



Antibacterial Activities in Upper River Benue Surface Water With *Diospyros Mespiliformis* Seed Extracts

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Abstract

In this study, the extracts of *Diospyros Mespiliformis*; crude seed, mucilage and seed was used to investigate the potential effect of microbial activity and percentage turbidity reduction on surface water. The Sample water were collected from Upper River Benue at Jimeta Bridge. The sample was collected in Jerry cans (50 liters) transported to wet Laboratory, Department of Fisheries, School of Agriculture, Modibbo Adama University of Technology Yola. Portion of the water fetched were transferred to microbiology laboratory of same University for antibacterial test where three bacteria wer selected for the analysis. Moreover, part of the sample were used for Turbidity Test. Data obtained were analyzed using Analysis of Variance (ANOVA). The Result shows the potency of *D. mespiliformis* extracts on surface water and percentage turbidity reduction. The following bacteria; *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* were used for the evaluation with *E. coli* recorded it highest mean of inhibition zone of 21 and zero as it lowest for crude seed at 15mg/l, 22 highest and zero is the lowest for seed mucilage at 15mg/l and 22 was the highest and zero lowest for seed extracts at 10mg/l. *B. subtilis* had highest mean of inhibition zone of 15 and zero as it lowest for crude seed, 20 highest and zero is the lowest for seed mucilage and 20 was the highest and zero lowest for seed extracts. *S. aureus* had highest mean of inhibition zones of 24 and zero as it lowest for crude seed, 20 highest and zero it lowest for seed mucilage and 22 was the highest and zero is the lowest for seed extracts. *D. mespiliformis* extracts succeeded by reducing very high percentage of the turbid water. *D. mespiliformis* extracts should be used hence forth use as primary coagulants for treatments of raw water for its ability to inhibit bacteria growth.

Keywords: Upper River Benue; West African Ebony; Seed Extracts; Surface Water

Abbreviations: ANOVA: Analysis of Variance; NTU: Nephelometric Turbidity Units; WHO: World Health Organization; SON: Standard Organization of Nigeria.

Introduction

Waste waters and man-made or naturally occurring surface waters can be unsuitable for consumption and irrigation and/or the health and proliferation of naturally

occurring freshwater organisms due to the presence of excessive macronutrients and toxic heavy metals and organic xenobiotic compounds. Plant such as Duckweeds can improve water quality by removing or facilitating the removal of the deleterious substances from the water [1].

Diospyros Mespiliformis (kanya) Hochst (Ebenaceae) is used in ethnomedical practice against malaria in northern Nigeria [2]. Beyond pharmacological value, *Diospyros spp.*

have distinct and complementary important qualities, namely valuable wood, and edible fruits, which provide significant economic benefits and are recognized and utilized in various industrial and commercial sectors [3,4]. Previous studies in laboratory showed that the plant has analgesic, anti-inflammatory and antipyretic effects Adzu B, et al. [5], with CNS activity that is sedative in nature [6]. The plant was also reported to have potent antibacterial Adeniyi BA, et al. [7] and Lajubutu BA, et al. [8], anti-trypanosomal activities [9]. While separate studies have been conducted on identifying the antibacterial activity of many plants, little work was reported to elucidate their combined bactericidal effects [10]. Plant species also showed promising result in antimicrobial effect. Comparing with coagulation properties of plant species, little is known about the potential existence of natural disinfectants (i.e., substances with the ability to kill or inactivate pathogenic microorganisms), even though many herbs and plant extracts are used in traditional medicine and as pesticides in developing countries [11]. Researchers reported antimicrobial activity of *Dolichos lablab*, *Moringa oleifera*, *Azadirachta indica* and other plant species. Yongabi Kenneth A [12], Found that *Moringa oleifera*, *Jatropha curcas* and Hibiscus showed a better coagulation and disinfection activity with the methanol extracts. The antimicrobial effect could be attributed to flocculation Nwaiwu NE, et al. [13] and bactericidal action [14].

Generally, *Diospyros spp.* are tree shrubs or subshrubs with entire alternate leaves, solitary flowers, and fleshy fruits (berries) with usually two or more seeds. The characteristics of the leaves and flowers of these species are often used to identify fossil casts [15-17]. *Diospyros* species are predominantly distributed between the tropics, and the most notable diversity of this botanical genus occurs in Africa [15-18]. As confirmed in the Plant List [19], the WFO Plant List currently contains 1575 species related to the genus *Diospyros*, of which 734 have accepted scientific names [20]. Regarding the Mozambican flora, the genus is represented by 31 species, corresponding to 18 accepted scientific name species, seven accepted subspecies (subsp.), three species that are considered synonyms, and three species that are not yet in the WFO [20], Flora of Mozambique [21] and Da Silva MC, et al. [22].

The hexane fraction of *D. mespiliformis* leaves has anti-inflammatory properties (inhibits stronger the LOX), and that the methanolic extracts of different plant parts showed wound healing effects. On the other hand, the butanol and ethyl acetate fractions activate LOX activity. However, it show that *D. mespiliformis* extract can have pro-inflammatory and anti-inflammatory effects [23]. This study is based on the hypothesis that a solution of the antibacterial agents would have a greater range of bactericidal activity than the solutions of other biocides.

Materials and Methods

Sample Location

Water sample was collected from Upper Benue River Adamawa Yola. Adamawa State is located within the climate of Northern Guinea Savannah zone and lies between latitude 80 and 110N and longitude 11.50 and 130E and climate is tropical with two distinct seasons; the dry and rainy seasons. The experiment was conducted at wet laboratory in the Department of Fisheries, School of Agriculture Modibbo Adama University of Technology Yola.

Experimental Design

The experiment was design as a factorial experiment. The crude seed, mucilage and seed extracts were randomly assigned to three (3) treatments with one control and all samples were replicated each to determine the effectiveness of the *Diospyros Mespiliformis* and is one contained containing 0g concentration, treatment 2, 3 and 4 constitute 5g, 10g and 15g respectively. Plastic buckets was used for the experimental set up. The experiment lasted for twelve weeks in every week a liter of water sample from each sample was taken to the laboratory for analysis. In the laboratory Sensitivity test and Turbidity analysis were conducted and results were recorded.

Process of the Extract of *Diospyros Mespiliformis* Crude Seed

Diospyros Mespiliformis seed was dry, dehusk and broken using pestle and mortar the dry crude seeds was grounded to powder form using grinding machine. Process for *Diospyros Mespiliformis* mucilage and seed extracts was conducted at Damaturu, Yobe State.

Sensitivity Test

Serial dilution technique was used three drops of the samples were pipetted into each set of petri-dish containing 20 ml of solidified nutrient agar. The nutrient agar was prepared with distilled water it was heated to boil to dissolve the agar completely. Autoclave at 121°C for 15 minutes. The dispense agar was inverted and incubated at 37°C for 24 hours. The plates containing treatments were removed from the incubator after 24 hours and ruler was used to measure the zone of inhibition in milli-meter.

Statistical Analysis

All the experiments were conducted in triplicate to ensure reproducibility of results. The final results were the average of the three. Data obtained were analyzed using Analysis of Variance (ANOVA).

Results

Antimicrobial Activity of Surface Water Treated with *Diospyros Mespiliformis* Crude Seed, Mucilage and Seed Extracts.

Table 1 is the laboratory analysis which shows the mean for zone of inhibition against the three test organisms of *Diospyros Mespiliformis* seed extracts recorded with the

highest mean inhibition zone of 0 mm², 18 mm², 21 mm² and 24 mm² while *Bacillus subtilis* had the lowest mean inhibition zone of 0 mm², 11 mm², 12 mm² and 15 mm² for crude seed. The mean inhibition zone of *Diospyros Mespiliformis* seed extracts indicated that *Staphylococcus aureus* had the highest mean for zone of inhibition as: 0 mm², 18 mm², 22 mm² and 21 mm² while *E.coli* had the lowest mean for inhibition zone of 0 mm², 10 mm², 15 mm² and 15 mm² respectively.

Bacteria	Crude seed (T1)				Mucilage (T2)				Seed extracts (T3)				LSD
	T1x	T1a	T1b	T1c	T1x	T1a	T1b	T1c	T1x	T1a	T1b	T1c	
	(mgL ⁻¹)	(mg L ⁻¹)	(mg L ⁻¹)	(mg L ⁻¹)	(mg L ⁻¹)	(mg L ⁻¹)	(mg L ⁻¹)	(mg L ⁻¹)	(mg L ⁻¹)	(mg L ⁻¹)	(mg L ⁻¹)	(mg L ⁻¹)	
<i>E. Coli</i>	0	12 ^{cd}	9 ^a	1 ^a	0	11 ^d	1 ^a	2 ^a	0	0 ^d	15 ^b	5 ^b	3.11***
<i>Bacillus</i>	0	11 ^c	2 ^c	15 ^b	0	2 ^c	9 ^a	0 ^a	0	12 ^c	0 ^a	0 ^a	2.15***
<i>taph</i>	0	18 ^d	21 ^{bc}	24 ^a	0	19 ^{cd}	19 ^{cd}	20 ^{bcd}	0	8 ^d	22 ^b	21 ^{bc}	3.20***

Means in same row with different superscripts are significantly different at ($p < 0.05$). Key: T1x = Control, T1a = 5 mg L⁻¹, T1b = 10 mg L⁻¹ and T1c = 15 mg L⁻¹

Table 1: Culture Inhibition Zones (mm²) exhibited by Three Test Organisms Treated with *Diospyros Mespiliformis* Extract.

Effect of *Diospyros Mespiliformis* Dosage on Percentage Turbidity Reduction

Table 2 gives information on the weekly results of *Diospyros Mespiliformis* dosage on percentage turbidity reduction of crude seed measured as lowest at 15m/l in the first week with it highest at 68.42%. For the second weeks 17.91% recorded lowest at 10m/l of crude seed 50.75%

was the highest of the seed extract at 15m/l. at the third weeks of the experiments 11.11% recorded lowest at 15m/l of *Diospyros Mespiliformis* mucilage while 47.62% was its highest at 15m/l of seed extract. In the fourth weeks 47.62% recorded as lowest at 5m/l of crude seed whereas 85.71% was the highest at 5m/l of *Diospyros Mespiliformis* mucilage. Moreover 50% recorded lowest at 15m/l of crude seed and 81.58% was its highest at 10m/l of seed extract.

Dosage in mg L ⁻¹	% turbidity reduction of crude seed	% turbidity reduction of mucilage	% turbidity reduction of Seed extract
WK1 0	0	0	0
5	12.5	18.75	20
10	16.25	8.75	7.5
15	6.25	15	31.25
WK2 5	26.87	37	38.81
10	17.91	25.37	44.78
15	20.9	20.9	50.75
WK3 5	28.57	33.33	34.92
10	17.46	15.87	41.27
15	12.7	11.11	47.62
WK4 5	47.62	85.71	80.95
10	52.38	83.33	71.43
15	57.14	80.95	54.76
WK5 5	55.26	78.94	78.95
10	68.42	71.05	81.58
15	50	76.32	57.89

Key: WK1= Week 1, WK2 = Week 2, WK 3= Week 3, WK4 =Week 4 and WK5 = Week 5

Table 2: Effect of *Diospyros Mespiliformis* Dosage on Percentage (%) Turbidity Reduction.

Discussion

Antimicrobial Activity of Surface Water Treated with *Diospyros Mespiliformis* Crude Seed, Mucilage and Seed Extracts

The crude seed, mucilage and seed extracts of *D. mespiliformis* were tested against *E. coli*, *S. aureus* and *B. subtilis*. In a similar work reported by Shekhar C, et al. [24] in which crude ethanol extract of *M. oleifera* was tested against *E. coli*, *S. typhi*, *V. cholera*, *Shigella dysenteriae* and *Pseudomonas aeruginosa*, showed its activity against *E. coli* only. This study showed that all the treatments had antibacterial activity against *E. coli*, *S. aureus* and *B. subtilis*. With *B. subtilis* recorded the mean 20 mm at 15mg/l dosage of *D. Mespiliformis* Mucilage and 20 mm at 15mg/l dosage of the Seed Extracts. Similarly, biogenic silver nanoparticles demonstrated excellent antibacterial activity against a broad range of bacteria, with the highest antibacterial activity observed against *E. faecalis* (17.77 mm) and *B. subtilis* (20 mm), also demonstrating good hemocompatibility against humans and rat red blood cells [25].

In humans, *Salmonella* are the cause of two diseases called salmonellosis: enteric fever (typhoid), resulting from bacterial invasion of the bloodstream, and acute gastroenteritis, resulting from a food borne infection/intoxication. It was shown that *D. mespiliformis* crude seed, mucilage and the seed extracts has antimicrobial properties against *E. coli*, *Bacillus subtilis* and *Staphylococcus aureus* bacteria. It was observed that *S. aureus* was the most sensitive to the seed extracts as compared to *B. subtilis* and *E. coli*. This is an indication that the seed extracts can be effectively used to treat water contaminated with *S. aureus*. Meningitis is potentially epidemic and life-threatening and transmission of *S. aureus* in humans can occur through contaminated water body. To prevent it from spreading, water if possible food as well must therefore be kept clean and the domestic water can be treated using *D. mespiliformis* crude seed, mucilage and the seed extracts. In this study, inhibition activity against *S. aureus* for *D. mespiliformis* extract was observed at the highest concentrations of 15 mg/l. This indicated that *S. aureus* was the most tolerant bacteria species to *Diospyros* extract as compared to *E. coli* and *B. subtilis*.

Turbidity

The declared World Health Organization guideline for turbidity for safe drinking water is 5 NTU (log100.700NTU) [26]. This is in consonance with the result obtained in this study with almost same value of 6 NTU of surface water treated with 5g of *D. mespiliformis* mucilage. Since groundwater has aquifers, the streams water is filtered by normal process, while the surface water encounter

immediate effect as there are aquifers to channels the contaminants. The most significant result of using *D. mespiliformis* as coagulant agent is its reduction ability from the initial reading at 5 mg/L 12.5% turbidity reduction of crude seed and 85.71% turbidity reduction of seed mucilage same concentration but different treatment. The important of dosage on turbidity has been emphasized by Zand AD, et al. [27]. Over dosage should be avoided because there is a probability of destabilization of the destabilized particles to occur due to the saturation of the polymer bridge [28]. The settling of the particles is disturbed making it impossible to reach the desired turbidity needed. This is the reason why both coagulants used showed similar trend of increasing turbidity after achieving their optimum dosage. The effects of *Diospyros* extracts on percentage turbidity reduction is as shown in table 2 at *D. mespiliformis* extracts dosage of 0, 5, 10 and 15mg/L in the first week, the percentage reduction in turbidity of the sample water were 0, 12.5, 16.25 and 6.25% respectively. This shows that 5 mg/L dosage is sufficient enough to control turbid water, it is in line with the finding of Muyibi SA, et al. [29] who shows Moringa oleifera extract is less efficient in low turbid water. However, the maximum allowable turbidity value for safe drinking water as declared by WHO and SON guideline is 5 NTU [30,31].

Conclusion

This study provided valuable insights that *D. mespiliformis* extracts is very effective in its ability for antibacterial functions and reduction of turbid water. At maximum dose 5 mg/l of *D. mespiliformis* mucilage mostly observed to be more effective. *D. mespiliformis* mucilage and seed extract are more effective to *S. aureus*, *E. coli* and *B. subtilis* when compared with *D. mespiliformis* crude seed. This plant species have made the requirements of drinking water quality in terms of maximum permissible limit of turbidity (≤ 5 NTU) and in its capacity to inhibit the activities of *S. aureus*, *E. coli* and *B. subtilis* if there are used for rural area household water treatment.

Mohammed AM, et al. [32] reported the presence of flavonoids in *D. mespiliformis*, this may be the main reason for the antibacterial activity of the extract. In similar study Castillo-Lopez RI, et al. [33] reported that Flavonoids compounds such as quercetin and kaempferol in *M. oleifera* to be the main reason for the antioxidant activity of the plant. Flavonoids compound are known to have antioxidant, antibacterial, antifungal; and antiviral activity.

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