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Original Research Article

POTENTIAL USES OF Moringa oleifera AND AN EXAMINATION OF ANTIBIOTIC EFFICACY CONFERRED BY M. oleifera SEED AND LEAF EXTRACTS USING CRUDE EXTRACTION TECHNIQUES AVAILABLE TO UNDERSERVED INDIGENOUS POPULATIONS

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ABSTRACT:

Moringa oleifera, a pantropical plant, is one of approximately thirteen species belonging to the monogeneric Moringaceae family. Ethnobotanical studies conducted in Guatemala found that one of the primary medicinal purposes of M. oleifera was its use for the treatment of infectious skin and mucosal diseases. As it is common practice for researchers to scientifically validate the efficacy of traditional medicine, it is less common for researchers to scientifically validate simple, reproducible means of conferring therapeutic benefits of plant parts. This study was conducted to investigate pragmatic extraction techniques for seed and leaf extracts of M. oleifera, a plant species for which numerous studies have demonstrated its antimicrobial efficacy. M. oleifera seeds and leaves were extracted using three different solvents (de-ionized water, inorganic ethanol, organic ethyl acetate) and two different extraction methods (crude, sophisticated). Sensitivity disks impregnated with the various extracts were used for antibiotic susceptibility testing of fourteen bacterial species: seven representative Gram-negative and seven representative Gram-positive. De-ionized water was the only solvent capable of extracting plant constituents which conferred bacterial inhibition. Seed extracts were found to inhibit a broader range of organisms (4) than leaf extracts (1). 75% of the organisms inhibited by seed extracts were Gram-positive bacteria. A single parameter, the zone of inhibition, was used to compare antibacterial efficacy between extraction methods, trials, and controls. No difference was observed between the zone of inhibition of crude and sophisticated extracts. Seed extracts demonstrated a zone of inhibition comparable to that of penicillin and tetracycline.

Keywords:Moringa oleifera,Crude extraction,Phytomedicine

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INTRODUCTION:

Antibiotic drugs are one of the many tools in our current arsenal of medical defense. Penicillin is a prime example of how modern antibiotics revolutionized our capacity to combat morbidity and mortality resulting from bacterial infections. Despite great strides and advances in medical equipment and therapies, infectious diseases have long remained the leading cause of morbidity and mortality in the developing world [1]. A sober approach to examining the underlying cause for such disparities requires acknowledging that within the current climate of market-driven economies and for-profit pharmaceutical practices, people often suffer from curable and preventable diseases due to the expenses associated with research, development, and delivery of healthcare [2].

Resources elucidating current research efforts and statistical data for the ‘big-three’ communicable diseases – malaria (resulting from infection with Plasmodium species), AIDS (resulting from infection with HIV), and tuberculosis (resulting from infection with Mycobacterium species) - abound the annals of scientific literature. Nevertheless, documentation of research aimed toward pragmatic and reproducible antibiotic therapies for residents of resource-poor settings is comparatively small. According to Osrin et al. [3], the individuals who define the majority of infectious disease mortality, namely neonates and infants of rural communities in low-income countries, do so in fact because they fall beneath the research and health services radar.

Plant Overview

A pantropical plant of the Moringaceae family, Moringa oleifera, is one of approximately thirteen species in the monogeneric family [4] [5] [6] [7]. Native to the sub-Himalayas of India, M. oleifera has been naturalized in various tropical and sub-tropical regions of the world, including the Middle East, Africa, the Americas, Asia, the Philippines, Cambodia, and the Caribbean islands [4] [5] [6] [7]. A wide range of common names for the tree are documented, including benzolive tree, drumstick tree, horse-radish tree, kelor tree, mother’s best friend, never die tree, mlonge, moonga, mulangay and numerous others [5] [6] [7].

Due to its tolerance of drought and nutrient deficient soils, the perennial softwood is a tree with minimal growth needs [6]. As such, it can withstand climate conditions that range from the high humidity found in the tropics to the arid lands of sub-Saharan Africa, parts of Asia, and the Middle East [4] [5]. Physically, it reaches a maximum height of 7-12 meters and a diameter of 20-40 cm at roughly 2 meters of height [6].

Nutritive Properties

Asserting its multi-faceted value, the plant is utilized for its highly nutritive, medicinal, and water purification properties [4]. The plant’s nutritive properties are ubiquitous throughout the plant, resulting in the observation that most plant parts attain nutrition and can be eaten: leaves, seeds, bark, roots, exudates, flowers, and pods [8]. After discovering the plant’s edible nature, organizations such as Trees for Life, Church World Service, and Educational Concerns for Hunger Organization enacted its widespread use as a nutritive supplement for the malnourished and underserved populations of the tropics and subtropics [8].

Regarding human micronutrient and macronutrient needs, M. oleifera quantitatively provides more nutrients per gram of plant material than many other plant species. For example, gram-for-gram

comparisons of M. oleifera leaves (fresh and dried) and other common nutritional plant sources reveals that M. oleifera provides more than seven times the vitamin C found in oranges, 10 times the vitamin A found in carrots, 17 times the calcium found in milk, nine times the protein found in yogurt, 15 times the potassium found in bananas and 25 times the iron found in spinach [9] [10]. The plant also has high concentrations of phosphorus, copper, α-tocopherol, riboflavin, nicotinic acid, folic acid, pyridoxine, and β-carotene [11]. Furthermore, the plant's leaf structure contains significant quantities of the 10 essential amino acids [9] [10].

Water Purification Properties

M. oleifera has been shown to contain water-soluble proteins that act as coagulants. Numerous studies have demonstrated that M. oleifera seed pumice, when added to contaminated water supplies, effectively precipitates mineral particulate and various organics out of solution. The mechanism of action that facilitates precipitation is attributed to the ability of charged protein molecules to bind and flocculate soluble particulate matter [6] [12] [13].

Medicinal Properties

Chemical compounds isolated from M. oleifera have been shown to contain useful pharmacological properties with prospective medicinal applications. A list of possible medical applications conferred by M. oleifera plant parts includes, but is not limited to, antihypertensive, anticancer, antispasmodic, antitumor, antiulcer, cholesterol lowering, diuretic, hepatoprotective, and hypoglycemic capabilities, as well as treatment of infectious skin and mucosal diseases [1] [4] [14]. Leaf extracts have been used to treat hyperthyroidism and currently have application as an anti-Herpes Simplex Virus Type-1 medicine [15] [16].

Phytochemical Constituents

Numerous antibacterial compounds have been isolated from M. oleifera, including: glucosinolates, rhamanose, pterygospermin, and isothiocyanates. Specifically, these compounds include 4-(4'-O-acetyl-a-L-rhamnopyranosyloxy)benzyl isothiocyanate (1), 4-(a-L-rhamnopyranosyloxy)benzyl isothiocyanate (2), niazimicin (3), pterygospermin (4), benzyl isothiocyanate (5), and 4-(a-L-rhamnopyranosyloxy)benzyl glucosinolate (6) [8].

Since extensive and scientifically rigorous studies pertaining to the antibacterial activity of M. oleifera seeds began, a number of biochemical agents have been purported as the component responsible for the observed bacterial inhibition. During the 1940s and 1950s, prior to isolation and identification of a specific antibacterial agent from the seeds, it was hypothesized that pterygospermin was responsible for the seeds' antibacterial activity [8]. Pterygospermin, however, was later found to dissociate into two benzyl-isothiocyantes, compounds known to possess antimicrobial properties [8]. Current literature cites the isothiocyanate structure and its precursor, glucosinolate, as primary constituents from M. oleifera seed extracts that confer antibacterial activity [17].

Research

It is common practice for researchers to scientifically validate the efficacy of traditional medicine. It is less common for researchers to scientifically validate efforts to employ a simple, reproducible means of conferring the therapeutic benefits of an agent with pre-existing evidence that substantiates its putative health benefits.

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Martin Price, executive director of Educational Concerns for Hunger Organization (ECHO), published an article calling for the development of an antibiotic ointment from the seeds of Moringa oleifera, noting that the research would be an undertaking of how the poor could benefit from science [18].

The purpose of this study was to investigate pragmatic extraction techniques for seed and leaf extracts of Moringa oleifera, a plant species for which numerous studies have demonstrated its antimicrobial efficacy [4] [5] [7]. It is hypothesized that bacterial inhibition would be conferred – to varying degrees – by both plant parts being studied.

Considering the populations for which this research was aimed to benefit (those that occupy resource-poor, low income, and remote regions of the developing world), the phrase 'pragmatic technique' is used in this paper to define methods that do not require sophisticated laboratory equipment (i.e. rotary evaporators, gas-/high performance liquid chromatography, etc.). Specifically, M. oleifera seeds and leaves were extracted using: three different solvents – de-ionized water (DI H_20), inorganic ethanol (95% EtOH), and organic ethyl acetate (EA); and two different extraction methods – crude and sophisticated. Sensitivity disks impregnated with the various extracts were used for antibiotic susceptibility testing of fourteen different bacterial organisms: seven representative Gram-negative (G-) species and seven representative Gram-positive (G+) species.

METHODS

I. Plant material preparation

Leaf powder and whole seeds were obtained from Educational Concerns for Hunger Organization (ECHO), located in Ft. Myers, Florida, U.S.A. Prior to extraction, seeds were pulverized via two methods: crude and sophisticated. The crude method, chosen for its ease in reproducibility and designed for use in the most unindustrialized regions of the developing world, utilized the crushing and grinding of the substrate with a mortar and pestle. Alternatively, the sophisticated method was propagated by means of an ordinary coffee bean grinder. Both methods were used until further agitation no longer decreased the size gradation of ground plant matter.

II. Antimicrobial constituent extraction

The extraction process included three components. The first consisted of plant part: leaf powder (obtained from Moringa Farms, California, U.S.A.), and seed powder/grinds (obtained from ECHO). The second dimension included solvent variability. Three different liquid media were used to facilitate the extraction process of antimicrobial constituents from leaf and seed materials: 1) DI H_2O, 2) 95% ethanol (EtOH), and 3) ethyl acetate (EA). The final dimension was created by the previously stated dual extraction techniques: crude and sophisticated.

For each extraction, 10 grams of raw plant material was macerated with 40 mLs of solvent; solvents were heated to boiling temperatures and then introduced to small containers (either sterilized glassware or tin cans depending on the extraction method) that contained the plant material. The plant-solvent mixture was agitated for 30 s to ensure thorough mixture of the components; the solution was then covered and allowed to steep with no heat stimulus for 15 mins. After 15 mins of steeping, the containers were each weighed to determine solvent loss from evaporation. As needed, excess solvent was added to the beakers to normalize the

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concentrations to 10 grams of plant material (PM) per 40 mLs of solvent (crude concentration equaling: 250 mg/mL).

The extraction process (heating solvent, then transferring solvent to PM, then macerating PM) for the crude technique was performed using empty tin cans that were sterilized with boiling water within the can and then decanting the water. Alternatively, sterile (autoclaved) laboratory glass beakers were used to facilitate the sophisticated technique.

After normalization of the plant, liquid extract was separated from solid PM by filtration using coffee filters. In light of the possibility of other modalities, this method was chosen because it was economical, effective, and extendable to the geographical areas of this study's interest.

III. Extract impregnation of sensitivity disks

Sterile sensitivity disks were crafted by autoclaving 6.5 mm circular disks punched from Whatman GF/D Glass Microfibre filter disks, using a standard 6.5 mm hole-punch. Sterile forceps were used to individually submerge sensitivity disks in extracts (experimental trials) or solvents (negative controls), thus impregnating them. After submersion, the disks were flicked to remove excess fluid and then placed upon aluminum foil plates. These plates were heated at 80°C for 45 minutes to dry the disks and evaporate residual solvent.

IV. Organism growth

Fourteen bacterial organisms were grown at 37.5°C to assist in determining plant constituent efficacy as an antimicrobial agent (Table 1).

Table 1. Three organisms (M. smegmatis, A. faecalis and S. aureus) exhibited susceptibility to M. oleifera seed extracts, while one organism (B. sphaericus) demonstrated susceptibility to both M. oleifera seed and leaf extracts.

Organism		Biological Classification		Inhibition			
				Seed Extracts		Leaf Extracts	
		Gram Stain	Morphology	Yes	No	Yes	No
1	Bacillus sphaericus	G+	Bacillus	X		X	
2	Bacillus subtilis	G+	Bacillus		X		X
3	Bacillus megaterium	G+	Bacillus		X		X
4	Bacillus ceresu	G+	Bacillus		X		X
5	Mycobacterium smegmatis	G+	Bacillus	X			X
6	Micrococcus luteus	G+	Coccus		X		X
7	Staphylococcus aureus	G+	Coccus	X			X
8	Alcaligenes faecalis	G-	Bacillus	X			X
9	Enterobacter aerogenes	G-	Bacillus		X		X
10	Escherichia coli	G-	Bacillus		X		X
11	Klebsiella pneumoniae	G-	Bacillus		X		X
12	Proteus mirabilis	G-	Bacillus		X		X
13	Proteus vulgaris	G-	Bacillus		X		X
14	Shigella flexneri	G-	Bacillus		X		X

Organisms were attained from American Type Culture Collection. Agar slants, incubated at 37.5°C were used as perpetual growth sites for the organisms. Seven days before plating, the organisms were transferred to tryptic soy broth (TSB), the medium in which they remained until susceptibility testing was performed. Transference of 75 µLs of TSB (containing the respective microorganism) to sterile LB agar plates was followed with a glass hockey-stick method of evenly distributing the organism across the plate and ensuring confluent bacterial growth.

Five sets of organisms were prepared to correlate with extract methods and plant part (4); a final set of organisms were prepared to examine positive and negative controls. Three agar plate replicates were prepared for each organism within all 5 sets.

V. Disk implantation

Immediately following dissemination of bacterial TSB onto LB agar plates, sensitivity disks were implanted onto the plate. Three sensitivity disks, each correlating to a specified extract solvent, were placed on each replicate within the four extraction sets. Six sensitivity disks, which correlated to the positive (Vancomycin, Penicillin, and Tetracycline) and negative (pure solvent without extract) controls, were placed on each replicate within the control set. A rectangular shape beneath the agar plate was used for standardized orientation of the plate and subsequent placement of each disk.

VI. Incubation and reading of dishes

Agar plates were incubated at 37.5°C for a total duration of 72 hrs, and zones of inhibition (mm) were recorded at 24, 48, and 72 hrs post disk-placement.

RESULTS

I. Plant part and solvent

Leaf and seed extracts prepared with 95% EtOH or EA conferred no inhibition among the 14 organisms. Extracts prepared using M. oleifera leaves with DI H_2O exhibited antibacterial inhibition against one of the fourteen bacteria: Bacillus sphaericus (Table 1). M. oleifera seed extracts prepared with DI H_2O conferred inhibition among four of the 14 bacteria: B. sphaericus, Mycobacterium smegmatis, Staphylococcus aureus, and Alcaligenes faecalis (Table 1).

II. Susceptible species

A total of four bacterial species were susceptible to inhibition by either leaf or seed extracts prepared from M. oleifera (Table 1). One species, B. sphaericus, showed susceptibility to both seed and leaf extracts. M. smegmatis, S. aureus, and A. faecalis were susceptible to seed extracts but were resistant to leaf extracts. Of the species susceptible to seed extracts, bacterial inhibition was phylogenetically and morphologically scattered; the extracts were effective against representative species of G+ bacillus (B. sphaericus and M. smegmatis), G+ coccus (S. aureus), and G- bacillus (A. faecalis). One common trend was the indication that 75% of the organisms inhibited by seed extracts were G+ while the remaining 25% were G-; further 75% were bacilli species, whereas the remaining 25% were cocci species.

III. Method variation

(Results are displayed as Average ± Standard Error)

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Each extraction for the given plant part and solvent was performed using two extraction methods: crude, and sophisticated. The leaf-water extract prepared using sophisticated methods demonstrated antibacterial efficacy against B. sphaericus (4.11mm ± 0.11), while the leaf-water extract prepared using crude methods did not inhibit the organism. Of the four organisms susceptible to seed-water extracts, two were susceptible to both crude and sophisticated extraction methods, while the remaining two were susceptible to only one method (Table 2).

Table 2. Utilizing sophisticated and crude methods, DI H2O M. oleifera seed extracts demonstrated statistically significant zones of inhibition and provided statistically similar results to penicillin and/or tetracycline with regard to four pathogens.

Organisms Inhibited by Seed DI H2O Extract		Bacterial Inhibition Method		Observed Antibiotic Efficacy	Antibiotics for Which Seed Extracts Demonstrated Statistically Comparable Results		
		Sophisticated	Crude		V	P	T
1	Bacillus sphaericus	X	X	V, P & T		X	
2	Mycobacterium smegmatis		X	T			X
3	Staphylococcus aureus	X		T		X	X
4	Alcaligenes faecalis	X	X	V, P & T			X

(Where: V = vancomycin, P = penicillin, and T = tetracycline)

B. sphaericus and A. faecalis demonstrated susceptibility to both extraction methods for the extracts. The sophisticated method produced a wider zone of inhibition (diameter) for B.sphaericus, crude (6.44mm ± 1.97) and sophisticated (11.22mm ± 0.29); the crude method, however, demonstrated an increased zone of inhibition (diameter) for A. faecalis (crude = 5.67mm ± 1.92 and sophisticated = 1.67mm ± 0.19). M. smegmatis was inhibited by crude extract (2.0mm ± 1.73), but showed no resistance to the sophisticated extract. S. aureus was susceptible to sophisticated extracts (1.78mm ± 0.91), but not crude seed-water extracts. For all organisms, no significant difference in zone of inhibition was observed between the two extraction methods ($P > 0.1$ or $P > 0.2$).

IV. Controls versus extracts

Each organism examined was subjected to susceptibility tests with negative and positive controls (NCs and PCs). Susceptibility to NCs, PCs, and plant part extracts were compared using zone of inhibition (ZOI) measurements. NCs consisted of sensitivity disks soaked in pure solvent: DI H2O, 95% EtOH, or EA. None of the NCs were found to confer inhibition among the fourteen organisms.

PCs consisted of three known antibiotic agents: penicillin, tetracycline, and

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vancomycin. Species susceptible to both penicillin and the seed extracts, namely B. sphaericus and A. faecalis, did not demonstrate a significant difference between the average ZOI produced by penicillin or the seed extracts. Similar to penicillin, species susceptible to tetracycline and seed extracts, with the exception of B. sphaericus, demonstrated comparable average ZOIs, that is, ZOIs were not significantly different between tetracycline disks and seed extract disks. Alternatively, for organisms susceptible to vancomycin and seed extracts – B. sphaericus and A. faecalis – vancomycin disks resulted in significantly elevated ZOIs relative to the seed extracts.

DISCUSSION

I. Plant Part and Solvent

In discussing the general inhibitory efficacy of leaf and seed extracts from this research, regrowth must be considered. Regrowth is a biological phenomenon whereby organismal reproduction/growth is inhibited for a short duration, but is subsequently followed by proliferation of the organism. Because data collections were performed at 24-hr intervals, regrowth of organisms prior to the first data collection (24-hrs) or between each of the 24-hr intervals must not be ruled out and may account for the lack of observed organismal inhibition by leaf or seed extracts.

Plant Part

Of the fourteen organisms examined, seed extracts were more effective antibacterial inhibitors than leaf extracts. In fact, several trials exhibited microbial proliferation near and/or on the sensitivity disks soaked in leaf extract. It is suspected that proliferation was due to the nutrient dense structure of M. oleifera leaves [8]. Similarly, for organisms that were not inhibited by seed extracts, proliferation of bacterial colonies was also observed near and/or on the sensitivity disks soaked in seed extract. Again, this may also be attributed to the nutrient rich nature of the plant's seeds [8]. Thus, given the abundance of vitamins, minerals, and amino acids found within the leaves [4] [5] [7] [19], coupled with the populations this research aimed to address, it can be reasonably concluded that leaf material would be more appropriately allocated for use as a nutritional supplement rather than a source of antibacterial components.

Solvent

The polarity of water is markedly elevated relative to EtOH and EA. It is possible that variations in solvent polarity, as measured by dielectric constants, explains why EtOH and EA extracts did not attract, bind, and thus extract charged antimicrobial constituents. Further, it is also possible that the 95% EtOH extract used, may have degraded proteins implicated in inhibitory properties, thus debilitating the extract's ability to confer bacterial inhibition.

II. Method Variation

The primary objective underlying the experimental design for this study was the determination as to whether crude extraction methods were capable of inhibiting bacterial growth. The absence of statistical evidence pointing toward significant differences between ZOIs conferred by crude and sophisticated extraction methods implicates that for certain bacterial species, the crude method of extraction is just as effective as the sophisticated method. These findings support the prospect of crude extraction as a pragmatic method of extracting antibacterial agents from M. oleifera in resource-poor settings.

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III. Susceptible Species

Despite phylogenetic diversity among species susceptible to M. oleifera extracts, results demonstrated a common trend in that M. oleifera seed extracts were three times more effective against G+ species compared to G- species based upon the species observed. It is suspected that these differences were observed due to variation between the cell wall composition of G+ and G- bacteria. Perhaps the second phospholipid membrane bilayer of G- bacteria helped these species evade antibiotic degradation from the peptides within the seed extract [20].

IV. Controls versus Extract

The lack of significant difference between ZOIs produced by M. oleifera seed extracts compared to those produced by penicillin and tetracycline implicate comparable antibacterial efficacy in susceptible taxa between M. oleifera seed extracts and the aforementioned antibiotics. These results encouraged the researchers of this study to examine the biochemical mechanism of action for penicillin and tetracycline, and then use such activity to hypothesize possible mechanisms of action yielded by seed extracts.

Administered at clinical doses, antibiotics that inhibit the proliferation of bacteria are classified as either: bactericidal or bacteriostatic. The former prevent bacterial proliferation by killing the organism, whereas the latter prevents bacterial proliferation by preventing the organism from dividing, which in turn assists the individual's immune system to destroy the pathogen. Penicillin is a bacteriostatic β-lactam antibiotic derived from a fungi source, and is effective in destroying G+ bacteria. Its mechanism of action is such that it inhibits bacterial cell wall formation by blocking the cross-linking of structures within the cell wall (i.e. cell wall synthesis) [20]. Tetracyline, however, is a broad-spectrum bacteriostatic antibiotic, functioning as a G+ and G- antibiotic. Unlike penicillin, tetracycline inhibits bacterial growth intracellularly by binding to the bacterial 30S ribosomal subunit, thus disrupting protein synthesis [20].

The mechanism by which M. oleifera derived isothiocyanates result in bacterial inhibition is not understood to date. However, extensive research documenting the efficacy of synthetic, highly active antibacterial peptides derived from M. oleifera seed proteins has helped elucidate the mechanism by which the cationic peptide results in microbial inhibition [13]. The secondary structure of the antimicrobial polypeptide contains positively charged α-helices that are thought to bind to negatively charged phospholipid heads of the bacterial cell membrane; the charge attraction and stabilization allows for subsequent interaction between a loop region in the antibacterial peptide structure and aliphatic fatty acids of the bacterial membrane [13]. In turn, the bacterial membrane is destabilized by changes in permeability and lipid distribution, as well as disruption of its membrane potential. In sum, these effects result in breaks in the bacterial cell membrane, disaggregation of its components, and eventually cell death [12] [13].

With this understanding, antibiotic peptides of M. oleifera seed extracts were expected to exhibit inhibition comparable to that of penicillin; however, they were not expected to produce results similar to tetracycline. Given that tetracycline works intracellularly as a bacteriostatic antibiotic, it is possible that other constituents of M. oleifera seed

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extracts, such as the identified isothiocyanates, also work intracellularly in a concerted effort with the antibiotic peptides.

V. Future Research

In futures trials it would be desirable to repeat the leaf and seed DI H_2O extract experimentation, withholding the 80°C incubation that evaporated the solvent. It is suspected that motility afforded to microbial inhibiting molecules by the presence of DI H_2O will allow the molecules to disperse across the agar lawn with greater confluence. Repeated experiments could also rule out the possibility of regrowth by collecting data samples over durations shorter than 24-hrs, i.e. 3-hr, 6-hr, 9-hr, or 12-hr time intervals.

Although several of the organisms tested in this study are known to be common colonizers of human skin and gastrointestinal tracts, it is recommended that antibacterial susceptibility tests, using M. oleifera extracts, be performed against pathogenic bacteria endemic to resource-poor settings. Further, it is also suggested that MIC studies be conducted using the crude extraction technique. If the extracts prove to be efficacious inhibitors of pathogenic bacteria, then shelf-life studies should be performed to further qualify this pragmatic extraction technique that it may be used in destitute regions worldwide.

CONCLUSIONS

This study demonstrated that when M. oleifera seeds and leaves are prepared using very crude methods they can provide, to varying abilities, antimicrobial capabilities comparable to some contemporary remedies against common pathogens that cause human morbidity. This research is a reminder that heroic lengths and modern science are not always necessary to combat antimicrobial pathogens in remote regions where modern medicine is not available.

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