Short Research Article

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 favor 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. 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 rotary evaporator 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 activities in the order idea that MIC values range from 25 to 50 mg/ml while The MBC values of the two extracts ranged from 50 to over 50mg/ml concentrations on three bacteria studied.

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

1.INTRODUCTION

Plants, vital components of biological diversity, serve primarily 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. The effectiveness of these medicinal plants depends on their compounds, very numerous and very varied depending on the species, which are as many different active ingredients [4]. This is a wide variety of secondary substances such as essential oils, flavanes, tannins, and anthocyanins, which form and accumulate in plants [5]. Recent surveys have shown that 80% of rural populations in developing countries and 85% of populations south of the Sahara use medicinal plants as the main treatment; most therapies involve the exploitation of the active ingredient of medicinal plants [1]. Since their discovery in the early twentieth century, antibiotics have 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 any treatment, bacteria limit the range of antibiotics available in medical therapy [2]. In the face of these limitations, the development of new drugs becomes important. At present, the search for new drugs of natural origin involves the inventory of plants and the systematic examination of their biological activity. [6]. Among 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 favorably 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 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.

2. MATERIALS AND METHODS

2.1Chemicals

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

2.2 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).

2.3 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 chopped and ground.Then 480 grams of *Zea mays* stigma powder and 792 grams of *Carica papaya* seed powder were soaked for 48h 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 (%)
Carica papaya seeds	792	6000	33/250	56,5	7,13
Zea may's stigmas	480	3500	24/175	43,2	9

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 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, 50, 25, 12.5, 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, 25, 12.5, 6.75, 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 was made. As negative control, well containing only MHB, and other containing MHB and undiluted extract. The last control, which served as positive control is composed of MHB and 100 µl of inoculums.

2.5.2 Minimum inhibitory concentration (MIC) determination by agar dilution method

The MIC was defined as the lowest sample concentration that prevented color change of the medium and that resulted in the complete inhibition of bacterial growth. It was determined by observing the growth bacteria with our naked eyes.

2.5.3 Minimum bactericidal concentration (MBC) determination

The minimum bactericidal concentration (MBC) was defined as the lowest concentration of sample, which completely inhibited the growth of bacteria. The MBC of the sample 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

3.1 Phytochemical composition of the extracts

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)

Table 2. Phytochemical composition of the plant extracts

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

3.2 Determination of MIC and MBC

The results of the MICs and CMBs as well as that of the CMB / CMI ratio are set out in Table 3.

Table 3. Effect of *Carica papaya* seeds and *Zea mays*'s stigmas extracts on selected bacteria

Plant samples	*Carica papaya* seeds			*Zea may's* stigmas		
	E. coli ATCC	S. aureus ATCC	*K. pneumoniae* ATCC 13883	E. coli ATCC	*S. aureus* ATCC	*K. pneumoniae* ATCC 13883

Bacterial strains	25922	29213		25922	29213	
CMI (mg/ml)	50	25	25	50	25	25
CMB (mg/ml)	>50	50	50	>50	50	50
CMB/CMI	>1	2	2	>1	2	2
Effect	bacteriostatic			bacteriostatic		

The MIC values of *Carica papaya* seeds and *Zea mays'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 3**.**

4. DISCUSSION

The extracts of *Carica papaya* seeds and *Zea mays* stigmas have an antibacterial activity on the different reference strains studied.

These results confirm those of Brij B. T. and collaborators that medicinal plants represent a rich source of antimicrobial agents [12]. In fact, *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, followed by the strain of *Escherichia coli ATCC 25922* at a MIC Twice as large, equal to 50 mg / ml. these results are consistent with those of Jyotsna K. P. and collaborators [13]. According to these authors, the hydro-ethanolic extract of papaya seeds exerts bacteriostatic activity on numerous strains, in particular strains of *Staphylococcus aureus*, and *Escherichia coli.*

However, the results of some authors have shown a greater antibacterial activity of *Carica papaya seed* extracts. According to Brij B. T. and collaborators, any extract of *Carica papaya* fruit (endocarp, epicarp, seeds) showed antibactérial activity toward the gram positive bacteria strains of *Staphylococcus aureus* as well as gram negative bacteria strains of *Escherichia coli* [12]. This similary in antibacterial effect can be justified not only by the solvents used but also by the methods used. In the same way with these authors, we have used the ethanolic extract in the context of our experiments. Nevertheless, the microdilution method associated with spreading on agar medium was used in replacement of the method of diffusion in agar medium used by these authors. Evaluation of the antimicrobial activity of the *Zea mays* stigma extract showed antibacterial activity on the strain of *Escherichia coli ATCC 25922* with a MIC of 50 mg / ml; Then on strains of *Staphylococcus aureus ATCC 29213* and *Klebsiella pneumoniae ATCC 13883* with a MIC of 25 mg / ml. These results confirm those of Sawsan and collaborators [14]. These authors reported a bactericidal antibacterial activity in contrast to the bacteriostatic effect that we observed. Probably, this difference would result from the difference in the technique of implementing MICs and CMBs. Indeed, the microdilution method associated with spreading in agar medium was used in place of the method of diffusion in agar medium. In addition, these results are in accordance with those of Selim M. and S.M. Shahinul Islam, in which the extract of Zea mays stigmas showed antibactérial activity on strains of *Staphylococcus aureus, Klebsiella pneumoniae* and *Escherichia coli* in spite of the method of disk used by its authors [15]. Even though we obtained MICs resulting from the action of the two extracts on all the germs studied, we could not determine the CMB of the two extracts on the species *Escherichia coli ATCC 25922*. In reality, this CMB would probably be Greater than 50 mg / ml and determined a stock solution of extract with a concentration greater than 100 mg / ml was prepared. However, according to Rao, BTIC (benzylisothiocyanate), which is the predominant constituent of *papaya* seeds, is toxic at high doses (dose above 1.5 mg / l) [16]. Corn stigma also contains oxalic acid (Risk of renal lithiasis) which may lead to nephrotoxicity [17]. 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 Mpondo and collaborators, papaya seeds and corn stigma are used for the treatment of several pathologies of bacterial origin including urinary tract infections [18]. 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 groups are recognized as having antibacterial properties [19]. 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 [20]. Therefore, flavonoids and tannins inhibit the growth of different types of bacteria: *Staphylococcus aureus*, *Escherichia coli* [19].

Also, Eke O. N. and collaborators have shown that the presence of saponins, and alkaloids can elucidate the antibacterial activity [20]. The presence of these chemical groups makes it possible to justify, on the one hand, the antibacterial properties observed in this study and, on the other hand, to explain the traditional use of papaya seeds and corn stigmas in the treatment of pathologies of bacterial origin.

5. CONCLUSION

The antibacterial activity observed from plant extracts grown in our environment on bacterial strains responsible for community and nosocomial infections raises hope in the search for an alternative therapeutic for the treatment of bacterial infections. The fact that these extracts include 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.

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