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Antimicrobial Effects of The Leaf and Bark Extracts of Spondias Mombin Linn

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Abstract

Spondias mombin is a plant that is widely used in traditional medicine. Majority of its medicinal potential is yet to be uncovered. The aim of this study was to investigate the antimicrobial effects of the leaf and bark extracts of Spondias mombin on a human pathogenic bacterium and a fungus. The leaves and bark of Spondias mombin were obtained, washed, and extracted with sterile distilled boiled water for 24 hours. The antimicrobial sensitivity of the extracts was determined against a bacterium (Staphylococcus aureus) and a fungus (Candida albicans) using the "Poison Food Plate Technique". The results obtained revealed that both extracts reduced the colony diameter of Staphylococcus aureus and the mycelial growth of Candida albicans. The Spondias mombin bark extract showed the highest activity against Staphylococcus aureus and Candida albicans at 30 g/ml with diameters of 12.33±1.86 and 16.67±2.19 mm, respectively. The findings of this study indicate that the Spondias mombin leaf and bark extracts possess antibacterial and antifungal properties in which the potency is concentration dependent. The decrease in potency of these extracts is as a result of phytochemical evaporation on long exposure to the environment. Therefore, when these phytochemicals are fully harnessed based on environment conditioning, antimicrobial and antifungal effects of Spondias mombins will be further enhanced in treating some highly infectious clinical diseases.

1.0. Introduction

Spondias mombin Linn. is a flowering plant and belongs to the Anacardiaceae family. The plant is endemic to Brazil and it is also found in Nigeria and widespread through tropical regions in America, Africa, and Asia. In Brazil, it is usually found in the North and Northeast region [1]. The fruit is commonly known as "Caja in brazil. This natural fruit is mostly used in northeastern Brazil to create jelly, juice, jams, and ice cream. The leaves of *Spondias mombin* are used in folk medicine to cure a variety of systemic disorders such as inflammation of the mouth and throat, prostatitis, and herpes labialis. The ripe fruit is covered by a leathery skin and a thin coating of pulp cover the ripe fruit. The seed has an oil content of 31.5% [2]. It is called (Ogheghe) among the Esan people of Edo State, Nigeria. The tree has corky bark, white flowers, and green fruits which become yellow when ripe. The color of the flowers, leaves and ripe fruits gives an aesthetic property to the plant. The flowering and eventual fruiting takes place between March-April and July-August". The fruit is high in vitamins A and C, as well as vitamin C carotenoids and flavonoids, which are known as nutraceuticals. Fruit quality varies greatly from region to region, with some being sweeter than others [3]. Moreover, the fruits are edible and sometimes called monkey plums, but the wood is of low quality and rarely used. *Spondias mombin* extracted juice is used to prepare ice cream, cool beverages, and jelly in Costa Rica and Brazil. It is used in Panama and Mexico in fairly large quantities as jams. The tree is commonly used in carpentry for matchsticks, matchboxes, physician's

spatulas, sweetmeats sticks, pencils, pen holders, packing cases, interior sheathing of buildings and boats, and as a cork alternative. Wood is solely used for fuel in Costa Rica and Puerto Rico. In Africa, ashes from burned wood are used in indigo dyeing [4]. *Spondias mombin's* stem bark is commonly used to carve amulets, statuettes, cigarette holders, and other decorative things.

1.1. Plants as antimicrobial agents

All parts of *Spondias mombins* have been reported in Nigeria traditional medicine in treatment of gonorrhea [5]. Advancement in research development over the years identifies new phytochemicals and the role they play in medicine [6]. Recent studies have demonstrated the importance of molecular structure in predicting the effects of certain phytochemicals [7]. The antimicrobial effects of these plant parts have been confirmed by numerous phytochemical studies [8]. Studies shows that plant derivatives have been used as medicine in ancient times before contemporary medicine [9]. Presence of some phytochemical in *Spondias* species demonstrates pharmacological properties, such phytochemicals include phenolics, that have antidiabetic and antioxidant properties, flavonoids, sterols, saponins, tannins and triterpenes [10, 11]. These phytochemicals plays active role in defense mechanism of the plant against predators, flavours and medicinal properties of the plants [12]. [13] Studies show the microbial activity of *Spondia mombins* with methanolic ethanolic, and acetone leaf extracts activity against *Enterobacter aerogens*, *Staphylococcus aureus*, *Salmonella typhi* and *Klebsiella* pneumonia. *Serratia marcescens* on aqueous leaf extract demonstrate antibacterial properties. Mombintane I and Mombintane II isolated from methanolic stem bark extracts of S. mombin demonstrate antimycobacterial properties in vitro against Mycobacterium tuberculosis [14]. Fruit juice of *Spondias mombin* is drunk as a diuretic and febrifuge. The decoction of the astringent bark serves as an emetic, a remedy for diarrhea, dysentery, and hemorrhoids, and a treatment for gonorrhea and leucorrhea. *Spondias mombin* is believed to expel calcifications from the bladder in case there is a bladder infection. The powdered bark of *Spondias mombin* is applied on wounds in case there is wound infection, especially during the African traditional treatment of infection. A tea made from the flowers and leaves of *Spondias mombin* extract is taken to relieve stomach aches, biliousness, urethritis, cystitis, and eye and throat inflammations [15]. *Spondias mombin* leaves juice and the powder of dried leaves is used as poultices on wounds and inflammations [16]. The gum is employed as a cough expectorant and to expel tapeworms [17]. Many of the commercially established medications utilized in contemporary medicine were first used in rudimentary forms in traditional or folk healing methods, or for other uses that implied biological activities. The key advantages of adopting plant-derived medications are that they are generally safer than synthetic counterparts and that they provide significant therapeutic benefits at a lower cost. Antimicrobial plants have a critical role in lowering the worldwide burden of infectious diseases [18]. However, the growth and spread of multi-drug resistant (MDR) strains in pathogenic bacteria, fungi, viruses, and protozoa has become a major public health concern, as there are fewer, or possibly no, effective antimicrobial medicines available for pathogenic bacterial infections [19]. A large variety of medicinal plants have been identified as excellent sources of natural antibacterial substances as an alternative to synthetics antibiotics in the treatment of various bacterial and fungal diseases. Secondary metabolites present in plants include tannins, alkaloids, phenolic chemicals, and flavonoids, which have been shown to have antimicrobial activities in- vitro [20]. Numerous studies have documented the effectiveness of flavonoids such as swertifrancheside [21], glycyrrhizin (from diglicorice) and chrysin against HIV [22]. The chewing stick is widely used in African countries as an oral hygiene aid in place of toothpaste. Chewing sticks come from a variety of plant species, and the chemically active component within each stick may be diverse [23].

Figure 1: Ripe Fruits of *Spondia mombis*

Figure 2: Tree of *Spondia mombin*

Figure 3: Leaves and flowers of *Spondia mombin*

Figure 4: Bark of *Spondia mombin*

2.0. Materials and Method

2.1. Plant materials used

The plant materials used were the leaves and the bark of *Spondias mombin* and were collected at the Faculty of Life Sciences University of Benin, Benin City, Edo State, Nigeria.

2.2. Pathogens used

The test pathogens used for this study are *Staphylococcus aureus* (bacterium) and *Candida albicans* (fungus). They were isolated from the tooth of a patient at the Medical Microbiology Laboratory, University of Benin Teaching Hospital (UBTH), Benin City, Edo State.

2.3. Control Used

Ketoconazol (150mg) for *Candida albican* (fungus), and Chloramphenicol (250mg) for *Staphylococcus aureus* (bacterium).

2.4. Preparation of plant extracts

The fresh leaf and bark of *Spondias mombin* were washed under running water before surface sterilizing with 70% ethanol. The fresh leaves and bark of *Spondias mombin* were sliced into a piece and extracted with boiled water. The plant extract from both fresh leaves and bark was prepared by soaking the following weights 10 g, 20 g, and 30 g of each in 100 ml of boiled sterilized distilled water in the beakers and covering the beakers with cotton wool wrap in aluminum foil paper and was allowed to stand for 24 hours. Thereafter, the suspensions of the fresh leaf and bark were filtered using sterile muslin cloth aseptically.

2.5. Preparation of culture media

The media used were:

a. Nutrient agar (NA)

The medium was prepared according to the instructions of the manufacturer. This is as follows; 7g of the nutrient agar powder was suspended in 250 ml of distilled water in two conical flasks and shaken to dissolve properly. The conical flasks were sealed with cotton wool wrap in aluminum foil paper and autoclaved at 121oc for 15 minutes. The medium was allowed to cool down to body temperature before pouring into plates aseptically (about 20 ml) and covered with cover plates. Then the plates were allowed to solidify.

b. Potato dextrose agar (PDA)

The medium was prepared according to the manufacturer's instructions by suspending 9.7g of potato agar powder in 250 ml of distilled water in conical flasks and shaking it to dissolve properly. The conical flasks were sealed with cotton wool wrap in aluminum foil paper and autoclaved at 121^{o}c for 15 minutes. The medium was allowed to cool to body temperature before pouring into plates aseptically (approximately 20ml). The plates were allowed to solidify.

2.6. Standardization of the test pathogens

The pathogens were standardized using a serial dilution technique i.e., the stock sample on a slant was introduced into a prepared nutrient broth and incubated overnight (24 hours). 0.1 ml of the broth was introduced into 9.9 ml of sterile distilled water to make a dilution of 1:1000 and also from the dilution; another 0.1 ml was pipetted into 9.9 ml of sterile distilled water to make a dilution of 1:10,000. It was then standardized by adding approximately 85 ml of 1% sulfuric acid (H_2SO_4) to a 100ml volumetric flask. Using a volumetric pipette, add 0.5ml of 1.175% anhydrous barium chloride ($BaCl_2$) drop wise to the 1% sulfuric acid (H_2SO_4) while constantly swirling the flask. Bring the volume to 100ml with 1% H_2SO_4. Stir or mix for approximately 3 to 5 minutes while examining visually, until the solution appears homogeneous and free of clumps and Store it at room temperature (25 °C). The same procedure was repeated for the fungi using potato dextrose broth [24]. The same procedure was repeated for the fungi using potato dextrose broth and its turbidity compared with the McFarland standard of 0.5 which was approximately (1.0 × 106 Cfu /ml).

2.7. The determination of antifungal effect on leaf and bark extracts of *Spondias mombin*

The antifungal efficacy of the leaf extract and bark extract was determined by the "poisoned food technique". Three concentrations of the extract of leaf and stem bark (10g in 100 ml, 20g in 100ml, and 30g in 100 ml) each of the concentrations were poured into 90ml Petri plates at 1ml/plate using a sterile pipette. The prepared Potato Dextrose Agar (PDA) medium was added and shaken gently

and allow to solidify. A Cork borer measuring 5mm in diameter was used to cut an agar block from 7day old culture of *Candida albicans* grown on PDA and placed at the center of the Petri plates and incubated at room temperature (approximately 28^{o}c). Three replicates and control were prepared. The diameter of the mycelia growth (mm) of the pathogen was measured and recorded after 72 hours, thereafter daily measurements were taken for four days. The antifungal effects of the extract were calculated with the following formula.

Inhibition of mycelia growth (%) =C-T/C) x 100 (1)

Where C is the average diameter of the mycelial growth of Candida albicans and the diameter of the colony of *Staphylococcus aureus* in control plates and T is the average diameter of the mycelia growth of *Candida albicans* and the diameter of the colony of *Staphylococcus aureus* in poisoned food plates.

2.8. Data Analysis

Statistical Package for Social Science (SPSS version 29, IBM), Duncan`s multiple range tests P=0.05 was used for comparing results.

3.0. Results

Table 1: The effects of bark of *Spondias mombin* extract on bacterium *Staphylococcus aureus* at concentration of 10, 20, 30g/ml and control (Choloramphenicol) after 24h inoculation.

Treatment	Day 1	Day2	Day3	Day 4
Chloramphenicol	90.00±0.00^{b}	90.00±0.00^{b}	90.00±0.00^{b}	90.00±0.00^{b}
10g/ml	56.33±19.06^{b}	57.67±18.48^{b}	58.00±18.19^{b}	60.00±17.32^{b}
20g/ml	14.67±9.17^{a}	22.33±7.36^{a}	22.67±7.69^{a}	28.00±7.51^{a}
30g/ml	12.33±1.86^{a}	18.00±1.16^{a}	21.67±2.73^{a}	25.00±2.89^{a}

Value with different alphabets within the same column are significantly different at the P=0.05 Duncan`s multiple range tests

Table 2: The effects of leaf extract of *Spondias mombin* on bacterium *Staphylococcus aureus* at concentration of 10, 20, 30g/ml and control (Chlorophenicol) after 24h inoculation

Treatment	Day 1	Day2	Day3	Day 4
Chloramphenicol	90.00±0.00^{b}	90.00±0.00^{b}	90.00±0.00^{b}	90.00±0.00^{b}
10g/ml	55.67±0.67^{a}	59.67±0.88^{a}	63.33±3.33^{a}	64.33±3.38^{a}
20g/ml	55.00±0.58^{a}	58.67±1.33^{a}	59.00±1.00^{a}	60.67±1.45^{a}
30g/ml	54.67±2.91^{a}	55.67±3.18^{a}	56.00±3.22^{a}	57.67±3.53^{a}

Values with different alphabets within the same column are significantly different at the P =0.05 Duncan`s multiple range tests.

Table 3: The effects of bark extract of *Spondias mombin* on fungus *Candida albicans* at concentration 10, 20, 30g/ml and control (Ketoconazol) after 24h.

Treatment	Day 1	Day2	Day3	Day 4
Ketoconazol	36.00±6.66^{b}	37.33±7.54^{a}	38.33±7.22^{a}	44.33±10.71^{b}
10g/ml	25.33±3.76^{ab}	29.67±3.84^{ab}	32.00±4.16^{a}	33.67±3.84^{a}
20g/ml	26.67±2.60^{ab}	28.33±2.33^{ab}	31.33±1.33^{a}	33.00±1.53^{a}
30g/ml	17.00±2.08^{a}	21.33±0.33^{a}	25.33±1.45^{a}	30.00±2.89^{a}

Value with different alphabets within the same column are significantly different at the P=0.05 Duncan`s multiple range tests.

Table 4: The effects of leaf extract of *Spondias mombins* on fungus *Candida albicans* at concentration 10, 20, 30g/ml and control (Ketoconazol) after 24h

Treatment	Day 1	Day2	Day3	Day 4
Ketoconazol	28.33±0.88^{b}	34.33±3.38^{b}	37.00±5.57^{b}	42.33±7.97^{b}
10g/ml	22.67±2.91^{ab}	25.33±3.28^{ab}	27.00±3.51^{ab}	29.00±4.04^{ab}
20g/ml	18.67±3.18^{a}	22.33±3.38^{a}	25.33±4.67^{ab}	27.67±4.98^{ab}
30g/ml	16.67±2.19^{a}	20.67±2.33^{a}	21.00±2.08^{a}	22.33±1.86^{a}

Value with different alphabets within the same column are significantly different at the P=0.05 Duncan`s multiple range tests.

Table 5: The effects of bark extract of *Spondias mombin* on *Staphylococcus aureus and Candida albicans* at concentration 10, 20 and 30g/ml and control (Chlorophenicol) after 24h

Treatment	Organism	Day 1	Day 2	Day 3	Day 4
Control	Staph	90.00±0.00^{d}	90.00±0.00^{c}	90.00±0.00^{c}	90.00±0.00^{c}
	Candida	44.33±10.71^{bc}	36.00±6.66^{ab}	37.33±7.54^{ab}	38.33±7.22^{ab}
10g/ml	Staph	56.33±19.06^{c}	57.67±18.48^{b}	58.00±18.19^{b}	60.00±17.32^{b}
	Candida	25.33±3.76^{ab}	29.67±3.84^{a}	32.00±4.16^{a}	33.67±3.84^{a}
20g/ml	Staph	14.67±9.17^{a}	22.33±7.36^{a}	22.67±7.69^{a}	28.00±7.51^{a}
	Candida	26.67±2.60^{ab}	28.33±2.33^{a}	31.33±1.33^{a}	33.00±1.53^{a}
30g/ml	Staph	12.33±1.86^{a}	18.00±1.16^{a}	21.67±2.73^{a}	25.00±2.89^{a}
	Candida	17.00±2.08^{ab}	21.33±0.33^{a}	25.33±1.45^{a}	30.00±2.89^{a}

Value with different alphabets within the same column are significantly different at the P= 0.05 Duncan`s multiple range tests.

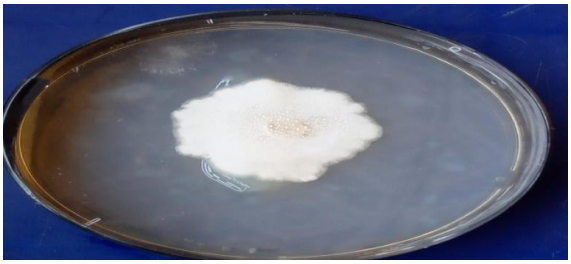

Figure 5: *Candida albicans (S.mombin bark control)*

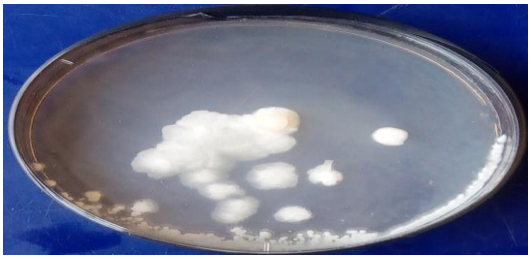

Figure 6: *Candida albicans (10g/ml bark treatment)*

Figure 7: *Candida albicans (20g/ml bark treatment)*

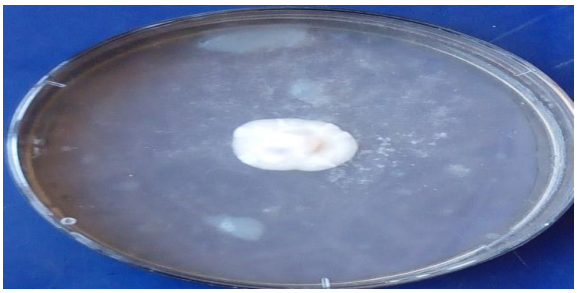

Figure 8: *Candida albicans (30g/ml bark treatment)*

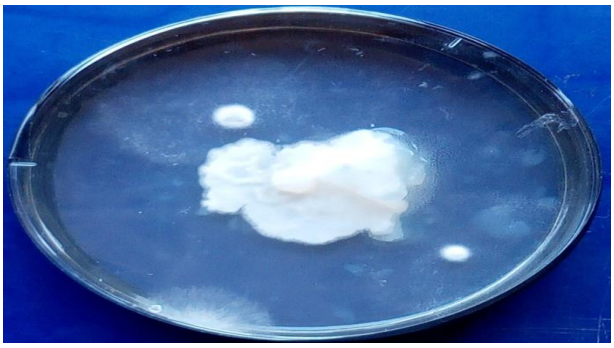

Figure 9: *Candida albicans (leaf extract control)*

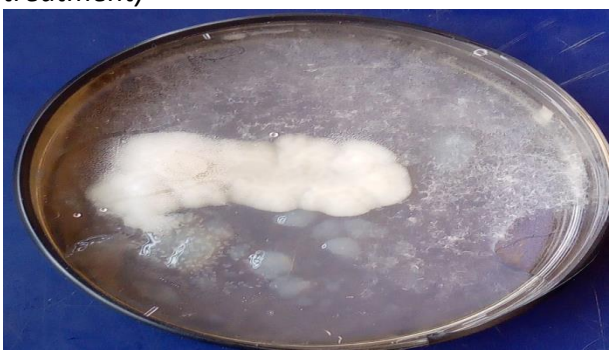

Figure 10: *Candida albicans (10g/ml leaf treatment)*

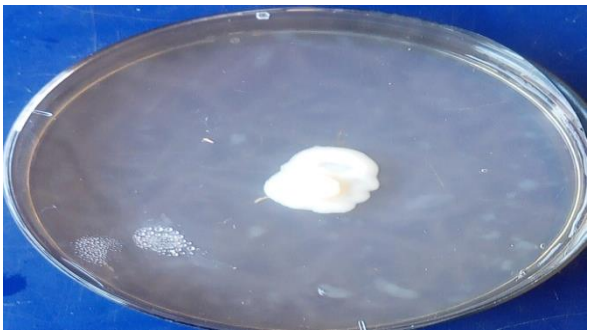

Figure 11: *Candida albicans (20g/ml leaf treatment)*

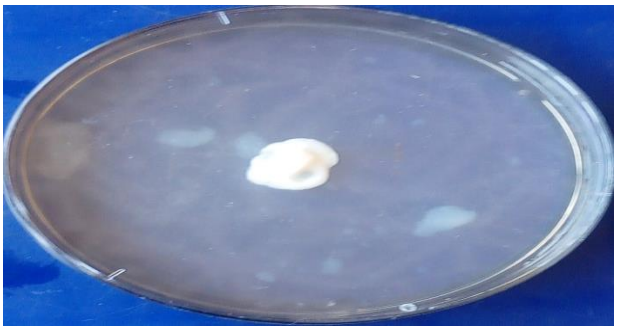

Figure 12*: Candida albicans (30g/ml leaf treatment)*

Figure 13*: Staphylococcus aureus (leaf extract control)*

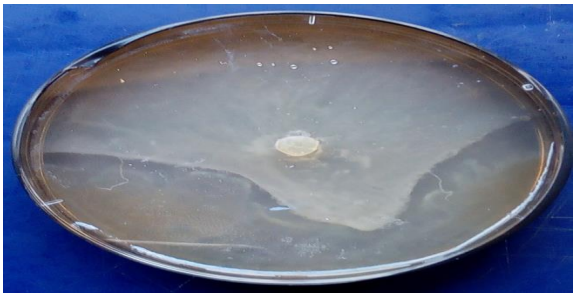

Figure 14*: S. aureus (10g/ml leaf treatment)*

Figure 15*: S. aureus (20g/ml leaf treatment)*

Figure 16*: S. aureus (30g/ml leaf treatment*

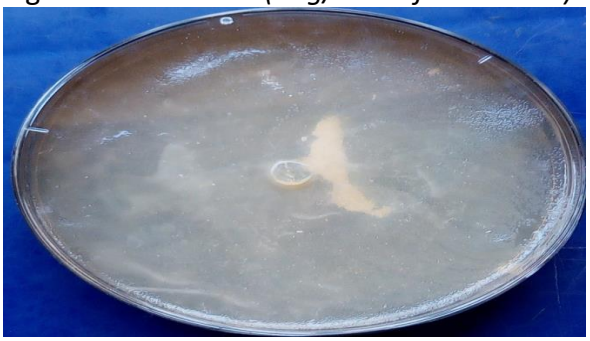

Figure 17: *Staphylococcus aureus (bark extract control)*

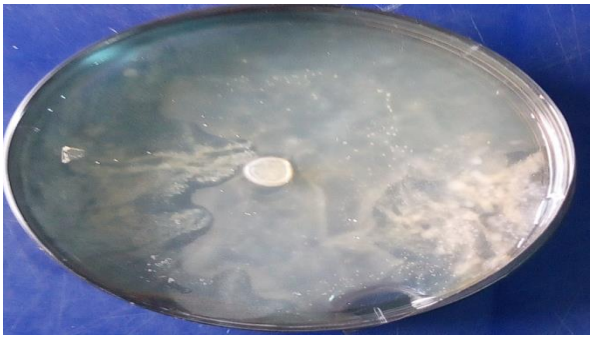

Figure 18**:** *S. aureus (10g/ml bark treatment*

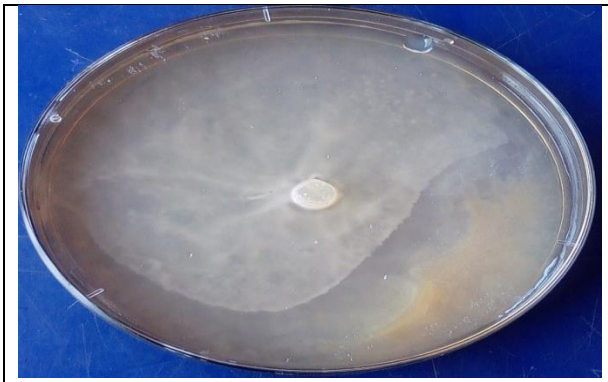

Figure 19*: S. aureus (20g/ml bark treatment)*

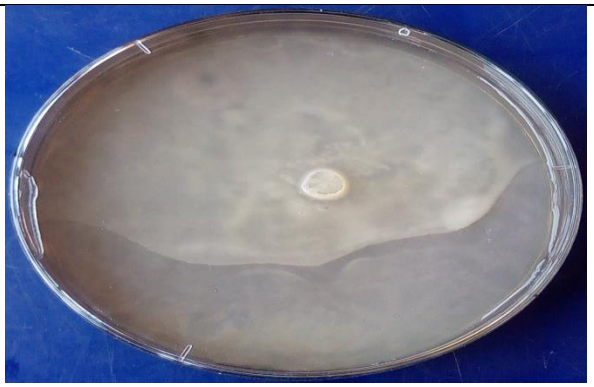

Figure 20: *S. aureus (30g/ml bark treatment)*

Table 6: The antimicrobial effect of leaf extract of *Spondias mombin* on *Staphylococcus aureus* and *Candida albicans* at concentration 10, 20,30g/ml and control (Ketoconazol) after 24h.

Treatment	Organism	Day 1	Day 2	Day 3	Day 4
	Staph	90.00±0.00^{d}	90.00±0.00^{d}	90.00±0.00^{d}	90.00±0.00^{d}
Control	Candida	28.33±0.88^{b}	34.33±3.38^{b}	37.00±5.57^{b}	42.33±7.97^{b}
	Staph	55.67±0.67^{c}	59.67±0.88^{c}	63.33±3.33^{c}	64.33±3.38^{c}
10g/ml	Candida	22.67±2.91^{ab}	25.33±3.28^{a}	27.00±3.51^{ab}	29.00±4.04^{a}
	Staph	55.00±0.58^{c}	58.67±1.33^{c}	59.00±1.00^{c}	60.67±1.45^{c}
20g/ml	Candida	18.67±3.18^{a}	22.33±3.38^{a}	25.33±4.67^{a}	27.67±4.98^{a}
	Staph	54.67±2.91^{c}	55.67±3.18^{c}	56.00±3.22^{c}	57.67±3.53^{c}
30g/ml	Candida	16.67±2.19^{a}	20.67±2.33^{a}	21.00±2.08^{a}	22.33±1.86^{a}

Value with different alphabets within the same column is significantly different at the P= 0.05 Duncan`s multiple range tests.

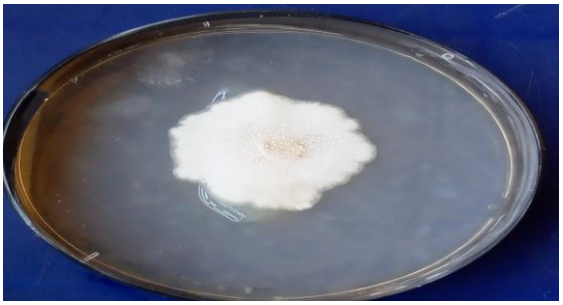

Figure 21: *C Candida albicans (S.mombin bark control)*

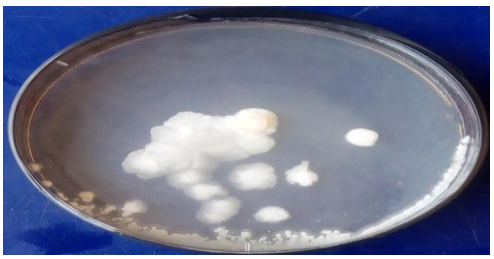

Figure 22: *Candida albicans (10g/ml bark treatment*

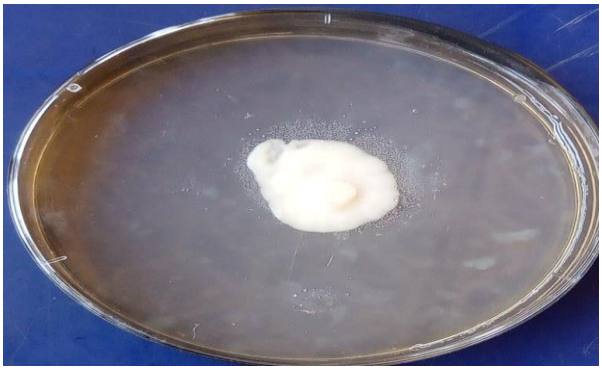

Figure 23: *Candida albicans (20g/ml bark treatment)*

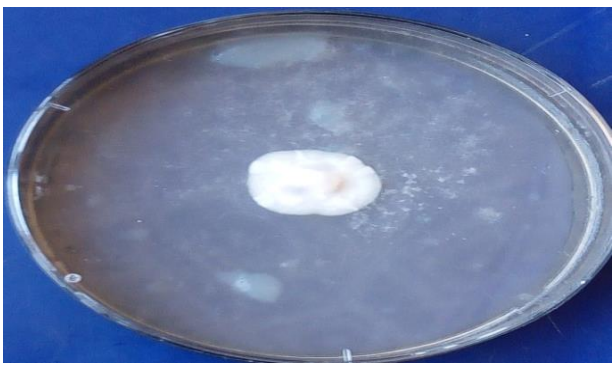

Figure 24: *Candida albicans (30g/ml bark treatment)*

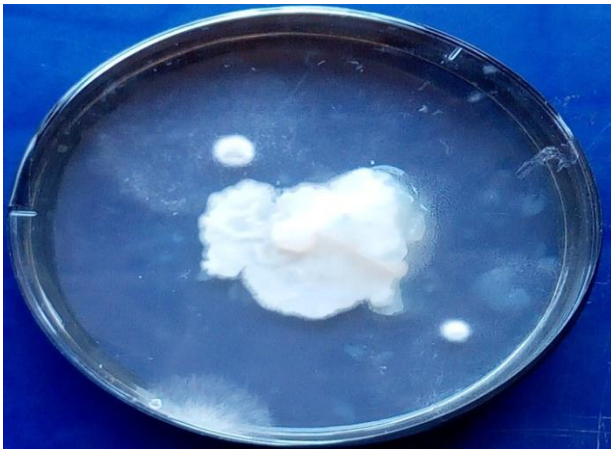

Figure 25: *Candida albicans (leaf extract control)*

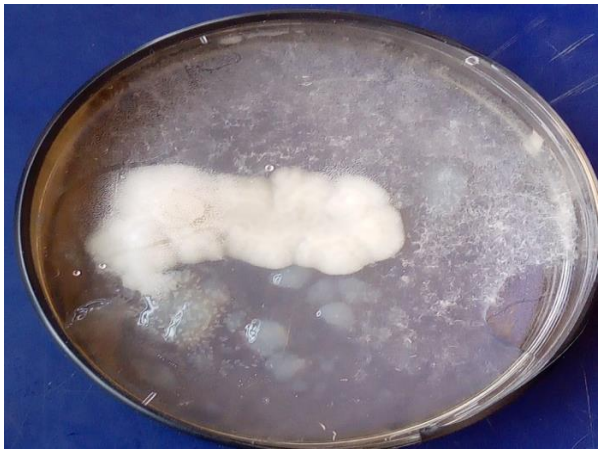

Figure 26: *Candida albicans (10g/ml leaf treatment)*

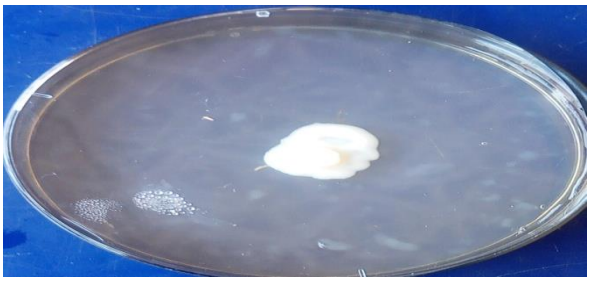

Figure 27: *Candida albicans (20g/ml leaf treatment)*

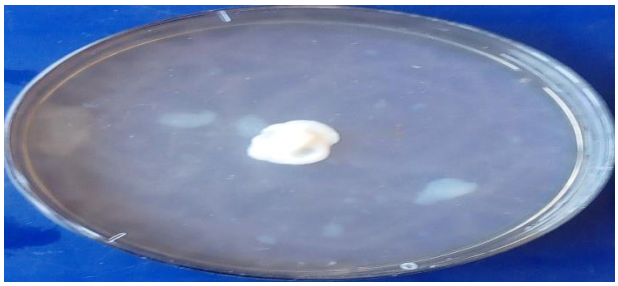

Figure 28*: Candida albicans (30g/ml leaf treatment*

Figure 29*: Staphylococcus aureus (leaf extract control)*

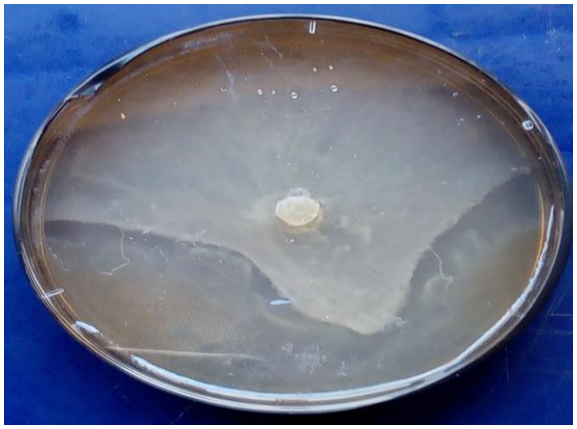

Figure 30*: S. aureus (10g/ml leaf treatment)*

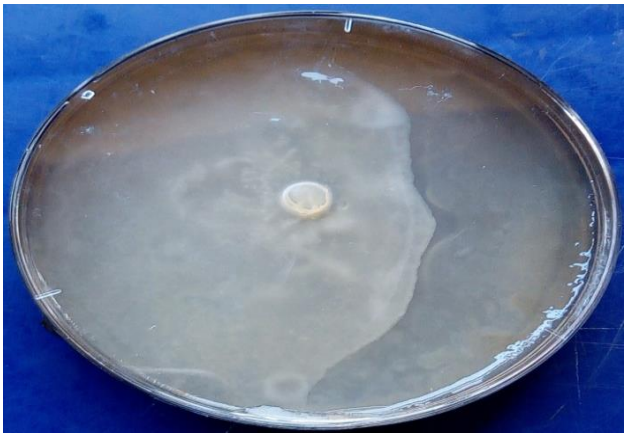

Figure 31: *S. aureus (20g/ml leaf treatment)*

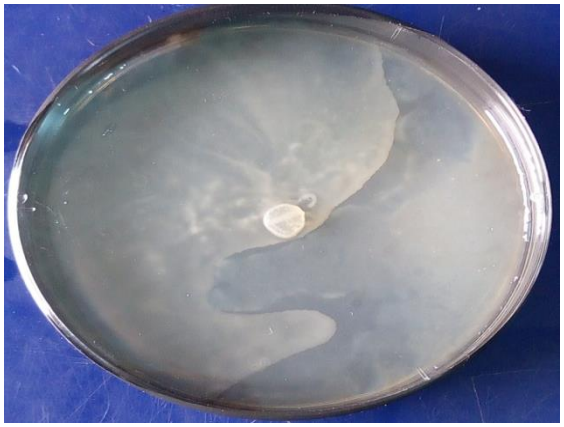

Figure 32: *S. aureus (30g/ml leaf treatment)*

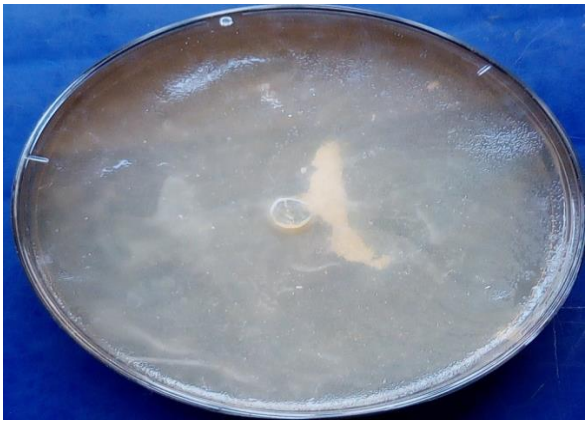

Figure 33: *Staphylococcus aureus (bark extract control)*

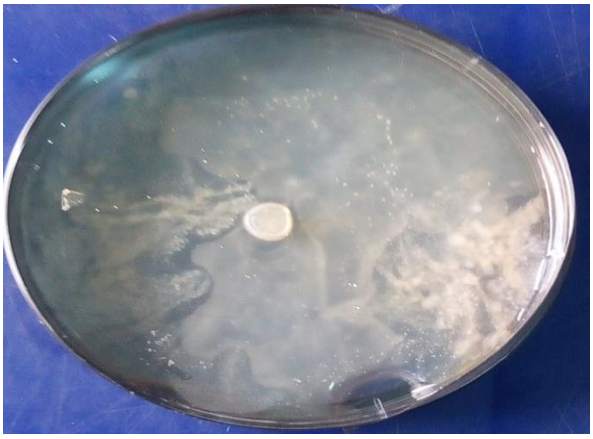

Figure 34: *S. aureus (10g/ml bark treatment)*

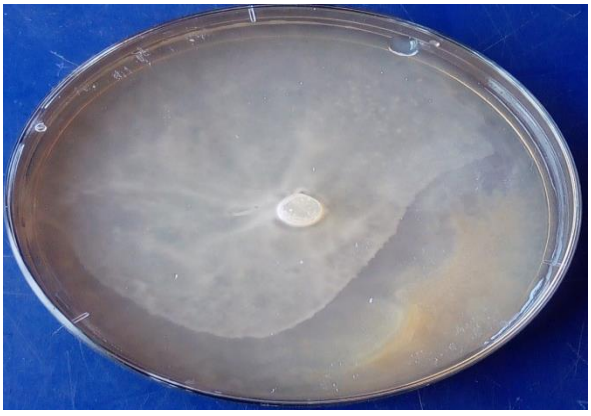

Figure 35: *S. aureus (20g/ml bark treatment*

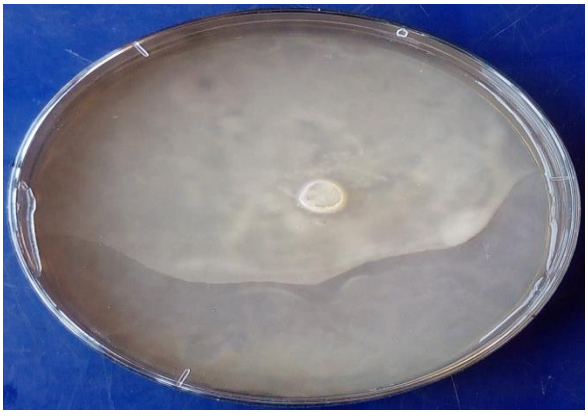

Figure 36: *S. aureus (30g/ml bark treatment)*

3.1. Discussion

Investigations on the antimicrobial effect of the leaf and bark extracts of *Spondias mombin* show that the extracts have an adverse effect on the organisms used which corresponds with the findings of [25]. The inhibitory effect of leaf extract of *Spondia momnbin* on micro-organism supports the report of [26]. This is an indication that this plant can be used as antibiotics in treatment of human

infections. The susceptibility of these pathogens to leaf and bark extract of *Spondias mombin* is due to the present of phytochemicals, this correspond with the results of [27] in which Mombintane I and Mombintane II have been isolated from methanolic stem bark extracts of *Spondias mombin* that have antimycobacterial properties in vitro against Mycobacterium tuberculosis. Other phytochemicals have also been identified to possess antimicrobial and antifungal properties. Findings of [28]. (Sharma, 2006) shows that Flavanoids have anti-oxidant, anti-cancer, anti-microbial, anti-aging, anti-lukemic, radical scavenging and vasodilatory properties. The study of [29] shows that *Spondias mombin* leaves had beta-lactamase, an enzyme produced by certain bacteria that inactivate penicillin and results in response of antibiotics. There are variations in the levels of growth at the different concentrations used in this study. In both cases, higher concentrations have more adverse effects on the microorganism and this indicates that the extract is concentration dependent. The leaf extracts of *Spondias mombin* had more adverse effect on the fungus (*Candida albicans*) while the bark extract had more advert effect on the bacterium (*Staphylococcus aureus*) at all concentrations used. The susceptibility of these organisms to *Spondias mombin* leaf and bark extract at 10, 20, and 30 g/ml from day1-4 indicates that *Spondias mombin* is a medicinal plant with antifungal and antibacterial properties but there was decrease in medicinal properties on exposure for day 1-4. This shows that, improved environment on exposure will further increase the potency of the extracts. Generally, the bark extract was more effective than the leaf extract. This could be attributed to the presence of higher bioactive compounds in the bark extracts of *Spondias mombin* [30]. According to [31] *Spondias mombin* stem bark revealed the presence of fifteen (15) compounds, which include flavonoids, tannins, saponins, alkaloids, other phenols, and steroids. The most prevalent flavonoids were flavone, rutin, flavanones, naringin, anthocyanin, epicatechin, proanthocyanin, and naringenin. The alkaloids present in high concentrations were ribalindine and linamarin. Some other phenolics, such as resveratrol, ellagic acid, chlorogenic and pyrogallic, were in high concentration. [32] and [33] identified a similar pattern of metabolites to those found in their work. Medicinal plant's therapeutic efficacy is based on the chemical elements they contain. Plants high in tannins have antibacterial potential because of their ability to combine with proteins to generate stable water-soluble chemicals, killing bacteria by destroying their cell membrane directly [34]. It has been reported that the phytochemical study of *Spondias mombin* showed that the plant contains alkaloids, flavonoids, tannins, saponins, and phenolic compounds, anthraquinones, caffeoyl ester, 6-alkenylsalcyclic acids, and proanthocyanins [35]. The current study shows that effectiveness of the leaf and bark extract of *Spondias mombin* is concentration dependent and the longer the organisms are exposed to the environment the more the potency of the extract of *Spondias mombin* is reduced . This result justifies the use of bark and leaves extracts of *Spondias mombin* by traditional medicine to treat tooth ache.

4.0. Conclusion and Recommendation

Spondias mombin is known to be effective against pathogenic organisms. Therefore, it is a plant with high medicinal value. It has become a vital raw material in the manufacturing of antibiotics in the pharmaceutical industry. More studies on environmental conditioning *Spondias mombin* extract to further increase efficacy in the treatment and management of other diseases is recommended. This will not only aid in the treatment of various diseases but complete eradication of such diseases as HIV, breast cancer, and liver infections, to name a few. *Spondias mombin* is therefore a potential plant in the manufacturing of drugs for various diseases.

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