The Pharmaceutical and Chemical Journal, 2016, 3(4):186-191

Available online www.tpcj.org

Research Article

ISSN: 2349-7092
CODEN(USA): PCJHBA

Effects of crude solvent extracts of flower and stalk of male *Carica papaya* (paw paw) on ten pathogenic bacteria

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Abstract Three solvents extracts of flower and stalk of male *Carica papaya* were used to inhibit ten pathogenic bacteria. The ten bacteria investigated in this research were: *Shigella Sonei, Streptococcus Pyrogenes, Escherichiacoli, Pseudomonas aeruginosa, Staphybcpccus aureus, Salmonella enteridis, Basilius cereus, Streptococcus faecalis, Neisseria gonorrhoeae,* and *Shigella dysenteriae.* Three solvents were used in the extraction of active ingredientsin flower and stalk of male *Carica papaya* and they are distilled water, ethanol and *n*-hexane. Aqueous extract of flower and stalk of male *Carica papaya* could inhibit the ten test bacteria. These are: *Fusarium verticilloids* and *Fusarium oxysporum*. Ethanolic extract could not inhibit one out of ten test bacteria (*Salmonella enteriditis*). *n*-hexane extract could not inhibit three out of the ten test bacteria (*S. Pyrogenes, S. enteriditis* and *N. gonorrhoeae*). The minimum inhibitory concentrations (MIC) of ten bacteria investigated as mentioned above using three solvents were: aqueous extract, 0.05±0.01 mg/ml, 0.05±0.01 mg/ml, 0.05±0.01 mg/ml, 0.35±0.01 mg/ml, 0.05±0.01 mg/ml, 0.05±0.01 mg/ml, 0.05±0.01 mg/ml, 0.05±0.01 mg/ml, 0.05±0.01 mg/ml and 0.05±0.01 mg/ml respectively. The MIC for ethanolic extract were: 0.05±0.01 mg/ml, 0.25±0.01 mg/ml, 0.25±0.03 mg/ml, 0.05±0.01 mg/ml, 0.05±0.01 mg/ml, no inhibition, 0.05±0.01 mg/ml, 0.05±0.01 mg/ml, 0.05±0.01 mg/ml and 0.05±0.01 mg/ml respectively. The MIC for n-hexane extract are: 0.05±0.01 mg/ml, no inhibition, 0.05±0.01 mg/ml, 0.35 0.01, 0.05±0.01 mg/ml, no inhibition 0.05±0.01 mg/ml, 0.05±0.01 mg/ml no inhibition and 0.05±0.01 mg/ml respectively. The minimum bactericidal concentration (MBC) for ten test bacteria as mentioned above were: for aqueous extract 0.05±0.01 mg/ml, 0.30±0.01 mg/ml, 0.03±0.01 mg/ml, 0.15±0.01 mg/ml, 0.03±0.01 mg/ml, 0.03±0.01 mg/ml, 0.03±0.01 mg/ml, 0.05±0.01 mg/ml, 0.05±0.01 mg/ml, 0.03±0.01 mg/ml, and 0.03±0.01 mg/ml respectively. The MBC for ethanolic extract were: 0.05±0.01 mg/ml, 0.3±0.01 mg/ml, 0.02±0.01 mg/ml, 0.03±0.01 mg/ml, 0.05±0.01 mg/ml, no inhibition, 0.03±0.01 mg/ml, 0.02±0.01 mg/ml, 0.03±0.01 mg/ml and 0.02±0.01 mg/ml respectively. The MBC for *n*-hexane extract were: 0.05±0.01 mg/ml, no inhibition, 0.05±0.01 mg/ml, 0.02±0.01 mg/ml, 0.03±0.01 mg/ml no inhibition, no inhibition, 0.05±0.01 mg/ml, 0.05±0.01 mg/ml, no inhibition, and 0.03±0.01 respectively. Hence, crude solvent extracts of flower and stalk of male *Carica papaya* are antibiotic in nature.

Keywords Male *Carica papaya*, flower and stalk, bacteria, zone of inhibition, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

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. *Carica papaya* belongs to the genus carica. Preliminary qualitative and quantitative phytochemical analysis of ethanol and aqueous extracts of *C. papaya* showed the presence of many phyto-

compounds [2]. 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 [3]. In this present work, the authors intend 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 determine 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 bacteria. *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 [4].

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 enzymes. Other enzymes present are glycosyl hydrolases (β-1, 3-glucanases, chitiriases and lyzozynus) protease inhibitors (cystertin and ghtaminylcyclotrunsferces and lipases [5]. 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 [6]. *Carica papaya* (pawpaw) contains the enzyme papain, a protease used for tendering meat and other proteins [1]. The fruits are popularly used and processed into juice and wine, and also cooked as vegetable [7]. 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 [8]. Due to its antioxidant and fibre content, it is used in treatment of ailments such as chronic indigestion, overweighing, obesity, high blood pressure [9].

Sample Collection and Preparation

Flower and 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, 1973 [10]. The antifungal activity of flower and stalk of male *C. papaya* was determined by agar well diffusion method [11]. The zone of inhibition was recorded to the nearest size in mm [12]. 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 18 hours at 37 °C. Then 0.1, 0.2, 0.3, 0.4, and 0.5 mg/ml of ampicilin was used as positive control and distilled water as negative control respectively. The MIC value was determined, macroscopically after incubation in comparison with the growth and sterility control. MBC; 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 MBC were recorded. Three replicates were done for each extract concentration and control against the bacteria.

Results

Table 1: Anti-Bactericidal Activities of Crude Extracts of Flower and Stalk of Male *Caraca papaya*

Test Organisms (Bacteria)	**Conc. of Strait (mg/ml)**	**Average diameter (mm) of inhibition zone**			**+ve control using ampicilin**	**-ve control distilled water**
		Distilled H_2O	EtOH	N-Hexane		
Shigelia	0.1	3.211±0.3	3.48±0.03	2.78±0.2	25.22±0.01	NA
Sonei	0.2	3.33±0,19	3.69±0.25	3.21±0.03	27.15±0.01	NA
	0.3	3.86±0.3	4.02±0.03	4.41±0.3	31.24±0.01	NA
	0.4	4.57±0.18	4.69±0.10	5.03±0.1	31.24±0.01	NA
	0.5	5.08±0.25	4.87±0.10	5.41±0.10	32.00±0.01	NA
Streptococcus	0.1	2.33±0.5	3.26±0.20	NA	22.6+0.1	NA
Pyrogenes	0.2	2.77±0.30	4.00±0.01	NA	22.88±0.01	NA
	0.3	2.98±0.02	5.34±0.01	NA	23.19+0.01	NA
	0.4	3.16±0.10	6.20±0.2	NA	23.57±0.01	NA
	0.5	3.45±0.01	7.11±0.01	NA	23.92±0.02	NA
Escherichia coli	0.1	2.93±0.05	2.33± 0.05	3.22±0.01	28.54±0.1	NA
	0.2	3.66±0.03	3.00±0.1	3.22±0.01	29.31±0.3	NA
	0.3	4.31±0.25	4.00±0.25	4.84±0.01	33.00±0.3	NA
	0.4	4.78±0.05	5.21±0.1	5.56±0.3	33.96±0.3	NA
	0.5	5.57±0.03	6.00±0.1	6.91±0.02	33.96+0.3	NA
Pseudomonas	0.1	NA	8.69± 0.01	NA	32.51±0.3	NA
Aeruginosa	0.2	NA	0.01± 9.12	NA	34.00±0.2	NA
	0.3	2.00±0.25	9.54±0.3	1.70±0.1	36.14±0.2	NA
	0.4	2.28±0.02	10.03±0,01	2.60±0.3	39.4±0.4	NA
	0.5	2.66±0.02	10.77±0.02	3.20±0.10	39.67±0.1	NA
Staphylococcus aureus	0.1	3.81+0.02	3.21±0.1	1.44±0.1	25.00±0.02	NA
	0.2	6.49±0.05	3,90±0.03	3.90±0.03	2.18±0.2	NA
	0.3	7.23±0.21	4.20±0.02	3.32±0.3	30.24±0.2	NA
	0.4	8.00± 0.05	4.90± 0.01	3.81±0.02	31.16± 0.3	NA
	0.5	8.51± 1 0.05	5.23 1±0.03	4.18± 0.2	31.16± 0.3	NA
Salmonella	0.1	2.08± 0.01	NA	NA	25.22± 0.01	NA
Enteriditis	0.2	2.87±0.3	NA	NA	27.15± 0.11	NA
	0.3	3.12±0. 02	NA	NA	30.00± 0.02	NA
	0.4	3.61± 0.1	NA	NA	31.24± 0.02	NA
	0.5	4.18± 0.05	NA	NA	32.00± 0.02	NA
Bacillus	0.1	8.00+ 0.01	5.31 ± 0.02	1.40 0.1	22.6±0.1	NA
Cereus	0.2	8.22± 0.1	5.53±0.01	1.93±0.1	22.88±0.0	NA
	0.3	9.00+ 0.02	5.87±0.02	2.31±0.2	28.19±0.2	NA
	0.4	9.13+ 0.3	6.00±0.2	2.86±0.1	23.57±0.1	NA
	0.5	8.70± 0.1	6.72±0.1	0.2±3.12	23.92±0.02	NA
Streptococcus faecalis	0.1	15.41± 0.1	5.50±0.02	2.55±03	28.54±0.1	NA
	0.2	16.00±0.1	7.00±0.01	3.21 ±0.02	29.31±0.3	NA
	0,3	16.78±0.2	8.87±0.01	4.08±0.02	31.00±0.4	NA
	0.4	18.22± 0.1	9.08±0.1	4.25±0.01	3.00±0.3	NA
	0.5	20.08±0.3	9.47 ±0.3	5.11 +0.1	33.96±0.3	NA
Neisseria	0.1	1.85±0.1	1.11±0.30	NA	32.51±0.3	NA
Gonorrhoeae	0.2	2.00±0.2	1.60±0.20	NA	34.00±0.2	NA
	0.3	3.18±0.1	2.26±0.10	NA	36.14±0.2	NA
	0.4	3.55 ±0.2	2.97±0.2	NA	39.4±0.4	NA
	0.5	4.10±0.2	3.10±0.3	NA	39.47±0.1	NA

Shrigella Dysenteriae	0.1	4.7±0.1	3.21±0.1	1.08±0.3	25.00±0.02	NA
	0.2	3.97±0.1	3.78±0.4	1.22±0.2	26.0±0.01	NA
	0.3	4.03±0.3	4.22±0.2	1.69± 0.02	28.00±0.01	NA
	0.4	4.65± 0.2	5.03±0.01	2.11±0.2	30.24±0.2	NA
	0.5	4.49±0.1	5.24±0.3	2.91±0.01	31.16±0.3	NA

Key; (mm) = Millimeter;
NA = No Action.

Table 2: Minimum Inhibitory Concentration (MIC)

Test organisms	**Minimum Inhibitory Concentration (mg/ml)**		
	Water extract	**Ethanol extract**	**Hexane extract**
Shigella sonnei	0.05±0.01	0.05±0.01	0.05±0.01
Streptococcus pyrogenes	0.05±0.01	0.05±0.01	Nil
Escherichia coli	0.05±0.01	0.05±0.01	0.05±0.01
Pseudomonas aeruginosa	0.35±0.01	0.05±0.01	0.35±0.01
Staphylococcus aureus	0.05±0.01	0.05±0.01	0.05±0.01
Salmonella enteriditis	0.05±0.01	Nil	Nil
Basillus cereus	0.05±0.01	0.05±0.01	0.05±0.01
Streptococcus faecalis	0.05±0.01	0.05±0.01	0.05±0.01
Meisseria gonorrhoeae	0.05±0.01	0.05±0.01	Nil
Shigella dysenteriae	0.05±0.01	0.05±0.01	0.05±0.01

Table 3: Minimum Bactericidal Concentration (MBC) Of Ten Bacteria

Test Organisms	**Water extract (mg/ml)**	**Ethanol extract (mg/ml)**	**Hexane extract (mg/ml)**
Shigella sonnei	0.05+0.01	0.05±0.01	0.05±0.01
Streptococcus pyrogenes	0.3±0.01	0.3±0.01	Nil
Escherichia Coli	0.3±0.01	0.2±0.01	0.05±0.01
Pseudomonas aeruginosa	0.15±0.01	0.03±0.01	0.20±0.01
Staphylococcus aureus	0.03±0.01	0.05±0.01	0.03±0.01
Salmonella enteriditis	0.03±0.01	Nil	Nil
Basillusceresu	0.03±0.01	0.03±0.01	0.05±0.01
Streptococcus faecalis	0.05±0.01	0.02±0.01	0.05±0.01
Neisseria gonorrhoeae	0.03±0.01	0.03±0.01	Nil
Shigella dysenteriae	0.03±0.01	0.02±0.01	0.03±0.01

Discussion and Conclusion

Table 1 portrayed the result of antibacterial activities of three solvent extracts of the flower and stalk of male *Carica papaya* on ten pathogenic bacteria investigated in the research. They are: *S. sonnei, S. Pyrogenes, E. coli, P. aeruginosa, S. aureus, S. enteriditis, B. cereus, S. facaelis, N. gonorrhoeae, S. dysehteriae.* 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 the ten bacteria. These ten bacterial are *S. pyrogenes, S. sonnei, E. colli, P. aeruginosa, S. aureus, S. enteriditis, B. cereus, S. faecalis, N. gonorrhoeae and S. dysenteriae*. At 0.1-0.5mg/ml concentration ethanol extract showed some inhibitory effect on nine out of ten test bacteria. They were *Shigellasonnie, S. pyrogens, E. coli, P. aeruginosa, S. aureus, B. cereus, S. faecalis, N. gonorrhoeae* and *S. enteriditis*. At 0.1-0.5 mg/ml *n*-hexane extract indicated some inhibitory effect on six bacteria namely; *S. sonnie, E. coli, S. aureus, B. cereus, S. faecalis*, and *S. dysenteriae.* At 0.3-0.5mg/ml concentration, ethanolic extract had no action on three bacteria which were; *S. pyrogenes,*

S. enteriditis and *N. Gonorrhoeae* . Table 1 also showed the commercial drugs used as positive and negative control. Positive control using two different antibiotics specially showed some inhibitory effects on the ten test bacteria while negative control showed no action against the ten bacteria.

Table 2 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 bacteria. The least MIC of the aqueous extract 0.05±0.01mg/ml was shown on nine test bacteria. These were; *S. sonnie, S. pyrogenes, E. coli, P. aeruginosa, S. aureus, B. cereus, S. faecalis, N. gonorrhoeae and S. dysenteraie.* MIC of the n-hexane extract 0.05±0.01mg/ml was shown on six test bacteria. These were *S. sonnei, E. coli, S. aerues, B. cereus, S. faecalis* and *S. dysenteriae.*

Table 3 showed the results of minimum bactericidal concentration (MBC) of the aqueous, ethanolic and *n*-hexane extracts of male flower and stalk of male *Carica papaya* on ten test bacteria. For aqueous extract, the least MBC 0.03±0.01mg/ml was shown on seven test bacteria. They were: *S. pyrogenes, E. coli, S. aereus, S. enteriditis, B. cereus, N. gonorrhoeae* and *S. dysenteriae.* For ethanolic extract, the MBC 0.02±0.01mg/ml was shown on three test bacteria. These were *E. coli, S, faecalis* and *S. dysenteriae*. For *n*-hexane extract, the least MBC 0.03±0.01mg/ml was shown on two test bacteria: *S. aereus* and *S. dysenteriae*.

Conclusion

The analytical investigation showed that, the crude extracts of flower and stalk of *S. aureus* and *S. dysenterial* male Carica papaya had anti bacterial effect on these ten test organisms which are; *Shigella Sonei, Streptococcus Pyrogenes, Escherichiacoli, Pseudomonas aerugino, Staphybcpccus aureu, Salmonella enteridis, Basilius cereus, Streptococcus faecalis, Neisseria gonorrhoeae, and Shigella dysenteriae*.

This implied that crude solvent extracts of flower and stalk of male Carica papaya could be used to cure disease caused by the above mentioned micro organisms. The result of this investigation also portrayed the fact that, traditional medicinal use of flower and stalk of male *Carica papaya* should continue and bioactive ingredients responsible for the antimicrobial properties of the flower and stalk should be elucidated.

Flower and Stalk of male *Carica papaya Male C. papaya* Plant

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