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Phytochemical Screening and Evaluation of Antibacterial and Antifungal Activity of the Ethanolic Extracts of *Moringa oleifera* Lam leaves and *Hyphaene thebaica* (Doum) fruit Grown in Sudan

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Keywords

Moringa oleifera, *Hyphaene thebaica*, *in vitro* antibacterial activity, *in vitro* antifungal activity, minimum inhibitory concentration

Abstract

Aim: The aim of this study is to assess the antibacterial and antifungal activity and to determine the zone of inhibition, MIC, MBC and MFC of extracts against some pathogenic bacterial and fungal strains. ***Methodology:*** In the present study, the antimicrobial activity of ethanolic extracts of leaves of *Moringa oleifera*Lam (Moringaceae) and *Hyphaene thebaica* (Doum fruit) was evaluated for potential antimicrobial and antifungal activities against medically important bacterial and fungal strains. The antimicrobial activity was determined using Agar diffusion well-variant method. The antibacterial activity of plant extracts at concentrations (5, 25, 50, 100, 250 µg/ml) were tested against two Gram-positive-*Bacillus anthracis Staphylococcus aureus*; three Gram-negative-*Escherichia coli, Streptococcus pyogenes, Pseudomonas aeruginosa* human pathogenic bacteria; and three fungal strains- *Aspergillus niger, Aspergillus clavatus, Candida albicans*. Moreover,

preliminary phytochemical analysis of the plant extracts was carried out and the secondary metabolites are evaluated. ***Results:*** Zones of inhibition of extracts were compared with that of different standards like ampicillin, ciprofloxacin together with norfloxacin for antibacterial activity and nystatin together with griseofulvin for antifungal activity. *Moringa oleifera* extract produced a remarkable antibacterial activity against *S. pyogenes* and *Bacillus anthracis* at a concentration of 250µg/ml. *Hyphaene thebaica* extract possessed a significant antibacterial activity against all the tested strains at concentrations more than 5 µg/ml. *H. thebaica* fruit extractshowed higher antifungal activity compared to *Moringa oleifera* extract which mild. The preliminary phytochemical analysis of the plant extracts revealed the presence of alkaloids, tannins, flavonoids, saponins, triterpenoids, steroids, glycosides and anthraquinones. ***Conclusion:*** The microbial activity of the leaves of *Moringa oleifera* and fruit of *Hyphaene thebaica* may be attributed to the presence of various secondary metabolites. Hence, these plants can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals.

1. Introduction

Antibiotics are one of our most important weapons in fighting bacterial infections and have greatly benefited the health-related quality of human life since their introduction. However, over the past few decades, these health benefits are under threat as many commonly used antibiotics have become less and less effective against certain illnesses not, only because many of them produce toxic reactions, but also due to emergence of drug-resistant bacteria. It is essential to investigate newer drugs with lesser resistance. Drugs derived from natural sources play a significant role in the prevention and treatment of human diseases. In many developing countries, traditional medicine is one of the primary healthcare systems(Elujoba, Odeleye, and Ogunyemi 2005; Newman, Cragg, and Snader 2003). Herbs are widely exploited in the traditional medicine and their curative potentials are well documented (Sheng-Ji 2001; Srivastava, Shankar, and Gupta 2010). About 63% of new drugs developed between 1981 and 2012 were based on natural products and they have been very successful, especially in the areas of infectious disease and cancer (Hou et al. 2012). Recent trends, however, show that the discovery rate of active novel chemical entities is declining (Lam 2007). Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanisms of action (Parekh and Chanda 2007; Newman, Cragg, and Snader 2003). The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world (Rauha et al. 2000). Much work has been done on ethnomedicinal plants in India (Jain 1994; Chopra 1933; Katewa, Chaudhary, and Jain 2004). Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, glycosides, etc., which have been found *in vitro* to have antimicrobial properties (Freeland and Janzen 1974; Sher 2004).

Herbal medicines have been known to man for centuries. Therapeutic efficacy of many indigenous plants for several disorders has been described by practitioners of traditional medicine (Prakash and Gupta 2005). Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The World Health Organization estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population (Adigüzel et al. 2005). The harmful microorganisms can be controlled with drugs and these results in the emergence of multiple drug-resistant bacteria and it has created alarming clinical situations in the treatment of infections. The pharmacological industries have produced several new antibiotics;

resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to synthetic drugs which are utilized as therapeutic agents (Alanis 2005).

To expand the spectrum of antibacterial and antifungal agents from natural resources, *Moringa oleifera* and *Hyphaene thebaica* have been selected for investigation. *Moringa oleifera* miracle tree, drumstick tree, horseradish tree and other names all refer to one species of 14 from family of Moringaceae. Sudan, tropics and subtropics Africa, India, Pakistan, Bangladesh, Afghanistan, South America, and different other places are native place of it (Fahey 2005; Wadhwa 2013). It has an impressive range of medicinal uses with high nutritional value. Different parts of this plant contain a profile of important minerals, and are a good source of protein, vitamins, β-carotene, amino acids and various phenolics (Bukar, Uba, and Oyeyi 2010). *Moringa oleifera* leaf ethanolic extract was found to possess a considerable antibacterial activity (Jaiswal et al. 2009; Goyal et al. 2007).

Hyphaene thebaica (Doum fruit) is a desert palm native to Egypt, Sub-Saharan Africa, and Western India (Hsu, Coupar, and Ng 2006). Doum fruit is a good source of essential minerals such as potassium, sodium, calcium, magnesium, and phosphorus(Aboshora et al. 2014). Furthermore, Doum fruit contains B-complex vitamins, carbohydrates, and fiber, which are essential for good nutrition. The antibacterial activity of *Hyphaene thebaica* has been reported by (Nwosu, Dosumu, and Okocha 2008; Elegami et al. 2001). In the current investigation carried out, a screening of ethanolic extracts of *Moringa oleifera leaves* and *Hyphaene thebaica* (Doum fruit) against pathogenic bacteria is done in order to detect new sources of antimicrobial agents.

2. Materials and Methods

2.1. Collection of Plant Materials

The fresh leaves of *Moringa oleifera* and the fruit of *Hyphaene thebaica* were collected between November and December, 2021 from various areas of Western and Northern districts, Sudan respectively. The plant specimens were identified in department of Pharmacognosy, Medical Research Centre, Khartoum, Sudan. Plant parts were collected based on the information provided in the ethnobotanical survey of Sudan. Each specimen/plant material was labeled, numbered, a noted with the date of collection, locality, and their medicinal uses were recorded.

2.2. Preparation of Plant Extract

The extraction of the *Moringa oleifera* leaves and *Hyphaene thebaica* fruit was carried out using known standard procedures (Florence, Adeboye, and Stephen 2014). The plant materials were dried in shade and powdered in a mechanical grinder. The powder (30.0 g) of *Moringa oleifera* and (35.0 g) of *Hyphaene thebaica* were initially defatted with petroleum ether (60-80°C), followed by 900 ml of ethanol by using a Soxhlet extractor for 72 hours at a temperature not exceeding the boiling point of the solvent. The extracts were filtered using Whatman filter paper (No.1) while hot, concentrated in vacuum under reduced pressure using rotary flask evaporator, and dried in a desiccator. The ethanolic extracts yield dark greenish solid residue weighing 8.50 g (24.28% w/w) from *Moringa oleifera* leaves and dark brownish highly viscous semi-liquid residue weighing 7.2 g (24.0% w/w) from *Hyphaene thebaica* fruit. More yields of extracts were collected by this method of extractions. The extracts were then kept in sterile bottles, under refrigerated conditions, until further use. The dry weight of the plant extracts was obtained by the solvent evaporation and used to determine concentration in mg/ml. The extract was preserved at 2- to 4°C. This crude extract of ethanol was used for further investigation for potential of antimicrobial properties.

2.3. Preliminary Phytochemical Screening

The extracts were subjected to preliminary phytochemical testing to detect for the presence of different chemical groups of compounds. Air-dried and powdered plant materials were screened

for the presence of saponins, tannins, alkaloids, flavonoids, triterpenoids, steroids, glycosides, anthraquinones, carbohydrates, as described in literatures (Shenoy et al. 2009).

2.3.1 Test for tannins:

To 2 ml ethanolic extract, 2 ml of 10% ferric chloride solution was added in a test tube. Blue-black precipitate indicates the presence of tannins.

2.3.2 Test for alkaloids:

To 2 ml ethanolic extract, 1 ml of 1 % hydrochloric acid was added in a test tube, and heated in a water bath for 10 minutes. 1 ml from the acidified solution was taken and 6 drops of Dragendorff's reagent / Wagner's reagent / Mayer's reagent were added and mixed separately. Orange precipitate / brownish-red precipitate / creamish precipitate respectively indicated the presence of alkaloids.

2.3.3 Test for saponins:

To 0.5 ml ethanolic extract, 5 ml distilled water was added in a test tube and vigorously shaken. Persistent froth volume produced, checked each 10 minutes for 30 minutes, and indicates the presence of saponins.

2.3.4 Test for cardiac glycosides (Keller-Kiliani test):

To 2 ml ethanolic extract, 1 ml glacial acetic acid, 6 drops of 10% ferric chloride solution and 6 drops of concentrated Sulfuric acid were added in a test tube. Green-blue color indicates the presence of cardiac glycosides.

2.3.5 Test for steroids and terpenes (Liebermann-Burchard reaction):

To 2 ml ethanolic extract of, 2 ml acetic anhydride and few drops concentrated Sulphuric acid were added in test tube. Blue-green ring between layers indicates the presence of steroids and pink- purple ring indicates the presence of terpenes.

2.3.6 Test for flavonoids:

To 2 ml ethanol extract, 1 ml of 1% potassium hydroxide solution was added in a test tube. Dark yellow color indicates the presence of flavonoids.

2.3.7 Test for carbohydrate (Molisch's test):

In this method, to 2 ml ethanol extract, 2 drops of Molisch s' test reagent (α-naphthol in ethanol) was added in a test tube and mixed thoroughly. Gently 5 ml of concentrated Sulfuric acid were added. Purple color at the interface indicates the positive test.

2.3.8 Test for reducing sugars (Fehling's test):

In this method, to 2 ml of Fehling's reagent (copper sulphate/sodium potassium tartrate in water) in an empty test tube, 3 drops ethanol extract were added and heated in a water bath at 60 °C. Green suspension and red precipitate indicates the positive test.

2.3.9 Test for anthraquinones:

To 2.5 g powdered material, 10 ml of 20% sulfuric acid and 2 ml of 2% ferric chloride solution were added in a test tube, boiled in a water bath (refluxed) for 30 minutes, allowed to cool, and filtered. The solution then extracted with 10 ml chloroform in a separating funnel. Chloroform layer separated and concentrated to about 4 ml and 2.5 ml of 10 % ammonia solution added. Pink-red color acquired by the alkaline layer indicates the presence of anthraquinone glycosides.

2.3.10 Test for cyanogenic glycosides:

To 3 ml ethanol extract, 2 ml sterile water was added in a conical flask. Freshly prepared sodium picrate paper was placed at the stopper and the solution was heated to boil. Change of color of sodium picrate paper from yellow to different shades of red indicates the presence of cyanogenic glycosides.

2.4. Test Microorganisms and Growth Media

The following microorganisms *Bacillus anthracis (MTCC 444), Staphylococcus aureus* (MTCC 446), *Escherichia coli* (MTCC 334), *Streptococcus pyogenes* (MTCC 396), *Pseudomonas aeruginosa* (MTCC 551) human pathogenic bacteria and fungus strains *Aspergillus niger* (MTCC 2211), *Aspergillus clavatus* (MTCC 1126), *Candida albicans* (MTCC 1345) were chosen based on their clinical and pharmacological importance. The bacterial and fungal strains obtained from Laboratory of Microbiology, Faculty of Dentistry, Jazan University were used for evaluating antimicrobial activity. The bacterial and fungal stock cultures were incubated for 24 hours at 37°C on nutrient agar and potato dextrose agar (PDA) medium (Laboratory of Microbiology, Faculty of Dentistry, Jazan University), respectively, following refrigeration storage at 4°C. The bacterial strains were grown in Mueller-Hinton agar (MHA) plates at 37°C (the bacteria were grown in the nutrient broth at 37°C and maintained on nutrient agar slants at 4°C), whereas the yeasts and molds were grown in Sabouraud dextrose agar and PDA media, respectively, at 28°C. The stock cultures were maintained at 4°C.

2.5. Antimicrobial Activity

2.5.1. Determination of zone of inhibition method

In vitro antibacterial and antifungal activities were examined for ethanolic extracts. Antibacterial and antifungal activities of plant part extracts against five pathogenic bacteria (two Gram-positive and three gram-negative) and three pathogenic fungi were investigated by the agar disk diffusion method (Mahesh and Satish 2008; Khan et al. 2009). Antimicrobial activity testing was carried out by using agar cup method. Each purified extracts were dissolved in dimethyl sulfoxide, sterilized by filtration using sintered glass filter, and stored at 4°C. For the determination of zone of inhibition, pure Gram-positive, Gram-negative, and fungal strains were taken as a standard antibiotic for comparison of the results. All the extracts were screened for their antibacterial and antifungal activities against the *Bacillus anthracis, Staphylococcus aureus, Escherichia coli, Streptococcus pyogenes, Pseudomonas aeruginosa* and the fungi *Aspergillus niger*, *Aspergillus clavatus*, *Candida albicans*. The sets of five dilutions (5, 25, 50, 100, and 250 µg/ml) of *Cassia fistula* extract and standard drugs were prepared in double-distilled water using nutrient agar tubes. Mueller-Hinton sterile agar plates were seeded with indicator bacterial strains (10^8 cfu) and allowed to stay at 37°C for 3 hours. Control experiments were carried out under similar condition by using ampicillin, ciprofloxacin, and norfloxacin for antibacterial activity and nystatin and griseofulvin for antifungal activity as standard drugs. The zones of growth inhibition around the disks were measured after 18 to 24 hours of in incubation at 37°C for bacteria and 48 to 96 hours for fungi at 28°C. The sensitivities of the microorganism species to the plant extracts were determined by measuring the sizes of inhibitory zones (including the diameter of disk) on the agar surface around the disks, and values <8 mm were considered as not active against microorganisms.

2.5.2. Minimum Inhibitory concentration

The minimum inhibitory concentration is defined as the lowest concentration able to inhibit any visible bacterial growth on the culture plates. This was determined from readings on the culture plates after incubation. The most employed methods are the tube dilution method and agar dilution methods. Serial dilutions are made of the products in bacterial growth medium. The test organisms are then added to the dilutions of the products, incubated, and scored for growth. This procedure is a standard assay for antimicrobials. Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and to monitor the activity of new antimicrobial agents. MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism. Clinically, the minimum inhibitory concentrations are used not only to determine the amount of

antibiotic that the patient will receive but also the type of antibiotic used, which in turn lowers the opportunity for microbial resistance to specific antimicrobial agents.

2.5.3. Preparation of Inoculum Test for antibacterial activity

The antibacterial assay was carried out by microdilution method in order to determine the antibacterial activity of the extracts tested against the pathogenic bacteria. The bacterial suspensions were adjusted with sterile saline to a concentration of 1.0 X 10^7 CFU/ml. The inoculants were prepared and stored at 4°C. All experiments were performed in duplicate and repeated three times.

2.5.4. Determination of MIC

The minimum inhibitory concentrations (MIC) and MBC were performed by a serial dilution technique using 96-well microtiter plates. The different ethanolic plant extracts were taken (1 mg/ml) and serial dilution of the extract with Luria broth for bacterial culture was used. The microplates were incubated for 72 hours at 28o. The lowest concentrations without visible growth (at the binocular microscope) were defined as MICs.

2.5.5 Determination of MBC

The MBCs were determined by serial sub-cultivation of 2 µl into microtiter plates containing 100 µl of broth per well and further incubation for 72 hours. The lowest concentration with no visible growth was defined as the MBC, indicating 99.5% killing of the original inoculum. The optical density of each well was measured at a wavelength of 655 nm by Microplate reader (Perlong, ENM8602) and compared with the standards Ampicillin for Bacteria (Hi-media lab, KSA) as the positive control. All experiments were performed in duplicate and repeated three times.

2.5.6. Determination of MFC

The fungicidal concentrations (MFCs) were determined by serial sub-cultivation of a 2 µl into microtiter plates containing 100 µl of broth per well and further incubation 72 hours at 28o Observation and Result C. The lowest concentration with no visible growth was defined as MFC indicating 99.5% killing of the original inoculum. A commercial standard, Fluconazole (Sigma), was used as positive controls (1–3000 µg/ml) for fungi. All experiments were performed in duplicate and repeated three times.

3. Results

3.1. Preliminary phytochemical screening

It was found that ethanolic extracts of both of *Moringa oleifera* leaves and *Hyphaene thebaica* fruit contained alkaloids, tannins, flavonoids, saponins, triterpenoids, steroids, glycosides and anthraquinones.

Table 1: Levels of phytochemicals present in the two ethanolic extracts of the two plants as a preliminary screening

Functional groups	Screened plants	
	Ethanolic extract of *M. oleifera* (leaves)	Ethanolic extract of *H. thebaica* (fruit)
Alkaloids	+++	+++
Flavonoids	++	+
Tannins	+++	+++
Saponins	+++	++
Anthraquinone	+	-
Coumarins	-	+
Steroids	-	-
Glycosides	-	-

Key: +++ = Appreciable amount; ++ = Moderate amount; + = Trace amount; - = Completely absence.

3.2. Antimicrobial activity

The antimicrobial activity of the extracts of *Moringa oleifera* leaves and *Hyphaene thebaica* fruit were studied in different concentrations (5, 25, 50, 100, and 250 µg/ml) against five pathogenic bacterial strains, two Gram-positive *Bacillus anthracis (MTCC 444), Staphylococcus aureus* (MTCC 446), three Gram-negative *Escherichia coli* (MTCC 334), *Streptococcus pyogenes* (MTCC 396), *Pseudomonas aeruginosa* (MTCC 551) human pathogenic bacteria and fungus strains *Aspergillus niger* (MTCC 2211), *Aspergillus clavatus* (MTCC 1126), *Candida albicans* (MTCC 1345). These strains have been selected based on their application purposes pathogenic importance.

Antibacterial and antifungal potential of extracts were assessed in terms of zone of inhibition of bacterial and fungal growth as well as their minimum inhibitory concentrations. The results of the antibacterial and antifungal activities are presented in tables (2 & 3) together with Figures (1-8).

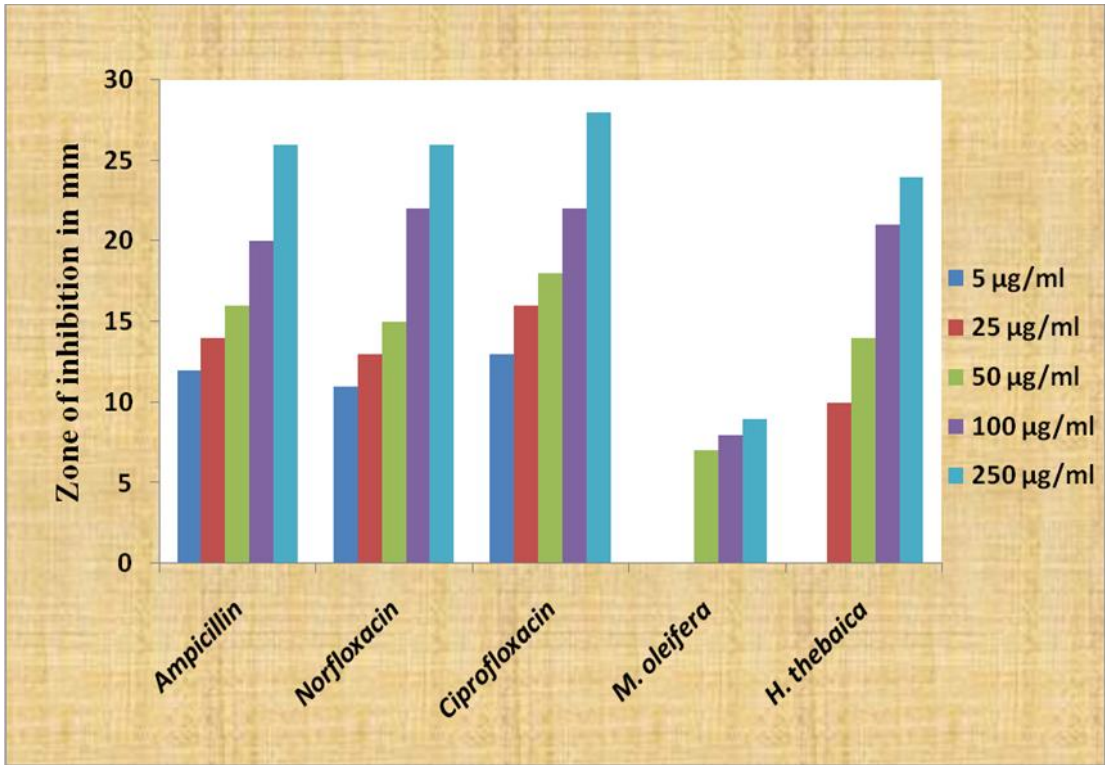

Fig. 1: Antibacterial activity of ethanolic extracts of *M. oleifera* leaves and *H. thebaica* fruit against *P. aeruginosa*(MTCC 551) compared to those of standard drugs

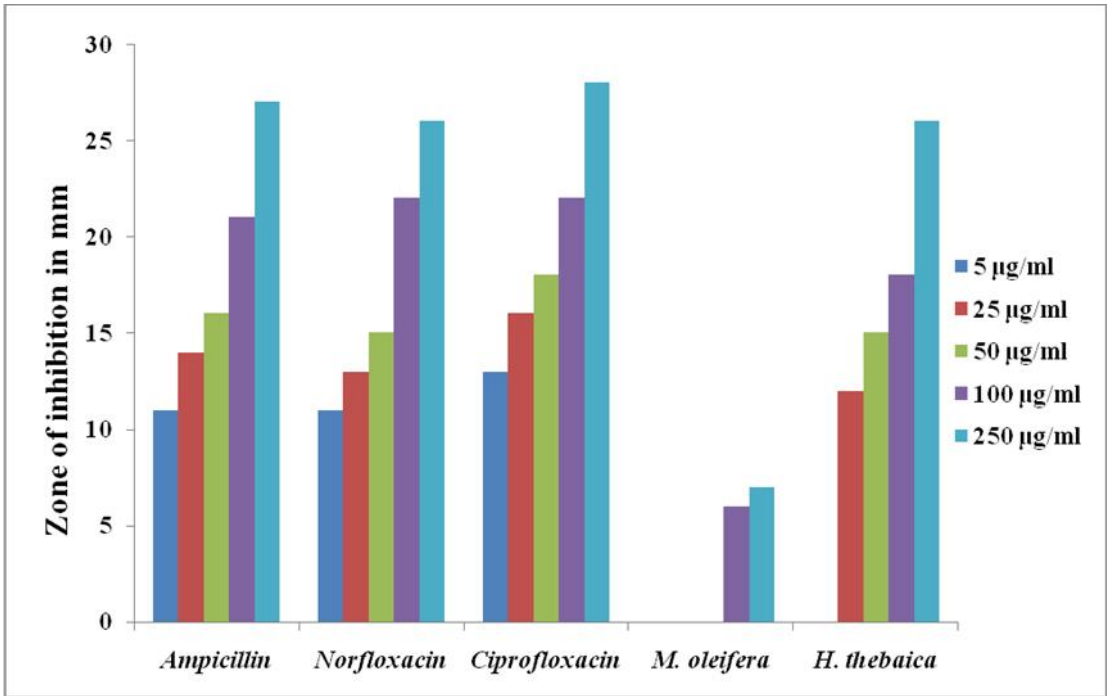

Fig. 2: Antibacterial activity of ethanolic extracts of *M. oleifera* leaves and *H. thebaica* fruit against *E. coli* (MTCC 334) compared to those of standard drugs

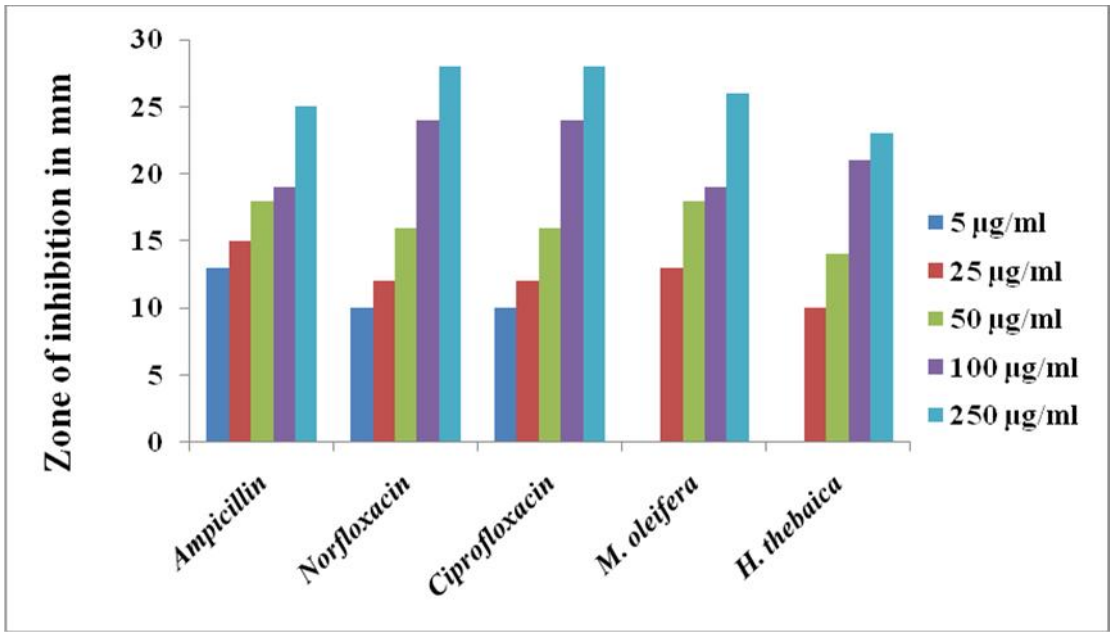

Fig. 3: Antibacterial activity of ethanolic extracts of *M. oleifera* leaves and *H. thebaica* fruit against *S. pyogenes* (MTCC 396) compared to those of standard drugs

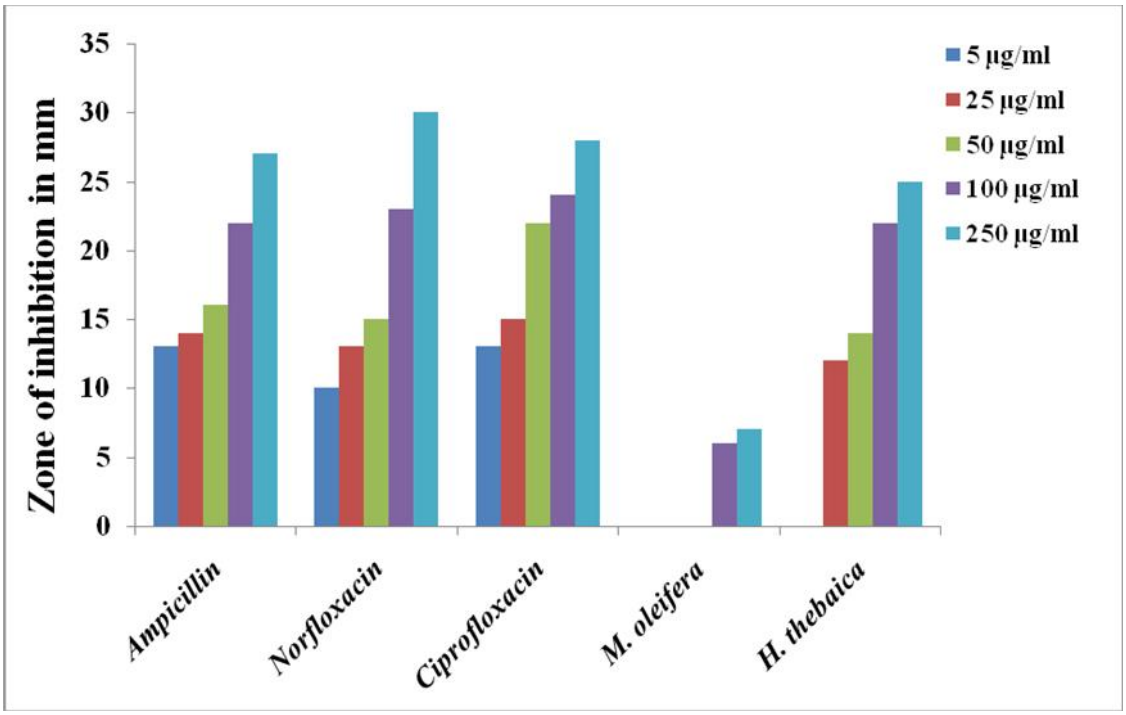

Fig. 4: Antibacterial activity of ethanolic extracts of *M. oleifera* leaves and *H. thebaica* fruit against *S. aureus* (MTCC 444) compared to those of standard drugs

The antibacterial and antifungal activities of the extracts increased linearly with increase in concentration of extracts (µg/ml). As compared with standard drugs, the results revealed that in the extracts for bacterial activity, *S. pyogenes and S. aureus* were more sensitive as compared with *E. coli* and *P. aeruginosa*, and for fungal activity, *C. albicans* shows good result as compared with *A. niger* and *A. clavatus*. The growth inhibition zone measured ranged from 10 to 30 mm for all the sensitive bacteria, and ranged from 14 to 20 mm for fungal strains [Figures 1 to 8].

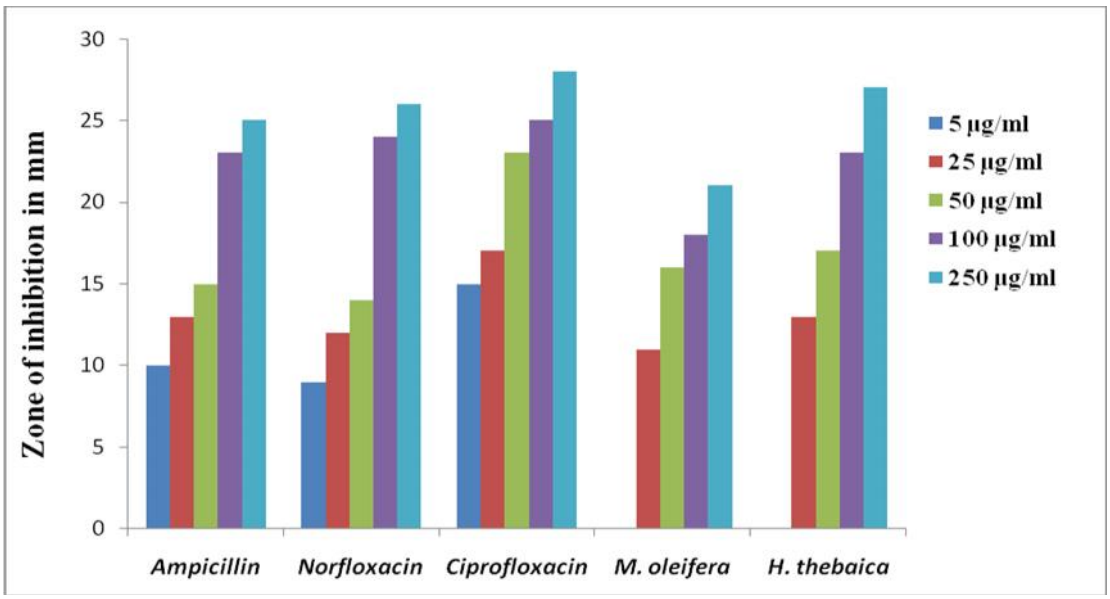

Fig. 5: Antibacterial activity of ethanolic extracts of *M. oleifera* leaves and *H. thebaica* fruit against *B. anthracis* (MTCC 446) compared to those of standard drugs

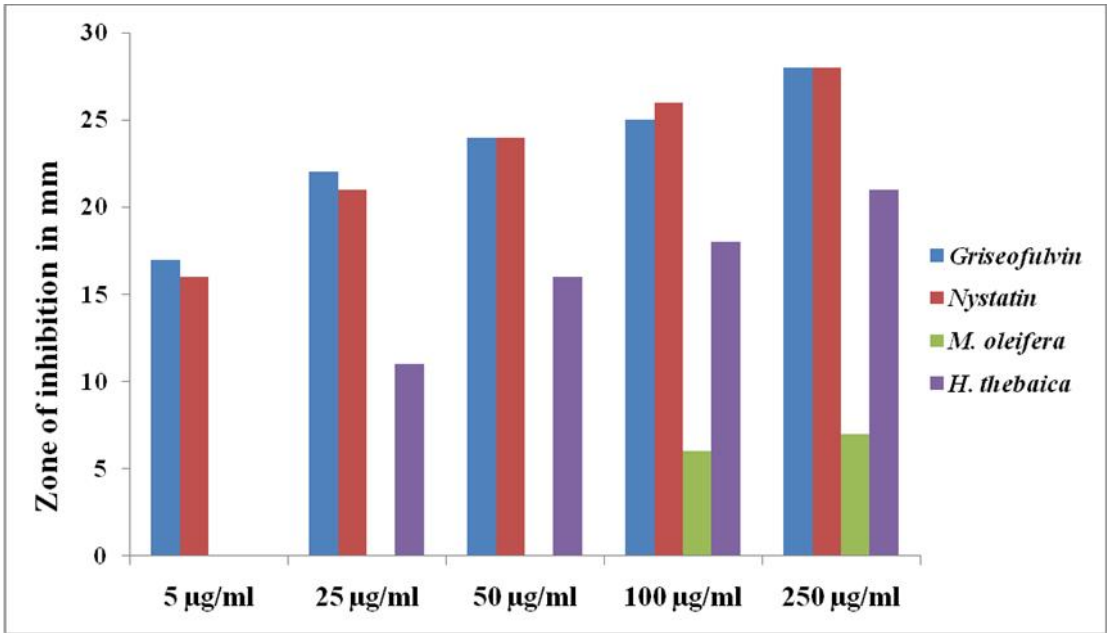

Fig. 6: Antifungal activity of ethanolic extracts of *M. oleifera* leaves and *H. thebaica* fruit against *C. albicans* (MTCC 1345) compared to those of standard drugs

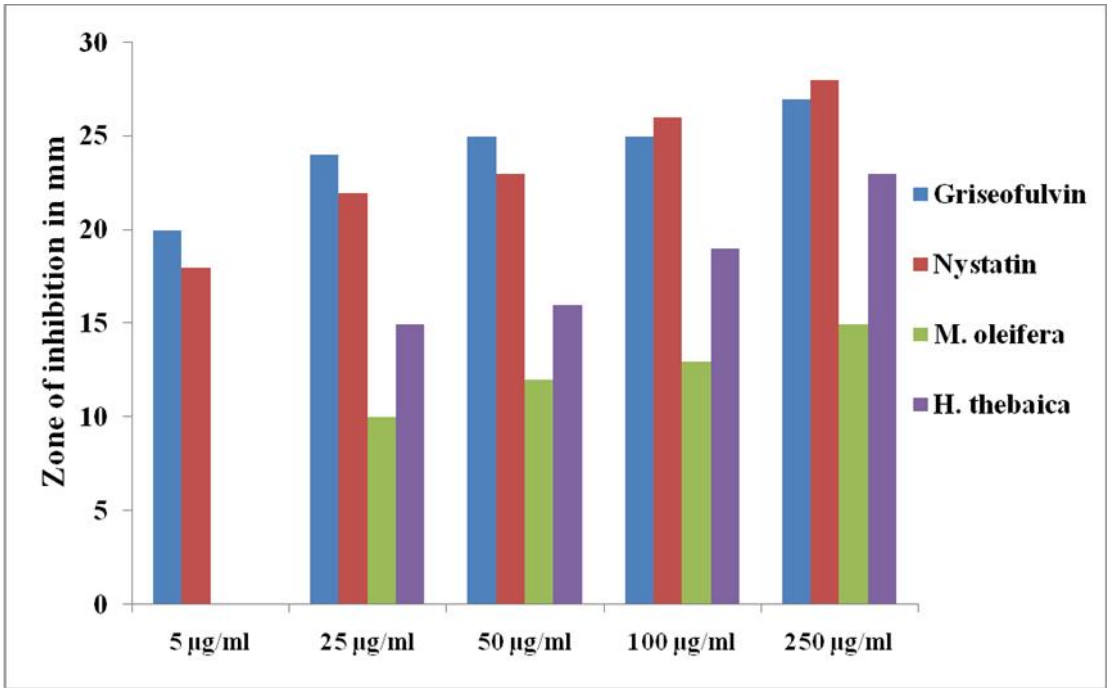

Fig. 7: Antifungal activity of ethanolic extracts of *M. oleifera* leaves and *H. thebaica* fruit against *A. niger* (MTCC 2211) compared to those of standard drugs

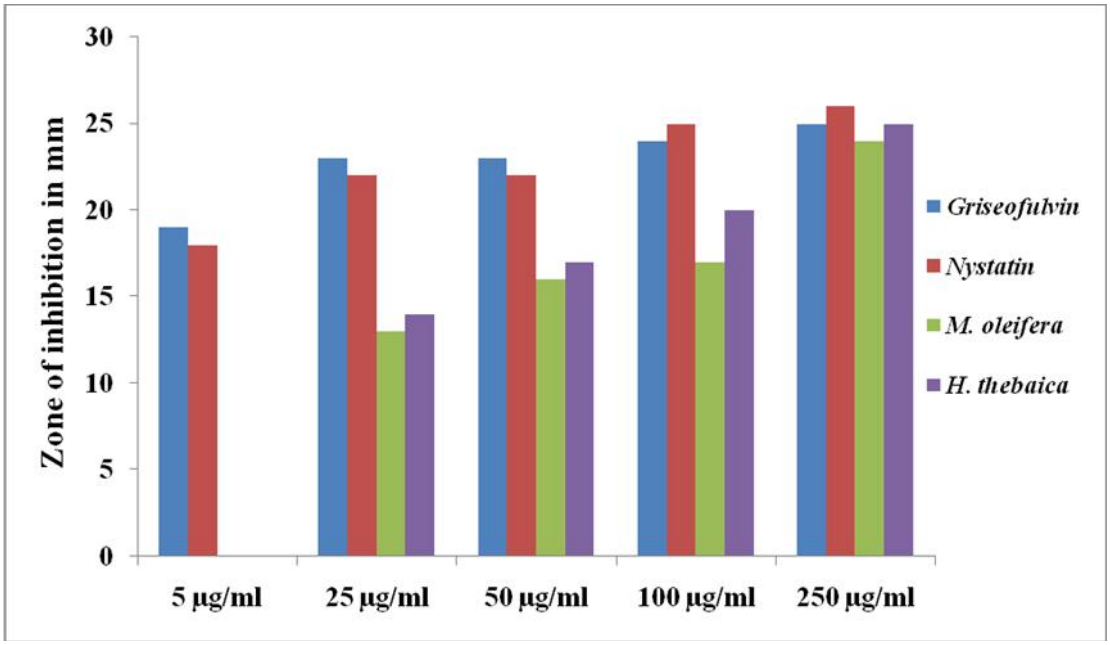

Fig. 8: Antifungal activity of ethanolic extracts of *M. oleifera* leaves and *H. thebaica* fruit against *A. clavatus* (MTCC 1126) compared to those of standard drugs

Table 2: Minimum inhibitory and bactericidal concentrations of ethanolic plant extracts of *M.oleifera* leaves and *H. thebaica* fruit on *S. pyogenes* and *B. anthracis*.

Plant tested	*S. pyogenes*		**(µg/ml)***B. anthracis*	
	MIC	MBC	MIC	MBC
M.oleifera leaves	44.4	52.6	22.0	33.0
*H. thebaica*fruit	28.6	37.5	23.6	36.5

Table 3: Minimum inhibitory and fungal concentrations (MFC) of ethanolic plant extracts of *M.oleifera* leaves and *H. thebaica* fruit on *A. niger* and *A. clavatus*.

Plant tested	*A. niger*		**(µg/ml)***A. clavatus*	
	MIC	MFC	MIC	MFC
M. oleifera leaves	44.2	56.0	28.6	42.2
H. thebaica fruit	32.4	38.8	33.3	46.3

The results show that the extracts of *M. oleifera* leaves and *H. thebaica* fruit were found to be more effective against all the microbes tested.

4. Discussion

Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world (Ahmad and Beg 2001; Cushnie and Lamb 2005; Adigüzel et al. 2005; Das, Tiwari, and Shrivastava 2010). The World Health Organization estimates that plant extract or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population (Verma and Singh 2008; Rates 2001). In the present work, the ethanolic extracts obtained from *H. thebaica* show strong activity against all the tested bacterial and fungal strains while the ethanolic extracts obtained from *M. oleifera* exert strong to moderate to no activity towards the tested bacterial and fungal strains. The results were compared with standard antibiotic drugs. In this preliminary phytochemical screening work of the two ethanolic extract on eight different phytoconstituents shown in table 1, alkaloids and tannins were found to be the most abundant (appreciable amounts) phytoconstituents in both extracts (Taha et al. 2020). The two extracts also showing no presence of steroids or glycosides as parts of their secondary metabolite content (Amauche, Chiubeze, and Liliam 2022).

In terms of antimicrobial activity, the results show that the activity of ethanolic extracts of *Moringa oleifera* leaves and *Hyphaene thebaica* fruit shows significant antibacterial and antifungal activities. Antibacterial activity of extracts of *M. oleifera* leaves and *H. thebaica* fruit against *P. aeruginosa* (MTCC 551) shown in **Fig.1.** explains the high capacity of the later extract (12.5-27 mm) inhibition compared to the former extract (less than 10 mm) inhibition. The results agreed with the review study conducted by (Ghotekar et al. 2020). The activity of the extracts generally increases by the increase of extract concentration. As shown in **Fig.2.**, antibacterial activity of extracts of *M. oleifera* leaves and *H. thebaica* fruit against *E. coli* (MTCC 334), only in the higher doses (125 & 100 µg/ml)*M. oleifera* extract showed low inhibition (less than 10 mm) towards *E. coli*, while all concentrations (5-125 µg/ml) of *H. thebaica* showed a considerable range of inhibition (12-28 mm) towards the pathogenic *E. coli* strains. Similar results obtained from studies on other species of Moringaceae & Palmae to which *M. oleifera* and *H. thebaica* belong respectively (Abdalla and Abdallah 2016b, 2016a; Ekor 2014).

As shown in **Fig.3.**, antibacterial activity of ethanolic extracts of *M. oleifera* leaves and *H. thebaica* fruit against *S. pyogenes* (MTCC 396) is noticeable by the clear variation in zones of inhibition directly proportional to the increase in concentration of extracts. This reflects the availability of the phytoconstituents on both extracts that exert the varied concentration dependent microbial activities of the extracts towards *S. pyogenes* bacterial strains (Ramya 2008; Khameneh et al. 2019). It is also obvious as in **Figs. 4and5,** another concentration dependent variation in antimicrobial activity of the two extracts towards *S. aureus* and *B. anthracis* is present. Only *H. thebaica* fruit extract shows strong to moderate antibacterial activity towards *S. aureus***(Fig.4.)**, while both *M. oleifera* leaves and *H. thebaica* fruit extracts showed strong to moderate activities towards both pathogens **(Figs 4 & 5)**. *M. oleifera* shows low zone of inhibition towards *S. aureus* indicating its inactivity **(Fig. 4.).**The minimum inhibitory and minimum bactericidal concentrations of ethanolic plant extracts of *M. oleifera* leaves and *H. thebaica* fruit on *S. pyogenes* and *B. anthracis*, **Table 2** reveals the correlation between the extracts activity and the inhibition efficiency as a confirmation factor. The results of antifungal activity of the extracts **(Figs. 6, 7, 8 and Table 3)**, indicate that the extracts show abroad range of antifungal activity towards the different fungal strains compared to antibacterial activity which is limited to most but not all bacterial strains. This inform us that, our extracts are more biological active for fungi than for bacteria.

This study also shows the presence of different phytochemicals with biological activity that can be of valuable therapeutic index. The result of phytochemicals in the present investigation showed that the plant contains same components

like saponin, triterpenoids, steroids, glycosides, anthraquinone, flavonoids. The study also showed that, our extracts are more active than the standard drugs used as controls towards most of the pathogens.

5. Conclusion

In the current investigation, the ethanolic extracts have been selected after study of such selected plants with ethanol extracts, ethanolic extracts gave higher yield of chemical constituents expected for this research work; the originality of this work is that good results have been found with hydroalcohol ratio, and it will be helpful to carry out other data with MIC and other formulation study, because in comparison of methanol or water extracts, hydroalcohol is more suitable for clinical study. The ethanolic extracts of *Moringa oleifera* leaves and *Hyphaene thebaica* fruit were found to be active on most of the clinically isolated microorganism, as compared with standard drugs. The present study justified the claimed uses of leaves in the traditional system of medicine to treat various infectious disease caused by the microbes. However, further studies are needed to better evaluate the potential effectiveness of the crude extracts as the antimicrobial agents. The present results will form the basis for selection of plant species for further investigation in the potential discovery of new natural bioactive compounds. Further studies which aimed at the isolation and structure elucidation of antibacterial active constituents from the plant have been initiated.

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