

Antimicrobial properties of some plant extracts on organisms associated with fish spoilage

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ABSTRACT

Antimicrobial activities of five concentrations (0.1, 0.2, 0.3, 0.4 and 0.5g/ml) each of ethanolic, cold and hot water extracts of Black pepper (*Piper guineense*), Grape (*Citrus paradisa*) peel and pawpaw (*Carica papaya*) seed on spoilage organisms of catfish, *Clarias gariepinus* (Burchell, 1822) were assessed by measuring inhibition zones, using the cup plate diffusion method. Inhibition zones were significantly different ($P < 0.001$) based on extraction method; plant material extracted and extract concentration. Results indicated that the best extraction method was hot water with a mean inhibition zone of $4.42 \pm 0.38\text{mm}$; followed by ethanolic and cold water extraction methods with $3.55 \pm 0.47\text{mm}$ and $0.60 \pm 0.15\text{mm}$ respectively. Among the plant materials evaluated, grape peel had the best antimicrobial activity with a mean inhibition zone of $3.70 \pm 0.40\text{mm}$ against all microorganisms tested, followed by black pepper ($2.68 \pm 0.42\text{mm}$) and pawpaw seed ($2.19 \pm 0.32\text{mm}$) respectively. Microbial inhibition was in the order *Enterobacter cloacae* ($5.24 \pm 1.18\text{mm}$); *Klebsiella pneumoniae* ($4.69 \pm 1.15\text{mm}$); *Citrobacter freundii* ($2.87 \pm 0.43\text{mm}$); *Proteus mirabilis* ($2.84 \pm 0.49\text{mm}$); *Staphylococcus aureus* ($2.47 \pm 0.60\text{mm}$); *Acinetobacter* species ($2.44 \pm 0.75\text{mm}$); *Klebsiella oxytoca* ($2.29 \pm 0.53\text{mm}$); *Bacillus megaterium* ($2.16 \pm 0.67\text{mm}$); *Bacillus subtilis* ($2.16 \pm 0.50\text{mm}$); *Escherichia coli* ($2.13 \pm 0.69\text{mm}$) and *Pseudomonas lundensis* ($2.13 \pm 0.48\text{mm}$) respectively. This study confirms the efficacy of some plant extracts as natural antimicrobials and suggests the possibility of employing them in fish preservation where spoilage is caused mainly by microbial activity.

KEY WORDS: Antimicrobial activity, cup plate diffusion, *Piper guineense*, *Citrus paradisa*, *Carica papaya*, inhibition zone, fish spoilage.

Introduction

Spoilage is a metabolic process that causes food to be undesirable or unacceptable for human consumption due to changes in sensory and nutritional characteristics (Doyle, 2007). Fish is a major source of food, providing a significant portion of the protein intake in the diets of a large proportion of the people, particularly in developing countries. In Nigeria Fish is the preferred source of high quality animal protein compared to poultry, beef, mutton, pork and veal. It is cheap and highly acceptable, with little or no religious bias, which gives it an advantage over pork or beef (Eyo, 2001; Ligia, 2002). Fish has the highest profile of essential sulphur-containing

amino acids such as cysteine, methionine and lysine which are limiting in some legumes and most cereal-based diets (Borgstrom, 1962). In spite of the high demand of fish in Nigeria, only about 70% of needs are met through local production, the rest being imported. Fish is highly perishable, being a high-protein food with typically high levels of free amino acids which microbes metabolize, 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 trimethylamine (TMA), the compound 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. The high temperatures of the tropics, lack of basic infrastructures and the unsanitary production conditions prevailing in most developing countries predispose fish to spoilage. In addition to deficits in demand and supply of fish in Nigeria, post harvest fish losses often reach 30 -50% in rural fishing communities especially during peak production periods (Eyo, 2001). In view of the demand-supply deficits in Nigerian fisheries it is pertinent to find ways of reducing post-harvest losses in the Nigerian fisheries.

In the global food industry today, 'natural' is a powerful force as there is increasing resistance at regulatory and consumer levels against chemical food preservatives (Halliday, 2007; Agatemor, 2009). Numerous naturally occurring antimicrobials are present in animal and plant tissues and many studies have evaluated the antimicrobial activities of several plant extracts, including *Sesamum radiatum* (Shittu *et. al*, 2007), *Allium cepa* (Agatemor, 2009), olives (Pazos *et. al*, 2008); Chardonnay grapes and black raspberries (Luther *et. al*, 2007) and orange essential oils (Matos *et. al*, 2007). The present study was carried out to evaluate the potential of three plant extracts as natural antimicrobials for use in organic fish production and preservation.

Materials and methods

Collection of plant materials

Grape fruits (*Citrus paradise*), pawpaw (*Carica papaya*) and Black pepper (*Piper guineense*) were collected from Kuto market in Abeokuta, Ogun State, Nigeria.

Preparation of plant extracts.

Plant materials were washed individually with clean sterile water and oven-dried for one hour at 160°C. 300g each of respective dry plant material was blended into fine powder and soaked in 150mls of distilled water (cold water extract), boiling water (hot water extract) or 95% ethanol (ethanolic extract) for 24hrs. The slurry obtained was left in clean, sterile glass container and shaken vigorously to allow for proper extraction. The slurry was filtered using a sterile muslin cloth after which the extract obtained was air dried and stored at 4°C until required according to the method of Azu and Onyeagba, 2007.

Antimicrobial screening tests

The sensitivity of the isolated and sub-cultured test organisms to black pepper and pawpaw seed extracts, and grape peel extract 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 the broth culture of the organism to an already prepared medium. 25ml of the cooled agar was poured into sterile Petri dishes and the top of the conical flask flamed prior to dispensation onto other plates. Holes of 15mm in diameter were made in the seeded agar using sterile cork borer.

Different dilutions of the plant extracts was prepared in the order of 0.1g/ml, 0.2g/ml, 0.3g/ml, 0.4g/ml and 0.5g/ml respectively in five test tubes and placed on a test tube rack. 0.5ml of each concentration was 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°C for 24hrs. The resulting inhibition zones were measured in millimeters and recorded against the corresponding concentration.

Statistical analysis

Data obtained in the study were statistically analyzed using Analysis of Variance (ANOVA). The means were separated using Fisher's Least Significant Difference (LSD) (Sanders, 1990).

Results

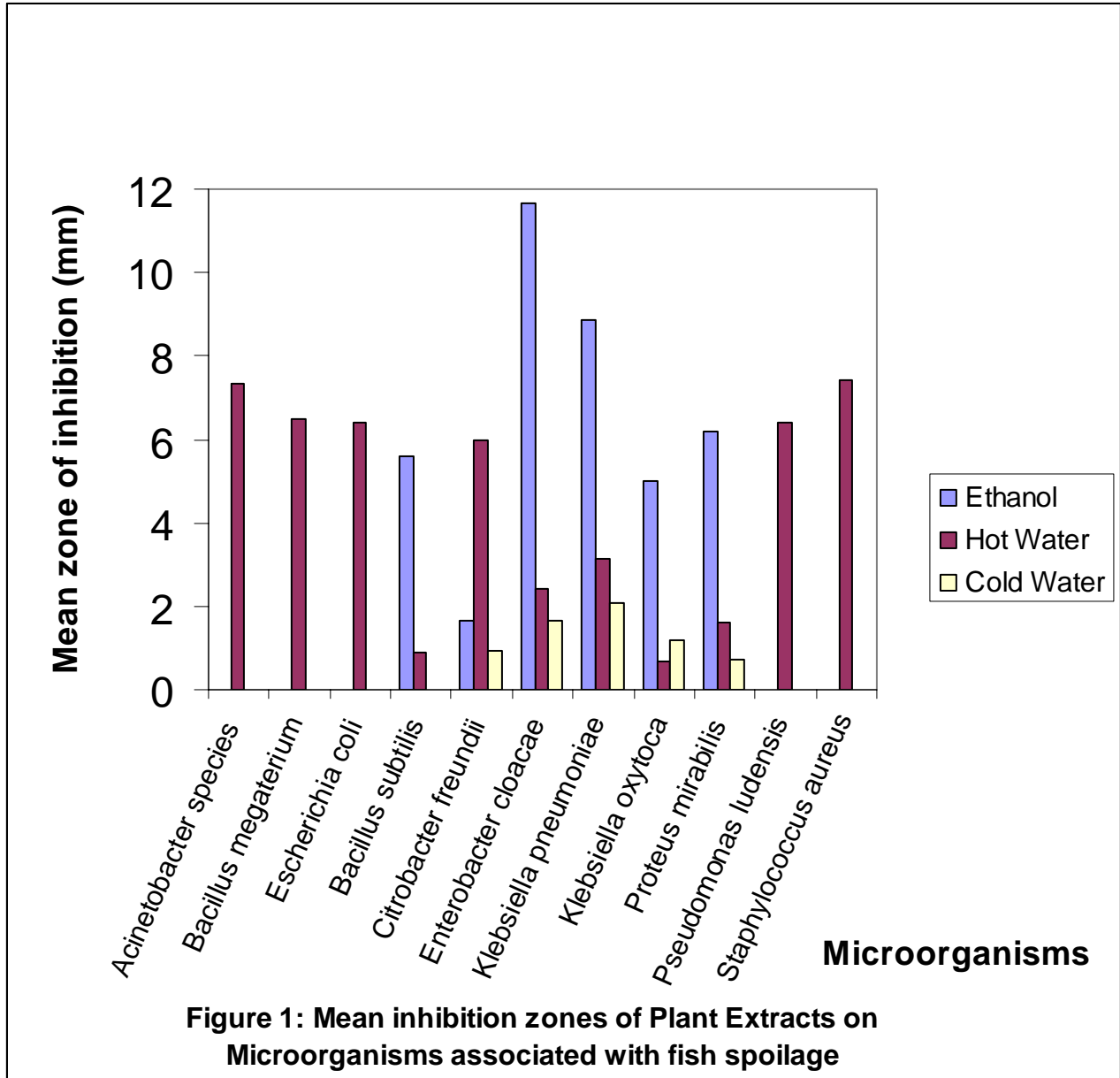


Table 1: Effect of extraction methods on microorganisms

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

Table 2: Mean inhibition zones (mm) of ethanolic, cold and hot water extracts of Grape peel, Pawpaw seed and pepper on microorganisms associated with *Clarias gariepinus* spoilage.

Plant extract	Mean zone of Inhibition (mm)			
	Extraction Method			
	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	

Table 3: Effects of extract concentration on the sensitivity of fish spoilage organisms

<u>Concentration (g/ml)</u>	<u>Mean Zone of Inhibition (mm)</u>
0.1	1.74 ± 0.35^c
0.2	2.44 ± 0.43^{bc}
0.3	2.86 ± 0.45^{abc}
0.4	3.34 ± 0.51^{ab}
0.5	3.90 ± 0.65^a

Means with the same letter are not significantly different

Discussion

Plant products, particularly spices and extracts of various plant parts have been used extensively as natural antimicrobials and antioxidants. In the commercial preservation of fish and fish products, natural antioxidants from plant sources have been found to extend shelf life and prevent fishy taste and flavour (Pazos et al., 2008; Luther et. al. 2007, Martos et. al. 2007). In the present study, 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 microorganism with a mean inhibition zone of 5.24 ± 1.18 mm, while *Pseudomonas lundensis* was the least inhibited with a zone of 2.13 ± 0.48 mm. In general, all the microorganisms associated with *Clarias gariepinus* spoilage were inhibited by one or more of the plant extracts used in this study. While hot water extracts of all plants tested inhibited all eleven microorganisms, ethanol extracts inhibited six, having no effect on *Acinetobacter species*, *Bacillus megaterium*, *Escherichia coli*, *Pseudomonas lundensis*, and *Staphylococcus aureus*; and cold water extracts inhibited five, not inhibiting *Acinetobacter species*, *Bacillus megaterium*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas lundensis*, and *Staphylococcus aureus*. Thus the efficacy of plant extracts evaluated as antimicrobial agents was dependent on the solvent of extraction. This finding is in agreement with the results of Agatemor (2009) who found that ethanolic extracts of some Nigerian spices were more potent than the aqueous extracts against common food borne microorganisms including *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Streptococcus faecalis*.

Extract concentration was found to have a direct effect on the efficacy of plant extracts evaluated. Generally, a concentration of 0.5g/ml of plant extracts evaluated exhibited the highest potency against spoilage organisms of *Clarias gariepinus*. However, no significance difference was established between the potency of extract concentration of 0.3g/ml and higher concentration, therefore, it may be economical to work within this concentration.

Antimicrobial activity varied significantly ($P < 0.001$) between plant extracts, with grape peel having the highest efficacy and pawpaw seed the least.

This study confirms the efficacy of ethanolic, hot and cold water extracts of grape peel, black pepper and pawpaw seeds and their potential as organic preservatives in fisheries and aquaculture.

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