Medicinal Usage of Fenugreek Seeds

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***Abstract*- Fenugreek (Trigonella foenum-graecum L.) is one of the most promising medicinal herbs known from ancient times having nutritional value. Fenugreek seeds are known to exhibit potent antioxidant, antimicrobial, hypoglycemic, and nephroprotective activities. This study aimed at determining the physicochemical properties of fenugreek seeds in order to ascertain its quality as a food and medicinal agent. The purpose of this study was to screen phytochemical compounds present in crude fenugreek seeds extracts and evaluate their potential activity against an E. coli, Bacillus cereus, and Staphylococcus aureus.Organic (hexane, acetone, ethanol and methanol) extracts were prepared from powdered fenugreek seeds. Bacterial growth inhibitory effects were evaluated by measuring the diameter of the inhibition zone (IZ) using an agar-disc diffusion method. The organic extracts prepared with chloroform, acetone or methanol showed low to moderately high growth inhibitory effect. Phytochemical screening of the extracts revealed the presence of the major compounds known to have anti-bacterial activity such as tannins and flavonoids in the aqueous and methanolic extracts. The results indicate that fenugreek seeds crude extracts may have anti-bacterial potential against selective micro organism.**

Index Terms- Antimicrobial activity, Fenugreek, Extraction, Phytochemicals, Inhibition zone.

INTRODUCTION

From ancient time 80% of world people use traditional medicines which are made up from medicinal plants and its parts, Indian literatures Ayurveda, Unani, and Siddha have description on this.(Ajay Kumar *et al*., 2009) In India people usesspices, health foods and home remedies as herbal medicines.(Ashok *et al*.,2007)When resistance bacteria attacked, this is challenge for antimicrobial therapy of infectious diseases to cure these type of infectious disease and Plants have Vitamins, Antimutagenic, Nitrogen containing compounds, Antioxidant, Phenolic compound, Phytochemical, Minerals, Anti tumor and Diuretic activities so these materials of plants used as plant extract to made traditional medicines and therapeutic treatments to cure the problems of multi drug resistance organisms.(Shanmugasundaram *et al*., 2005)

Many bacteria like *Salmonella*(from unpasteurizedmilk and other dairy products), *Clostridium perfringens*(from not originally cooked food, reheated food or food not kept at the appropriate temperature), *Staphylococcus aureus* (fromunpasteurized dairy products and salty foods), *E. coli*(fromeating raw or undercooked ground beef or drinking unpasteurized beverages or dairy products), *Listeria monocytogenes*(from refrigerated, ready-to-eat foods such as hot dogs, deli meats, unpasteurized milk, raw sprouts, dairy products and raw and undercooked meat, poultry and seafood) cause food borne disease. Minority of food borne illness cause nausea, diarrhea, and vomiting by *Bacillus cereus*.(BaronS.*et al*.,1996)*Staphylococcus aureus* causes osteomyelitis, bacteramia, cellulitis, pneumonia, most community associated infections that affect soft tissues and skin.(DeLeoF.R. *et al*.,1557-1568)*Salmonella typhimurium* causes diarrhea and typhoid fever in human.(BartlettJG,2002)

To control this type of microorganisms we take synthetic antibiotics like Ampicillin, Cefixine, Amoxicillin-clavulanate, Tetracycline, Clarithromicine, Azithromycin, Erythromycin, Fluoroquinolones, Cephalosporins, Penicillins, etc. But because of theses antibiotics, side effects like Antibiotic associated diarrhea, Allergic reactions-Anaphylaxis, Toxic Epidermal Necrolysis (TEN), Stvens-Johson Syndrome (SJS), Diarrhea, Bloody diarrhea, Vomiting, Serious and Rare allergic reaction, Mucous membrane and Skin disorder, etc may produce.(McCormick *et al*.,1995;ShehabN *et al*.,2008; MercedesE.Gonzalez *et al*.,2017)

The agents or drugs or molecules have the capacity to suppress the multiplication or kill the microbes is known as antimicrobial agent.(T.Musumeci *et*

al.,2013)In plants secondary metabolites have many active principals of drugs and in a variety of different components antimicrobial activity of plant extract may reside.(LaiPK *et al*.,2004) World's oldest medicine herbs belong to the family Fabaceae which is Fenugreek(Trigonella foenum-graecum), can lower blood sugar level in Diabetes and its seeds are rich in Dietary fiber, It is used to increase milk supply in lactating women and cure breast cancer because it used as Galactagogue.(Amin A. *et al*.,2005) In boils, abscesses, crabunles, diabetes, constipation, hyper triglyceridemia, tuberculosis, atherosclerosis, high cholesterol-Fenugreek seeds are used and sometimes also as externally poultice.(Nadkarni *et al*.,1976) Fenugreek seeds posses volatile oil, toxic oils and alkaloids which are toxic to parasites, fungi and bacteria.(Fraenkel *et al*.,2007)To prepare powder for medicinal uses and prepare extracts, we used leaves and seed of Fenugreek.(WagnerH *et al*.,1993)

Here we studied about antimicrobial activity of Trigonella foenum-graecum. This antimicrobial activity is due to phytochemicals of Fenugreek seeds so here also we studied qualitative analysis of phytochemicals. For this purpose we collect Fenugreek seeds from Mehsana market, Gujarat.

MATERIALS AND METHOD

Microorganisms:

A total three microorganisms were used to assess the antibacterial properties. All strain identified by microscopic identification and biochemical test for specific micro organism. Two of three organisms are gram positive *Bacillus cereus, Staphylococcus aureus* and one is gram-negative *Salmonella typhi*. All strain was preserved in nutrient agar slants.

Plant materials:

- Seeds of Trigonella foenum-graecum

Solvent system for extraction:

In this study two solvent systems were used for plant extraction these were methanol and acetone.

Preparation of plant extracts:

It is very essential to extract desired chemical components from medicinal plants to study the antibacterial activity. The steps include pre-washing, grinding in the process of obtaining homogeneous sample. To enhance the kinetics of analytic extraction, the surface of sample and solvent system contact should be maximized. All the potential active components should be retained during the extraction procedure. The extraction of plant by traditional method can elevate the hypothesis of obtaining the plant as 'herbal 'drug.

The plant material , Seeds of Trigonella foenum-graecum were collected,by using mortar ground to powdered form and packaged in an air tight plastic container until used. Sample of the tested medicinal plant was prepared into methanol and acetone extracts. Extracts were prepared by dissolving 20gm of fine powder of Trigonella foenum-graecum in 100ml Methanol and acetone. The contents were kept for 48 h. Then extracts were obtained by using Soxhlet extraction method. Then concentrated to dryness under reduced pressure using rotatory evaporator and the residues were stored at 4ºC.

These extract was used in this study for in vitro antimicrobial analysis.(WangL.*et al*.,2006)

Antimicrobial activity:

- Disk diffusion method is used for in vitro anti microbial screening for primary selection of the therapeutic agent. About 20ml nutrient agar was platted in Petri dishes and allowed to solidify for 30 minutes. The test microorganisms were seeded into medium by melted agar method. From 0.5 gm extract sample each active ingredient was weighted separately and dissolved in 20 ml DMSO so that final concentration of active ingredient may reach 25 mg/ml. this extract was used to saturate 100 Whatman filter paper No.1 discs.(El Mahmood *et al*.,2008)Then after prepared disk of different plant extract were placed on Petri plate having media and selective micro organism. These plates were incubated at37˚C for 24-48 hours to allow maximum growth of the microorganism. After incubation, the plates were observed for clear and distinct zone of inhibition surrounding the disc. The diameter of zone of inhibition produced by the extract was measured that expressed in millimeter (mm) and compared with the standard. The work was carried out in laminar flow.(Vander *et al*., 1999)

Phytochemical analysis:

Chemical tests for the screening and identification of bioactive chemical constituents in the medicinal plants under study were carried out in extracts as well as powder specimens using the standard procedures as described by qualitative analysis.

1. Test for Tannins:
About 1 ml extract was mixed in 3ml water and heated on water bath for 5 minute and then filtered and 1 ml ferric chloride added to the filtrate and observed dark green color and blue- black color was formed. It indicates that the presence of tannins.(EdeogaHO *et al*. 2005)

2.Test for Flavonoids:
0.5 gm of various extract was shaken with petroleum ether to remove the fatty
materials (lipid layer). The defatted residue was dissolved in 20ml of alcohol and filtered. The filtrate was used for the this test.3 ml of the filtrate was mixed with 4 ml of 1% potassium hydroxide in a test tube and the color was observed. A dark yellow color indicates the presence of flavonoids.

3. Test for Alkaloids:
0.5 to 0.6 gm of various extract was mixed in 8 ml of 1 % HCL, warmed and filtered. 2 ml of the filtrate were treated separately with both reagent (Mayer' and Wagner's). After which it was observed whether the alkaloids were present or absent in the turbidity or precipitate formation.
[Mayer's reagent: - dissolve 1.36 gm $HgCl_2$ in 60 ml water and pour into a solution of 5 gm KI in 10 mlofwater. Add sufficient water to make 100 ml. that give cream or -pale yellow precipitate in dictated the positive result].(Evans.W.C *et al*.,1997)
[Wagner's reagent: - dissolve 2 gm iodine and 6 gm of KI in 100 ml water. That give brown or reddish brown precipitate indicates the positive result].(Wagner.H *et al*.,1993)

RESULT

ZONE OF INHIBITION:

ZONE OF INHIBITION (mm)			
	Methanol Extract	Acetone Extract	Aqueous Extract
Salmonella typhi	12.0 ± 1.0	10.0 ± 1.0	0.0
Bacillus cereus	28.0 ± 2.0	25.0 ± 2.0	0.0
Staphylococcus aureus	22.0 ± 2.0	19.0 ± 2.0	0.0

PHYTOCHEMICAL ANALYSIS:

Name of Phytochemical	Methanol Extract	Acetone Extract
Tannin	+++	++
Flavanoid	++	+
Alkanoid	++	++

Discussion
Microorganisms produce enterotoxin to damage human cell. A protein exotoxin released by a microorganisms that targets the intestines is called enterotoxins.("enterotoxin"atDorland'sMedicalDictionary) It is related to aerolysin with haemolytic, cytotoxic and enterotoxic activities. First a pre-protein with a single peptide synthesized and after crossing the membrane they are separated and work as toxin. The toxin binds to a glycoprotein on the surface of the target cell and form pore in host cell membrane, as a result degradation of villi and crypt and cell death occurred.(DongyouLiu,2015) To induce pathogenesis a virulence factor is designed.(Blomster-HautamaaDA et al.,1986)
To control this type of microorganisms some antimicrobial drugs are discovered and established but some are hidden by nature. So as a source of Human Disease Management from last decade investigation on plants is done.(AiyelagabeOO *et al*.,2001, PrashanthD *et al*.,2001;MounishwamyV *et al*.,2002; WoldemichaelGM *et al*.,2003) Plants posses Phytochemicals (phyto, meaning plant from Greek word) which are biologically active, naturally occurring chemical compounds, provides health benefits for humans further than those attributed to macronutrients and micronutrients.(HaslerCM *et al*.,1999)Phytochemicals contribute to the plant's color, aroma and flavor and also protect plants from disease and damage.(GibsonEL *et al*.,1998;MathaiK.*et al*.,2000) Recently, more than 4,000 phytochemicals have been cataloged which have roles in the protection of human health by their dietary intake. Phytochemicals have possible role in preventing and treating heart disease and

cancer.(Mathai K. *et al*., 2000) Also they have been promoted for preventing and treating of macular degeneration, diabetes, high blood pressure, etc.

Fenugreek seeds posses alkaloid, flavanoid, carbohydrate, protein, amino acids, vitamins, and minerals.(EvanceWC.*et al*.,2002) It also contain flavonoid quercetin and luteolin as antioxidant(ShyamGupta *et al*.,2012)and two alkaloid trigonelline and choline.(Mehrotra *et al*.,1970-1979)To analysis presence of Phytochemical, acetone, methanol and aqueous seed extracts of Fenugreekwas carried out to test for the presence of proteins, tannins, phenols, terpenoids, flavonoids, saponins and alkaloids.(Upadhyay *et al*.,2008)

CONCLUSION

In this experiment we perform the qualitative analysis of Fenugreek seeds. As a result we got zone of inhibition for selective microorganisms and this saw the antimicrobial activity of Fenugreek seeds. Also we find out the phytochemicals which are present in Trigonella foenum-graecum. So we concluded that phytochemicals are responsible for antimicrobial activity of Fenugreek seeds. And also further study will be on quantitative analysis of Fenugreek seeds.

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