



# “EVALUATION OF ANTIMICROBIAL ACTIVITY OF AQUEOUS CLOVE AND FENUGREEK SEED EXTRACT COMBINATION”

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## ABSTRACT

The clove bud (*Syzygium aromaticum*) extract used to be performed with the aid of using Soxhlet equipment and fenugreek seed (*Trigonella foenum-graecum*) extract was once executed via maceration technique by way of the usage of distilled water as solvent. Evaluation of antimicrobial undertaking of both extract was carried out by means of agar nicely diffusion method in opposition to gram tremendous bacterium *S. aureus* and gram negative bacterium *E.coli*. Minimum inhibitory concentration (MIC) of combine extracts was once decide by way of using broth dilution method. TLC bioautography and phytochemical evaluation had been also performed.

**Key words:** Maceration, Bioautography, Phytochemical, Evaluation, Extract

## INTRODUCTION

### Clove

The symbol of dignity that is what “Clove” truly means. It is a valuable and treasured Spice of the world. Cloves (*Syzygium aromaticum*, *Eugenia aromaticum* or *Eugenia caryophyllata* ) are the fragrant dried flower buds , which are frequently used in biryanis , pickles , salads and garam masala.



Fig: 1 clove

### Planting material

The seeds ought to be amassed from completely ripe fruits for elevating seedlings. Fruits for seed collection recognised oftentimes as “mother of clove” are allowed to ripe on the tree and drop down naturally.

### Botanical Name

*Eugenia caryophyllus*, *Syzygium aromaticum*

### Common Names

Cloves, Carophyllus, Clovos, Caryophyllus

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

**Family:** Myrtaceae

**Geographical Source:** It is indogenous to Amboyna & Molucca islands. It is now cultivated chiefly in Zanzibar, Pemba, Penang, Madagascar, Caribbean islands, Sri Lanka & India.

### Collection and cultivation

The seeds should be accrued from absolutely ripe fruits for elevating seedlings. Fruits for seed collection regarded commonly as “mother of clove” are allowed to ripe on the tree and drop down naturally.

### Chemical Constituents

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

### Volatile Constituents

Clove yields distinctive sorts of unstable oil [oil extracted from i. leaves, ii. the stem, iii. the buds and iv. the fruit.] The chief factor of all the sorts of oil is eugenol.

**Bud Oil** <sup>[2]</sup>, **Leaf Oil** <sup>[1][4]</sup>, **Clove Stem Oil** <sup>[1]</sup>, **Fruit Oil** <sup>[2]</sup>

### Non-volatile Constituents

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

**Tannins** <sup>[5]</sup>, **Triterpenes** <sup>[6]</sup>, **Sterols**, **Flavonoids**

### Uses

Antiseptic, Carminative, Flavouring agent, Stimulant, Local anaesthetics, Spice, Oil in perfumery, dental preparation in mouth washes, expectorant, reduce dental pain

## Fenugreek

Fenugreek is used historically as demulcent, laxative, lactation stimulant and famous hypocholesterolemic, hypolipidemic and hypoglycemic exercise in healthful and diabetic animals and humans. The defatted seeds of fenugreek reduce gastrointestinal absorption of glucose and cholesterol and bile acids secretion.



Fig no: 2

**Fenugreek:** *Trigonella foenum-graecum*

**Family:** Fabaceae (Leguminosae)

### Collection and Cultivation

The finely grinded seed powder was taken. From the whole extract 10 gm. of seed powder was taken and 50 ml of ethyl alcohol was introduced to that extract stirred it continuously 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, a steroid sapogenin found in fenugreek is the beginning compound for over 60% of the complete steroid manufacturing through the pharmaceutical industry. Other sapogenins found in fenugreek seed encompass yamogenin, gitogenin, tigogenin, and neotigogenins.

### Uses <sup>[8][10]</sup>

1. Fenugreek is used in the treatment of wound, abscesses, arthritis, bronchitis, ulcer and digestive problem.
2. It is additionally used traditionally as a demulcent, laxative, lactation stimulant and famous hypocholesterolemic, hypolipidemic, and hypoglycemic activity in healthful and diabetic animals and human.

## AIMS AND OBJECTIVE

### Aims

- 1) To check the antimicrobial activity of Fenugreek seeds and clove.
- 2) To give the knowledge about chemical constituents of Fenugreek seeds and clove.
- 3) To study the effect of antimicrobial agents on microbes.

4) To study the various methods of extraction of Fenugreek seeds and clove.

## Objectives

- 1) To accumulate the plant material.
- 2) To authenticate the plant material.
- 3) To extract plant fabric with the aid of the usage of soxhlet apparatus.
- 4) Phytochemical screening of chemical components existing in both extracts.

## METHODOLOGY

### Drying

The fenugreek seeds and clove buds had been added to lab in air tight bag and seeds and buds were sun-dried so as to minimize the moisture content.

### Grinding

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

### Preparation of extracts

#### Preparation by Aqueous extraction of clove:-

Clove buds (75gram) had been washed, dried and then weighed. The buds are then reduced to finely divided measurement by using the system of grinding. Powdered clove are fed inside the Soxhlet apparatus and the assembled apparatus was once allowed to work for 24-48 hours. After extraction of clove bud by means of Soxhlet equipment then listen the extract in hot air oven at 40<sup>0C</sup>.

#### Preparation by Aqueous extraction of fenugreek:-

The aqueous extraction of fenugreek are organized of fenugreek plant fabric for which weighed 25gm of seeds powdered, add 250ml of sterile distilled water to it, stored the combination for 7 days and filtered it with muslin cloth, filtrate used to be allowed for warm extraction method on water tub at 40<sup>0C</sup>.

### Qualitative estimation of phytochemicals:

#### Phytochemical screening:

The Phytochemical screening of unique extracts was carried out to determine the presence of lively secondary metabolites. Fresh inventory solution of each extract used to be organized for each scan at different concentration. For the detection of alkaloids and glycosides, 50mg of extract used to be dissolved in 5 ml of dilute HCl and then filtered. The filtrate was used for the detection of alkaloids and glycosides; where as detection of phenolics, tannins, phytosterols, phytosteroids, carbohydrate, flavonoids, proteins and amino acids, 50 mg of extract used to be dissolved in 5 ml of distilled water and then filtered. <sup>[11]</sup>

#### Chemical tests for clove bud extract

- 1] Test for tannins
  - 2] Test for terpenoids
  - 3] Test for saponins
  - 4] Test for alkaloids
- a) Dragendorff's test b) Hager's test c) Mayer's test d) Wagner's test

**Chemical tests for fenugreek seed extract:**

1. Test for Cardiac glycosides 2. Test for Flavonoids 3. Test for Saponins 4. Test for Tannins 5. Test for Terpenoids 6. Test for reducing sugars 7. Test for Glycosides

**Antimicrobial assay**

The agar well diffusion method used to be adopted for the antimicrobial sensitivity test. For antibacterial studies, the microbial strains *Staphylococcus aureus*, *Escherichia coli*.

**Preparation of Inoculums**

Bacterial suspensions have been geared up from in a single day cultures by using ability of the direct colony method. Colonies had been taken at once from the plate and suspended broth. These pre culture broth had been allowed to stand in a single day in a rotary shaker at 37°C, after which these cultures had been maintained on broth in freeze for in a similar way use

**Preparation of growth media**

Nutrient agar used to be used for preparation of medium for increase of above stated organisms. Nutrient agar were taken (2.3 gm. with 100ml of distilled water) for instruction of growth media. Prepared nutrient agar was autoclaved at 121°C and 15 lb. pressure and then nutrient agar was poured in petri plates beneath the laminar float with suitable sterile conditions. After solidification, plates have been saved in incubator at 24 hours for checking of infection in media, followed via using the plates for similarly testing the antibiotic susceptibility of the remoted 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 well diffusion method for antimicrobial activity:-**

The seed extract of *Trigonella foenum-graecum* and bub extract of *Syzygium aromaticum* was once examined through agar well diffusion method. Antimicrobial assignment was once checked toward Gram-advantageous *S.aureus*, Gram –negative *E.coli*. Figure5: Principle of antimicrobial assay:

By the use of Agar appropriate diffusion assay, showing quarter of clearance spherical the wells

- 1) wells
- 2) solvent alone and used as a awful control,
- 3) boom of micro-organism round the plates,
- 4) antibacterial drug alone and used as a brilliant control,
- 5) area of inhibition spherical the drug,
- 6) plant extract and additionally a quarter of inhibition round plant extract<sup>[11]</sup>

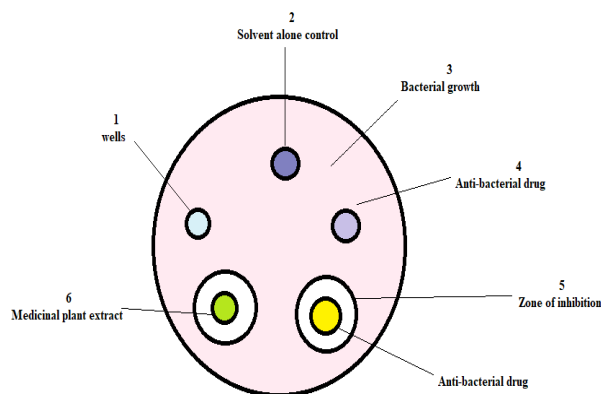


Fig no.3

**Table no.1 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 probably to advance in opposition to substances having more than one kind of modes of action. Combinations of the spices in several instances proven synergistic or additive consequences on microorganisms and confirmed lower FICs (Table 3). Combinations like aqueous extract of clove and fenugreek showed synergistic endeavor towards E.coli and additive effects against Staphylococcus aureus.

**Table no.2 Measurement of MIC of crude drug extract:-**

Clove(cl)			Fenugreek(Fe)			Combination		
MI	RC	Cmax	MIC	RC	Cmax	MIC	RC	Cmax
E.coli								
28	28	181	19	19	55	91	91	91
S.aureus								
28	28	91	37	28	137	55	37	126

Minimum inhibitory concentrations (MICs) had been determined via broth dilution method in culture tubes. Various concentrations (50, 40, 30, 25, 20, 15, 10, 7.5, 5, 2.5, 1.25 mg dry weight/ml) of the extracts were delivered to broth immediately after inoculating with sparkling 0.2 ml culture of the strain, preserving final volume at 5 ml. The cultures were incubated on a rotary shaking incubator at 37°C for forty eight hours.



The lowest awareness of the spice or natural extracts showing no visible growth was once considered as the MIC.

## RESULT:

### 1. Preparation of sample for clove & Fenugreek seeds

Conc. of clove & Fenugreek seeds	Frequency	Mean diameter of inhibition zone for clove	Mean diameter of inhibition zone for Fenugreek seeds
10%	5	8.1	No Inhibition
11%	5	8.6	No Inhibition
12%	5	9.1	No Inhibition
13%	5	9.7	5.1
14%	5	10.2	5.6
15%	5	11.2	6.6
16%	5	13	7.1
17%	5	15	7.6
18%	5	16	7.8
19%	5	18.6	8.1
20%	5	19	8.6

Table summarized the antibacterial pastime of clove aqueous extract and the results showed that clove extract exhibited antibacterial pastime towards *Staphylococcus aureus* at 9 of the ten concentrations used in this study and the mean of the diameter of inhibition area were (8.1mm), (8.6 mm), (9.1 mm), (9.7 mm), (10.2mm), (11.2 mm), (13 mm), (15mm), (16mm) for the concentrations of (10%), (11%), (12%), (13%), (14%), (15%), (16%), (17%), (18%) of clove with recognize to 100mg/ml. Each extract was examined in opposition to the five isolates of *Staphylococcus aureus*. The antibacterial endeavor of the extracts had been recorded as the suggest diameter of the resulting inhibition zones of increase measured in (millimetres).

The antibacterial activity of fenugreek aqueous extract was proven in Table. The effects revealed that fenugreek exhibited antibacterial exercise against *Staphylococcus aureus*. As the suggest of the diameter of inhibition zone were (5.1mm), (5.6mm), (6.6mm), (7.1mm), (7.6mm), (7.8mm), (8.1mm), (8.6mm) for the concentrations of (13%), (14%), (15%), (16%), (17%), (18%) , (19%), (20%) respectively whilst the concentrations of (10%), (11%), (12%) did not supply any inhibition.

## DISCUSSION

Natural antimicrobial agent have been extra famous due to their efficacy in opposition to antibiotic resistant microorganism and campaign for consumption of herbal product according to previous reports, extract of each crude tablets Clove (*Syzygium aromaticum*) and Fenugreek (*Trigonella foenum-graecum*) established antimicrobial activity towards specific microorganisms. Combination of aqueous extract of clove and fenugreek confirmed antibacterial pastime against *S.aureus* and *E.coli* with MIC being 91 and 55, respectively. Comparatively, in this learn about *S.aureus* exhibited decrease Minimum inhibitory attention (55) for aqueous extract of *Syzygium aromaticum* and *Trigonella foenum-graecum* than *E.coli* (91).The antibacterial endeavor of formulation F2 and F3 shows the maximum recreation as in contrast to F1

## CONCLUSION

From the present study, it is concluded that ANTMICROBIAL ACTIVITY OF AQUEOUS CLOVE AND FENUGREEK SEED EXTRACT COMBINATION used to be evaluate. Natural resources which are section of every day weight loss plan dietary supplement with antimicrobial property constitute a new supply of herbal drugs.

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