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Competence of Benzoil Tree (*Moringa Oleifera* L.) as Antibacterial and Antifungal Agent

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Abstract

Background: A plant's activity towards biological properties is the first step to consider it for medicinal and therapeutic purposes. To evaluate the medicinal properties, we have determined the anti-bacterial and anti-fungal potential of *Moringa oleifera* L. (Benzoil tree). The focus was to obtain and isolate certain chemical substances that can neutralize the effect of common and pathogenic selected bacterial and fungal species collected from local hospitals of Khyber Pakhtunkhwa.

Methods: A total of five fractions were selected i.e. crude methanolic extracts, n-hexane, chloroform, ethyl acetate and aqueous extracts were prepared and their activity checked against four bacterial strains including *Streptococcus mutans, Staphylococcus aurous,* MRSA (methicillin resistance *Staphylococcus aurous*) and *Serratia marcescens*; and four fungal strains i.e. *Fusarium oxysporum, Aspergillus flavus, Polysphondylium pallidum* and *Alternaria alternata.*

Results: The highest anti-bacterial activity shown by crude methanolic extract fraction i.e. (48-38%) and the lowest activity was exhibited by aqueous extract (15-0%) against all the selected bacterial strains. Similarly, the highest anti-fungal activity indicated by crude methanolic extracts (60-45%) against the four selected fungal species and lowest activity shown by the aqueous fractions (26-0%). Reasonable activity was also exhibited by others fractions as well.

Conclusion: The outcomes of our experiment strongly supports that *Moringa oleifera* has significant antibacterial and antifungal activities, so the plant is effective antibacterial and antifungal agent.

Introduction

Moringa oleifera is among the highly investigated and distributed species in tropical and subtropical countries of the world belonging to a single genus containing family Moringaceae [1,2]. The tree varies from 5 to 10 meters vertically and can be found wildly or cultivated in well humid and hot climate and is normally resistant to considerable ratio of drought. It is native to Asian countries like India, Pakistan and is now cultivated all over the world [3]. The Benzoil tree has many common names like kelor tree or drumstick or horseradish tree in different regions of the world while in Pakistan it referred to as 'Sohanjna' [4]. It is a highly valued medicinal plant and its every part is used medicinally giving resilient results. It has an extraordinary range of nutritional value and many useful minerals along with biomolecules like phenols, proteins, a wide range of vitamins, amino acids and *β*-carotene have been reported [5]. The seeds of *Moringa oleifera* contain water-soluble substances and edible oils, which comprises extraordinary thickening characteristics for purifying wastewater [6]. The reproductive part of *Moringa oleifera* mainly seeds been reported to prevent the growth of pathogenic microorganisms like *Staphylococcus aureus* and *Pseudomonas aeruginosa* during extraction when the temperature rises above 56°C [7]. Due to the extraordinary high value of medicinal and nutritional properties found in the plant discovered and reported with time, the numbers of its spreaders and purveyors classifying it as "beneficial and healthful" food are now supporting it as miracle plant or magic potion. Much of this recent interest has been termed acceptable but still it is very important, to clearly differentiate between tough scientific results and suggestions from the sketch presented by the spreaders [8]. As the plant is highly medicinal, it's in vitro anti-fungal activities in ethanol extract has been assayed upon dermatophytes like *Trichophyton rubrum* and the results have been found good [9,10]. For investigating anti-bacterial and anti-fungal activity, *Moringa oleifera* seed crude extract was applied to several bacterial species (*Bacillus subtilis, Pasteurella multocida Staphylococcus aureus* and *Escherichia coli*) and some fungal species like (*Rhizopus solani* and *Fusarium solani*) and has repressed growth of all strains up to certain limit in all samples i.e. dialyzed, residue, supernatant and crude form. The repression of growth zones reflects better inhibition for bacterial species than fungal species [11].

The aqueous and ethanol leaf extract of said plant was extremely active for *Candida tropicalis* and *Saccharomyces cerevisiae* but was inactive for *Candida albicans* according to [12]. The fungal inhibition zones in plates containing leaf extract observed that the colony reduced in diameter inside the plates when compared with the control plates. The same leaf extract when applied to bacteria, results displayed more repression for *Escherichia coli,* which trailed by *Staphylococcus aureus,* then followed *by Klebsiella pneumonia, Pseudomonas aeruginosa,* and was least for *Bacillus subtilis* [13]. By paper disc diffusion method, slight inhibition was caused by all leaf extracts on entero-pathogens, while methanolic and aqueous extracts have more repressive influence on bacteria found in wounds [14,15]. Sprouting of spores, mycelium and radial growth of all pathogenic fungus been efficiently inhibited by the leaves, roots and the protective layer of pod extract [16]. The seeds of the said plant have an active microbial chemical compound 4(α-l-rhamnosyloxy)-benzyl isothiocyanate along with roots, which also contain benzyl. The former chemical compound produced in 8 to 10% during water extraction when ascorbic acid added to it. It can act against many bacterial and fungal species for which in vitro least bacterial preventing concentration is 56 tmol/l for *Bacillus subtilis* and 40 tmol/1 for *Mycobacterium phlei* [17]. Keeping in view the uses and importance of this plant the current research work was undertaken to evaluate the anti-bacterial and anti-fungal activities of this plant against the pathogenic species prevailing in the local hospitals.

Methods

Antibacterial activity

Collection and processing of plant specimen

Moringa oleifera L was collected from different parts of Khyber Pakhtunkhwa. The collected plant material was washed, dried and powdered.

Preparation of extraction and fractions

For the extraction of active metabolites, Cold maceration method performed. Seventy-five percent plant material in powdered form dissolved in two liter of ethyl alcohol and kept on incubation at 40°C for five days. The plant material afterwards filtered three times carefully and as a result, a clean filtrate obtained. The filtrate then undergoes through rotatory evaporator at 40°C for the removal of liquid portion and a semi dried extract obtained, which was then dried and liquefied in 100 ml double distilled water. Five different solvents were then used to dissolve fractionates namely n-hexane, chloroform, ethyl acetate, methanol and ethanol respectively with the help of separating funnel. All the solvents again passed through rotatory evaporator and concentrated solvent fraction obtained and labeled for further procedure.

Media used

For anti-bacterial activity, agar well diffusion method proves to be suitable. Potato dextrose broth (PDB) was used for this purpose.

Test for bacterial strains

The selected four bacterial strains have pathogenic nature, out of which one specie was gram negative and 3 species were gram positive e.g. of gram negative is *Serratia marcescens* and that of gram positive is *staphylococcus aurous, streptococcus mutans* and MRSA (*Methicillin resistance staphylococcus aurous*) respectively. These strains were obtained from Khyber Teaching Hospital, Peshawar.

Measurement of zones of inhibition

For comparison of zones of inhibition to normal extract, a negative control has been designed containing Dimethyl sulfoxide 20mg/ml and extracts were dissolved

in a similar manner and cefotaxime (standard antibiotics) was used as a positive control. The plant fraction about 75µl introduced into the wells of petri dish and then for uniform growth of the strains put the plates for 24 hours inside an incubator at 37°C. When the incubation period was completed, then transverse lines been drawn on the plates and diameter growth zone was measured with the help of a ruler. The experiment repeated repeatedly to calculate the standard data.

Test for fungal strains

During antifungal activity, the four fungal strains were selected i.e. *Fusarium oxysporum, Aspergillus flavus, Polysphondylium pallidum,* and *Alternaria alternate.*

Results

Due to vast sources of therapeutic substances present in *Moringa oleifera*, which can act as anti-microbial agents, for the said reason world health organization has advised both established and underdeveloped countries to utilize indigenous knowledge [18]. Different medicinal plants have different complex substances which are utilized for specific diseases and their results turn out to be outstanding. The chemical substances in these plants are separate and utilized in crude or processed or modified form throughout the world. Plants having biological and anti-microbial efficiency have got their identity [19-21].

Anti-bacterial activity of *Moringa oleifera*

For optimizing the anti-bacterial prospective of the medicinal plant, four bacterial strains have been selected based on their pathogenicity and harmful nature, we selected these species from different hospitals of Khyber Pakhtunkhwa. These strains include *Staphylococcus aureus, Streptococcus mutans, Serratia marcescens* and MRSA (Methicillin resistance *staphylococcus aureus*). The doctors present in these hospitals were complaining about their pathogenicity therefore we selected these species to optimize the effect of plants extracts and develop efficient drugs against these bacterial strains. *Moringa*'s extract utilized in a tested amount of 6 mg/ml against organisms under experiments [22,23]. By observing the results, it was clear that some extracts have positive results and some fractions are completely inactive against the bacterial strains. Crude methanolic extract proved to be the best inhibitory fraction which repressed the growth of MRSA up to 48% (highest) followed by *S. mutans* and *S. marcescens* with 42% each and lowest inhibition was exhibited for *S. aureus* i.e. 38%. N-Hexane fraction prevented the growth of MRSA up to 44% and showed no activity against *S. aureus*, *S .mutans* and *S. marcescens* was 35 and 33% respectively. Chloroform was most active against *S. mutans* and MRSA with 46 and 40% but was inactive against *S. mutans* and *S. marcescens* with 0%. Ethyl acetate repressed the growth of *S. marcescens* with 47%, *S. mutans* with 42% and was inactive for the remaining two strains. Aqueous extract was less active against *S. aureus* with 15% and was inactive against remaining three species.

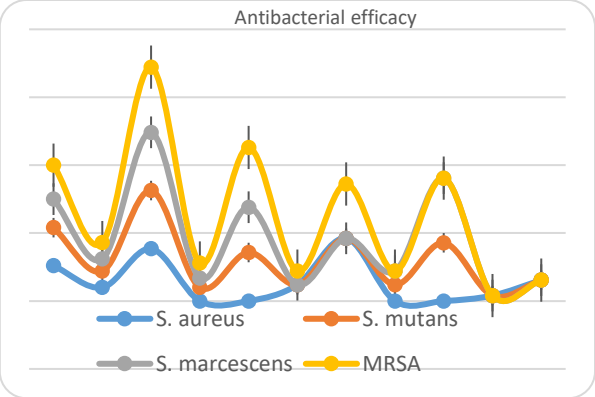

Figure 1: Antibacterial efficacy of *M. oleifera* against the tested species

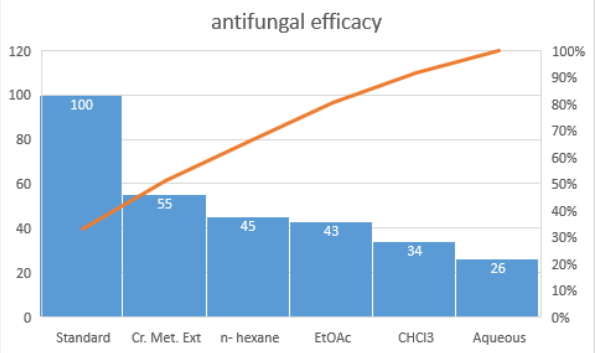

Figure 2: Antifungal efficacy of *M. oleifera* against the tested species

Anti-fungal activity of *Moringa oleifera*

Moringa extract proved to be very efficient against selected fungal strains. Crude methanolic extract repressed the growth of *A. alternate* with maximum percentage i.e. 60% and least percentage for *P. pallidum* and *F. oxysporum* with 45%. N-hexane was most active against *A. alternate* 53% and was least active against *P. pallidum* with 0%. Chloroform repressed the growth of *A. alternate* up to 60% ranging towards least for *A. flavus* and *P. pallidum* with 34%.

Ethyl acetate and aqueous extract was moderately active overall, the former inhibited the maximum growth of *Aspergillus flavus* with 43% and minimum inhibition for *P. pallidum* with 23%. Aqueous extract was most active against *A. alternate* with 30% and was completely inactive against *P. pallidum*.

Discussion

Since the age of mankind, has utilized plants for the food, medicinal purposes and many other purposes but its use as medicines was regarded as one of the best source ever [24]. Microorganism are the cause of almost 90% of all human's diseases, which needed to be stopped [25]. Plants have been used for this purpose and with time, evolution is occurring in microorganisms due to which their attacks have become more severe and harmful and the traditional medicines cannot cope with problem

completely [26,27]. To find new solution to prevent these modified microorganisms, humans need to rethink and discover new and efficient drugs [28]. Medicinal plants have certain metabolites due to which it attract this view to themselves and serve as an easy and cheap way of curing diseases that could be fatal for people living in remote areas of the world [29]. For the said purposes, we experimented anti-microbial activity of a medicinal plant *Moringa olifera,* in search to discover new and efficient anti-microbial drugs. [30]. Four strains of both bacterial and fungal species been selected based on their pathogenic nature reported from hospitals and their growth was checked against extracts of the plants in five different fractions i.e. Crude methanolic extract, n-hexane, chloroform, ethyl acetate and aqueous extract. The bacterial strains include *Streptococcus aureus, Staphylococcus mutans, Serratia marcescens* and Methicillin-resistant *Staphylococcus aureus* (MRSA) and fungal strains were comprised of *Aspergillus flavus, Alternaria alternate, Fusarium oxysporum* and *Polysphondylium pallidum.*

By observing the results of anti-bacterial activity, it was quite clear that growth stopped by extracts in different fractions but in some fractions, the bacterium did not get any harm from the extracts [31]. Crude methanolic extract showed best activity against all bacterial strains and was most active against MRSA with 48% and least active against *Streptococcus aureus* with 38%. n-hexane fraction stopped the growth of MRSA upto 44% and moderately stopped the growth of *S. mutans* and *S. marcesens* with 35% and 33% but was inactive against *S. aureus.* Chloroform inhibited the growth of *S. aureus* with 46% followed by MRSA with 40% but was inactive against the other two species. Ethyl acetate repressed the growth of *S. marcesens* and *S. mutans* but was inactive for the remaining two species. Aqueous extract proved to be least active and only inhibited the growth of *S. aureus* upto 15% and was inactive for the remining species.

High anti-fungal activity exhibited by the plant extract in mainly two fractions i.e. crude methanolic extract and chloroform but overall it proved to have very efficient results. The repression of growth in crude methanolic extract was maximum for *A. alternata* with 60% followed by *A. flavus* with 55% and least for *F.oxysporum* and *P.pallidum* with 45%. In chloroform, maximum zone of inhibition produced by *A.alternate* with 60% followed by *F. oxysporum* with 45% and minimum zone of inhibition exhibited by *A.flavus* and *P.pallidum* with 34%. n-hexane fraction mostly affect the growth of *A. alternata* with 53% and did not affect *P. pallidum* at all. Moderate activity then shown by both ethyl acetate and aqueous extracts ranging from highest of 43% for A.*flavus* and lowest of 20% for *F.oxysporum* [32, 33]. The overall results were very significant with p value for antibacterial activity = 1.57×10^{-06} and p= $1.19X\ 10^{-07}$for antifungal activities.

Competing interest

All the authors declare that they have no competing interest that can affect the current study.

Authors' Contribution

Khushnood ur Rehman: Conducted the experiments and wrote the paper

Muhammad Hamayun: Provided chemicals and reviewed the paper

Sumera Afzal Khan: Designed the experiments and reviewed the data

Shahab Saeed Khan: Conducted experiments and wrote paper

Sher Wali: Reviewed and formatted the paper.

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