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Moringa oleifera as natural coagulant in water treatment and production of antifungal soap

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Abstract. The use of *Moringa oleifera* seed in water purification has reduced the use of chemical-based coagulants which is detrimental to both human and livestock. This project aimed at testing the microbial properties of *M. oleifera* seed oil extract on some selected pathogens (Bacterial and fungi). The oil was extracted using Soxhlet apparatus with ethanol as solvents. Gas-chromatography-mass spectrometry (GCMS) analyses were carried out for the identification of active components in the oil extract. The zone of inhibition test carried out showed that this particular plant seed oil extract has antifungal property with *Candida albicans* and *Rizopus stolonifera* with highest zone of inhibition. The raffinate was used for water purification and the oil for the production of an antifungal soap.

Keywords: *Moringa oleifera*, coagulant, antifungal, GCMS, microorganism

1. Introduction

Portable water treatment is a source of some problem to man's health. For water to be fit for drinking and consumption standards, treatment is mandatory [1]. Coagulants can be classified into synthetic organic polymer, inorganic coagulant (PAC-polyaluminium chloride), and naturally occurring coagulant. Most of the coagulants used such as PAC and inorganic salt which is popularly known as 'alum' (aluminum sulfate) are not biodegradable products with coagulant effect depending on pH and the residual concentration which is a concern to the public health officers [2-6]. A major component of PAC and alum has been reported to induce Alzheimer's disease, various intestinal challenges and also has strong carcinogenic properties [7-12]. Interest on the use of natural or synthetic coagulant has been on the increase recently since they are safe and biodegradable for human consumption [13, 14]. The efficacy of *M. oleifera* seed as a natural coagulant in the treatment of residual and surface water has been studied by several researchers [15-17]. Seeds of *M. oleifera* according to [18] is a good alternative as a coagulant replacing aluminum salts used in the treatment of water. In counties like Sudan, *M. oleifera* seeds have been used instead of alum to treat high turbid Nile water by the rural dwellers [19-22].

The *Moringaceae* is a family of oilseed trees categorized as single genus trees with about 14 reputable species [23]. *Moringa* spp. is cultivated for its numerous functional values as it provides various practical application in developing countries due to its economic, nutritional and medicinal resource over the past few years [24]. Every part of the plant has been found useful; extracts from the roots have been reported to possess antimicrobial functions [25]. *M. oleifera* seed comprise of 19-47 % of oil. It is commercially referred to as Ben oil, its rich in oleic, behenic, steric and palmitic acids [26].

It has been noted to be composed of 70 % oleic acid, and it has a nice fragrance [27]. The presence of this high content of oleic acid makes it suitable for frying [28]. The oil is applied in hair and body care

products as a skin conditioner and air moisturizer; it has an incredible cosmetic value. It has been used in the past for skin ointments and preparations by Egyptians [29]. *Moringa* and other plants extract have been reported to possess antibacterial and antifungal properties [30-34]. Various techniques have been used to extract oil from seed kernels of various sources; these include aqueous enzymatic and Soxhlet extraction [35].

2. Materials and methods

2.1.Source of water: Waste water was obtained from the cafeteria in Covenant University Ota, Ogun State, Nigeria.

2.2.Extraction: Method of [36, 37] was used and the raffinate collected for water purification.

2.3.Microorganisms: The clinical isolates of *Bacillus subtilis, Staphylococcus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Candida albican* and *Rhizopus* were obtained at the Department of Biological Sciences Covenant University, Ota, Ogun using method of [31, 38, 39].

2.4. Determination of Antimicrobial analysis: This was done by the method of [29, 30-31][38, 40].

2.5. Water Purification Procedure

1000 ml of both unclean and clean water was measured for microbial analysis. 10 g of the seed Raffinate was poured into the unclean water and allowed to stand for 1 hour. 200 ml of water sample was removed from the 1000 ml of unclean water.

2.6. Most Probable Number (MPN)
The method of [41, 42] were used.

2.7. Soap Production Procedure

***Reagents required*:**
Caustic Soda: It is an inorganic compound having the chemical formula NaOH. It is a strong base with numerous applications in different industries. It was used to saponify the oil to soap.
Sodium Sulphate: It is strong base with chemical formula Na_2SO_4. It is usually used for the manufacture of detergents and paper pulping.
Soda Ash: It is also known as washing soda and usually occurs as a crystalline decahydrate. It is used in a number of household products especially cleaning agents. It prevents hard water from bonding with the soap and is effective in removing grease stains.
Sodium Silicate: It is a salt which is usually obtain from Salic acid and is stable in alkaline and neutral solutions. It is used in soap making as it increases the soaps emulsifying power
Perfume: It is an aromatic compound which is volatile in nature. It is usually added to give a pleasant odour to the soap.
Saponification: The process involves reacting the triglyceride with a strong alkali to produce glycerol and fatty acid salts. Alkali refers to a soluble base such as; potassium or sodium hydroxide. NaOH is usually used for solid or bar soaps while KOH is used for liquid soaps.

Method
41g of caustic soda, soda ash and 20g of sodium sulphate was dissolved in 120ml of distilled water in a 1000ml beaker and label solution A. The mixture was left for 48hrs before use to lose its heat of dissolution. 100ml of palm kernel oil, 20ml *M. oleifera* oil was measured and 0.156g of colorants was

added, the mixture was pour into solution A. 2.34g of sodium silicate was then added and stirred vigorously, after 30 seconds, 0.156g perfume was also added to the oil mixture. The mixture was stirred until trace was reached. It was then poured into a mould and left to solidify for 48hrs.

3. Results and discussion

3.1. Antimicrobial Test

Table 1: Antimicrobial Activity

ZONE OF INHIBITION (mm)		
ORGANISMS	EXTRACT	CONTROL
Bacillus subtilis	5	15
Staphylococcus	5	12
Escherichia coli	4	5
Klebsiella pneumoniae	4	10
Pseudomonas aeruginosa	5	11
Candida albicans	41	_
Rhizopus stolonifera	15	_

3.2. Antimicrobial Analysis

Table 1 show that the ethanol extract of *M. oleifera* seed has antimicrobial property. Hence it can be used to inhibit the growth of microorganism. The antibacterial activities of the extract were low compared to the zone of inhibition of the fungal isolates which were on the high side, for *Candida albicans* and *Rizopus stolonifera.* Extract has more antifungal activity than antibacterial activity. The soap was made as a result of the antimicrobial properties of *M. oleifera* seed oil extract. The soap can be used for bathing and disinfecting hands against fungal infections such as athlete's foot (redness, cause peeling, burning, itching and blisters), ring worm, jock itch and yeast infection.

3.3. M. oleifera Seed Oil Soap

Plate 1: *M. oleifera* seed oil soap

3.4. Water Purification

Plate 2: Sample A- Before water purification
Sample B - After water purification

Table 2: Culture Appearance

Sample A	Sample B
Nutrient Agar: culture yielded mixed growth of bacteria specie Plate count Agar: culture, yielded heavy growth of bacteria species (1900 colony forming unit/ per ml) EMB: yielded mixed heavy growth of coliform species. There was no growth of *E. Coli*	Nutrient Agar: culture yielded little growth of bacteria specie Plate Count Agar: culture yielded moderate growth of bacteria species (150 colony forming unit/ per ml) EMB: yielded moderate mixed growth of *klebsiella* and *Salmonella* specie. There was no *E. coli*

The representative samples were picked from the tubes and inoculum were standardized by 0.5 Macfarland Standard and 0.5 ml of the standardized inoculum were seeded into sterile petri dishes and each of the EMB, PCA, NA plates were poured using pour plate technique and were incubated for 24 hours at 37^{o} C. The cultural appearances on the inoculated plates were read and the result was recorded as shown in the table 2. The number of microorganisms present in the untreated water was reduced in the treated water but it was still not safe. This might be as a result of the quantity of *M. oleifera* raffinate for the quantity of water.

Table 3. Microbial analysis of the Water.

Sample	10ml	1ml	0.1ml	MPN (per 100ml)
Untreated water	111 (3)	100 (1)	000 (0)	25
Treated water	101 (2)	110 (2)	100 (1)	10

Key: (1) represents gas production, (0) represents no colour change.

Microbial analysis was done to ascertain the safety of water for usage by checking for the microorganisms in the water and what quantity was eliminated after contacting the water with *M. oleifera* seed. The method used was the Most Probable Number method [MPN]. The result was negative as there was no colour change but there was gas production. This means that the water is partially unsafe for drinking and cooking, but can be used for washing clothes or solid surfaces.

With the aid of two sterile containers, samples were taken from two sources. The first was from an untreated sewage and the other was the treated sample from the untreated source. They were taken to the laboratory for standard microbial analysis. Most probable number analysis was done using 3 tubes method in which MacConkey broth was used as the substrate and the samples were labelled A for the untreated water and B for the treated water. Sterile MacConkey broth were prepared according to the manufacturer's guide and inoculated with the test sample. The first three tubes were inoculated with 0.1ml of the sample, the second three were inoculated with 1ml each and the last three were inoculated with 10ml each of the sample, each has a Durham tubes inverted in the tubes to indicate the production of gas. They were all inoculated at 37^{o} C for 24hours and the tubes were examined after 24hours of incubation for the presence of gas and turbidity of the broth as shown in table 3.

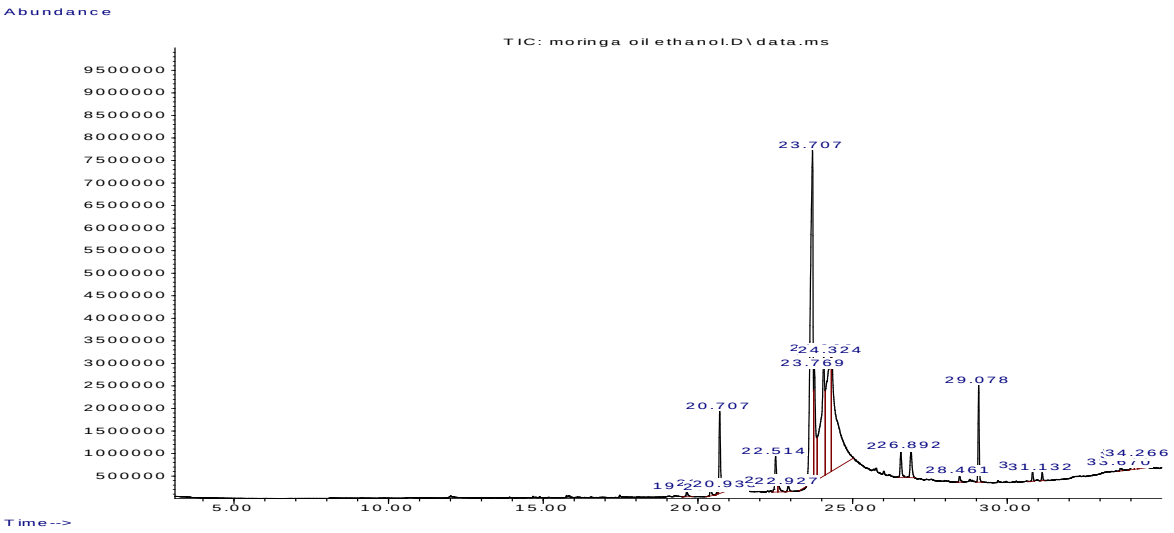

Figure 1: GCMS composition of ethanol extracted oil from *M. oleifera seed*.

Table 4: Composition of *M. oleifera* seed oil extract using GCMS

Retention time	Area %	Composition
20.705	3.98	Hexadecanoic acid, ethyl ester
23.709	26.81	Ethyl, (E)-9-Octadecenoic acid ethyl ester, Ethyl Oleate
23.766	6.56	(E)-9-Octadecenoic acid ethyl ester, trans-9-ctadecenoic acid, pentyl ester , Ethyl Oleate
24.069	14.51	cis-Vaccenic acid, Octadecanoic acid, ethyl ester, trans-13-Octadecenoic acid
24.304	15.54	Oleic Acid, 9-Octadecenoic acid, Oleic Acid
24.327	19.39	Oleic Acid
29.076	3.23	Nonadecanoic acid, ethyl ester

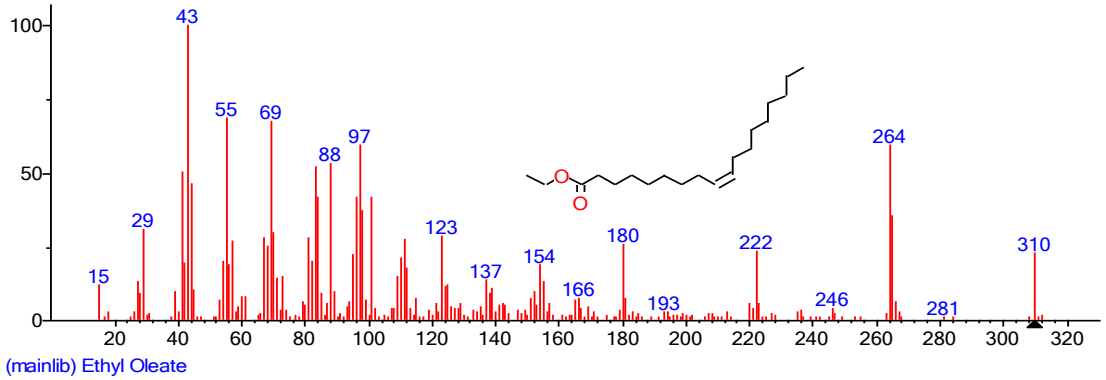

Figure 2: Mass Spectrum of fragments of most abundant chemical constituent of *M. oleifera* seed oil Figure

About 30 components were identified from the ethanol extract of *M. oleifera* seed, but only 7 components had appreciable quantity in the extract with Ethyl Oleate as the most abundant components with 26.81%.

4. Conclusion: The antimicrobial test carried out with the zone of inhibition confirmed that oil extract form *M.oleifara* seed can be used as a good substitute to synthetic antifungal soap and cream. *Candida albicans* and *Rizopus stolonifera* had highest zone of inhibition. The GCMS analysis showed that *M. oleifera* oil can be used for many health benefits and also in pharmaceuticals. Culture appearance and microbial analysis of both water samples [treated and untreated] shows that *M. oleifera* raffinate can be used for water purification.

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Conflicts of Interest: The authors affirm that there is no conflict of interest with respect to the publication of this paper.

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