NI	NI	NI
Aspergillus parasiticus	0.05± 0.01	NI	0.05 ± 0.01
Fusarium oxysporum	NI	NI	NI

Candida glabrata	0.05± 0.01	0.05± 0.01	0.05 ± 0.01
Candida parasilosis	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
Candida tropicalis	0.05 ± 0.01	0.25 ± 0.01	0.05 ± 0.01

NI: No Inhibition

Tables 5: Minimum fungicidal Concentration (MFC) of Ten Fungi

	Minimum fungicidal concentration (mg/ml)		
Test organisms (Fungi)	**Water extract (MFC)**	**Ethanol extract (MFC)**	**N-Hexane extract (MFC)**
Aspergillus niger	0.04±0.01	0.15±0.03	0.23±0.02
Microsporum gypseum	0.30±0.01	0.25±0.01	NI
Candida albican	0.02±0.01	0.02±0.01	0.02±0.02
Aspergilus flavus	0.05±0.01	0.05±0.02	NI
Fusarium verticilloides	NI	NI	NI
Asperigillus parasiticus	0.05±0.01	NI	0.03±0.01
Fusarium oxysporum	NI	NI	NI
Candida glabrata	0.05±0.03	0.03±0.01	0.02±0.01
Candida parasilosis	0.05±0.01	0.03±0.01	0.05±0.02
Candida tropicalis	0.03±0.01	0.05±0.01	0.05±0.01

Key: NI: No Inhibition

Discussion

Table 1 and 2 showed both qualitative and quantitative estimate of phytocompounds present in the flower and stalk of male *Carica papaya*. The phytochemical constituents of the flower and stalk are as follows: alkaloids 0.53 ± 0.01%, floaonoid 0.08 ± 0.02% saponins 0.37 ± 0.02% tannins 2.06 ± 0.01%, terpenoid 0.02 ± 0.01%, steroids 0.08 ± 0.01, phenol nil, cardiac glycoside 1.87 ± 0.02%. The flower and stalk of male *Carica papaya* contain an appreciable quantity of alkaloids. It could be used to remedy some diseases, depending on the type of alkaloids it contains. The presence of alkaloids signified the possession of medicinal values within the flower and stalk. Pure isolated alkaloids are used as basic medicinal agents and fungicidal properties. The presence of steroids in the sample indicates that it could be used to decrease the presence of cholesterol in the bloodstream. Saponins have been used in the treatment of cardiovascular disorders, they facilitate and ease the process of digestion and encourage the growth of beneficial bacteria within the intestine. Saponins also contribute to health and efficiency of the immune system by binding to germs and other pathogens as they enter the body. In this sense they function as natural anti-bodies. They are found to

be antimicrobial and are particularly effective when used to treat yeast a infections. The presence of cardiac glycosides in the flower and stalk of male *Carica papaya* indicates that it could be used, in the treatment of congestive heart failure and cardiac arrhythmia.

Table 3 portrayed the result of antifungal activities of three solvent extracts of the flower and stalk of male *Carica papaya* on ten micro-organisms (fungi) investigated in this work. They are: *A. niger, M. gyspseum, C. albican, A. flavus, F. verticilloides, A. paraciticus, F. oxysporum, C. glabrata, C parasilosis and C. tropicalis*. Five different concentrations of aqueous, ethanolic, and normal hexane extracts were used. At 0.1-0.5mg/ml concentration, aqueous extract showed some inhibitory effect on eight out of the ten test fungi. The fungi are; *A niger, M. gypseum, C. albican, A flavus, C. parasilosis, A. parasiticus, C. glabrata and C. tropicalis*. No action was recorded on the two remaining test fungi: *F. verticilloides and F. oxysporum.* At 0.1-0.2mg/ml concentration, ethanol extract shows some inhibitory effect on seven out of the ten test fungi. These are: *A, niger, M. gypseum, C. albican, A. flavus, C. glabrata, C. parasilosis and C. tropicalis*. At 0.1-0.2mg/ml, ethanolic extract shows no action against three fungi. These are; *F. verticilloides, A. parasiticus, and F. oxysporum*. At 0.1-0.5mg/ml, n-hexane extract indicated some inhibitory effect on six out of the ten test fungi. These are: *A, niger, C. albican, A. parasiticus, C. glabrata, C.*

parasilosis and C. tropicalis. It had no action on four fungi. These are: *M. gypseum, A. flavus, F. verticilloides and F. oxysporum.*. Table 3 also showed the commercial drugs used as positive and negative control. Positive control using two different antibiotics specifically showed remarkable inhibitory effects on the ten test fungi while the negative control showed no action against the ten test fungi.

Table 4 showed the results of the minimum inhibitory concentration (MIC) of the aqueous, ethanolic and n-hexane extracts of flower and stalk of male *Carica papaya* on the ten test fungi. The least MIC of the aqueous extract 0.05 ± 0.01mg/ml was shown on five test fungi: These are *A. niger, A. parasiticus, C. glabrata, C. parasilosis and C. tropicalis*. MIC of the ethanolic extract 0.05 ± 0.0lmg/ml was shown on four test fungi. These are, *C. albican, A. flavus, C. glabrata, and C. parasilosis*. MIC of the n-hexane extract 0.05 ± 0.0lmg/ml was shown on five test fungi. These are: *C. albican, A. parasiticus, C. glabrata, C. parasilosis and C. tropicalis*.

Table 5 showed the result of minimum fungicidal concentration (MFC) of the aqueous, ethanolic and n-hexane extract of flower and stalk of male *Carica papaya* on ten test fungi. For aqueous extract, the least MFC 0.2 ± 0.01mg/ml was shown on one test fungi,*Candida albican*. For ethanolic extract, the MFC 0.02 ± 0.0lmg/ml was shown on one test fungi *Candida albican*. For n-hexane extract the least MFC 0.02 ± 0.01mg/rnl was shown on one test fungi: *Candida glabrata.*

Conclusion

The analytical investigation showed that, the crude solvent extracts of flower and stalk of male *Carica papaya* have antigfungal effect on these microorganisms; *Aspergillus niger, Microsporum gypseum Candida albican, Aspergillus flavus, Aspergillus parasiticus, Candida glabrata, Candida parasilosis and Candida tropicalis*. This implies that crude solvent extracts of flower and stalk of male *Carica papaya* can be used to cure the diseases caused by the above mentioned microorganisms. Bioactive ingredients responsible for the antimicrobial properties of the flower and stalk of male *C. papaya* should be elucidated.

Male C. papaya Plant

Flower and Stalk of male Carica papaya

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