"EVALUATION OF ANTIMICROBIAL ACTIVITY OF AQUEOUS CLOVE AND FENUGREEK SEED EXTRACT COMBINATION"

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ABSTRACT

OBJECTIVES:-

The present investigation was performed to evaluate antimicrobial activity of aqueous clove bud and fenugreek seed extract combination.

METHODS:-

The clove bud (Syzygium aromaticum) extract was done by using Soxhlet apparatus and fenugreek seed (Trigonella foenum-graecum) extract was done by maceration process by using distilled water as solvent. Evaluation of antimicrobial activity of both extract was carried out by agar well diffusion method against gram positive bacterium S. aureus and gram negative bacterium E.coli. Minimum inhibitory concentration (MIC) of combine extracts was determine by using broth dilution method.TLC bioautography and phytochemical analysis were also performed.

RESULT:- The Evaluation of Antimicrobial activity of aqueous clove & fenugreek seed extract combination was evaluated . By Determination of MIC from individual extract & its combination.

CONCLUSION:- We conclude that formulation of clove & fenugreek seed extract combination effective in antimicrobial activity.

ANTIMICROBIAL ACTIVITY

An antimicrobial agents are the agent that kills microorganisms or inhibit their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. For example, antibiotics are used against bacteria and antifungals are used against fungi.

Table no.1. Some Herbal drugs which gives the Antimicrobial activity:-

Common Name	Scientific Name	Components Present	Therapeutic action
Thyme	Thymus vulgaris	Thymol	Antibacterial, Antioxidant
Oregano	Origamumvulgare	Carvacrol	Antimicrobial, Antiviral
Tulsi	Ocimum sanctum	Eugenol	Anti-inflammatory, Antimicrobial
Ginger	Zingiberofficinale	Phenylpropanoid-derived compounds, particularly gingerols and shogaols	Antimicrobial, Anti - inflammatory
Turmeric	Curcuma longa	Circumin	Antimicrobial, Anti-neoplastic
Cinnamon	Cinnamomumverum	Eugenol, cinnamaldehyde,beta - caryophyllene,linalool, methyl chavicol	Antimicrobial , Anticancer
Clove	Syzygium aromaticum	Phenylpropanoides such as carvacrol,thymol,eugenol and cinnamaldehyde	Antibacterial, Anti-inflammatory
Fenugreek	Trigonella foenum-graecum	Diosgenin, alkaloids, including trigonelline, gentianine	Antimicrobial , Antifungal

Why we use?

In India, clove and fenugreek are mostly cultivated and easily available in market in cheap cost. These are more affordable than conventional medicine. They have fewer side effects and are cost effective. Both drugs can be extracted by simple method. Therefore, we used these drugs in combination to increases their synergistic activity.

INTRODUCTION

CLOVE:

The symbol of dignity that is what "Clove" actually means. It is a precious and valuable spice of the world. It is an unopened flower bud growing on a tree belonging to the family Myrtaceae which is same as that of guavas. Cloves (Syzygium aromaticum, Eugenia aromaticum or Eugenia caryophyllata) are the aromatic dried flower buds ,

which are commonly used in biryanis , pickles , salads and garam masala. The tree that creates the miracle of nature originated from the Moluccas Islands located in Indonesia , actually known as Spice Island. It is common product found in the spice rack around the world. Clove buds posses intense fragrance and burning taste. They have deep brown in color, powerful fragrant odour which is warm , pungent, strongly sweet and slightly astringent. In India itis used in almost all spicy rich dishes. Indonesia uses half the world production of cloves to make kretek cigarettes in the proportion of one part of clove mixed with two parts of tobacco. In 2009 clove cigarettes were banned in the U.S. however they are still marketed with the newlabel as filtered clove cigars.

Fig. 1 : CLOVE

Scientific classification: Kingdom

	Plantae
Clade	*Tracheophytes*
Clade	*Angiosperms*
Clade	*Eudicots*
Clade	*Rosids*
Order	*Myrtales*
Family	*Myrtaceae*
Genus	*Syzygium*
Species	*Syzygium aromaticum*

Planting material

The seeds should be collected from fully ripe fruits for raising seedlings . Fruits for seed collection known commonly as "mother of clove" are allowed to ripe on the tree and drop down naturally. Such fruits are collected and sown directly in the nursery or soaked in water over night and then pericarp removed before sowing. The second method gives quicker and higher percentage of germination. Only fully developed and

uniform sized seeds, which show the signs of germination by the presence of pink radicle, are used for sowing . It is advisable to sow the seeds immediately after harvest .Heaping the fruits or keeping them tied up in air tight bags hastens the death of the seeds . Beds of 15- 20 cm height, 1m width and conventional length are prepared for sowing seeds. The fertilizers must be applied in two equal split doses during the months of May- June and September-October in shallow drenches dug around the plant about 1-11/m away from thebase.

- ***Botanical Names***
  Eugenia caryophyllus , Syzygium aromaticum

- ***Common Names***
  Cloves, Carophyllus, Clovos, Caryophyllus

Table no .2 SYNONYMS

Language	Names	Language	Names
Hindi	Laung,	**Greek**	Garifalo
Marathi	Luvang	**Chinese**	Ding xiang
Sanskrit	Lavangam	**French**	Giroflie
Tamil	Kirampu	**German**	Gewürznelk e
Telugu	Lavangalu	**Japanese**	Girofla
Gujarati	Lavang	**Indonesian**	Cengke

Biological source**: Clove consist of dried flower bud Eugenia Caryophyllus, **Family:

Myrtaceae

***Geographical Source:** Its is indogenous to Amboyna & Molucca islands. It is now cultivated chiefly in Zanzibar, Pemba, Penang, Madagascar, Caribbean islands, Sri Lanka & India. In India, Cloves are grown in Nilgiri, Tenkasi-hills & in Kanyakumari district of Tamil Nadu state. It is also cultivated in Kottayam & Quilon districts of Kerla.*

COLLECTION AND CULTIVATION :

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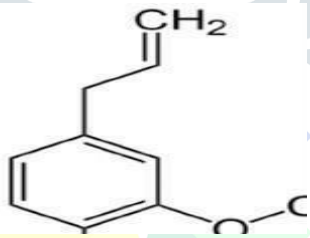
from the base. The trees begin to flower in 6 years. Full bearing is achieved by about 20 years and the production continues for 80 years or more. Bearing between years shows much variation. Clove clusters are handpicked, when the buds reach full size and turn pink but before they open. At this stage, they are less than 2 cm long. They are spread thinly on mats and stirred frequently for uniform drying. Well dried cloves will snap cleanly with a sharp click across the thumb nail and weigh about one third of the green weight. The opened flowers are not valued as a spice. Harvesting has to be done without damaging the branches, as it adversely affects the subsequent growth of the trees. On an average, a clove tree yields 3.5-7.0 kg/year, depending upon the age, size and condition of the tree.

CHEMICAL CONSTITUENTS

Clove comprises of volatile as well as non-volatile constituents.

Volatile Constituents

Clove yields different types of volatile oil [oil extracted from i. leaves, ii. the stem, iii. the buds and iv. the fruit.] These oils differ considerably in yield and quality. The yield and composition of the oil obtained are influenced by its origin, season, variety and quality of raw material, maturity at harvest, pre and post-distillation treatments and method of distillation . The chief component of all the types of oil is eugenol



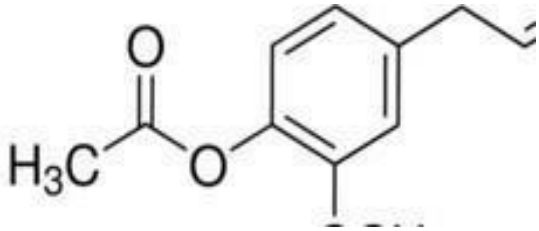
Eugenol

Bud Oil

Good-quality clove buds contain 15-20% essential oil. The oil is dominated by Eugenol (70– 85%), eugenylacetate (15%) and B-caryophyllene (5–12%), which together make up 99% of the oil. The constituents of the oil also include methylamylketone, methyl salicylate.

and B-humulene, benzaldehyde, B-ylangene and chavicol. The minor constituents like methylamylketone,methylsalicylate etc.are responsible for the characteristic pleasant odour of cloves. The clove bud and stem oils from Madagascar were also dominated by eugenol,eugenyl acetate and B-caryophyllene. The stem oil ccontained a higher level of eugenol, whereas the eugenyl acetate content was higher in the bud oil.

The oil fromclove bud contained 73.5–79.7% eugenol and 4.5– 10.7% eugenyl acetate, while the stem oil contained 76.4–84.8% eugenol and 1.5–8.0% eugenyl acetate. Both contained 7.3–12.4% B-caryophyllene and 1.0–1.4% a-humulene3 Pino et al. identified 36 compounds from the volatile oil of clove buds. Clove buds from India contained 12.9–18.5% oil, of which 44–55% was eugenol, wherea the pedicels contained 3.0–7.7% oil with 60.0–72.4%eugenol



Leaf Oil

Clove leaves yield 3.0–4.8% essential oil. The essential oil content during the different stages of leaf growth revealed that the eugenol content in the leaves increasedfrom 38.3 to 95.2% with maturity, while the contents of eugenyl acetate (51.2 to 1.5%) and caryophyllene (6.3 to0.2%)

decreased 4. Clove bud and leaf oil contain various classes of compounds, e.g. monoterpenes, sesquiterpenes, aldehydes and ketones.

Clove Stem Oil

Clove stem yields 6% volatile oil1. The oil is a pale tolight yellow liquid containing 80.2% eugenol and 6.6% B-caryophyllene, besides several minor components.

Fruit Oil

Ripe fruits yield 2% of oil, which is comprised of 50-55% eugenol.

Non-volatile Constituents:-

A few non-volatiles have been isolated from clove , which include tannins, sterols triterpenes and flavonoids.

Tannins

Cloves contain 10–13% tannins, which have the same chemical composition as gallotannic acid . Eugenin and ellagitanninwere isolated from cloves .Eugenol glucoside gallate, a chromone C-glycoside, galloyl andhexahydroxy diphenyl esters of 2, 4, 6-trihydroxyacetophenone- 3-glucopyranoside were isolated from clove leaves6. Further, two ellagitannins, namely ,syzyginin A (1, 2, 3-tri-O-galloyl–4, 6-(S) - tergalloyl-B-D-glucoside) and syzyginin B, were also isolated from the leaves.

Triterpenes

Cloves contain about 2% of the triterpene, oleanolic acid.Narayanan and Natu (1974) isolated maslinic acid from clove buds7. From clove, 2a-hydroxyoleanolic acid was also isolated,

Sterols

Sterols isolated from clove include sitosterol, stigmasteroland campesterol.

Flavonoids

A chromone C-glucoside, isobiflorin (5, 7-dihydroxy-2-methoxychromone-8-C-B-D-glucopyranoside) and biflorin were isolated from the ethanolic extract of cloves. From the ethanol extract of the seeds, apigenin6-C-[B-D-xylopyranosyl-(1→2″)-B-

Dgalactopyranoside]-7-O-B-D- glucopyranoside and apigenin-6-C-[B-D-xylopyranosyl- (1→2″)-B-Dgalactopyranoside]-7-O-B- D-(6-O-pcoumarylglucopyranoside) were isolated.

USES

- *Antiseptic*
- *Carminative*
- *Flavoring agent*
- *Stimulant*
- *Local anesthetics*
- *Spice*
- *Oil in perfumery*
- *Use in toothache dental preparation in mouth washes*

FENUGREEK:

Natural products have been a major source of new drugs Plants possess medicinal and drug activities. Medicinal plants are used by 80% of the world population and in developing countrie. Current study on natural molecules and products primarily uses on plants since they can be sourced more easily and be selected based on their ethno- medicinal uses. They can be extracted and used for chronic and infectious diseases. Clinical microbiologists have great interestin screening of medicinal plants for new therapeutics. The active drugs which play role are secondary metabolites. The antimicrobial activities of plant extract which produces different components including aldehyde and Phenolic compounds Fenugreek is used traditionally as demulcent, laxative, lactation stimulant and exhibits hypocholesterolemic, hypolipidemic and hypoglycemic activity in healthy and diabetic animals and humans.The defatted seeds of fenugreek reduce gastrointestinal absorption of glucose and cholesterol and bile acids secretion.

The herb fenugreek (Trigonellafoenum-graecumL,Fabaceaefamily) is anannual herb isused both in cooking and for the treatment of diabetes in many parts of the world especially in China, Egypt, India and middle eastern countries. In India, it is widely used in Bangladesh. Active compounds of fenugreek included soluble fiber trigonellediosgenin and 4-hydroxyisoleucine. Hypoglycemic activities have mainly been attributed to dietary fiber and Saponin. Fenugreek is a widely used herbal medicine for diabetes, but its efficacy for glycemic control remains unclear. Fenugreek(Trigonellafoenum-graecum) being rich in phytochemicals has traditionally been used as a food, forage and medicinal plant. It contain lysine and L-trytophan rich proteins, mucilaginous fiber and other rare chemical constituents such as saponins, coumarin,fenugreeke, nicotinic acid, sapogenins phytic acid ,scopoletinandtrigonelle which has therapeutic effects. The component called fenugreek in asteroidal sapogenin peptideester has hypoglycemic properties. Thus it is best use is to control blood sugar in both insulin dependent (Type-1) and non insulin dependant (Type 2) diabetes.

Fig.3:- FENUGREEK

Table no. 3. Scientific Classification:

Kingdom	*Plantae*
Division	*Magnoliophyta*
Class	*Magnoliopsida*
Order	*Fabales*
Family	*Fabaceae*
Genus	*Trigonella*
Species	*foenum-graecum*

***Fenugreek**: Trigonellafoenum-graecum.*

Family**: Fabaceae (Leguminosae) **Other

Names :

Language	*Names*
French	*FenugrecSenegre,Trigone ll*
German	*Bockshornklee, GriechischesHeu*
German-Italian	*FienoGreco*
Spanish	*Alholva,Fenogreco*
Indian	*Mayti,Methe,Methi*
Indian-Tamil	*Venthium*
Malay	*Alba*
Sinhalese	*Uluhaal*

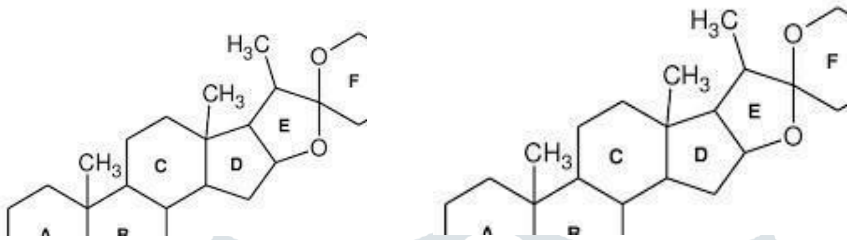
COLLECTION AND CULTIVATION

The finely grinded seed powder was taken. From the total extract 10 gm of seed powder was taken and 50 ml of ethyl alcohol was added to that extract stirred it constantly for 30min and the solution was kept in room temperature for this and then filter The filtered solution is again filtered with whatman's filter paper no 3.

CHEMICAL CONSTITUENTS

Diosgenin, asteroid sapogenin found in fenugreek is the starting compound for over 60% of the total steroid production by the pharmaceutical industry. Other sapogenins found in fenugreek seed include yamogenin, gitogenin, tigogenin, and neotigogens. Fenugreek seeds contain alkaloids, including trigonelline, gentianine and carpaine

compounds.The seeds also contain fiber hydroxyl is oleucine and fenugreekine,a component that may have hypoglycemic activity. Other constituents of fenugreek include mucilage, bitter fixed oil, volatile oil, and the alkaloids choline and trigonelline. Extract of fenugreek is obtained by alcoholic extraction. The chemical composition of Fenugreek seeds and defatted Fenugreek seeds is given inTable1. These seeds are a rich source of fiber and protein. The fiber may be further classed as gum (gelfiber) and neutral detergent fiber. Whole Fenugreek seeds also contain 4.8% saponins. Fenugreek seeds saponins are of steroidal nature (type furostanol saponins) with diosgenin as the principal steroidal saponin.



Diosgenin yamogenin

Table1:Proximate Composition(%) of Fenugreek Seeds

COMPOUND	***Whole Seeds***	***Defatted Seeds***
Moisture	*9.0*	*9.0*
Ash	*3.0*	*3.5*
Lipids	*8.0*	*Negligible*
Protein	*26.0*	*28.3*
Starch	*6.0*	*6.5*
Total Fiber	***48.0***	***51.7***
Gum	***20.0***	***19.2***
Neutral DetergentFiber	***28.0***	***32.5***

Benefits of Fenugreek:

1. *25 - 100 grams of fenugreek seeds eaten daily can diminish reactive hyperglycemia in diabeticpatients.*
2. *Fenugreek leaves and seeds help in blood formation. They are good for preventing anemia and rundown conditions.*
3. *Including fenugreek seed in lactating mothers increases the flow of milk.*
4. *A paste of the fresh fenugreek leaves, applied on the face prevents pimples, blackheads, dryness of the face and early appearance of wrinkles.*
5. *For removal of dandruff inhair.*
6. *If you add half a teaspoon of fenugreek seeds to the lentil and rice mixture while soaking, dosas will be more-crisp.*

USES

1. *Fenugreek is used in the treatment of wound, abscesses, arthritis, bronchitis, ulcer and digestive problem.*
2. *It is also used traditionally as a demulcent, laxative, lactation stimulant and exhibits hypocholesterolemic, hypolipidemic, and hypoglycemic activity in healthy and diabetic animals and human.*
3. *The seeds of fenugreek have been reported to have anti-diabetic, anti-*

cancerous, anti- inflammatory and antioxidant activity.

4. *Its leaves have been reported to possess potential antibacterial activity, antifungal activity, anti-diabetic and antioxidant property.*

OBJECTIVES

- *To collect the plant material*
- *To authenticate the plant material*
- *To extract plant material by using soxhlet apparatus*
- *Phytochemical screening of chemical constituents present in both extracts*
- *To study antimicrobial activity of both clove and fenugreek powdered extracts*
- *To find out the synergistic effect of both plant extract against gram positive S.aureus and gram negative E.coli*
- *To measure the minimum inhibitory concentration (MIC) of the selected plant extracts against gram positive S.aureus and gram negative E.coli*

LITERATURE REVIEW:

Cloves (S. aromaticum) are dried aromatic unopened floral buds of an evergreen tree 10-20 m inheight, belongingtothe family Myrtaceae, indigenousto India, Indonesia, Zanzibar, Mauritius and Ceylon.They are esteemedas a flavoringagent and also used as a spice for scenting ,chewing tobacco and an ingredient of betel chew. Cloves have many therapeutic uses, they control nausea and vomiting, cough, diarrhea, dyspepsia, flatulence, stomach distension and gastro intestinal spasm, relieve pain, cause uterine contractions and stimulate the nerves. In addition, the cloves are highly antiseptic antimutagenic , anti- inflammatory , antioxidant , antiulcerogenic , antithrombotic , antifungal, antiviral and antiparasitic. Spices have been traditionally used since ancient times ,for the preservation of food products as they have been reported to have antiseptic and disinfectant properties. S. aromaticum has been shown to be a potent chemo preventive agent, used by the traditional Ayurvedic healers of India since ancient times to treat respiratory and digestive ailments. Eugenol is the main volatile compound extracted from clove bud (S. aromaticum) and used in traditional medicine, as a bactericide, fungicides and anesthetic

Trigonella foenum-graecum commonly known as fenugreek is an annual herb indigenous to the countries touching on the eastern shores of the Mediterranean and widely cultivated in India,Egypt, and Morocco. The plant parts like leaves and seeds are widely consumed in Indo-Pak subcontinent as well as in other oriental countries as a spice in food preparations and as an ingredient in traditional medicines. A wide range of uses was found for fenugreek in ancient times. Fenugreek being rich in antioxidants and phytochemicals has been traditionally used as food, forage and medicinal plant.

Medicinally it was used in the treatment of wounds, abscesses, arthritis, bronchitis, ulcer and digestive problems. Thus, fenugreek is food and a spice commonly eaten in many parts of the world for many years. Fenugreek is also used traditionally as a demulcent, laxative, lactation stimulant and exhibits hypocholesterolemic, hypolipidemic and hypoglycemic activity in healthy and diabetic animals and humans. The pharmacological uses of different plant parts of fenugreek have been reported by different researchers. The seeds of fenugreek have been reported to have anti-diabetic, anti-cancerous, anti-inflammatory and antioxidant activity. Its leaves have been reported to possess potential antibacterial activity, antifungal activity , anti-diabetic and antioxidant

property. Fenugreek is an ancient medicinal plant as the plant contains active constituents such as alkaloids, flavonoids, steroids, saponins, etc

Microscopical characters of Fenugreek seed:-

Transverse section of fenugreek seed shows Testa, Endosperm, Cotyledons

1. ***Testa**- the outermost layer is covered with cuticle and composed of thick wall, cylindrical, lignified cells with conical projections, through which a white line, linea lucida ,extends across; middle layer of testa composed of cells with thick-walls and wide intercellular spaces near their tops; inner section of testa composed of a few layers of narrow, tangentially elongated, compact, thin-wall parenchyma cells.*

2. ***Endosperm**-outer layer consists of rectangular to polygonal thick-wall cells, full of aleurone grains; multiple layers of cells of various shapes, large, full of mucilage.*

3. ***Cotyledons**- a layer of epidermal cells with thick walls; 3-4 layers of cells containing oil.*

METHODOLOGY

Drying

The fenugreek seeds and clove buds were brought to lab in air tight bag and seeds and buds were sun-dried so as to reduce the moisture content. After sample were drying stored in a safe place till further use. These seeds and buds were further used for qualitative, quantitative, antimicrobial and other methods.

Grinding

Once the seeds and buds were properly dried, then the seeds and buds grinded to make a fine powder and used for making aqueous extract.

Preparation of extracts

Preparation by Aqueous extraction of clove:-

Clove buds (75gram) were washed, dried and then weighed .The buds are then reduced to finely divided size by the process of grinding. Powdered clove are fed inside the Soxhlet apparatus and the assembled apparatus was allowed to work for 24-48 hours. After extraction of clove bud by Soxhlet apparatus then concentrate the extract in hot air oven at 40^0 C

Preparation by Aqueous extraction of fenugreek :-

The aqueous extraction of fenugreek are prepared of fenugreek plant material for which weighed 25gm of seeds powdered, add 250ml of sterile distilled water to it, kept the mixture for 7 days and filtered it with muslin cloth, filtrate was allowed for hot extraction process on water bath at 40^0 C

Qualitative estimation ofphytochemicals:

Phytochemical screening:

The Phytochemical screening of different extracts was carried out to determine the presence of active secondary metabolites. The phytochemical research based on ethnopharmacological information is generally considered as an effective approach in the discovery of new anti-infective agents from higher plants (Duraipandiyan et al.,2005). Methanol, ethyl acetate, chloroform, acetone and aqueous extracts of seeds and buds were tested for the presence of various secondary metabolites such as phenols, carbohydrates, flavonoids, tannin, proteins, alkaloids, amino acids, phlobatanin, steroids and terpenoids according to establish procedures (Trease and Evans, 1989; Sofowora, 1993; Jigna et al., 2006 and Harborne,1973, Aguinaldo et al., 2005). Fresh stock solution of each extract was prepared for each experiment at different concentration. For the detection of alkaloids and glycosides, 50mg of extract was dissolved in 5 ml of dilute HCl and then filtered. The filtrate was used for the detection of alkaloids and glycosides; whereasdetection of phenolics, tannins, phytosterols, phytosteroids, carbohydrate, flavonoids, proteins and amino acids, 50 mg of extract was dissolved in 5 ml of distilled water and then filtered. The filtrate was used for detection of phenolics, tannins, phytosterols, phytosteroids, carbohydrate, flavonoids, proteins and amino acids.

CHEMICAL TESTS FOR CLOVE BUD EXTRACT

TEST FOR TANNINS:-

2ml of each extract was added separately to 4ml of water and a few drops of 0.1% FeCl3 were added to the extracts to form a blue colored solution (fig.1).

TEST FOR TERPENOIDS:-

Salkowaski test was used. 5ml of various extracts was taken in different test tubes.To each of them 2mlof chloroform was added, along with it 3ml of concentrated sulphuric acid was added slowly to form a layer (fig.2).

TEST FOR SAPONINS:-

1ml of the extract was added to 20ml of distilled water in a test tube and was shaken vigorously for15minutes. Formation of the foamy layer indicated the presence of saponins (fig.3).

TEST FOR ALKALOIDS 1.DRAGENDORFF'S TEST

To 2.0ml filtrate of plant drug extract, 2.0ml of Dragendorff's reagent was mixed. Formation of reddish brown precipitate indicated the presence of alkaloids(fig.4).

2. HAGER'S TEST

To 2.0ml filtrate of plant drug extract, 2.0ml of Hager's reagent was mixed. Formation of yellow colour indicated the presence of alkaloids(fig.5).

3. *MAYER'S TEST*

To 2.0ml filtrate of plant drug extract, 2.0ml of Mayer's reagent was mixed. Formation of reddish brown precipitate indicated the presence of alkaloids(fig.6).

4. *WAGNER'S TEST*

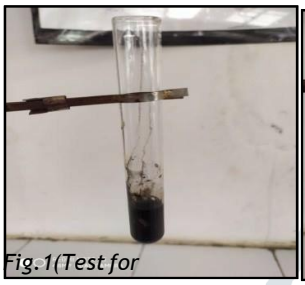


Fig.1(Test for

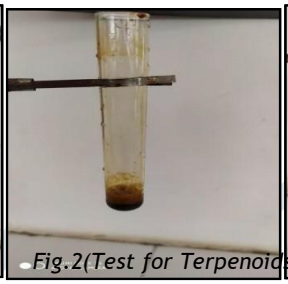
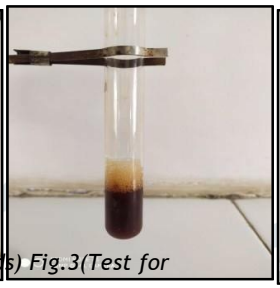


Fig.2(Test for Terpenoids)



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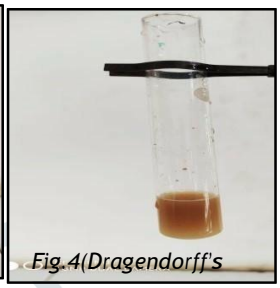


Fig.4(Dragendorff's

Fig.5(Hager'sTe

Fig.6(Mayer's

Fig.7(Wagner's

To 2.0ml filtrate of plant drug extract, 2.0ml of Wagner's reagent was mixed. Formation of reddish brownprecipitate indicated the presence of alkaloids(fig.7)

CHEMICAL TESTS FOR FENUGREEK SEED EXTRACT

TEST FOR ALKALOIDS :

To identify the presence of alkaloids in the extract 2ml of extract is taken and to that 2ml of Wagner's reagent is added. A brownish precipitation formation is observed. Thus it indicates the presence of alkaloids(Fig.1)

1. *Test for Cardiac glycosides:*

   To test the presence of glycosides, 2ml of extract is dissolved with 2ml of chloroform then carefully add concentrated sulphuric acid to form a layer. Deep reddish brown colour at the interface of steroid ring indicates the presence of cardiac glycosides(Fig.2)

2. *Test for Flavonoids:*

   To know the presence of Flavonoids in the seeds, 2ml of extract is added to 2ml of 10% lead acetate. Yellowish green colour indicates the presence of flavonoids.(Fig.3)

3. *Test for Saponins:*

   For this, 2ml of extract is dissolved with 2ml of Benedict's reagent. Blue black ppt indicates the presence of saponins(Fig.4)

4. *Test for Tannins:*

   To know the presence of tannins, 2ml of extract is treated with 0.1% of Ferric chloride. Brownish green layer indicates the presence of tannins(Fig.5)

5. *Test for Terpenoids:*

   To identify the presence of Terpenoids , 2ml of extract is dissolved with 2ml of chloroform and concentrated sulphuric acid is carefully added to form a layer. A reddish brown colour is observed which indicates the presence of terpenoids(Fig.6)

6. *Test for Reducing sugars:*

   The extract was shaken with distilled water and filtered. The filtrate is boiled with Fehling's solution A and B for few minutes an orange red precipitate indicates the presence of reducing sugars(Fig.7)

7. *Test for Glycosides:*

To identify this, extract is hydrolysed with HCL solution and neutralized with NaOH solution.

Few drops of Fehling's solution A and B are added, Red precipitateindicates the presence of glycosides(Fig.8)

Fig.1(Test for alkaloides)

Fig.2(Test for cardic glycosides)

Fig.3(Test for flavonoids)

Fig.4(Test for saponins)

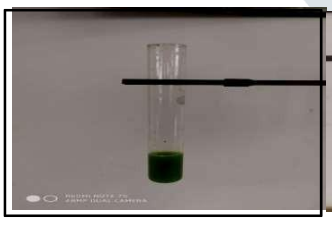


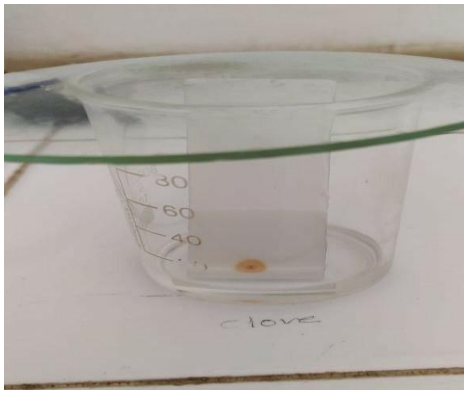
Fig.5(Test for Tannins)

Fig.6(Test for Terpenoids)

Fig.7(Test for Reducing Sugars)

Fig.8(Test for Glycosides)

PHYTOCHEMICAL SCREENING AND IDENTIFICATION OF MEDICINALLY ACTIVE SUBSTANCES FOUND IN AQUEOS EXTRACT OF CLOVE AND FENUGREEK SEEDS :-



Thin layer Chromatography of clove

Comparison between the essential oil of clove and concordance of R.f Value was found to be 0.65 with mobile phase hexane/acetone 9:1

Thin layer Chromatography of Fenugreek

Comparison between the essential oil of fenugreek and concordance of R.f Value was found to be 0.65 with mobile phase methanol/hexane/acetone 4.5:4.5:1

ANTIMICROBIAL ASSAY

The agar well diffusion method described by Zaria, (1955) was adopted for the antimicrobial sensitivity test. For antibacterial studies, the microbial strains Staphylococcus aureus, Escherichia coli was obtained from D B Science College ,Gondia

Preparation of Inoculums:

Bacterial suspensions were prepared from overnight cultures by the direct colony method. Colonies were taken directly from the plate and suspended broth. These pre culture broth were allowed to stand overnight in a rotary shaker at 37°C, after which these cultures were maintained on broth in freeze for further use.

Preparation of growth media:

Nutrient agar was used for preparation of medium for growth of above said organisms. Nutrient agar were taken (2.3 gm with 100 ml of distilled water) for preparation of growth media. Prepared nutrient agar was autoclaved at 121°C and 15 lb pressure and then nutrient agar was poured in petri plates under the laminar flow with suitable sterile conditions. After solidification, plates were kept in incubator at 24 hours for checking of contamination in media, followed by using the plates for further testing the antibiotic susceptibility of the isolated strains.

Preparation of sample from extract :-

To prepare the samples for the evaluation of antimicrobial activity

Preparation of sample from aqueous extract of clove

10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20% of nine to ten concentration were dissolve in 1ml of dimethyl sulfoxide (DMSO) solution with respect to 100mg/ml.

Preparation of sample from aqueous extract of fenugreek

10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20% of nine to ten concentration were dissolve in 1ml of dimethyl sulfoxide (DMSO) solution with respect to 100mg/ml.

Agar welldiffusion method (Bauer et al., 1966) for antimicrobialactivity:-

The seed extract of Trigonella foenum-graecum and bub extract of Syzygium aromaticum was tested by agar well diffusion method.
Antimicrobial activity was checked against Gram- positive S.aureus , Gram −negative E.coli . Figure 3.1: Principle of antimicrobial assay:
By the use of Agar well diffusion assay, showing zone of clearance around the wells

1) *wells*
2) *solvent alone and used as a negative control,*
3) *growth of bacteria around the plates,*
4) *antibacterial drug alone and used as a positive control,*
5) *zone of inhibition around the drug,*
6) *plant extract and also a zone of inhibition around plantextract*

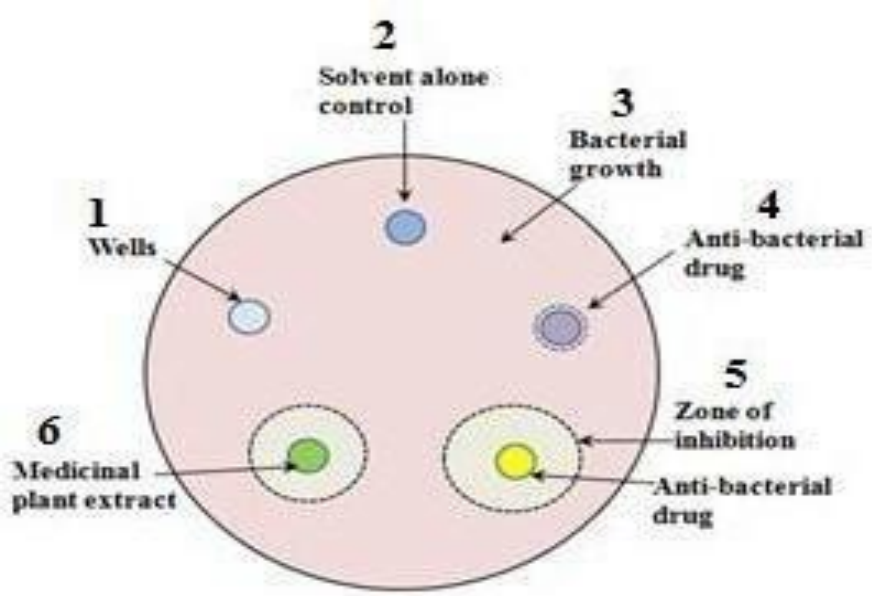


Fig:-3

Firstly the entire agar surface was streaked with the swab for 4 times with different bacteria using a sterile cotton swab in different plates respectively. The inoculums was allowed to dry for 5 minute . Then a wells with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borerora tip and different volume (100-400 mL) of the antimicrobial agent or extract solution at desired concentration is introduced into the well.Different concentration of extracts i.e. aqueous, methanol, ethylacetate, chloroform, acetone extract of seeds with different volumes, antibiotics (positive control- Ampicillin (10 µg)) and DMSO (negative control) were poured into 6mm well and plates were incubated at 37°C aerobically for 18 hours. The test materials having antimicrobial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the medium. Appearance of growth was considered as bacteriostatic, whereas no growth was considered as bactericidal effect.

***Preparation of combine formulation of crude extract** :-*

Formulation	*Clove : Fenugreek*
F1	*1:1*
F2	*4:5*
F3	*5:6*

Combined antimicrobials are preferred as microbial tolerance is less likely to develop against substances having more than one type of modes of action. Combinations of the spices in several cases demonstrated synergistic or additive effects on microorganisms and showed lower FICs (Table 3). Combinations like aqueous extract of clove and fenugreek showed synergistic activity against E.coli and additive effects against Staphylococcus aureus.

Measurement of MIC of crude drug extract:-

Clove (Cl)			*Fenugreek (Fe)*			*Combination*		
MIC	*RC*	*Cmax*	*MIC*	*RC*	*Cmax*	*MIC*	*RC*	*Cmax*
E.coli								
27	*27*	*180*	*18*	*18*	*54*	*90*	*90*	*90*
S.aureus								
27	*27*	*90*	*36*	*27*	*136*	*54*	*36*	*125*

Minimum inhibitory concentrations (MICs) were determined by broth dilution method in culture tubes (Jorgensen et al., 1999). Various concentrations (50, 40, 30, 25, 20, 15, 10, 7.5, 5, 2.5, 1.25 mg dry weight/ml) of the extracts were added to broth immediately after inoculating with fresh 0.2 ml culture of the strain, keeping final volume at 5 ml. The cultures were incubated on a rotary shaking incubator at 37ºC for 48 hours. The lowest concentration of the spice or herbal extracts showing no visible growth was considered as the MIC.

RESULT

1. ***Procurement of crude drug :-*** *Fenugreek (Trigonellafoenum-graecum) and Clove (Syzygium aromaticum)plantswerecollectedfrom localmarket of Gondia,Maharashtra, India. The taxonomicidentities of these plants were confirmed by the taxonomist.*

2. ***Preparation of aqueous extract of drug :-***

Clovebuds(75gram)were washed, dried and then weighed. The buds are then reduced to finely divided size by the process of grinding. Powdered clove are fed inside round bottom flask of Soxhlet apparatus and the assembled apparatus was allowed to work for 24-48 hours.

The aqueous extraction of fenugreek are prepared of fenugreek plant material for which weighed 25gm of seeds powdered, add 250ml of sterile distilled water to it, kept the mixture for 7 days and filtered it with muslin cloth, filtrate was allowed for hot extraction process on water bath at 40^0 C.

3. ***Preparation of sample:-***

Conc. Of clove	*Frequency*	*Mean diameter of inhibition zone*
10%	*5*	*8*
11%	*5*	*8.5*
12%	*5*	*9*
13%	*5*	*9.5*
14%	*5*	*10*
15%	*5*	*11*
16%	*5*	*14*
17%	*5*	*16*
18%	*5*	*17*
19%	*5*	*18.5*
20%	*5*	*19*

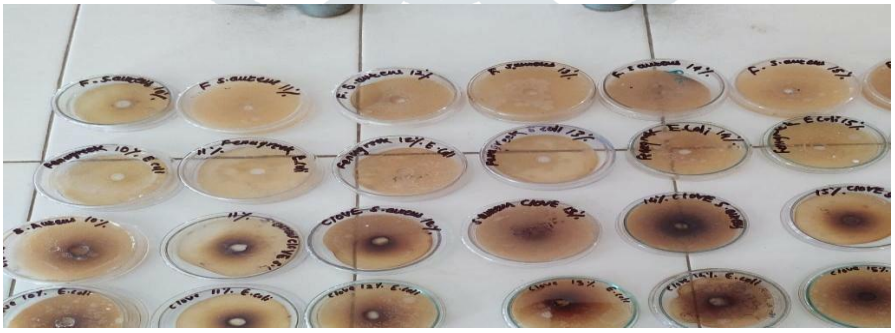
Table(1) summarized the antibacterial activity of clove aqueous extract and the results showed that clove extract exhibited antibacterial activity against Staphylococcus aureus at nine of the ten concentrations used in this study and the mean of the diameter of inhibition zone were (8 mm), (8.5 mm), (9 mm), (9.5 mm), (10 mm), (11 mm), (14 mm), (16mm),
(17mm)for the concentrations of (10%), (11%), (12%), (13%), (14%), (15%), (16%), (17%), (18%) of clove with respect to 100mg/ml.

Each extract was tested against the five isolates of Staphylococcus aureus. The antibacterial activity of the extracts were recorded as the mean diameter of the resulting inhibition zones of growth measured in (millimeters).The antibacterial activity of fenugreek aqueous extract was shown in Table 2.The results revealed that fenugreek exhibited antibacterial activity against Staphylococcusaureus.

As the mean of the diameter of inhibition zone were (5mm), (5.5mm), (6.5mm), (7.0mm), (7.5mm), (7.8mm), (8.0mm), (8.5mm) for the concentrations of (13%), (14%), (15%), (16%), (17%), (18%) , (19%), (20%) respectively while the concentrations of (10%), (11%), (12%) did not give any inhibition .

4. ***Agar well diffusion method:-***

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts. Similarly to the procedure used in disk-diffusion method, the agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or a tip, and a volume (20–100 mL) of the antimicrobial agent or extract solution at desired concentration is introduced into the well. Then, agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested.



5. ***Preparation of combine formulation of crude extract :-***

Formulation	*Clove : Fenugreek*	*Effect*
F1	*1:1*	*Less*
F2	*4:5*	*More*
F3	*5:6*	*More*

Combined antimicrobials are preferred as microbial tolerance is less likely to develop against substances havin g more than one type of modes of action. Combinations of the spices in several cases demonstrated synergistic or additive effects on microorganisms and showed lower FICs (Table 3). Combinations like aqueous extract of clove and fenugreek showed synergistic activity against E.coli and additive effects against Staphylococcus aureus.

6. ***Measurement of MIC of crude drug extract:-***

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MIC	*RC*	*Cmax*	*MIC*	*RC*	*Cmax*	*MIC*	*RC*	*Cmax*
E.coli								
27	*27*	*180*	*18*	*18*	*54*	*90*	*90*	*90*
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27	*27*	*90*	*36*	*27*	*136*	*54*	*36*	*125*

Minimum inhibitory concentrations (MICs) were determined by broth dilution method in culture tubes (Jorgensen et al., 1999). Various concentrations (50, 40, 30, 25, 20, 15, 10, 7.5, 5, 2.5, 1.25 mg dry weight/ml) of the extracts were added to broth immediately after inoculating with fresh 0.2 ml culture of the strain, keeping final volume at 5 ml. The cultures were incubated on a rotary shaking incubator at 37ºC for 48 hours. The lowest concentration of the spice or herbal extracts showing no visible growth was considered as the MIC.

DISCUSSION

Natural antimicrobial agent have been more popular due to their efficacy against antibiotic resistant microorganism and campaign for consumption of natural product according to previous reports, extract of both crude drugs Clove (Syzygium aromaticum) and Fenugreek (Trigonella foenum-graecum) demonstrated antimicrobial activity against different microorganisms.Combination of aqueous extract of clove and fenugreek showed antibacterial activity against S.aureus and E.coli with MICbeing 90 and 54 , respectively. Comparatively, in this study S.aureus exhibited lower Minimum inhibitory concentration (54) for aqueous extract ofSyzygium aromaticum andTrigonella foenum-graecum than E.coli (90).The antibacterial activity of formulation F2 and F3 shows the maximum activity as compared to F1.

CONCLUSION

From the present study, it is concluded that ANTMICROBIAL ACTIVITY OF AQUEOUS CLOVE AND FENUGREEK SEED EXTRACT COMBINATION was evaluate. Natural substances which are part of daily diet nutritional supplement with antimicrobial property constitute a new source of herbal drugs. The antimicrobial potential of this plant is a remarkable relevance asEugenia caryophyllus andT. foenum-graecum extract are edible and generally consumed and no extra processing is needed for its administration. Further isolation and exploration on the isolated chemical constituents on antimicrobial activity may lead to

chemical entities for clinicalapplication.

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