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# Antibacterial Activities of Moringa Oleifera Plant Bark, Leaf, and Seed Extracts on Abattoir Waste Water

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**Abstract:** Microbes including *Escherichia coli, Staphylococcus aureus, Klebsiella* spp and *Salmonella* spp were isolated from the abattoir water and were identified by carrying out Gram's reaction and some biochemical tests. The seed powder was able to clear the abattoir waste water more compared with the leaf and bark indicating that the seed has high coagulation activity and as such can be used to replace chemical coagulants such as Alum (Potassium aluminium sulphate). The bacterial count of the abattoir waste water treated with the bark showed more decrease in bacterial count when compared with that of the leaf and seed. The antibacterial effect of extracts of the seed, bark and leaf of *Moringa oleifera* was determined *in vitro* by the Kirby Bauer method and were used on microorganisms such as *Escherichia coli, Klebsiella* spp and *Salmonella* spp. The bark was found to be more effective with a zone of inhibition of 20mm. Phytochemical analysis of the extracts showed that the leaf and seed extracts contained reducing sugars and tannins while the leaf, seed and bark extracts contained anthraquinones.
**Keywords:** Antibacterial; Abattoir waste water; Moringa oleifera, Extracts.

## 1. Introduction

Abattoirs are places where animals are slaughtered and possibly sold. These places are usually dirty as a result of the processes involved. Of utmost environment concern is the water or effluent as they contain microbes that could accumulate and cause infection and even cause environmental pollution. This abattoir water or effluents result from the cleaning of the carcasses of the slaughtered and washing of the slaughterhouse floor. It contains a high concentration of whole blood from the slaughtered animals and suspended particles of semi-digested and undigested food within the stomach and intestine of slaughtered and dressed food animals [1]. These effluents often times are not treated before they are discharged into water bodies [2].

Previous studies have shown that zoonotic diseases (i.e. diseases that can be transferred from animals to humans and vice versa) are yet to be fully controlled or totally eliminated in most of the abattoirs in Nigeria [3, 4]. Often times, the effluents are drained into rivers that may serve as one of the sources or the only source of drinking water to the community around it. It can also lead to deoxygenation of rivers [5] and as such kill the aquatic animals. The BOD (biochemical oxygen demand) of such waters can be high as high as 8000mg/L as they contain blood, feather, bone, manure, hair and fat [6]. Waters with a high concentration of total dissolved solids can add a laxative effect or cause the water to have an unpleasant mineral taste. The salts dehydrate the skin of especially aquatic animals [7].

Thus these waters are of serious public health concern as they can cause diarrhoea, cholera and typhoid fever especially in developing countries [8]. Accumulation of toxins in the biological system can increase as a result of excessive availability of nutrients caused by the disposal of animal feaces inappropriately [9].

Another concern is the growing emergence of antibiotic resistant organisms. One of the well-known is Methicillin-Resistant *Staphylococcus aureus.* Most microbes are no longer sensitive to antibiotics especially the broad spectrum antibiotics. There is therefore a gradual shift from conventional medicines. Also, the increased cost and reduced efficiency of conventional medicines has prompted and encouraged the search for plant-based antimicrobials [10].

*Moringa oleifera* is a tropical multipurpose tree that naturally grows in India, South-Saharan Africa and South-America [11]. It is popularly called the "miracle tree". Every part of the plant (leaves, flowers, seeds, roots and bark) can be used as food or for medicinal and therapeutic purposes [12]. Its seeds also contain between 30-35 % (w/w) of vegetable oil [13]. They are also used in the preparation of cosmetics, mechanical lubricant, and lately for potential biofuel production. *M.oleifera* seeds are also used as a primary coagulant in drinking water clarification and wastewater treatment due to the presence of a water-soluble cationic coagulant protein that is able to reduce turbidity of the water treated [14]. The leaves of this plant have also been used for water treatment and have been found to be non-toxic and a biodegradable [15]. The seeds can act as absorbent for heavy metal removal [15-17]. The leaves

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have also been found to have antitumoural, antioxidant, antihepatotoxic, hypotensive, hypoglycemic, hycholesterolemic and anti-inflammatory/diuretic actions [18].

This research was carried out to determine the compare the antibacterial activity of the bark, leaf and seed of *Moringa oleifera* and anticoagulation effect on abattoir waste water.

At the end of this study, knowledge on the choice part of the plant with the best antibacterial effect will be known. The part that will be better useful for water purification and clearing will also be known.

# 2. Materials and Methods

## 2.1. Collection and Identification of the Leaf, Seed and Bark of *Moringa oleifera*

The seeds and bark were obtained from the farm house of Babcock University, Ilisan-Remo, Ogun State. The leaves were collected from a backyard in Ijebu-Ode, Ogun State. All three parts of the plant was verified by botanical standards in the Department of Biology, Babcock University.

## 2.2. Preparation of the Seeds, Bark and Leaves

The seeds were unshelled to remove the shell. The leaf, unshelled seeds and bark were then air-dried to a constant weight to remove its moisture for four days. After drying, they were blended using a sterile blender to fine, smooth powder. The bark was broken into small fine pieces by pounding.

## 2.3. Leaf, Seed and Bark Extraction

200ml of 70% methanol (70ml of methanol in 30ml of distilled water) was put in three separate sterile conical flasks and 50g of each of the three blended parts of the plant were separately weighed and put in the three conical flasks individually and allowed to stand at room temperature for 48hours. At the end of the 48hours, the extract was obtained by filtering the mixtures separately using Whatsman's filter papers. The filtrates were heated at $60^0$C till the aqueous extract was obtained which was stored and used for the phytochemical analysis.

## 2.4. Phytochemical Analysis

This was done according to the method of Kasolo, *et al.* [19] and was as follows:

i. Test for reducing sugar: 1mg of the leaf and seed extract was dissolved separately in 2ml of water and then 1ml of Fehling solution and then the mixture was heated. A brick red precipitate indicated the presence of reducing sugars.

ii. Tannins: 1mg of the leaf and seed extract was dissolved separately in 1.5ml of water, 3 drops of dilute ferric chloride was added to the mixture. A black-blue colour indicated the presence of tannins.

iii. Anthraquinones: 2ml of 25% Ammonia solution was added to 1mg of each of the extracts and shaken. A red solution indicted the presence of anthraquinones.

## 2.5. Abattoir Waste Water Sample Collection

The sample was collected from a pig abattoir in Iperu, Ogun State. A sterile bottle was used to collect the water running off while the slaughtered pig was being washed. The water sample was immediately transported to the laboratory for physicochemical and microbial analysis.

## 2.6. Measurement of Physicochemical Parameters

The pH of the wastewater was measured using a pH meter and found to be 5.56 and the temperature was within 30-$34^0$C.

## 2.7. Wastewater Treatment Using the Blended Leaves and Seeds

1g each of the blended leaf and seeds was added to 200ml of abattoir water in two separate conical flasks and incubated at $37^0$C for 24hours. A control that did not contain either of the plants was also incubated. After five days, 1ml of each of them was in used for serial dilution and then cultured on Nutrient Agar (NA-general purpose bacteria medium), Salmonella-Shigella agar (SSA) Eosin Methylene Blue (EMB) and MacConkey agar (MCA).

## 2.8. Culture Media Preparation

The culture media used in this study included Nutrient Agar (NA-general purpose bacteria medium), Salmonella-Shigella agar (SSA) Eosin Methylene Blue (EMB) and MacConkey agar (MCA). All the agar media were prepared according to manufacturers' instructions and sterilized using an autoclave at $121^0$C for 15 minutes at 15psi (pounds per square inch), allowed to cool and about 9ml of each medium was poured into sterile petri dishes and allowed to solidify.

## 2.9. Bacteria Cultivation

Serial dilution of the wastewater was carried out by taking 1ml of the sample and transferring to 9ml of distilled water (diluent) in a test tube ($10^{-1}$). 1ml was taken from the $10^{-1}$ tube and subsequently dispensed into another test tube containing 9ml of diluent ($10^{-2}$). This was done until the sample had been diluted in 10 tubes obtaining $10^{-1}$, $10^{-}$

$^{2}$, $10^{-3}$, …, $10^{-10}$ dilutions. The essence of diluting was to obtain decreased microbial population that can be easily counted and will be within the range of 30-300 colonies.

Spread plate method was used in the cultivation and this was done by taking 0.5ml of sample from $10^{-9}$ and $10^{-10}$ tubes respectively and dropping on NA respectively in duplicates and then spread using a plastic spreader to obtain uniform distribution of the sample on the medium. The inoculated plates were incubated at $37^{0}$C for 24hours and after which observed for colonial characteristics and colony counting was done. Discreet colonies were thereafter subcultured to obtain pure isolates of bacteria on the four different agars.

### 2.10. Identification of Isolates

Gram stain, microscopic examination and biochemical tests (citrate utilization, sugar fermentation, catalase, oxidase) were carried out on the test isolates.

### 2.11. Antibiotic Sensitivity Testing of Leaf, Seed and Bark Extracts

Using a sterile swab, test organisms (*Klebsiella* spp, *Escherichia coli, Salmonella* spp) were picked and swabbed onto NA medium and then a 9mm cork borer was used to bore four holes in the medium and then aliquots of the three extracts were placed on each of the three holes separately and on the fourth hole, methanol was placed. A control using Gram negative antibiotic sensitivity disc was also done for each of the three organisms and incubated at a $37^{0}$C for 24hours. The zone of clearance was measured to show antibacterial activity of the extracts.

## 3. Results

This growth of the organisms for the primary culture was observed after 24 hours and then subcultured onto three selective and differential media to obtain pure cultures.

**Table-1.** Bacterial count

| Dilution | Abattoir water + leaf | Abattoir water + seed | Abattoir water + bark | Control |
|---|---|---|---|---|
| $10^{-9}$ | $8.0x10^{10}$ | $9.1x10^{10}$ | $6.0x\ 10^{10}$ | $1.28x10^{11}$ |
| $10^{-10}$ | $6.3x10^{11}$ | $7.45x10^{11}$ | $3.8x10^{11}$ | $6.2x10^{11}$ |

**Table-2.** Phytochemical Analysis

| **Phytochemicals** | **Leaf** | **Seed** | **Bark** |
|---|---|---|---|
| Reducing sugars | + | + | - |
| Tannins | + | + | + |
| Anthraquinones | - | + | - |

**Key:** + = Present, - = Absent

The zone of clearance around the bark was measured and found to be 20mm against *Escherichia coli*, while the other extracts showed no antibiotic activity against the test isolates.

## 4. Discussion

Antibacterial effect of the bark extract proved to be more effective against *Escherichia coli, Klebsiella* sp and *Salmonella* spp compared with the seed and leaf extracts. The seed and leaf extracts were also effective against the test organisms in agreement with the work of Akinyeye, *et al.* [10]. Abattoir waste water treated with ground seed, leaf and bark samples showed reduced bacterial count compared with control that was untreated as shown in Figure 1.

The seeds of *Moringa oleifera* have better coagulation activity when compared with the leaf and bark as evident in the fact that the ground seeds were able to cause clumping of the solids in the wastewater and as such reduce the total dissolved solids (TDS) after five days. Thus, its seeds can be used to replace other chemicals used in water treatment.

Phytochemical analysis of the seed, leaf and bark showed the presence of anthraquinones, tannins and reducing sugars. This agrees with the findings of Akinyeye, *et al.* [10] and Kasolo, *et al.* [19].

## 5. Conclusion

The bark of *Moringa oleifera* in addition to its seeds and leaves have antibacterial effect and as such can be packaged, preserved and sold as drugs with the appropriate means of administration prescribed. The presence of phytochemicals in these plant parts indicates that they are nutritious and as such can provide the needed nutritional needs of humans. The use of its ground seeds as a coagulant should be encouraged, rather than the use chemical such as Alum (Potassium aluminum sulphate) which is dangerous to human health.

## 6. Recommendation

Studies on the best means of preserving *Moringa oleifera* extracts should be carried out. Every household should be encouraged to plant at least one tree of this plant so as to provide quick and safe supply of its healthy and nutritious parts.

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