Antimicrobial Properties of Grape Fruit, Pawpaw and Black Pepper Extracts on Organisms associated with Fish Spoilage

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On average, Nigeria loses 30-50% of its fish harvest to spoilage. This exacerbates her fish demand-supply gap; hence the need to devise means of mitigating the spoilage. This paper reports the findings of a study that delved into the antimicrobial properties of Grape Fruit (*Citrus paradisa*), Pawpaw (*Carica papaya*) and Black Pepper (*Piper guineese*) extracts on organisms associated with fish spoilage. In the study, the antimicrobial effect of five concentrations (0.1, 0.2., 0.3, 0.4 and 0.5g/ml) of ethanolic, cold and hot water extracts of these plants on spoilage organisms associated with catfish (*Clarias gariepinus*) were assessed, through measuring inhibition zones, using the cup plate diffusion method. The inhibition zones were found to significantly differ, across extraction method; plant material and extract concentration. Hot water was found to be the best extraction method, with a mean inhibition zone of 4.42 ± 0.38mm; followed by ethanolic and cold water methods, with 3.55 ± 0.47mm and 0.60 ± 0.15mm respectively. Among the plant materials, grape peel had the best antimicrobial activity, with a mean inhibition zone of 3.70 ± 0.40mm against the eleven microorganisms tested, followed by black pepper (2.68 ± 0.42mm) and then pawpaw seed (2.19 ± 0.32mm).

Key words: Antimicrobial activity; *Piper guineese*; *Citrus paradisa*; *Carica papaya*

Introduction

Fish is a major source of food that provides a significant proportion of the protein intake in the diets of many people. In Nigeria, it is the preferred source of animal proteins. It is relatively cheap and widely accepted, which gives it an advantage over other sources of animal proteins like pork (Eyo, 2001; Ligia, 2002). It has the highest profile of essential sulphur-containing amino acids (e.g. cysteine, methionine and lysine) that are limiting in some legumes and most cereal-based diets (Borgstrom, 1962). Nevertheless, fish is highly perishable—being a high-protein food with high levels of free amino acids that microbes metabolize, thereby producing ammonia, biogenic amines (putrescine, histamine and cadaverine), organic acids, ketones, and sulphur compounds (Dalgaard *et al.*, 2006; Emborg, *et al.*, 2005). Lipid degradation in fatty fish produces rancid odours and, in addition, marine and some freshwater fish contain trimethylamine oxide (TMAO)—the precursor of trimethlamine (TMA), which is responsible for fishy odours generated through microbial degradation of TMAO. Prior to microbial spoilage, enzymatic and chemical deteriorative changes occur in fish because of the high content of unsaturated fatty acids, free amino acids and other highly reactive compounds in fish. In the tropics, the high temperatures, lack

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of basic infrastructure and unsanitary production conditions that prevail in most developing countries predispose fish to spoilage—a metabolic process that causes food to be undesirable, or unacceptable, for human consumption, due to changes in its sensory and nutritional characteristics (Doyle, 2007). In Nigeria, where barely 70% of the demand for fish is met locally, post-harvest fish losses average between 30 to 50% (Eyo, 2001), which exacerbates the demand-supply gap. In view of this gap, it is pertinent that ways of reducing post-harvest fish spoilage are devised. To this end, this study was carried out to evaluate the potential of grape fruit, pawpaw and black pepper as natural antimicrobials for use in organic fish production and preservation. These were purposely selected because there is increasing resistance against chemical food preservatives (Agatemor, 2009). Besides, organism tissues possess inherent antimicrobials and, already, some studies (e.g. Shittu et al., 2007; Agatemor, 2009; Pazos et al., 2008; Luther et al., 2007; Matos et al., 2007) had evaluated the antimicrobial activities of several plants.

Materials and Methods

Grape fruits (*Citrus paradise)*, pawpaw (*Carica papaya)* and Black pepper (*Piper guineese*) were collected from Kuto market in Abeokuta, Ogun State, Nigeria, and washed with clean, sterile, water and oven-dried (at 160^{o}C), for one hour. Three hundred (300) grams of each of the plants were blended into fine powder and soaked in 150mls of distilled water, boiling water and 95% ethanol, for 24 hours. The slurry obtained were left in clean, sterile glass containers and vigorously shaken, to allow for proper extraction. The slurry were then filtered, using a sterile muslin cloth, after which the extracts obtained were air dried and stored at 4^{o}C, as required according to Azu and Onyeagba (2007)'s method. The sensitivity of the isolated and sub-cultured test organisms to black pepper, pawpaw seed and grape peel extracts was carried out using the cup plate diffusion method as described by Cruickshank *et al.* (1975). A sterile syringe was used to add 1ml per plate of broth culture of the organism to an already prepared medium. Twenty-five (25) ml of the cooled agar was poured into sterile Petri dishes and the top of the conical flask flamed prior to dispensation onto other plates. Fifteen (15) mm-diameter holes were made in the seeded agar using sterile cork borer. Different dilutions of the plant extracts were prepared in five test tubes in the order of 0.1g/ml, 0.2g/ml, 0.3g/ml, 0.4g/ml and 0.5g/ml and placed on a rack. 0.5ml of each concentration were introduced into each hole on the medium and allowed to stand on the bench for one hour—for proper diffusion—and, thereafter, incubated, at 37^{0}C, for 24hrs. The resulting inhibition zones were then measured, in millimetres, and recorded against the corresponding concentration. The data obtained were analyzed using Analysis of Variance (ANOVA) and the means separated using Fisher's Least Significant Difference as cited by Sanders (1990).

Results

The results obtained are shown in Figure 1 and Tables 1, 2 and 3.

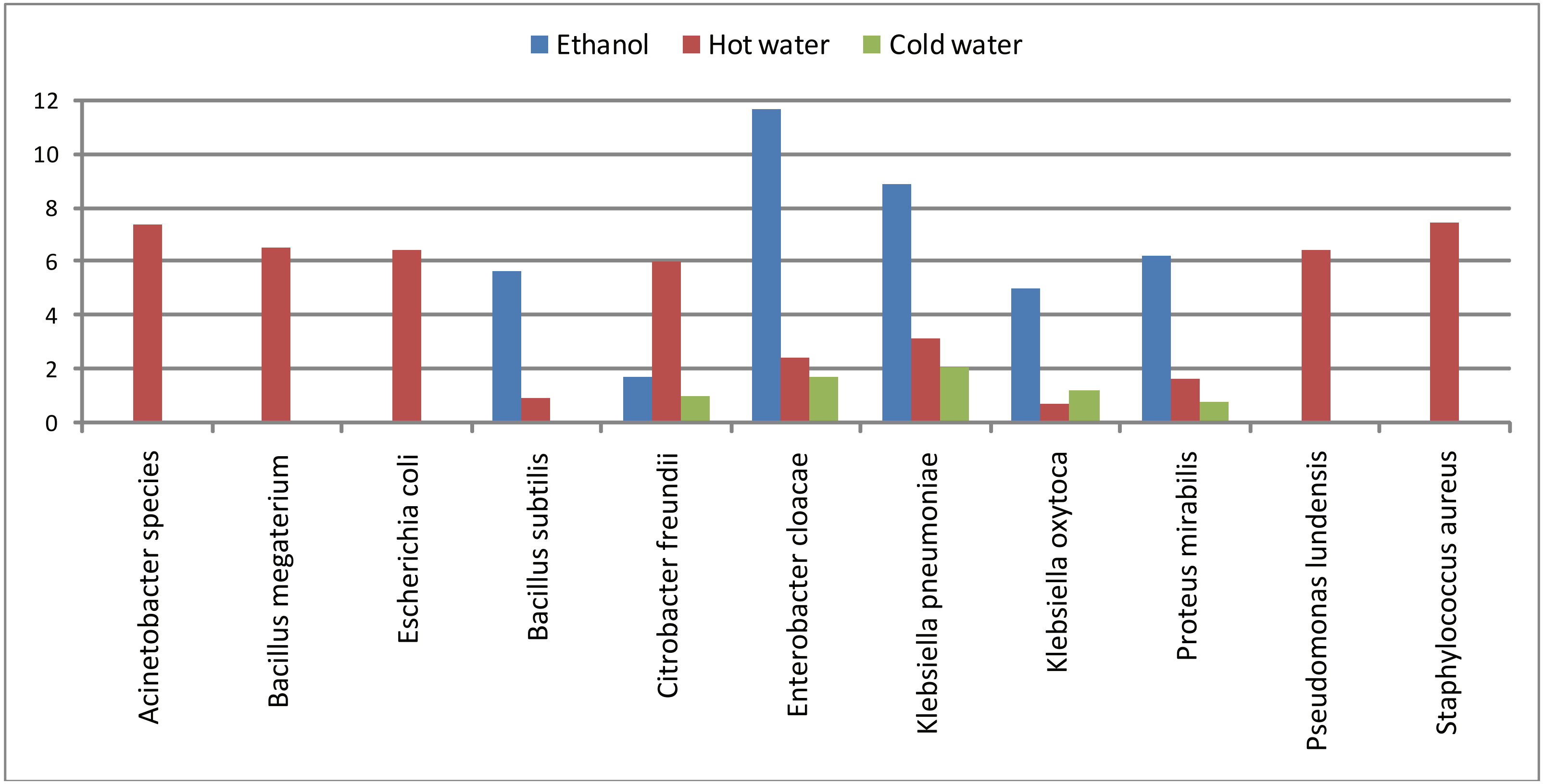

Figure 1: Mean Inhibition Zones of Plant Extracts on Micro organisms associated with Fish Spoilage (mm)

Table 1 shows the effect of extraction method on the micro organisms associated with fish spoilage.

Table 1: Effect of Extraction Method on Micro organisms associated with Fish Spoilage

Organism	Mean zone of Inhibition (mm) Extraction method		
	Ethanol	Hot water	Cold water
Acinetobacter species	0 ± 0	7.33 ± 1.64	0 ± 0
Bacillus megaterium	0 ± 0	6.47 ± 1.51	0 ± 0
Escherichia coli	0 ± 0	6.40 ± 1.6	0 ± 0
Bacillus subtilis	5.60 ± 0.85	0.87 ± 0.61	0 ± 0
Citrobacter freundii	1.67 ± 0.68	6.0 ± 0	0.93 ± 0.44
Enterobacter cloacae	11.67 ± 2.62	2.4 ± 1.15	1.67 ± 0.65
Klebsiella pneumoniae	8.87 ± 2.48	3.13 ± 1.74	2.07 ± 1.17
Klebsiella oxytoca	5.0 ± 1.15	0.67 ± 0.32	1.2 ± 0.62
Proteus mirabilis	6.2 ± 0.64	1.6 ± 0.67	0.73 ± 0.34
Pseudomonas lundensis	0 ± 0	6.4 ± 0.50	0 ± 0
Staphylococcus aureus	0 ± 0	7.4 ± 0.90	0 ± 0

The mean inhibition zones of Ethanolic, cold and hot water extracts on the micro organisms associated with fish spoilage are shown in Table 2.

Table 2: Mean Inhibition Zones of Ethanolic, Cold and Hot Water Extracts on Micro organisms associated with Fish Spoilage (mm)

	Mean zone of Inhibition (mm) Extraction Method			
Plant extract	Ethanol	Hot water	Cold water	
Grape peel	3.60 ± 0.79	5.71 ± 0.70	1.8 ± 0.41	**3.70 ± 0.40[a]**
Pawpaw seed	1.73 ± 0.37	4.84 ± 0.76	0 ± 0	**2.19 ± 0.32[c]**
Pepper	5.31 + 1.06	2.73 + 0.43	0 + 0	**2.68 ± 0.42[b]**
Means	**3.54 ± 0.74[ab]**	**4.42 ± 0.63[ab]**	**0.60 ± 0.12[c]**	

The results in Table 3 show the effect of extract concentration on the sensitivity of the organisms associated with fish spoilage.

Table 3: Effect of Extract Concentration on the Sensitivity of Organisms associated with Fish Spoilage

Concentration (g/ml)	Mean Zone of Inhibition (mm)
0.1	1.74 ± 0.35[c]
0.2	2.44 ± 0.43[b c]
0.3	2.86 ± 0.45[a b c]
0.4	3.34 ± 0.51[a b]
0.5	3.90 ± 0.65[a]

Note: Means with the same letter are not significantly different

Discussion

Grape peel, Pepper and Pawpaw seed extracts were found to exert antibacterial activities on eleven bacterial species associated with catfish (*Clarias gariepinus*) spoilage. *Enterobacter cloacae* was the best inhibited micro organism, with a mean inhibition zone of 5.24 ± 1.18mm, while *Pseudomonas lundensis* was the least inhibited, with a zone of 2.13 ± 0.48mm. In general, all the micro organisms associated with *Clarias gariepinus* spoilage were inhibited by one or more of the plant extracts tested. Hot water extracts of all the plants inhibited all the (eleven) micro organisms; ethanol extracts inhibited six; while cold water extracts inhibited five. This means that the efficacy of the extracts as antimicrobial agents was related to the solvent used. This is in agreement with the results of Agatemor (2009), who found that ethanolic extracts of some Nigerian spices were more potent, than aqueous extracts, against common food borne micro organisms like *Staphylococcus aureus, Klebsiella pneumoniae, Proteus vulgaris* and *Streptococcus faecalis*. Extract concentration was found to have a direct effect on the efficacy of the extracts. Specifically, concentrations of 0.5g/ml exhibited the highest potency. Nonetheless, the difference established between the potency of 0.3g/ml and higher concentrations was not statistically significant. Therefore, it may be economical to work with concentrations of 0.3g/ml. Antimicrobial activity significantly varied ($P < 0.001$) between plant extracts, with grape peel and pawpaw seed having the highest and least efficacy respectively.

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