Antimicrobial activity of some plant extracts against resistant pathogens

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Abstract

Resistance to antibiotics is one of the prime problems dealing with international health today, as well as significant reason of death over the globe. the aim of the study is to determine antibacterial effects of fresh Neem (*Azadirachta indica*) leaves and Methi (*Trigonella foenum-graecum)* extracts against common resistant pathogens of Methicillin resistant staphylococcus aureus, , ESBL producing bacteria *Escherichia coli*,*Klebsiella pneumonaie* and *Pseudomonas aerogenosa* using Kirby-Bauer disc diffusion and serial dilution method The antibacterial effects of aquous and methanolic plants extract of Azadirachta indica leaves extracts, were analysed and there was no zone of inhibition was found against MRSA at all dilution. zone of inhibition against *Escherichia coli* , at pure concentration was 28mm. zone of inhibition against *pseudomonas aerogenosa* at pure concentration was 16mm. zone of inhibition against *Klebsiella pneumonaie* at pure concentration was 13mm.Similarly we determined the antibacterial effects of aquous and methanolic plants extract of Methi (*Trigonella foenum-graecum*) was analysed and measured highest zone of inhibition at pure concentration against *pseudomonas aerogenosa* of 14mm and zone of inhibition of 12mm against *E.coli* was measured, zone of inhibition of 6mm at pure concentration was measured against MRSA and no zone of inhibition was measured against *klebsiella pneumonaie* at all concentration . These plant extracts which proved to be potentially effective can be used as natural alternative preventives to control infection.

Keywords

Azadirachta indica, Trigonella foenum-graecum, Antibacterial

Introduction

Resistance to antibiotics is one of the prime problems dealing with international health today therefore, it has become one of the significant reasons of death over the globe. At the present time it is far becoming the main reason of loss of life worldwide. Various micro-organism have attained and established mechanism of resistance, which creates a load on the system of health worldwide with the increasing economic cost. Number of fatalities associated with the resistance to antibiotics have been increased due to unavailability of alternative medicines. 23,000 people lost their life per annum in America owing to resistance to drug. Infectious illnesses emergency and the expansion of antibacterial resistance in micro-organism resultant in failure of present antibiotics , has led to an vital necessity for the determination of novel, harmless, and effective antibiotics (Quelemes et al., 2015).

Traditionally, the inspiration for new medicinal molecules has come from plants, as medications produced from plants that have significantly improved human health and wellbeing. Despite the fact that many naturally occurring medications have been replaced by synthetic ones that are highly strong, some drug components still come from trees.

For a long time, medicinal plants have played a significant role in the treatment of various illness conditions, including diabetes, malaria, and anemia. Despite that the use of higher plants for novel medications is still remaining. A thorough screening of them could lead to the discovery of brand-new, potent chemicals. (Harjai et al., 2013).

Plants containing secondary metabolites like flavonoids, tannins, steroidals, terpenoids, saponins, coumarins, xanthones, lignans, , alkaloids and iridoids are known to possess effects against microbes. (Saleem et al., 2010; negi, 2012; nunes et al., 2020).

The bark, flowers, fruits, seeds, roots and leaves of Neem tree are known to use in the past as anti-inflammatory, anti-septic and anti-pyretic. Also, it was used for dental problems, skin problems and fever.

Neem tree leaf is the principal part of the tree that has been utilized mainly. It consists of constituents that treat inflammation, hyperglycemia, ulcer, malaria and infections that are caused by bacteria, virus and fungi. Moreover, it has actions such as immunomodulation. It has effects against inflammation, oxidation, and cancer (Banna et al., 2014).

Trigonella feonum-graecum L. is called as Fenugreek (Family: Fabaceae). *Fenugreek* is advantageous therapeutic herb that is used from past years. The leaves as well as stem of Fenugreek has found to have marked action against bacteria (Sharma et al., 2016).

Studies on Anti-bacterial action of Azadirachta indica and *Trigonella feonum-graecum* L on resistant pathogens are rare in Pakistan. The investigation was done to evaluate the anti-bacterial action of extracts of Neem Leaves as well as *Trigonella feonum-graecum* L by using several significant human resistant infectious bacteria to investigate its potential to use as an antibiotic in upcoming years. The microbes that were used involved local clinical bacterial isolates like Methicillin resistant *staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, *Escherichia.coli*, and *Klebsiella pneumonaie*.

Material and Methods

Plants extraction preparation

Fresh Neem (*Azadirachta indica*) eaves were collected from Memon Goth area of Malir town karachi and Methi (*Trigonella foenum-graecum*) were purchased from nearby store in Karachi, Pakistan. They were cleaned by using water, decontaminated then rinsed using distill water. After that they were kept in shade for drying.

The dried out(*Trigonella foenum-graecum*) Methi as well as Neem leaves were comminuted and formed into powder having fine particle size that could pass sieve of 100mm. Maceration was done following comminution. Thirty five gram of Neem leaves powder and (*Trigonella foenum-graecum*) Methi powder were taken individually and macerated into solvent prepared by mixing 250 ml methanol and 250 ml water following 48-hour of agitation. After that they were strained using double coverings of muslin cloth. Then there were centrifuged at speed of 9000 rotations per minute for a period of ten minutes. The final step consisted of filtration by using Whatman filter paper No. (41). Clear filtrates were obtained.

The remnants were evaporated and dried at forty degree Celsius under reduced pressure. Rotatory vacuum evaporator was used for this purpose. The extract yields were weighed then kept in small bottles at storage temperature of five degree Celsius. The percentages of yield were estimated by formula: Extract yield% = R/S * 100 (where R; weight of extracted Neem leaves residues and S; weight of Neem leaves raw sample).

Antibacterial activity of the plant extracts

Bacterial strains

There were 4 bacterial strains used to investigate antibacterial potency of individual plant. The microbes were proven to be resistant pathogens. One strain of Gram positive (*S. aureus* and three strains of Gram negative (*E. coli*,

Klebsiella pneumoniae and *Pseudomonas aeruginosa*) bacteria. The resistant bacterial strains were obtained from the culture collection of a private hospital clinical laboratory of Karachi Pakistan.

Inoculums preparation

Pseudomonas aerogenosa ,MRSA, ESBL producing bacteria *E.coli* and *Klebsiella pneumonaie* clinical isolates obtained from a private hospital microbiology clinical laboratory , which were proven to be resistant, were used. They were then inoculated on Media specific for each strain were prepared (MSA for MRSA, and MacConkey for *E.coli* and *klebsiella pneumonaie* and nutrient agar for *pseudomonas aerogenosa*,each bacterial strain was subcultured overnight at 35 °C in their respective media. In order to harvest growth of bacteria 5 ml of sterile saline water was used. The adjustment of absorbance was done at 580 micro-meter and dilution was done to obtain viable cell count of 10^7 CFU/ml using spectrophotometer.

Preparation of concentrations for antibacterial assay

Serial dilution

Serial dilution of extracts of Neem (*Azadirachta indica*) leaves as well as (*Trigonella foenum-graecum*) Methi was done. Sterile distilled water was chosen as a diluent. 9ml of sterile distilled water was placed in a tube and 1ml of individual crude extract was included in a tube. A serial dilution was completed by using this tube. Dilution range was from 10^{-1} to 10^{-7} (Francine et al 2015).

Bacteria inoculation and disc-diffusion method

Media specific for each strain were prepared (MSA for staphylococcus aureus, and MacConkey for *Escherichia.coli* and *klebsiella pneumonaie* and nutrient agar for *pseudomonas aerogenosa*) and on each labeled plate that contains the medium, was inoculated 40 µl of standardized broth culture of the bacteria. In order to distribute microbes uniformly on surface of plates, spreader was utilized. Kirby-Bauer explained this procedure as disc diffusion method. Disc diffusion method is applying to evaluate action of chemical agents on bacteria, hence this method was applied for comparison of effects of various Neem leaves as well as (*Trigonella foenum-graecum*) Methi extracts concentration, which was attained by solvents water and methanol. Filter paper discs were made and subjected to sterilization. After that the discs were immerse in various extract concentration. After being aseptic discs were kept over the media having specified bacteria. Filter paper discs having 5mg of Gentamycin was utilized as positive control. The plates were stored at 5 degree Celsius in the fridge. Storage time was two hours. This was done to allow diffusion of plant extracts. After that incubation was done at 35 degree Celsius. Incubation time was twenty-four hours. Vernier caliper was used to measure existence of inhibition zones which was noted and considered as sign of action against bacteria.

Results

Table 1 ethnobotanical data of employed plant species and their extract yield percentage

Plant species	family	Common name	Local name	Plant part used	Extract yield%
Azadirachta indica	Meliaceae.	Margosa	neem	Leaves	8.57
Trigonella foenum-graecum	Fabaceae	fenugreek	Methy	seeds	5.714

Table 1 shows the ethanobotanical data of *Azadirachta indica* and *Trigonella foenum graecum* and their extracts yield percentage.Azadirachta indica extract yield percentage was 8.57% and trigonella foenum-graecum yield 5.714%.

Table 2 Antimicrobial screening test of Aquous and Methanolic plants extract of *Azadirachta indica* leaves extracts against some resistant pathogens

Dilution concentration of plants leaves extracts of *Azadirachta indica*	**Zone of inhibition (mm)**			
	Methicillin resistant *Staphylococcus aureus*	***Escherichia coli***	***Pseudomonas aerogenosa***	***Klebsiella pneumonaie***
pure	Growth	28mm	16mm	13mm
10^1	Growth	13mm	10mm	8mm
10^2	Growth	12mm	Growth	5mm
10^3	Growth	11mm	Growth	Growth
10^4	Growth	10mm	Growth	Growth
10^5	Growth	10mm	Growth	Growth
10^6	Growth	9mm	Growth	Growth
10^7	Growth	Growth	Growth	Growth

Note pure stands for 100% concentration.

Above table shows properties of aquous and methanolic plants extract of *Azadirachta indica* leaves extracts against bacteria. There was no zone of inhibition was found against MRSA at all dilution and at pure concentration,zone of inhibition against *Escherichia coli* were variable,at pure concentration the zone of inhibition was 28mm,and at 10^1 the zone of inhibition was 13mm and as the concentration decreases the zone of inhibition was also deceases.zone of inhibition against *pseudomonas aerogenosa* at pure concentration was 16mm and at 10^1 concentration the zone of inhibition was 10mm and at 10^2 dilution and onward no zone of inhibition was found against *pseudomonas aerogenosa*. zone of inhibition against *Klebsiella pneumonaie* at pure concentration was 13mm and at 10^1 dilution ,the zone of inhibition was 8mm and at 10^2 concentration the zone of inhibition was 5mm and there was no zone of inhibition was found on further dilutions.

Table 3 Antimicrobial screening test of Aquous and Methanolic plants extract of *Trigonella foenum-graecum* seeds extracts against some resistant pathogens

Dilution concentration of plants seeds extracts of *Trigonella foenum-graecum*	Zone of inhibition (mm)			
	Methicillin resistant Staphylococcus aureus(MRSA)	***Escherichia coli***	***Pseudomonas aerogenosa***	***Klebsiella pneumonaie***
Pure	6mm	12mm	14mm	Growth
10^1	4mm	10mm	10mm	Growth
10^2	2mm	9mm	9mm	Growth
10^3	Growth	8mm	8mm	Growth
10^4	Growth	7mm	7mm	Growth
10^5	Growth	7mm	Growth	Growth
10^6	Growth	6mm	Growth	Growth
10^7	Growth	6mm	Growth	Growth

Above table 3 shows the antibacterial effects of *Trigonella foenum-graecum* seeds extracts against some resistant pathogens. The highest zone of inhibition of 14mm was measured against *pseudomonas aerogenosa* at pure (100%) concentration an no zone of inhibition was found against *klebsiella pneumonaie*.

Zone of inhibition was measured 6mm against MRSA at pure concentration(100%) seeds extracts of *Trigonella foenum-graecum*,zone of inhibition was measured 4mm against MRSA at dilution of 10^1 ,zone of inhibition was measured 2mm against against MRSA at dilution of 10^2 and at dilution 10^3 and onward no zone of inhibition was measured.

Zone of inhibition was measured 12mm against Escherichia coli at pure (100%) concentration using methanolic extracts of seeds extracts of *Trigonella foenum-graecum*,zone of inhibition was measured 10mm at dilution of 10^1 . zone of inhibition was measured 9mm and 8mm at dilution of 10^2 and 10^3 ,at dilution 10^4 and 10^5 zone of inhibition was measured 7mm and dilution of 10^6 and 10^7 zone of inhibition was measured 6mm.

Zone of inhibition was measured 14mm against *pseudominas aerogenosa* which was highest among other pathogens used in this study , using methanolic extracts of seeds *of Trigonella foenum-graecum* at pure (100%) concentration.zone of inhibition was measured 10mm at dilution of 10^1 zone of inhibition was measured 9mm at dilution of 10^2 and zone of inhibition was measured 8mm and 7mm at dilution of 10^3 and 10^4.and at further dilution no zone of inhibition was found. No zone of inhibition was measured against any concentration and dilution against *klebsiella pneumonaei*.

Discussion

This study analyzing antibacterial effects of two plants parts neem leaves and *Trigonella foenum-graecum* seeds extracts against common resistant pathogens using aquous and methanolic solvent.Very rare studies are conducted in Pakistan on antibacterial effects of Pakistani neem leaves and *Trigonella foenum-graecum* seeds extracts using aquous and methanolic solvent on resistant pathogens.

Antibacterial effects of Neem leaves extracts

In the present study we use combination of methanol and water as extracts solvent to analyse antibacterial effects of Neem leaves,our results showed no zone of inhibition against MRSA. Comparing to other studies in a study conducted by Wendy C. Sarmiento et al in 2011 and found a zone of inhibition 7.67mm at 100% concentration using ethanolic extracts .Another Study conducted by (Naeem et al., 2021) and measured zone of inhibition 14.23±1.37 against MRSA ,another study conducted by (Fasher et al., 2022) and found no zone of inhibition at 25% concentration and at 50% concentration he found 3mm zone of inhibition against MRSA and also found 16mm zone of inhibition at 100% concentration using Neem petroleum ether and methanol extract as solvent.anothers study conducted by (Noor et al., 2023) and found zone of inhibition of 9.8mm both in methanolic extracts and ethanolic extracts against MRSA strains.

In the present study we found zone of inhibition 28mm against *Escherichia coli* using water and methanol solvent.Comparing to other studies conducted by (Sukanya et al., 2009) and found the zone of inhibition of 10mm and 9mm against *E.coli* using aquous and methanol solvent but the study conducted by (Yaseen, 2016) and found zone of inhibition 21mm against *E.coli* using cold aquous extracts he also found zone of inhibition of 6mm against *pseudomonas aerogenosa* using cold aqous extracts but in our study we found zone of inhibition of 16mm which is much hghe.In the present we found zone of inhibition of 13mm against *klebsiella pneumonaie* comparing to other studies in a study conducted by (Fasher et al., 2022) and found no zone of inhibition against *klebsiella pneumonae* using Neem petroleum ether and methanol extract.another study conducted by (Chaturvedi et al., 2011) and found zone of inhibition of 12.5mm against *klebsiella pneumonaie*. This study highlights the capability of neem extracts to compound medicinal agents that can overcome multidrug regimen resistant bacteria. Moreover, antimicrobials contain various adverse effects that can overcome by combining antibiotics and extracts as there would be reduced antibiotic exposure. Hence, economical as well as safe medicinal agents could be developed.

Antibacterial effects of *Trigonella foenum-graecum*

- In current study the properties of *Trigonella foenum-graecum* was found against different resistant pathogen Trigonella foenum-graecum. we measured the zone of inhibition of 6mm at pure 100% concentration against MRSA.
- Several studies in past evaluated the properties of fenugreek against microbes.
- one of the studies conducted by **(**Onyancha et al., 2021) and concluded that Methanol *Trigonella foenum-graecum* extract Showed marked antibacterial action against MRSA.
- another study conducted by (Alluri et al., 2014) and measured zone of inhibition of 12.5±0.28 mm against MRSA at concentration of 2mg/ml and he also determined zone of inhibition of 16.1±0.19 mm at 2mg/ml against E.coli and also found zone of inhibition 14.6±0.21 against pseudomonas aerogenos at 2gm/ml concentration.In the present study we determine zone of inhibition of 12mm aginst *E.coli* at pure 100% concentration and we also determined zone of inhibition of 14mm against *pseudomonas aerogenosa* at 100% concentration.Another study conducted by (Al-Timimi, 2019) anddetermined the antibacterial properties of *Trigonella foenum-graecum* and measured zone of inhibition of 3mm and 4mm in aquous and ethnolic solvent against *E.coli*. He further measured zone of inhibition of 14mm and 16mm against *pseudomonas aerogenosa* in aquous and ethnolic solvent of *Trigonella foenum-graecum*.Another study conducted by Babaei H.A et al 2018 and determined antibacterial effects of *Trigonella foenum-graecum* and measured no zone of inhibition against *Ecoli*,*Klebsiella pneumonae* and *pseudomonas aeogenosa* using aquous ,methanolic and ethanolic extracts of *Trigonella foenum-graecum.*
- One of the findings conducted by Norziah et al., concluded that fenugreek seed ethanolic extract revealed an inhibition zone of 70.3 mm on *Escherichia coli.* The inhibitory zone of methanolic extract was 44.1 mm. the inhibitory zone of ethanolic extracts of *Staphylococcus aureus* was 38.2 mm whereas, methanolic extracts for same microbe was 25.9 mm.
- The findings conducted by (Nandagopal et al., 2012) concluded that 100 micro-liter concentration of aqueous extract of fenugreek seed showed an inhibitory zone of 9.2 mm on Pseudomonas aeruginosa whereas, ethanolic extract showed an inhibitory zone of 16.3 mm on same microbe. The inhibition value of aqueous extract of fenugreek seed on *Staphylococcus aureus* was 6.45 mm whereas, ethanolic extract showed an inhibitory zone of 13.9 mm on same microbe.
- Our study revealed that difficulty can arise during process in preparation or diffusion of extract in culture media. Moreover, plants in various geographical areas contain various pharmacological properties. These factors have

significant contribution to their antibacterial properties. Hence, additional findings can evaluate antimicrobial effects of these extracts using high-performance liquid chromatography or gas chromatography techniques.

Conclusion

Our findings showed that aqueous as well as methanolic extracts of Neem leaves revealed no activity against MRSA strains and good results *E.coli* and comparable results against *pseudomonas aerogenosa* and *klebsilla pneumonaie*. Aqueous and methanolic extracts of *Trigonella foenum-graecum* have minor results against MRSA and no results *klebsiella pneumonae* and comparible reults against *E.coli* and *pseudomonas aerogenosa*.

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