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Assessing the curative property of Moringa oleifera and investigating its mechanism of action against urinary tract infection

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Abstract: The crude ethanolic, methanolic and ethyl acetate extracts were investigated against six uropathogens, where five were Gram negative and one Gram positive. All the isolates were multi drug resistant. Among a total of 746 isolates, *E.coli* was the predominant ranging about 324. The crude extracts showed activity against both gram positive and gram negative bacteria. Among the three extracts tested, the ethanol extract demonstrated remarkable activity. The extracts showed zones of inhibition ranging from 2mm to 11mm respectively. They were further subjected to investigation of minimum inhibitory concentration (MIC) against the same gram positive and gram negative bacteria. The minimum inhibitory concentration ranged between 6.25µg/ml and 100.0µg/ml concentration. The lowest activity of MIC was shown against *Pseudomonas aeruginosa* at 6.25µg/ml respectively. Pterygospermin, the reported active compound from *Moringa*, the miracle plant can not only serve as a drug, but also as nutritional supplement. However, further experimental and research efforts are under progress to study the activity of the fractions and isolate the active compound.
Key words: Pterygospermin, minimum inhibitory concentration, *Moringa,* uropathogens

Introduction

Infections are a serious health problem infects millions of people each year. Among the infections, Urinary Tract Infection (UTI) is one of the major infections caused, and its being the second most common type of infection in the body. Urinary tract infections account for about 8.3 million doctor visits each year. Women are especially prone to UTIs. These are treated withantibacterial drugs. Emergence of pathogenic microorganisms that are resistant or multi resistantto a major class of antibiotics has increased in recent years due to indiscriminate use of synthetic antimicrobial drugs. In addition, high cost and adverse side effects are commonly associated with popular synthetic antibiotics (such as hypersensitivity, allergic reactions, etc.), and are serious burning global issues in treating infectious diseases. Antimicrobial resistance amonguropathogens causing community and hospital acquired urinary tract infections is increasing (1). Despite the widespread availability of antibiotics, UTI remains the most common bacterial infection in the human population. Antibiotic resistance has increased rapidly during the last decade, creating a serious threat to the treatment of infectious diseases. Drug resistance is one of the most serious global threats to the treatment of infectious diseases (2). In addition to resulting in significant increases in costs and toxicity of newer drugs, antibiotic resistance is eroding our therapeutic armamentarium. Resistant strains of bacteria are continuing to increase, both in number and in variety, but not significantly different newer antibiotics are yet available. Treatment of infections caused by these resistant bacteria has become very difficult. Since they are resistant to many antibiotics, therapeutic options have become limited. Therefore, alternative methods of treatment are sought after.Plants are the oldest

source of pharmacologically active compounds, and have provided humankind with various medically useful compounds for centuries (3). Today it is estimated that more than two thirds of the world's population relies on plant derived drugs; In the USA approximately 25% of all prescription drugs used contain one or more bioactive compounds derived from vascular plants. Thus phytochemical screening of plant species, especially of ethnopharmaceutical use, will provide valuable baseline information in the search for new pharmaceuticals. Hence screening of antimicrobial plants for new agents poses an enormous challenge and is important especially with the emergence of drug resistant disease strains. It has only been in the past two decades or so that interest in higher plant antimicrobial agents has been reawakened world wide, and the literature in this area is becoming substantial.

Methodology

Collection of plant materials

Healthy disease free *Moringa oleifera* leaves were purchased from local supermarket at Tiruchirappalli District, Tamilnadu, India during the month of March and authenticated by the botanist and a voucher specimen was deposited in the herbarium of the department of Botany, Bishop Heber College, Tiruchirappalli 620 017, Tamil Nadu, India.. The leaves chosen for the study had been washed, shade dried macerated and lyophilised. The powder was extracted using ethanol in Soxhlet apparatus separately using 1 L of ethanol for 18h and then filtered. About 500g of *Moringa oleifera* leaves yielded 40g powder. The procedure was repeated to collect the needed quantity. The filtrates were evaporated to dryness under reduced pressure and at 40C in a Rotary evaporator (4). The dried residues were stored in airtight containers at -70C until further tests. The procedure was repeated with ethyl acetate and methanol respectively.

Specimens collected from urine were cultured on UTI agar /cystine lactose electrolyte-deficient medium, (Hi-Media, India) by the semi quantitative method and the specimens yielding colony counts = 10^4/ml were interpreted as diagnostic of UTI (5) and identification of isolates was done using standard microbiological techniques.

Detection of MRSA

All isolates in *Staphylococcus* species were tested for susceptibility to oxacillin by the agar screen method using 6 µg/ml oxacillin as recommended by the CLSI(6). Agar plates were incubated at 35°C and read at 24 hours and 48 hours incubation. Organisms growing on the plate were considered to be methicillin resistant.

Antimicrobial Susceptibility Testing

Disc Diffusion test (7)

All isolates were tested for susceptibility to the extracts and antimicrobial agents on Mueller Hinton agar (Hi-Media India) by the standard disc diffusion method recommended by the National Committee for Clinical Laboratory Standards. The diameter of the zone of inhibition of growth was recorded, and interpreted by the criteria of CLSI.

Determination of Minimal Bactericidal concentration (MBC)

The minimal bactericidal concentration (MBC) can be determined by subculturing the contents of the tubes onto extract-free solid medium and examining for bacterial growth. The Minimal Bactericidal Concentration (MBC) assay is performed as an adjunct to the MIC and is used to determine the concentration of the extract that is lethal to the target bacteria in vitro.

Docking

The structures of compounds were drawn using the online chemical compound drawing tool Marvin Sketch. The target protein was downloaded from PDB and docking of the compounds was done using the software GEM DOCK (a Generic Evolutionary Method for molecular docking).

GEMDOCK is a program for computing a ligand conformation and orientation relative to the active site of target protein.

Table1: Inhibitory effects of the different extracts of *Moringaoleifera*on ESBL-producing urinary pathogens by disc diffusion method (1000«g/10«l/disc)

Bacteria	Dia Of Zones (mm)		
	Methanol	Ethanol	Ethyl Acetate
Escherichia coli	3	6	4
Klebsiellapneumoniae	4	5	8
Pseudomonas aeruginosa	-	11	4
Proteus mirabilis	-	2	6
Citrobacterfreundii	-	4	9
MRSA	5	6	2

Table2: Antibacterial activity showingMinimum Inhibitory Concentration of the ethanolic extract of *Moringaoleifera* leaves

Name of Plant	Minimum Inhibitory Concentration(mg/ml)					
Moringaoleifera	*E.coli*	*Klebsiella*	*P.aeruginosa*	*P.mirabilis*	*C.freundii*	MRSA
	25	25	6.25	100	25	25

Results

Screening of Urinary Isolates

Urine samples from pregnant women suffering from urinary tract infection (UTI) were further processed by culturing on Mac Conkey agar and a special chromogenic UTI agar and were identified by various biochemical tests.

Screening for antibacterial activity by disc diffusion method

Activity of a given extract was determined by measuring the zones of inhibition (ZOI). Because zones of inhibition were often asymmetrical, experiments were repeated three times and the average was recorded.

Antibacterial activity of *Moringaoleifera*

Table 1 displays the results of the antibacterial testing for the various extracts of *Moringaoleifera* against the drug-resistant bacterial isolates and standard strains respectively. Measured inhibitory zones displayed in the table shows that among the three extracts used for the study, ethanol and ethyl acetate extracts demonstrated inhibitory activity against all the bacterial isolates under study and ranged between 2mm and 9mm. It is also seen that the extracts had no varied response to Gram positive and negative organisms. No zones were produced by the dimethyl sulfoxide (DMSO), the suspending solvent and the solvent only discs, indicating their non-involvement in the inhibitory role. Among the extracts of *Moringaoleifera*, ethanol and ethyl acetate extracst clearly demonstrated a far more superior effect than methanol.

Effect of the extracts on Methicillin-resistant *Staphylococcus aureus* (MRSA)

The MRSA strain selected for the study, in addition to being resistant to oxacillin was also resistant to other antibiotics as, ampicillin, amikacin, cefuroxime, cotrimazole, ciprofloxacin, gentamycin, nalidixic acid, ceftazidime, nitrofurantoin, ofloxacin and imipenem. This strain was effectively inhibited by the ethanol extract (ZIO=6mm) than the other two extracts.

Minimal Bactericidal Concentration of the extracts by broth dilution method

The MIC values of the two effective extracts of *Moringaoleifera*against the drug-resistant bacterial isolates and standard strains respectively are given in tables (Tables 2&3). As shown, the MBC values were lower for the ethanol extract than the other. Lower value of the MBC values of the ethanol and ethyl acetate extracts against these bacterial isolates fell in the range between 6.25mg/ml and 100mg/ml. A maximum activity by ethanol

extract was against *Pseudomonas sp* and the MBC was 6.25mg/ml, while it was 12.5 by the ethyl acetate extract against *C. freundii.*

Docking

From the output, the best docked poses were viewed and analyzed for the the binding energy, the H bonds and Vander Waals interaction values. The table showing the active site residues of the target protein interacting with the compounds was created and saved (fig 1, tables 3,4).

Fig 1 :Pterigospermin interacting with the active sites of the target protein.

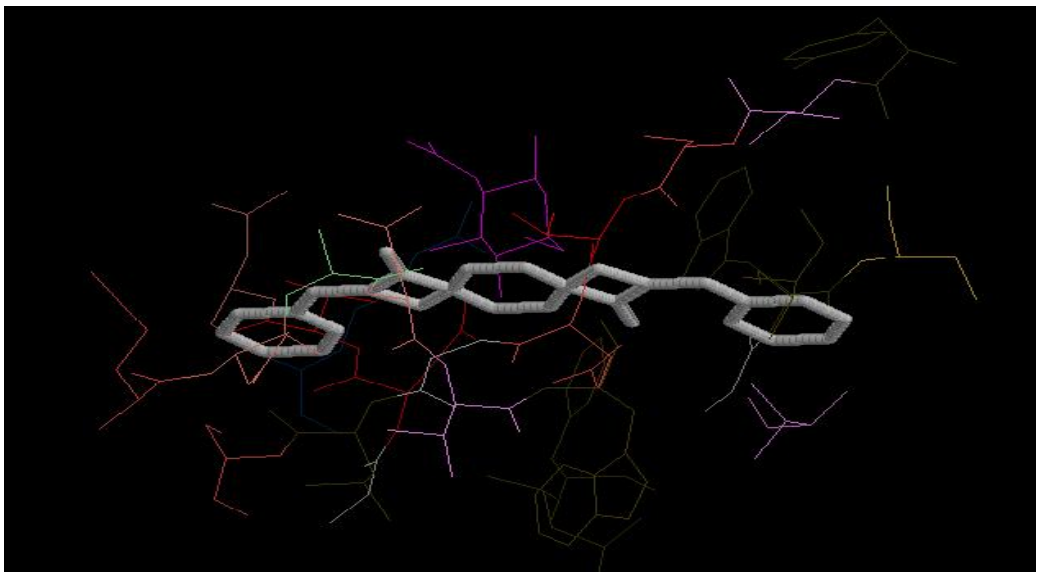

Table 3: Interaction of the ligand n-acetyl glucosamine with the target protein

		Compound	Energy	VDW	HBond	Elec
1	☑	cav1OIO_NAG-nag-lig-1.pdb	-75.55	-46.24	-29.31	0

Table 4: Interaction of the docked compound with the target protein

Compound	Energy	VDW	HBond	Elec
Pterygospermin	-105.58	-104.45	-1.13	0

Discussion

Every year, disease causing microbes and bacteria are getting more and more resistant to common antibiotics such as penicillin and amoxicillin. Superbug Drug-Resistant Health Threat Bacteria and microbes evolve to build up resistances because of the overuse of antibiotics. Studies have revealed that they have been systematically been overprescribed over the past decades, giving the most mundane strains of bacteria plenty of opportunities to build up defenses against them. Already, several antibiotic resistant strains of this once manageable disease have emerged, with doctors and scientists scrambling to find new kinds of antibiotics. Bacteria and microbes evolve to build up these resistances because of the overuse of antibiotics. Studies have revealed that they have been systematically been overprescribed over the past decades, giving the most mundane strains of bacteria plenty of opportunities to build up defenses against them. This limits therapeutic options. Hence alternative drug sources are sought after. Though several plants have been screened against bacteria, very few reports of plants against multi drug-resistant bacteria are available from India. Aqil*et al.*, (8)

have evaluated the ethanolic extracts of 10 Indian medicinal plants for their ability to inhibit chemical isolates of beta-lactamase producing methicillin resistant and methicillin sensitive *Staphylococcus aureus*and found flavonoids from the plants to be the active compounds with a MIC range of 1.3-8.2mg/ml.

Also Ahmad and Aqil (9) have reported that fractions from 15 traditionally used Indian medicinal plants were active against ESBL-producing multidrug-resistant enteric bacteria. They also had reported 45 Indian medicinal plants having activity against multi drug-resistant human pathogens. Also, certain bioactive plant extracts on beta-lactamase producing methicillin resistant *Staphylococcus aureus* was reported. Though many of the plants are reputed in the indigenous systems of medicine for their antimicrobial activities, yet, several are unknown in the medical community, since they remain to be scientifically established along with their active compounds.Among the several plants were screened for antibacterial activity, *Moringaoleifera,* a plant indigenous to India, attributed with several medicinal properties has not been screened against UTI. The Moringa plant provides a rich and rare combination of compounds which contribute to its therapeutic and and high nutritional value.*M.oleifera* is very important for its medicinal value. Various parts of this plant such as the leaves, roots, seed, bark, fruit, flowers and immature pods act as cardiac and circulatory stimulants, possess antitumor, antipyretic, antiepileptic, anti-inflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, cholesterol lowering, antioxidant, antidiabetic, hepatoprotective, antibacterial and antifungal activities, and are being employed for the treatment of different ailments in the indigenous system of medicine, particularly in South Asia. The active principle when isolated can not only serve as a drug, but also a nutritional supplement. This initial screening has revealed the potential of this plant as treatment for urinary infections.The isolated phytocompounds are known to be biologically active and therefore aid antimicrobial activities of the plant. However, further experimental and research efforts on the plants and their extracts are needed to be able to specify the pharmacological implication. Other details needed will include tests using other solvents, ultraviolet, infrared spectrometry, MS and NMR of the constituents of the fractions.

The ability of molecular docking methods to locate selective inhibitors reinforces our view of the structure-based drug discovery as a valuable strategy, not only for identifying lead compounds, but also for addressing receptor specificity. This study focuses on series of compounds that are screened for a successful candidate drug against the target GAFD (F17C-TYPE) FIMBRIAL ADHESIN FROM ESCHERICHIA COLI. With the active site residues of the target, the ligand N-acetyl glucosamine (NAG) was found to exhibit energy of -75.5 Kcal/mol. The interaction of the ligand with the target molecule, and the compound with the target molecule are shown in tables 3&4 respectively. The fitness score for the results can be calculated as follows:Fitness = Energy + VdW+ H bond+ Elec. The fitness value of pterigospermin were calculated as 211.16 respectively.From the results of fitness score and energy, pterigosperminis found to score well.

Conclusion

Moringaoleifera has been proved to possess activity against urinary tract infections and the active compound, pterigospermin was found to dock well with FimH, suggesting that the active compound can inhibit the binding of the bacteria with theuroepithelial cells. This promising result gives scope for further investigations leading to drug discovery.

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