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COMPARATIVE ANALYSIS OF ANTIBACTERIAL ACTIVITY IN DIFFERENT PLANT PARTS OF TRIGONELLA FOENUM GRACEUM(L.)

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ABSTRACT

Fenugreek (*Trigonella foenum graceum*) is one of the most promising medicinal herbs, known from ancient times and widely been consumed throughout the world as food, food additive and in traditional medicine since many years. Recent studies focus on plants research all over the world to extract appropriate and efficient antimicrobial drugs. The present study aimed preliminary to investigate comparative antimicrobial analysis of fenugreek leaves, stems, seeds of mature and immature plant, also dried plant (Kasoori methi) against gram-negative and gram-positive bacteria using different solvents Acteone and Methanol. The antimicrobial activities were evaluated using agar well diffusion method. The result showed that methanolic extract was more effective than acetonic extract.

KEYWORDS: Fenugreek (*Trigonella foenum graceum)*, cotyledonary stage, Antibacterial, *Bacillus subtilis, Salmonella typhii.*

INTRODUCTION

In recent years attention has been devoted to novel molecules derived from plant sources which has replaced vast chemically broad-spectrum antibiotics. The demand of plant-based therapeutics seems to be increasing due to their incredible ventures, being non-narcotic, having no side effects, easily available at affordable prices. Plants used in traditional medicines contain a wide range of ingredients that can be used to treat chronic as well as infectious diseases.[1] Over years, these herbal drugs have been shown to be effective.[2] Plant based system still play a vital role in health care and WHO has estimated about 80% of

worlds habitatants rely mainly on traditional medicine for their primary health care. Plants are known to be useful for human benefits and welfare. Of these benefits, is the capacity of some plant seeds, fruits, leaves and other parts exerting pharmacological activities such as analgesics, diuretics, antispasmodics, antimicrobial activity.[1]

Plants have limitless ability to synthesize a vast array of bioactive compounds which possess some bio efficacy. These substances serve as plant defense mechanism again predation of microbes, insects, herbivores. Living organisms are prone to infection by several microorganisms, especially bacteria and fungi.[3,4] Fenugreek has also been used as an external poultice to control inflammation and dandruff.[5] In general, bacteria have genetic ability to transmit and acquire resistance against the drugs used as therapeutic agents (7 remain). Researchers are now in search of effects of various plant extracts of bacteria.[5,6] Reports are available on in-vitro and in-vivo efficacy of plant extract against plant and human pathogens causing fungal infections.[7] Keeping this in the view, the present study has been evaluate the antimicrobial effects of various extracts of plant parts.

Antimicrobial Activity

Recently, multiple drug resistance has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases making it a global growing-problem. Isolation of microbial agents less susceptible to regular antibiotics and recovery of increasing resistant isolates during antibacterial therapy is rising throughout the world which highlights the needs of new principles. Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanism of actions.[8-12] Microorganisms' viz., bacteria, fungi, viruses, and protozoa, which have the capacity to cause diseases, are referred to as pathogenic microorganisms. Pathogenic microorganisms can be killed and inhibited by agents of biological or non biological origin, commonly referred as antimicrobials. It is estimated that the crude extract of the whole plant or plant parts showed growth inhibition, growth retardant and the killing mechanisms for the microorganisms. The plant extracts have been providing defense and are safety models to about 50% Western drugs.[13]

Aromatic and medicinal plants are known to produce certain bioactive molecules which react with other organisms in the environment and microbial growth, accelerating sites in biological organisms and nutrient media, which is most favorable, for preventing and inhibiting bacterial and fungal growth.[14] The substances that can inhibit the growth of

pathogens and have little toxicity to host cells are considered for developing new antimicrobial drugs.[15]

MATERIALS AND METHODS

1. Sample collections and preparation

Healthy plants& Stems (Both Mature & Immature Plant), dried fenugreek (Kasoori Methi) and seeds(both type) (*Trigonella foenum graceum. L*) were collected from the local market of Surat, Gujarat. The leaf & stem of the plant was kept for the sun drying. After 10 days the leaf and stem were dried and crushed in to powdered form and used for the analysis. The seeds were also crushed in to powdered form and used for the analysis.

Collection of Microbes

Bacterial strains of *Bacillus subtilis* and *Salmonella typhii* were used for the study and were collected from stock cultures of microbiology lab, Surat.

Bacterial strains

Microorganisms used for the determination of antibacterial activities of isolated compounds were gram positive *Bacillus subtilis* and *Salmonella typhii* .Different bacterial strains were maintained on nutrient agar and subcultures were freshly prepared before use. Bacterial cultures were prepared by transferring 2-3 colonies into tube containing 20ml nutrient broth and grown overnight at 37ºC.

Determination of Antibacterial activity

Antimicrobial activities of different extracts were determined by different methods. However, agar well diffusion methods[16,17] was used during the studies.[16,17]

Peptone – 5 gm

Beef Extract – 3 gm

Agar-Agar – 15 gm

Distilled water – 1000 ml

pH – 7.0

MATERIALS AND METHODS

Distilled water

Cup borer

Petriplates

Foreceps

Agar well diffusion assay

The Nutrient agar was melted and cooled to 48 - 50ºC and a standardized inoculums (1.5×108 CFU/mL, 0.5 McFarland) was then added aseptically to the molten agar and poured into sterile petridishes to give a solid plate. Wells were prepared in the seeded agar plates. The test compound (100 µl) was introduced in the well (6 mm in diameter). The plates were incubated overnight at 37ºC.

The antimicrobial spectrum of the chemical compounds was determined for the bacterial species in terms of zone sizes around each well. The diameters of zone of inhibition produced by the agent were compared with those produced by the commercial control antibiotics. The control zones were subtracted from the test zones and the resulting zone diameter was measured with antibiotic zone reader to nearest mm. The experiment was performed three times to minimize the error and the mean values are presented in Table.

RESULTS

In present study, different extract of different plant parts from *Trigonella foenum graceum* (L.) in different solvents were investigated for their antimicrobial potential. Results of antimicrobial studies using agar well diffusion been presented in table 1and 2. It has been revealed that methanol extract shows more antibacterial effect than acetone extract against both gram positive and gram negative bacteria.

Methanolic extract of *Trigonella foenum graceum* exhibit significant zone of inhibition against both *Bacillus subtilis* and *Salmonella typhi.*

It has been found that in methanolic and acetonic extract the kasoori methi(Methanol:IZ=3.46) &(Acetone:IZ=3.36) has shown more antibacterial assay against gram positive bacteria than any other plant parts such as seeds, leaves and stems of both stages of plants.

After kasoori methi it has been shown that maximum antibacterial asaay was found in Mature plant leaf in both Methanolic and Acetonic extracts (Methanol: IZ=3.22) and (Acetone: IZ=3.13).

While, in both Methanolic and Acetonic extract against gram negative bacteria Mature plant leaf (Methanol:IZ=3) &(Acetone:IZ=2.69) shows more antibacterial assay that any other plants parts such as seeds,leaves and stems of both stages of plants.

After Mature plant leaf it has been shown that maximum antibacterial asaay was found in Green Seeds in both Methanolic and Acetonic extracts (Methanol: IZ=2.56) and (Acetone: IZ=2.63).

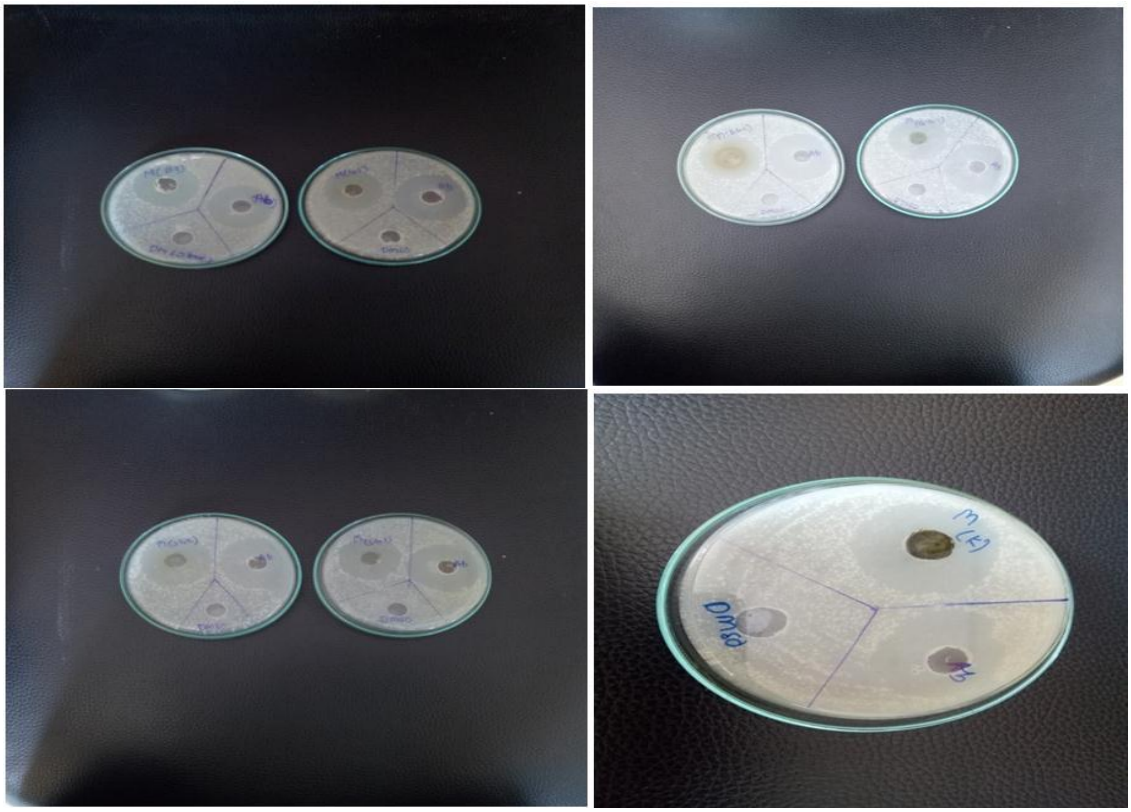
Table 1: Appendices.

Organism	**Plant Part**	**Diameter of Inhibition Zone in mm**	
(Standard deviation:4.2)		**Methanol Extract**	**Acetone Extract**
Bacteria			
Bacillus subtilis (ANTIBIOTIC: Penicillin)	Brown seeds	IZ = 2.96	IZ = 2.73
	Green seeds	IZ = 2.83	IZ = 3.06
	Mature Plant leaf	IZ = 3.22	IZ = 3.13
	Immature Plant leaf	IZ = 3.16	IZ = 3.13
	Mature Plant stem	IZ = 2.8	IZ = 2.86
	Immature Plant Stem	IZ = 2.96	IZ = 2.76
	Kasoori Methi	IZ = 3.46	IZ = 3.33

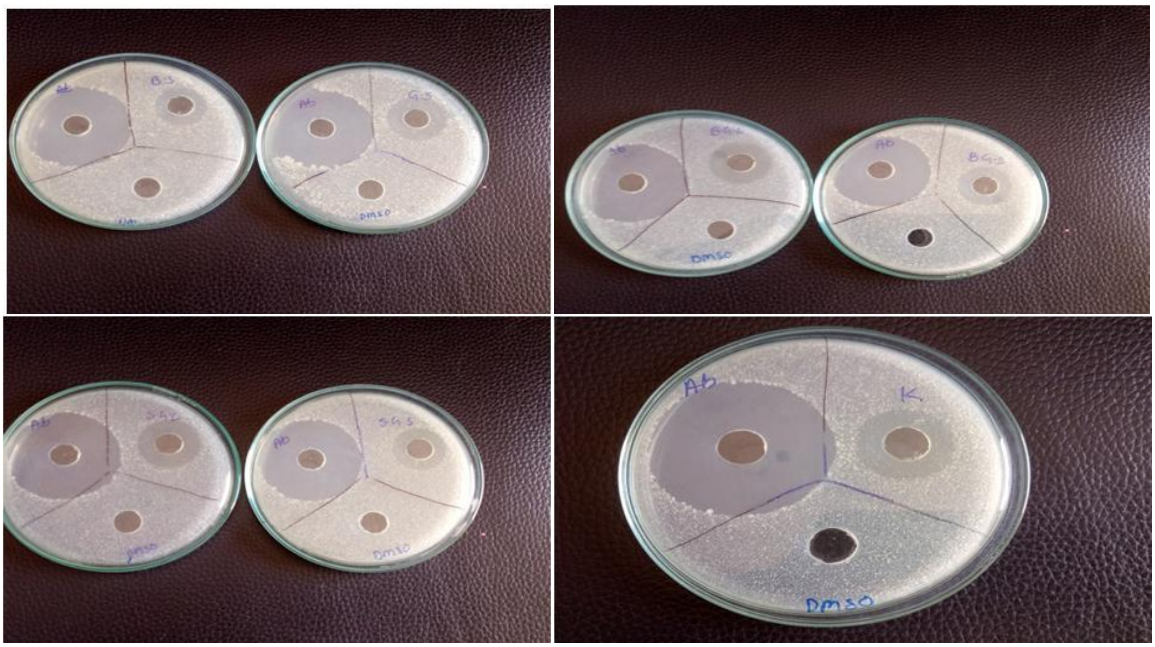
Table 2: Methanolic Extract (Gram Positive Bacteria).

Organism	**Plant Part**	**Diameter of Inhibition Zone in mm**	
(Standard deviation:3.9)		**Methanol Extract**	**Acetone Extract**
Bacteria			
Salmonella typhii ANTIBIOTIC(Tetracycline)	Brown seeds	IZ = 2.53	IZ= 2.23
	Green seeds	IZ = 2.56	IZ= 2.63
	Mature Plant leaf	IZ = 3	IZ= 2.69
	Immature Plant leaf	IZ = 2.76	IZ= 2.23
	Mature Plant stem	IZ = 2.56	IZ= 2.2
	Immature Plant Stem	IZ = 2.6	IZ= 2.23
	Kasoori Methi	IZ = 2.14	IZ= 2.16

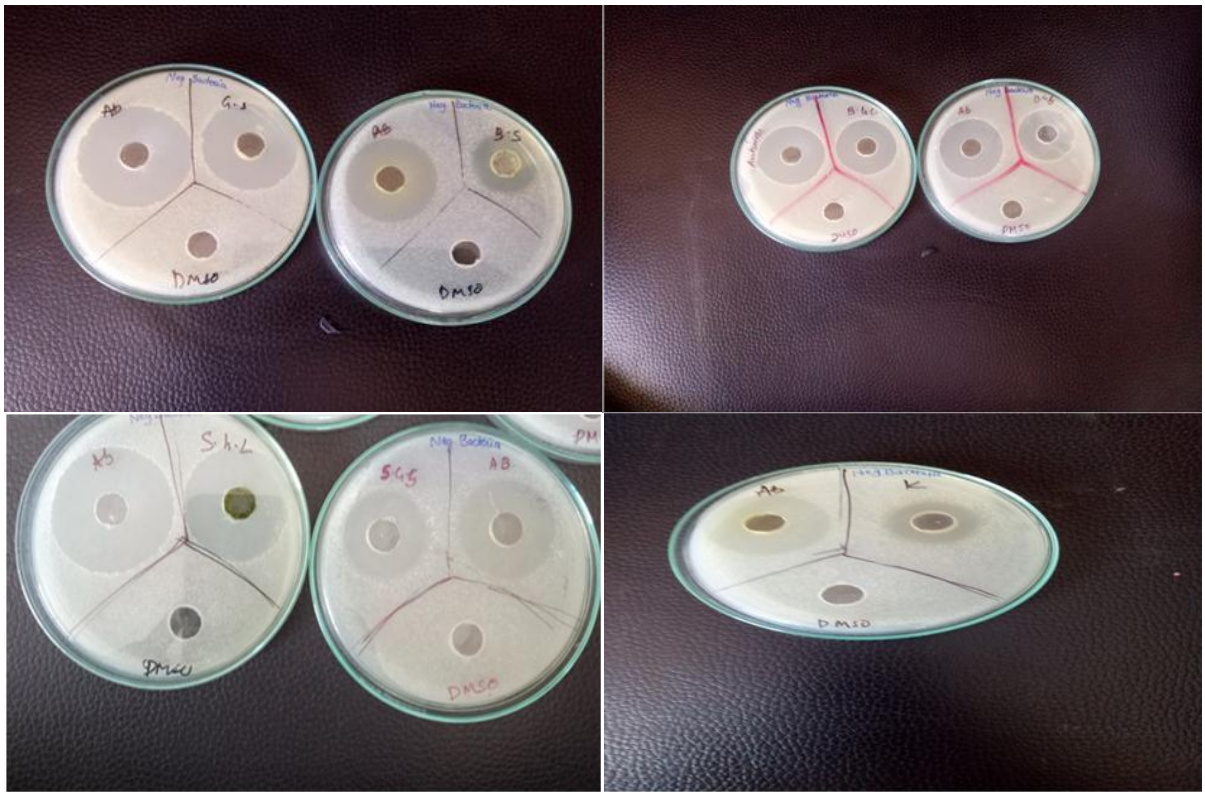
Methanolic Extract (Gram Positive Bacteria)



[Figure:1].



Gram Negative Bacteria



[Figure:2].

DISCUSSION

A number of investigators [Abdel-Massih R, Abdou E, Baydoun E and Z Daoud, Khanra R, Chatterjee A, Singh BP, Goel A and K Sen, Nandagopal S, Dhanalakshmi DP, Ganesh KA and D Sujitha, Premnath R, Sudesh J, Laxmi Devi N and SM Ardhya, Marzougui N, Boubaya A, Thabti I, Ferchichi A and A Bakhrouf] have evaluated the antibacterial efficacy of crude aqueous and organic extracts of seeds, leaves and stems of T. foenum-graecum against a variety of bacterial species including the antibiotic resistant ones and found to be significantly effective.[18-22]

Upadhyay, R. K., Ahmad, S., Jaiswal, G. Dwivedi, P. and Tripathi, R (2008) observed the Acetone extract of fenugreek showed promising inhibitory effect for *B. cereus, L. acidophilus* and *Pneumococcus.*[23]

Nandagopal et al., (2012) studies revealed that acetone extract of fenugreek has antibacterial activity against *Pseudomonas aerogenosa, E.coli, Salmonella paratyphi, Staphylococcus aureus.*[20]

In similarity, *Salmonella typhi* show antibacterial assay in both methanolic and acetonic extract.

BK Dash, S Sultana, N Sultana(2011) observed that Methanol extract of Fenugreek revealed antimicrobial activity against *Pseudomonas* spp.,whereas acetone extract of spices exhibited highest activity against *Escherichia coli*.[24]

Moradi N., Moradi K (2013) revealed that the Crude extracts of fenugreek seeds shows maximum growth in gram negative bacteria *Salmonella typhi* in acetonic extracts.[25]

Deans, S.G, and Barrata, M.T. (1998) revealed that in Acetonic extarct it was shown that it gives antibacterial effect in matureplant leaf and seeds.[26]

Sharma V, Singh P, Rani A. (2017) showed that the methanolic extract of fenugreek seeds give highest antibacterial activity.[27]

In similarity, Kasoori methi and mature plant leaf showed highest antibacterial activity in methanolic extract.

Kumar G, Karthik L, Bhaskara Rao KV.(2010) revealed that methanoilc extract show better antibacterial than acetonic against both gram positive and gram negative bacteria.[28]

Massih, R.A.; Abdou, E.; Baydaum, E. and Daoud, Z(2010) showed that methanol extract shows highest antibacterial activity.[29]

Premnath R, Sudesh J, Laxmi Devi N and SM Ardhya (2011) revealed that among acetone and methanol extract for seeds, stem and leaves the antibacterial activity was shown maximum in methanol extract.[21]

In present investigation methanolic and acetonic extract of in-vivo plant parts (leaves, stems. seeds and dried plant) of Trigonella foenum graceum were screened for their antibacterial activity againt Bacillus subtilis and Salmonella typhi.

In present study,dried plant i.e Kasoori methi showed largest zone of inhibition in bacterial culture of Bacillus subtilis (3.46)in methanolic extract, while no significant zone observed in the brown seeds.

Acetone Extract (Gram Positive Bacteria)

During this study, it was found that Leaf (both mature and cotyledonary) showed significant activity against Bacillus subtilis which may be due to the presence of alkaloids.

CONCLUSION

According to this study, plant based antibacterial drug have enormous therapeutic potential as they can serve the purpose with lesser side effects that are always associated with synthetic antimicrobial agents. The present study suggests that extract of fenugreek may have antibacterial activity against some human pathogen. According to present study, it was observed dried plant i.e. Kasoori methi was found to best effective as antibacterial agent. According to this study, methanol extract shows maximum antibacterial activity than acetone extract against both gram positive and gram negative bacteria. The higher antibacterial activity was observed in Kasoori methi i.e dried plant after Kasoori methi Mature plants shows maximum antibacterial activity. Chemical compounds like alkaloids, tannins, flavonoids etc of the plant may inhibit bacterial growth.

Further studies are needed to isolate and characterize the bioactive principles to develop new antibacterial drugs.

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