

In vitro evaluation of the antibacterial activities of **Zea mays**' stigma and **Carica papaya** seeds hydro-ethanolic extracts

ABSTRACT

Corn and especially maize stigma are traditionally used to facilitate urinary and digestive elimination functions that favour certain diseases such as urinary tract infections. Similarly, papaya seeds possess potent antibacterial and anti-inflammatory properties, which improve digestive health. The above activities of these parts of plants aforesaid might be associated with antibacterial activities. We aimed to evaluate the antibacterial activities of these two food crops. Each plant materials collected were air dried in shade, dried, and ground into fine powder, which were soaked in solvents (water: 30% - ethanol 70%) and shacked for 48 h. After filtering, every mixture was concentrated by using rotavapor and the extracts were prepared 100 mg/ml in sterile distilled water for antibacterial test. The antibacterial activities and the minimum inhibitory concentration (MIC) test of the extracts were assessed by microdilution method associated with spreading in agar medium. Both extracts showed bacteriostatic activity. The MIC values ranged from 25 to 50 mg/ml while the minimum bactericidal concentration (MBC) values of the two extracts were at least 50mg/ml on each of three bacterial species studied.

Key words: Carica papaya seeds, Zea mays stigmas, antibacterial activity, minimum inhibitory concentration, minimum bactericidal concentration.

INTRODUCTION

Plants, vital components of biological diversity serve primarily for human well-being [1]. Human populations have always used the elements of their environment, especially plants, to heal themselves [2]. According to the WHO in February 2013, traditional medicines whose quality, safety and efficacy are proven, contribute to achieving the goal of giving everyone access to care [3]. For many millions of people, herbal medicines, traditional treatments and traditional practitioners are the main or even the only source of health care. In developing countries, almost 80% of the rural population use medicinal plants to treat themselves [1]. The success of herbal treatments depends on components such as mineral oils, flavones, tannins, and anthocyanins, which form and accumulate in plants [4,5]. Since early twentieth century, antibiotics discovery has led to great advances in therapy and contributed to the rise of modern medicine. Unfortunately, the emergence of antibiotic-resistant bacteria has put an end to this wave of optimism. By becoming resistant to many treatments, bacteria limit the range of antibiotics available in medical therapy [2]. Face to these limits, the development of new drugs becomes important. At present, the search for new natural drugs involves the inventory of plants and the systematic examination of their biological activity [6]. Amongst the many under-studied plants that populate the rich African flora, an invaluable reservoir of bioactive molecules, are maize and papaya [6]. Papaya seeds have potent antibacterial and anti-inflammatory properties, which improve digestive health [7]. Stigmas of corn are traditionally used to facilitate the functions of urinary and digestive elimination acting favourably on certain diseases such as urinary infections thanks to their antioxidant and diuretic properties [8]. The present work deals with the study of the effect of hydroethanol extracts of Carica papaya seeds and corn stigmas on the in vitro growth of some of the germs involved in the pathologies for which these portions of plants are indicated.

MATERIALS AND METHODS

Chemicals

Ethanol (Reagent chemical Services Ltd., United Kingdom), Müller-Hinton Broth (MHB), Müller-Hinton agar (Oxoid LTD., Basingstoke, Hampshire, England), cotton swab (Nataso, India), sterile distilled water, were used for the study.

Plant collection and identification

Stigma of *Zea mays* were harvested in the summer of Corn. *Carica papaya* seeds were collected from natural papaya "solo". Both specimen were identified and authenticated by taxonomist at faculty of Natural Sciences of Lomé University. It was deposited at the Herbarium with voucher number *Zea mays* (15176) and *Carica papaya* (15175).

Preparation of solvent extraction

The plant materials were air dried in shade at room temperature (25-30°C). Afterwards, dried stigmas of *Zea mays* and dried *Carica papaya* seeds were dried, chopped and milled. 480 grams of *Zea mays* stigma powder and 792 grams of powder from the spraying of *Carica papaya* seeds were dispersed for 48 hours into respectively 3500 ml of the ethanolic mixture (1100 ml of water - 2400 ml of ethanol) and 6000 ml of the hydro-ethanol mixture (1800 ml of water - 4200 ml of ethanol) at room temperature. Then, the solutions were filtered by Whatman no. 1 filter papers and the solvent extracts were concentrated separately using rotary flash evaporator (Heavolph 94200 BIOBLOCK SCIENTIFIC German) at 45°C. After complete evaporation of the solvents each of the extract was weighed and the yield was calculate as showed on table 1.

Table 1. Percentage extract yield from the *Carica papaya* seeds and stigmas of *Zea may's* hydroethanolic extracts

	Dry powder (g)	70% Ethanol (ml)	Ratio	Yield (g)	Yield (%)
<i>Carica papaya</i> seeds	792	6000	33/250	56.5	7.13
<i>Zea may's</i> stigmas	480	3500	24/175	43.2	9.00

In order to obtain a solution of 100 mg/ml for bacterial assay, 4 g of each extract separately were distributed in 40 ml of sterile distilled water. The two extracts were sterilized by 0.45 nm millipore membrane filtration.

2.4 Phytochemical Screening of Extracts

The plant extracts were screened for the presence of major secondary metabolite classes such as alkaloids, flavonoids, phenols, saponins, sterols and triterpenes according to common phytochemical methods previously described by Trease and Evans [9] and Koudougou, [10].

2.5 Antibacterial Susceptibility test determination

The bacterial strains used are wild type phenotype reference strains (Confirmed by antibiogram) which were obtained from the American Type Culture Collection (ATCC). (*Escherichia coli* (ATCC 25922); *Staphylococcus aureus* (ATCC 29213) and *Klebsiella pneumoniae* (ATCC 13883)

2.5.1 Microdilution assay for MIC and MBC determinations

The microdilution **minimal** inhibitory concentration (MIC) of both extracts was determined using the microdilution method associated with spreading in agar medium [11]. Briefly, each sample solution of 100 mg/ml obtained were added to MHB and serially diluted two fold in a 96-well microplate which allowed at different concentration (**100 ml, 50 ml, 25 ml, 12.5 ml and 6.75 ml**) of each extract. One hundred microliters of inoculum (107 CFU/ml which were standardized with 0.5 Mac Farland) prepared in MHB were then added to come to different final concentration (**50 ml, 25 ml, 12.5ml, 6.75ml and 3.125 ml**). The plates were covered with a sterile plate sealer and then agitated with a shaker to mix the contents of the wells and incubated at 37°C for 24 h. Three witnesses **were** made: **one negative control well, containing only MHB, the second contains MHB and undiluted extract and The last control, a positive control is composed of MHB and 100 µl of inoculums.** MIC was determined by observing the growth bacteria with our naked eyes and the MBC of the samples was determined by sub-culturing the suspensions from the wells, which did not show any growth after incubation during MIC assays on solid nutrient agar by making streaks on the surface of the agar. The plates were incubated at 37°C for 24 h and the MBCs were determined after 24 h. Plates that did not show growth were considered to be the MBC for the extract.

3. RESULTS

Table 2. Phytochemical composition of the plant extracts

Plant Samples	Phytochemical composition					
	Alkaloids	flavonoids	Saponins	Tannins	Triterpenes	Sterols
Carica papaya seeds	+	+	+	+	+	+
Zea may's stigmas	+	+	+	+	-	-

The results of the qualitative phytochemical analysis indicated that alkaloids, **saponosides, gallic and catechic** tannins, flavonoids were present in both extracts; but terpenes and steroids were present only in *Carica papaya* seed extract. (Table 2).

Determination of MIC

The MIC has been defined as the minimum concentration of extract for which there is no visible growth to the naked eye.

Table3: Determination of MIC by inference from the primary full result

Plant samples	<i>Carica papaya</i> seeds			<i>Zea may's</i> stigmas		
	<i>E. coli</i> ATCC 25922	<i>S. aureus</i> ATCC 29213	<i>K. pneumoniae</i> ATCC 13883	<i>E. coli</i> ATCC 25922	<i>S. aureus</i> ATCC 29213	<i>K. pneumoniae</i> ATCC 13883
Extract concentration						
50mg/ml	Negative	Negative	Negative	Negative	Negative	Negative
25mg/ml	Positive	Negative	Negative	Positive	Negative	Negative
12.5mg/ml	Positive	Positive	Positive	Positive	Positive	Positive
6.25mg/ml	Positive	Positive	Positive	Positive	P	Positive
3.125mg/ml	Positive	Positive	Positive	Positive	P	Positive
MIC	50mg/ml	25mg/ml	25mg/ml	50mg/ml	25mg/ml	25mg/ml

Negative: no bacterial colonies after culture. Positive: Presence of bacterial colonies after culture

Determination of MBC

According to the determination of the low bactericidal concentration or MBC, we carried out 24 hours earlier, a bactericidal control test (a control which allows an assessment of the degree of inhibition relative to survival) by streaking in the stripe on a Petri dish, dilutions 10⁰, 10⁻¹, 10⁻², 10⁻³, and 10⁻⁴ of the starting inoculums, corresponding respectively to 100 %, 10 %, 1 %, 0.1 %, and 0.01 % of survivors. After reading the MIC, we made stripe stitching with 50 µl on a new MH agar from the pups without visible growth. These Culture media (MH agar) were then incubated at 37 ° C for 24 h. After that we compared the cultures to the bactericidal control. The MBC is the lowest concentration of extract leaving 0.01% of the bacterial inoculum alive. This corresponds in number, approximately to the population found in bactericidal control tube 10⁻⁴[12].

Table 4: Determination of MBC by inference from the second full result

Plant samples	<i>Carica papaya</i> seeds			<i>Zea may's</i> stigmas		
	<i>E. coli</i> ATCC 25922	<i>S. aureus</i> ATCC 29213	<i>K. pneumoniae</i> ATCC 13883	<i>E. coli</i> ATCC 25922	<i>S. aureus</i> ATCC 29213	<i>K. pneumoniae</i> ATCC 13883
Bacterial strains						
50µl stitching on MH agar from pups contain:						
50mg/ml	Positive	Negative	Negative	Positive	Negative	Negative
25mg/ml	Positive	Positive	Positive	Positive	Positive	Positive
MBC	>50mg/ml	50mg/ml	50mg/ml	>50mg/ml	50mg/ml	50mg/ml

Negative: no bacterial colonies after culture. Positive: Presence of bacterial colonies after culture

Table 5 : Determination of the effect of different extracts on several bacterial strains

Plant samples	<i>Carica papaya</i> seeds			<i>Zea may's</i> stigmas		
	<i>E. coli</i> ATCC 25922	<i>S. aureus</i> ATCC 29213	<i>K. pneumoniae</i> ATCC 13883	<i>E. coli</i> ATCC 25922	<i>S. aureus</i> ATCC 29213	<i>K. pneumoniae</i> ATCC 13883
Bacterial strains						
MIC	50mg/ml	25mg/ml	25mg/ml	50mg/ml	25mg/ml	25mg/ml
MBC	>50mg/ml	50mg/ml	50mg/ml	>50mg/ml	50mg/ml	50mg/ml
MBC/MIC	>1	2	2	>1	2	2
Effect	bacteriostatic			bacteriostatic		

The MIC values of *Carica papaya* seeds and *Zea may's* stigmas extracts range from 25 to 50 mg/ml while The MBC values of the two extracts ranges from 50 to over 50mg/ml concentrations on three bacteria as described in Table 4.

4. DISCUSSION

Extracts of *Carica papaya* seed and *Zea mays*'s Stigma showed antibacterial activity on the three reference bacterial strains we chose as controls for this study, which means that the extracts from these two plants have an activity that inhibits the growth of microorganisms. These findings are in line with the report of Brij et al. [13] who concluded that medicinal plants represent a rich source of antimicrobial agents. Indeed, *Carica papaya* seed extract inhibited the growth of strains of *Staphylococcus aureus* ATCC 29213 and *Klebsiella pneumoniae* ATCC 13883 at a MIC of 25 mg / ml. Inhibitory activity against *Escherichia coli* ATCC 25922 was found with a MIC twice as high, i.e. 50 mg / ml. Our results are similar to those found by Jyotsna et al. [14] According to these authors, the hydro-ethanolic extract of *Carica papaya* seeds wield a bacteriostatic activity on numerous strains, in particular strains of *Staphylococcus aureus*, and *Escherichia coli*.

However, findings of some authors have shown a greater antibacterial activity of *Carica papaya* seed extracts. According to Brij et al. [13], any extract of *Carica papaya* fruit (endocarp, epicarp, seeds) demonstrated antibacterial activity against Gram-positive bacteria strains of *Staphylococcus aureus* as well as Gram-negative bacteria strains of *Escherichia coli* [13]. This antibacterial effect can be justified not only by the solvents used, but also by the methods used. As these authors, we used the ethanol extract in our study. Nevertheless, the microdilution method associated with agar spreading was used to replace the agar diffusion method used by Brij et al. Assessment of the antimicrobial activity of the *Zea mays* stigma extract showed antibacterial activity on *Escherichia coli* ATCC 25922's strains with a MIC of 50 mg /ml, then on *Staphylococcus aureus* ATCC 29213 and *Klebsiella pneumoniae* ATCC 13883 with a MIC of 25 mg / ml. Our results are similar to those of Sawsan et al. [15] but in contrast, they observed a bactericidal effect. Probably, this difference would come from the difference between the operating procedure for determining MICs and MBCs. In our protocol, the microdilution method associated with spreading in agar medium was used in replacement of the method of diffusion in agar medium. Similar antibacterial activity of extract of *Zea mays* stigmas were found on strains of *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli*, despite the use of method of disk in agar plate by Morshed Selim and. Islam Shahinul S. M [16]. Even though, we obtained MICs resulting from the action of the two extracts on all the bacteria studied, we haven't been able to determine the MBC of the two extracts on *Escherichia coli* ATCC 25922. Probably, this MBC would be greater than 50 mg / ml but a stock solution of extract with a concentration greater than 100 mg / ml was prepared. However, according to Rao, [17], BTIC (benzylisothiocyanate), which is the predominant constituent of *papaya* seeds, is toxic at high doses (dose above 1.5 mg / l). Corn stigma also contains oxalic acid (Risk of renal lithiasis), which may lead to nephrotoxicity [18]. On the basis of the richness of the two tannin plants, these extracts have diuretic properties. Thus, corn stigma and *papaya* seeds would play a role in the elimination of toxins.

Also, according to Mpondo et al.[19], *papaya* seeds and corn stigma are used for the treatment of several pathologies caused by bacteria including urinary tract infections. This study has shown antibacterial activity on *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli*. In addition, the phytochemical study of the two extracts revealed the presence of alkaloids, flavonoids, saponosides, gallic and catechic tannins. Sterols and terpene compounds were found only in the *papaya* seed extract. All these chemical components are recognized to have antibacterial properties [20]. For example, flavonoids are known to inhibit the expression of DNA and the synthesis of certain enzymes and membrane proteins of microorganisms. Tannins are capable of inhibiting the growth of many microorganisms including bacteria [21]. Therefore, flavonoids and tannins inhibit the growth of different types of bacteria: *Staphylococcus aureus*, *Escherichia coli* [20].

In addition, Eke et al. [21] have shown that the presence of saponins and alkaloids may support the existence of antibacterial activity. The presence of these chemical groups could explain the antibacterial properties observed in this study and justify the traditional use of *papaya* seeds and corn stigmas in the treatment of bacterial diseases.

5. CONCLUSION

Extracts from the *Carica papaya* seeds and the stigmas of *Zea mays* each possess bacteriostatic antibacterial activity on the three reference strains of *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, and *Klebsiella pneumoniae* ATCC 13883 at MICs equal to 50mg /ml, 25mg/ml, and 25mg/ml, respectively. The observed antibacterial activity, from plant extracts grown in our environment, on bacterial strains responsible for community and nosocomial infections gives hope in the search for an alternative therapeutic for the treatment of bacterial infections. The fact that these extracts have several secondary metabolites such as tannins, triterpenoids, alkaloids, and flavonoids will help prevent the emergence of resistance. However, it is important to supplement our studies with

toxicity tests and to move towards the supply of improved traditional medicines to be offered in humans.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The company names used for this research are commonly and predominantly selected in our area of research and country. There is absolutely no conflict of interest between the authors and company because we do not intend to use these companies as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the company rather it was funded by personal efforts of the authors.

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