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EVALUATION OF ANTIBACTERIAL ACTIVITY IN *TRIGONELLA FOENUM GRAECUM* LEAVES

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ABSTRACT

Pharmacological industries have produced a number of new antibiotics in the last decades, resistance to these drugs by microorganisms has also increased. For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies.The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. The main objective is to study the anti-bacterial activity of *Trigonella* plant extract which was evaluated with antibiotic susceptible and resistant microorganisms. The highest antimicrobial potential was observed for the methanolic leaf extract of *Trigonellafoenumgraecum* when compared with the standard drug, Gentamycin. Steroidal and Phenolic constituents are found to be present in the leaf extract.

KEY WORDS:*Trigonellafoenumgraecum* ,antibacterial activity, Gentamycin.

INTRODUCTION

Herbal remedies are considered the oldest forms of health care known to mankind on this earth. As the age of the earth is increasing, the numbers of challenges that are faced by humans are also increasing. In present competitive busy world, pollution and prone infections are increasing day by day. Many synthetic drugs in this purpose of fighting against the infections caused by microbial organisms have occupied significant place in the field of medicine. synthetic drugs like Sulphonamides, quinolones(eg: Nalidixic acid) and antibiotics like Beta- Lactum antibiotics etc., have served this purpose in appreciable manner but on the other hand registered some unwanted side effects like hypotension, cardiac arrhythmia, renal failure ,mild nausea, vomiting, diarrhea etc., may occur on prolonged

usage. Now the world is looking forward for natural drugs with no or less side effects to treat many of the diseases, aliments, infections etc. Due to the resistance developed by microbial organisms there is a need of therapeutic agents against which resistance will not be developed easily. The present investigation is an attempt done to reveal the therapeutic activity of fenugreek which is well known as a condiment worldwide. As this Plant is used as neutraceutical also, there is less scope to develop undesirable effects. Fenugreek leaves are selected to evaluate the anti-bacterial effect against some microbes which fall in gram positive and gram negative category that causes infection in humans. Fenugreek is common name of *Trigonellafoenumgraecum* belonging to the family *leguminosaeis* an annual plant with leaves consisting of three small obovate to oblong leaflets. It is cultivated worldwide as a semi-arid crop[1]. The largest producer of fenugreek is India[2] where the major producing states are Rajasthan, Gujarat, Uttarakhand, Uttarpradesh, Madhyapradesh, Maharastra, Haryana and Punjab. It is reported that the chloroform extract of the leaves exhibits the scavenging activity[3]. The methanolic fraction of aerial parts showed activity against fungi species[4]. The aqueous extract of fenugreek leaves exhibit Anti-oxidant and Anti diabetic activity in heart tissue in alloxan induced diabetic rats [5].

The parts of plant used for medicinal purposes are seeds, dried or fresh leaves,sprouts. However, seeds are found most frequently used part.The seeds yield the whole of their odor and taste to alcohol and are employed in the preparation of emollient cataplasms, ointments and plasters. Externally it is used on carbuncles, abscesses, boils, substitute for cod-liver oil in scrofula, rickets, anemia[6] .The Fresh plant is used as an esculent[7].

Antibacterials[8] are the drugs that destroy or inhibit the growth of bacteria in concentrations that are safe for the host and can be used as chemotherapeutic agents[9] to prevent or treat bacterial infections. The objective of this study is to evaluate antibacterial activity of Fenugreek leaves in comparison with the standard drug, Gentamycin and determine its Potency from zone of inhibition obtained and calculating activity index.

MATERIAL AND METHODS

Collection of Plant materials

Fresh leaves of *Trigonellafoenumgraecum* (Family: Fabaceae) were used in this study. The fresh leaves are collected in the nearest market which was authenticated and confirmed by Professor Mrs B. Pratibha Devi, Head, Department of Botany, Osmania University,

Hyderabad. The leaves after collection were washed to remove the debris and then shade dried and the dried leaves were powdered to get a coarse powder.

Preparation of Extract

The dried powdered material of *Trigonellafoenumgraecum* leaves (TgL) (50gm) was taken and moistened with sufficient quantity of methanol and subjected to soxhlet extraction with 3 cycles of solvent and concentrated. Extraction solvent was of reagent grade and extract was dried in desiccator . All compounds were routinely checked by TLC on silica gel-G plates using different solvent systems by trial error method and Rf values were recorded in the Table 1. Phytochemical analysis for the natural compound in the TgL extract was performed and the results were noted in the Table 2.

Experimental Model

In vitro model - Disc plate method was adopted to evaluate the antibacterial activity of methanolic fenugreek leaf extract. Test organisms used for study are Gram positive bacteria: Staphylococcus aureus (MTCC740),Bacillus subtilis (MTCC441) and Gram negative bacteria: Pseudomonas aeroginosa (MTCC424), Escherichia coli(MTCC41), Proteus vulgaris(MTCC426) and Klebsiella pneumonia (MTCC423).All the strains were obtained from Institute of Microbial Technology ,Chandigarh, India. 24hr cultures of above were freshly prepared and spread on to the sterile nutrient agar plates which were prepared by pouring 20ml of the nutrient media into each sterile petri dish and left until Hardened. Inoculation of each bacterium in separate Petri plates was done by spreading using swab which was spread on to the plates uniformly and were incubated at 37^0C for 24 hr[10,11]. Discs of diameter 8.5mm were prepared and a dose of 10µl of concentrated *Trigonellafoenumgraecum* leaf extract(100mg/ml) taken as test sample against each microorganism inoculated in petri plates and Gentamycin (60mg/ml) is taken as standard concentration for experiment ,discs of diameter 8.5mm were prepared and a dose of 10µl of standard were placed on the agar media with inoculum in the petri plates and allowed to stand for an hour to ensure proper diffusion and thereafter incubated for 24hrs at 37^oC .Potency of plant extract was determined using six discs containing the extract against each microorganism. Antibacterial activity was determined by measuring the zone of inhibition around each disc in plate using Zone reader. Measured inhibition zones were recorded as mean diameter in mm .This was repeated for six discs and average diameters were recordedin the Table 3 and Activity Index was calculated to estimate the Potency of the test compound.

RESULTS AND DISCUSSION

Rf values of methanolicTrigonellafoenumgraecum extract using various solvent system

Table 1: Results of TLC

Extract	Mobile phase	Ratio	Rf values
Methanol	Pet ether: ethyl acetate: toluene	4:3:3	-----
Methanol	Ethyl acetate: pet ether	1:1	1
Methanol	Chloroform: methanol: water	4:3:3	1
Methanol	Chloroform: methanol: water	4:3:2	0.92
Methanol	Ethyl acetate: methanol: water	10:1.5:1	0.6
Methanol	Butanol: glacial acetic acid : water	4:5:1	0.88
Methanol	Formic acid: toulene: glacial acetic acid	9:3:1	0.95

TLC of Methanolic effluent fraction of *Trigonella* showed the Rf values ranging from 0.6 -0.95.Ethylacetate:methanol:water is best solvent system for separation of *Trigonella.* An idea about polarity of various phytoconstituents is also obtained while performing TLC analysis. Compound showing high Rf value in less polar solvent system have low polarity and with less Rf value have high polarity.

Table 2: Results of Phytochemical investigation of the extract.

TEST	OBSERVATION	INFERENCE
Test for carbohydrates Molisch's Test	violet ring is not formed at the junction of two liquids	Negative
Test for reducing sugars Fehlings Test:	Brick red ppt is not observed	Negative
Test for Monosaccharide's Barfoed's Test:	Red ppt is not observed	Negative
Test for non reducing poly saccharides Iodine test:	Blue color is not formed	Negative
Test for proteins Xanthoprotein test	White ppt is not formed	Negative
Test for amino acids Ninhydrin test:	Purple or bluish color is not formed	Negative
Test for Steroids Salkowski reaction:	Chloroform layer appear red and acid layer shows greenish yellow fluorescence	Positive
Liebermann-burchard reaction	First red then blue and finally green color appears	Positive
Liebermann reaction	Blue color appears	Positive
Test for cardiac glycosides	yellow to orange color is not	Negative

Baljet's Test:	observed	
Test for anthraquinone glycosides: Borntragers test:	Ammonical layer doesn't turn to pink or red	Negative
Test for alkaloids Dragondroff's Test:	Orange brown ppt was not formed.	Negative
Mayer's Test	No ppt	Negative
Test for tannins and phenolic compounds, 5% $FeCl_3$ solution	Deep blue black color	Positive
Lead acetate solution	White ppt	Positive
Bromine water	Decoloration	Positive
Acetic acid solution	Red color is not formed	Negative
Dil.$KMnO_4$ solution	No decoloration	Negative

Bromine water, Lead acetate solution ,Test for tannins and phenolic compounds, Test for Steroids - Salkowski reaction, Liebermann-burchard reaction , Liebermann reaction were positive for *Trigonellafoenumgraecum.*

Table 3: Results of Antibacterial activity

Test Organisms	**Mean diameters(cm) . Zone of inhibition**	
	***Trigonellafoenumgraecum* extract**	**Gentamycin standard**
Proteus vulgaris	3.4± 0.11	2.62 ± 0.11
Klebsiella pneumonia	3.23 ± 0.08	2.74 ± 0.10
Escherichia coli	3.23 ± 0.27	2.74 ± 0.09
Pseudomonas aeroginosa	3.11 ± 0.07	2.74 ± 0.107
Staphylococcus aureus	3.06 ± 0.08	2.26 ± 0.10
Bacillus subtilis	3.15 ± 0.10	2.64 ± 0.15

Diameter of zone of inhibition results are expressed as mean ± SEM from six observations of each strain of bacteria against each test group (i.e., plant extract and standard)

$$\text{Activity Index}^{12} = \frac{\text{Zone of Inhibition of the sample}}{\text{Zone of Inhibition of the standard}}$$

Table 4: values of activity index determined from Zone of Inhibition.

Micro organism	**Zone of Inhibition for each disc with extract**					**Average Activity Index**
	1	**2**	**3**	**4**	**5**	
Proteus vulgaris	1.31	1.11	1.27	1.5	1.37	1.31
K.pneumoniae	1.03	1.32	1.13	1.4	1.14	1.21
E.coli	1.0	1.1	1.03	1.31	1.67	1.22
P.aeruginosa	1.07	1.36	1.0	1.23	1.15	1.16
S.aureus	1.33	1.20	1.60	1.29	1.35	1.35
B.subtilis	1.27	1.11	1.12	1.43	1.06	1.20

Trigonellafoenumgraecum concentration 100mg/ml was found to be more potent than standard concentration 60mg/ml against *Proteus vulgaris, Klebsiella pneumonia, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Bacillus subtilis* The increase in Zone Of Inhibition was found to be one fold more than standard.(3.4cm; 2.6) against gram microorganisms and showed potent antibacterial activity against *Proteus vulgaris* . Activity index of *Trigonellafoenumgraecum* was found to be more highly potent may be due to the presence of phytochemical constituents like trigonelline, coumarins and flavonoids. Activity index clearly shows that TgL extract is more potent than standard and also draws out the observation that TgL showed broad spectrum activity with order of potency*S.aureus, Proteus vulgaris, E.coli, K.pneumoniae, B.subtilis, P.aeruginosa.*

CONCLUSION

Trigonellafoenumgraecum leaf has got more potent activity than standard drug Gentamycin against gram positive and gram negative microorganisms which contain principle phytochemical constituents like trigonellin, flavonoids. Phytochemical analysis has reported the presence of steroidal and phenolic compound in the extract.

This particular study has a wide scope in future for the development of herbal drugs with less or no side effects and there are some void in these three drugs which should be filled by research in near future

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