Phytochemical Screening, Antimicrobial Activities and Gas Chromatography Profile of the n-Hexane extract of *Trigonella foenum-graecum* "Fenugreek" Seed Oil

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***Abstract*: To evaluate the phytochemical constituents, antimicrobial activity, and GC profile of the seed oil extract of *Trigonella foenum-graecum*. In the present research, solvent extraction by maceration was performed by using 95% n-Hexane. Phytochemical analysis of the n-Hexane extract was carried out. Phytochemical screening showed that alkaloids, tannins, flavonoids, carbohydrates, balsam, and phenols were negative, while saponins, resins, terpenes, steroids and cardiac glycosides were positive. The Antimicrobial potential of *Trigonella foenum-graecum* seed oil extract was determined using the agar well diffusion method by the zone of inhibition on nutrient agar. MIC and MBC tests were performed to determine the relative antimicrobial properties of the extract. GC profile was also carried out to determine the constituents in the oil extracts. For the agar, well diffusion method of antimicrobial activity screening, all the concentrations of *Trigonella foenum-graecum* showed negative results in all the bacteria except for Bacillus subtilis. The extract is highly selective in terms of antimicrobial potency; the isolation of the active component(s) may present more insight. For the GC profile, 11 peaks were recorded.**

***Keywords*: *Trigonella foenum-graecum*, agar well diffusion method, bacterial strains, inhibition.**

I. INTRODUCTION

Plant extracts are treasured based on their ethnomedicinal values. Their various intrinsic pharmaceutical, cosmetic, and nutraceutical properties further define their economic roles for entrepreneur purposes. Fenugreek is one plant that possesses phytoconstituents such as flavonoids, alkaloids, terpenoids, steroids, saponins, anthocyanins, and tannins (Sumayya et al., 2012). The evolution of resistant strains of microorganisms to multiple antibiotics in combination therapy has generated great interest for research into plants with active compounds that elicit therapeutic, medicinal, aromatic, and aesthetic uses Gurinder and Daljit et al., (2009), Newman and Cragg et al., (2007). Phytochemicals are natural and non-nutritive bioactive compounds produced by plants, which act as protective agents against external stress and pathogenic attack. It could be administered singly or combined with other active agents in the formulation of drugs. Rashmi et al., (2011). Infectious diseases and drug resistance have raised serious concern for successful treatment Moniruzzaman et al., (2015). Food safety, food packaging, and storage shelf life are some burning issues in the food industry. The aspects of flavoring and preservation of food materials are yet to be well tapped. The global quest for new, effective, safe and environmentally friendly resources for preventing and curing infections has propelled this current work. Medicinal plants have remained the major source of highly effective and friendly drugs. Plants draw attention when they are discovered with molecular entities of untapped chemical diversity Malherbe et al., (2012). In recent times, the high cost of leaving gradually revived people's interest in the use of natural medicinal and aromatic plants in developed as well as developing countries. The measure of safety associated with the utilization of plant-derived drugs is yet another pull toward Phytomedicine as reported by Rai et al., (2003). Traditionally the people of India have a long-standing practice of using a wide variety of herbal products in the treatment of diseases or as preservatives in foods. Spices are indispensable components of Indian cuisines since ancient times. Spices are considered as a rich source of bioactive antimicrobial compounds Dash et al., (2011). Disasters from pathogens are managed by employing antibiotics, however, the misuse of antibiotics is leading to widespread antibiotic resistance thus creating alarming clinical situations where old remedies to infections are rendered ineffective. Recent trends show that many infections occur because microorganisms have mutated overcoming conventional therapy. Pathogens display the genetic ability to develop resistance to many antibiotics Fair et al., (2014), Landecker et al., (2015), Li et al., (2018). Currently, there has been a surge of interest in the antibacterial properties of plants extract. It is progressively acceptable that these phytochemicals will be prescribed by doctors as antibacterial medications Bhalodia et al., (2011). Many investigations were carried out on the therapeutic applications of various plant species on different diseases such as fungal, viral, and bacterial contagion. Approximately 33% of the world's population depends on conventional/ therapeutic plants and their extract to meet their essential needs. At the same time, the world health organization (WHO) reported that 80% of individuals worldwide are

accustomed to using manmade medications Kumar et al., (2012).

Fenugreek seeds

The fenugreek seed is known for its pleasantly bitter/slightly sweet taste. The seed is used to flavor many foods mostly curry powders, tea, and spice blend. Fenugreek seeds have a central hard and yellow embryo which is surrounded by a large layer of white-semitransparent endosperm. (Betty et al., 2008)

The chemical composition of fenugreek [such as seeds, husks, and cotyledons] showed that endosperm had the highest [4.63g/100g] saponins and [43.8g/100] protein content. The husk contains higher total polyphenols. The extract of endosperm and fenugreek seeds is about 200 g concentration the seeds of fenugreek contain about 0.1-0.9% of diosgenin which is extracted commercially. Naidu et al., (2010). The seeds also contain saponin [fenugrin B]. Fenugreek seeds have been found to contain coumarin compounds as well as a number of alkaloids such as trigonelline, gentianine, and carpanine. A large amount of trigonelline is degraded to nicotinic acid and similar pyridines during roasting. The major bioactive compounds in fenugreek seeds are believed to be polyphenol compounds such as rhaponticin and isovitexin. He et al., (2015) Small amounts of volatile oil and fixed oil have been found in fenugreek seeds. Sowmya et al., (1999).

Fenugreek oil

Fenugreek seeds from the plant are consumed for their therapeutic properties. The oil is extracted from the seed by different methods of extraction. Below are the benefits of fenugreek oil;

Aids digestion. Enhances physical endurance and libido. May improve diabetes. Enhances breast milk supply. May help to fight Acne and Promote Skin Health. Working as an expectorant May suppress appetite (Christine et al.,2020)

II. METHODOLOGY

Sample Collection

100g of seeds of *Trigonella foenum-graecum* (Fenugreek) were weighed and bought from a grocery store in terminus market, Jos plateau state on 17th July 2021.

Sample Preparation

The fenugreek seeds were washed with running water, spread, and allowed to dry at room temperature for the duration of one week. The seeds were placed at a shaded area without exposure to sunlight to avoid any evaporation of active constituents such as phenol. The seeds were crushed into seeds powder by using the crusher. The seeds, powder was then collected in the conical flasks. The mouth of conical flasks was sealed with polyethene film and covered with aluminum foil and kept in the cupboard.

Extraction of Essential Oil by Maceration Using N-Hexane as Solvent

1. 100g of seed powder was weighed.
2. 500 ml of n-Hexane was measured using a measuring cylinder.
3. 100g of seeds powder was immersed in the ethanol in a 1L conical flask.
4. The mouth of the conical flask was filmed with polyethene and the flask was covered with aluminum foil to avoid any evaporation of the ethanol and the active components of the extract.
5. The flask was swirled for 5 minutes to ensure an even and complete extraction of the seeds powder.
6. The flask was then kept in the dark in the cupboard for one week and swirling was done throughout the maceration process.
7. The mixture was filtered by using muslin cloth after the maceration process was completed.
8. The filtrate was collected in a beaker which was then transferred into a conical flask.
9. The filtrate, which was the macerated product was kept in a flask and covered with polyethene film and aluminum foil. The macerated extract was kept for further use in the phytochemical screening of the extract after evaporating the extract using a rotary evaporator. Qualitative phytochemical screening of fenugreek oil was carried out.

Phytochemical Analysis of the Plant Extract

The fenugreek seed extract was subjected to phytochemical screening to check for the presence or absence of plant secondary metabolites such as Saponins, tannins, alkaloids, flavonoids, steroids and terpenes, cardiac glycosides, balsam, carbohydrates, phenols, and resins.

1. *Test for alkaloids***:** To 2mL of extract, a few drops of Dragendorf's reagent were added; the appearance of an orange coloration is an indication of the presence of alkaloids.
2. *Test for flavonoids:* To 2mL of the extract, a few drops of 5% lead acetate were added to give a cream light color which indicated the presence of flavonoids.
3. *Test for tannins:* To 2mL of the extract, a few drops of 10% ferric chloride were added to give a deep bluish or greenish color which indicated the presence of tannins.
4. *Test for Saponins:* To 1ml of the extract, 4mL of distilled water was added and shaken vigorously. For ation of froth indicated the presence of Saponins.
5. *Test for terpenes and steroids:* To 1mL of the extract, 2mL of concentrated Sulphuric acid was added along-side of the test tube. Formation of reddish-brown ring at the interphase indicated the presence of terpenes and steroids.
6. *Test for cardiac glycosides (Salkowski's test):* 2mls of the extract was dissolved in 2mls of chloroform and Sulphuric acid was carefully added to form a lower layer. A reddish-brown color at the interphase indicated the presence of cardiac glycosides.
7. *General test for balsam:* 3drops of alcoholic ferric chloride were added to 2mls of the extract. A dark

green colour formation indicated the presence of balsam.

8. *Test carbohydrates:* 5drops of the extract were added to 2.0mls of Benedict's reagent, placed on a hot plate for 5 minutes, and was observed for the formation of brick red precipitation which indicated the presence of carbohydrates.
9. *Test for phenol:* To 2mls of the extract, 2mls of ferric chloride was added and observed for the formation of a deep bluish-green coloration which indicated the presence of phenol.
10. *Test for resins:* To 2mls of the extract, 2mls of acetic anhydride was added and drops of concentrated Sulphuric acid were added to observe for a violet color which indicated the presence of resins.

III. ANTIMICROBIAL ACTIVITIES

The antimicrobial activity of the plant extract was investigated by the agar well diffusion method. The MIC (minimum inhibitory concentration) was carried out with the extract of T. foenum-graecum and the MBC was determined to reconfirm the MIC (minimum inhibitory concentration). Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial agent required to inhibit the visible growth of a microorganism after overnight incubation. The antimicrobial activity screening procedures were performed based on the standard protocol by Iqbal and by Baydoun with slight modifications.

Agar well diffusion method

Agar well diffusion method is widely used to evaluate the antibacterial activity of plants or microbial extracts. The agar plate surface was inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 to 8mm was punched aseptically with a sterile metallic borer, and a volume (20–100μL) of the antimicrobial agent or extract solution at desired concentration was introduced into the well. Then, agar plates were incubated under suitable conditions depending on the test microorganism.18 the standard microorganisms used in this study were: Bacillus subtilis (ATCC 6633), Staphylococcus aureus (ATCC 29737), Escherichia coli (ATCC 8739), Pseudomonas aeruginosa (ATCC 27853). All of the processes of antimicrobial screening activity was done in a laminar air flow cabinet to avoid any contamination or cross-contamination occur especially during the processed of nutrient agar preparation and well diffusion test.

Serial dilution of macerated extract

4g of the gummy macerated extract and 40ml of distilled water was measured and distilled water was used for serial dilution and sent to the autoclave prior to use for sterilization. This gave a stock solution that contained a concentration of 100mg/mL. The solution was then transferred into a test tube and mixed well by using a vortex mixer and water bath sonicator. Six dilutions were prepared from the extract as 10mg/ml, 20mg/ml, 40mg/ml, 60mg/ml, 80mg/ml, and 100mg/ml.

Preparation of bacterial strains

0.65g of nutrient broth was weighed and dissolved in 50mL of distilled water. A magnetic stirrer was used to dissolve the nutrient broth completely. A total of four nutrient broths were prepared by following in the same way. The mouths of the four conical flasks were fitted with cotton and covered with aluminum foil. Then four conical flasks containing the nutrient broth were subjected to an autoclave for 2 hours at 121°C for sterilization and were allowed to cool down. Inoculation of bacteria strains into the nutrient broth was done in the laminar airflow cabinet. The 3 to 4 loops of bacterial culture of Staphylococcus aureus were inoculated into the nutrient broth. The nutrient broth with bacterial strain was incubated in an incubator at a temperature of 37°C for 24 hours. The same process was carried out with Bacillus subtilis, Escherichia coli, and Pseudomonas aeruginosa.

Preparation of nutrient agar plate

Twenty-one grams of nutrient agar powder was weighed. The nutrient agar powder was dissolved in 750mL of distilled water in a 1000mL conical flask. A magnetic stirrer was used to stir the mixture to ensure proper mixing. After stirring, the nutrient agar solution was sent into an autoclave for sterilization at a temperature of 121°C for 2 hours. After the autoclave, the hot sterilized nutrient agar solution was poured into the Petri plates in the laminar airflow cabinet. Each of the Petri plate contained approximately 25mL of nutrient agar solution which can only occupy 60-70% of the petri plate. The nutrient agar solution was allowed to solidify in the cabinet and the UV of the laminar airflow cabinet was on for 15mins to achieve sterilize

Agar well diffusion test

70% of ethanol was sprayed to sterilize both hands before the start of the test. Total of six concentrations of extract were used 10mg/L, 20mg/L, 40mg/L, 60mg/L, 80mg/L, and 100mg/L. Two plates were needed for each bacterial strain. 0.1mL of nutrient broth containing bacterial strain was transferred on the two plates by using a micropipette. The sterilized L- shaped spreader was used to spread the bacterial strain on the plate evenly. 3 wells were made on one of the two plates by using a sterilized borer. The 3 wells were separated with each other evenly forming an equal triangular distance. The well was filled until around 60 to 70% of the well's height. Next, the lid was shown to the flame for a while, and the plate was covered. The process was repeated for each bacterial strain and duplicate sets were made for the purpose of comparison. The plates with bacterial strain and extract were incubated in the incubator at a temperature of 37°C for 24 hours. The zone of inhibition of each plate was observed, measured, and recorded. For each bacterial strain, four plates were needed for the duplicate sets. One plate was used as a positive control plate for each bacterial strain and another extra plate was used as a negative control plate.

Preparation of standard McFarland bacterial culture

Four to five loops of the bacterial strain were cultured on sterile nutrient broth and incubated for 24 hours at a temperature of

37°C. This step was done for four bacterial strains. After incubation of 24 hours, the nutrient broth, and the bacterial culture were centrifuged at 5000 rpm for 10 minutes. Cell mass was obtained at the bottom of the centrifuge tube. The supernatant was discarded and the cell mass collected was re-suspended into another new sterile nutrient broth. The bacterial cell mass suspension was standardized to a value of 0.08 to 0.1 of the absorbance according to McFarland standard by using a UV-Visible spectrophotometer at a range of 625 nm.

Serial dilution procedure for MIC test

30mg of the dried gummy macerated extract was dissolved in 30mL of distilled water to obtain a concentration of 1000µg/mL. Two-fold serial dilutions were made to get 5 different concentration which was 1000, 500, 250, 125, and 62.5µg/ml. MBC (minimum bactericidal concentration)

The minimum bactericidal concentration (MBC) is the lowest concentration of an antibacterial agent required to kill a bacterium over a fixed, somewhat extended period, such as 18 hours or 24 hours, under a specific set of conditions. It can be determined from the broth dilution of MIC tests by sub-culturing agar plates that do not contain the test agent. The MBC is identified by determining the lowest concentration of antibacterial agent that reduces the viability of the initial bacterial inoculum by a pre-determined reduction such as larger than or equal to 99.9%.19 After 24 hours of incubation, two tubes that showed the clear appearance of solution and were the least turbid were chosen as a test sample for each bacterial strain. 0.1ml of the selected incubated tubes was transferred onto the agar plates and spread evenly for each bacterial strain. Duplicate sets of agar plates were made. 0.1ml from each positive tube that contains the bacterial strain was transferred onto an agar plate and spread evenly. The plates were incubated at 37°C for 24 hours and the results were observed and recorded. 3.5.6 GC analysis of fenugreek oil

Ultrafast GC Configuration

For this application, the TRACE GC Ultra is configured with n SSL injector, an Ultrafast option (including the analytical column), and a Fast Flame Ionization Detector (FFID) featuring 6ms time constant and acquisition frequencies up to 300 Hz. Such a high speed is, in fact, a compulsory requirement for the correct acquisition (15-20 points/peak) of the extremely narrow peaks (approx.100 ms PW1/2h) typical for this type of chromatography. The assembly allows the instrument to achieve heating rates up to 1200 °C/min, and fast cooling times occur rapidly, as well, taking about one minute to return to 50 °C from 350 °C compared to about 4 minutes in conventional mode. The column module, connected to the split-splitless injector and the FID detector as a removable accessory, is completely and directly controlled by the instrument's local user interface and electronics.

The TRACE GC Ultra was used to perform the same application in conventional mode with the ultrafast accessory removed. All of the components of the essential oils analyzed were identified through GC/MS technique. The injections were performed using a Thermo Scientific AS3000 autosampler, designed to achieve maximum detection.

IV. RESULTS AND DISCUSSION

Table 1: Summary of the Percentage Yield

of the oil = = 8.82±0.04%

Table 2: Phytochemical Screening

S/N	PHYTOCHEMICALS	CONCENTRATIONS
1	Alkaloids	-
2	Flavonoids	-
3	Tannins	-
4	Saponins	+
5	Terpenes and Steroids	+
6	Cardiac Glycosides	+
7	Balsam	-
8	Resins	+
9	Phenols	-

Key: + = Present &- = Absent

Table 3: Antimicrobial Activity of the extract by Agar well diffusion method

C = 10mg/ml

C = Positive Control Gentamycin 10mg/ml

Table 3a: Zone of Inhibition for Bacillus subtilis (Set 1)

Concentration (mg/ml)	Diameter Reading of Zone of Inhibition (Mm)								
	1st	2nd	3rd	4th	5th	6th	7th	8th	Mean
10	-	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-	-
40	-	-	-	-	-	-	-	-	-
60	10	4	6	4	4	6	6	6	5.75
80	10	10	10	6	6	4	8	8	7.75
100	10	10	8	8	8	10	8	8	8.75

Table 3b: Zone of Inhibition for Bacillus subtilis (Set 2)

Concentration (mg/ml)	Diameter Reading of Zone of Inhibition (Mm)								
	1st	2nd	3rd	4th	5th	6th	7th	8th	Mean
10	-	-	-	-	-	-	-	-	-
20	10	6	8	6	8	6	7	8	7.375
40	10	10	10	10	10	9	9	10	9.75
60	20	10	20	10	10	10	20	20	15.0
80	22	22	28	18	10	20	20	20	20.0
100	24	16	36	30	38	36	20	20	27.5

Table 3c: Zone of Inhibition for Staphylococcus aureus (Set 1)

Concentration (mg/ml)	Diameter Reading of Zone of Inhibition (Mm)								
	1st	2nd	3rd	4th	5th	6th	7th	8th	Mean
10	-	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-	-
40	-	-	-	-	-	-	-	-	-
60	-	-	-	-	-	-	-	-	-
80	-	-	-	-	-	-	-	-	-
100	-	-	-	-	-	-	-	-	-

Table 3d: Zone of Inhibition for Staphylococcus aureus (Set 2)

Conentration Hgfrcdc c v 6f (mg/ml)	Diameter Reading of Zone of Inhibition (mm)								
	1st	2nd	3rd	4th	5th	6th	7th	8th	Mean
10	-	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-	-
40	-	-	-	-	-	-	-	-	-
60	-	-	-	-	-	-	-	-	-
80	-	-	-	-	-	-	-	-	-
100	-	-	-	-	-	-	-	-	-

Table 3e: Zone of Inhibition for Escherichia coli (Set 1)

Concentration (mg/ml)	Diameter Reading of Zone of Inhibition (mm)								
	1st	2nd	3rd	4th	5th	6th	7th	8th	Mean
10	-	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-	-
40	-	-	-	-	-	-	-	-	-
60	-	-	-	-	-	-	-	-	-
80	-	-	-	-	-	-	-	-	-
100	-	-	-	-	-	-	-	-	-

Table 3f: Zone of Inhibition for Escherichia coli (Set 2)

Concentration (mg/ml)	Diameter Reading of Zone of Inhibition (mm)								
	1st	2nd	3rd	4th	5th	6th	7th	8th	Mean
10	-	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-	-
40	-	-	-	-	-	-	-	-	-
60	-	-	-	-	-	-	-	-	-
80	-	-	-	-	-	-	-	-	-
100	-	-	-	-	-	-	-	-	-

Table 3g: Zone of Inhibition for Pseudomonas aeruginosa (Set 1)

Concentration (mg/ml)	Diameter Reading of Zone of Inhibition (mm)								
	1st	2nd	3rd	4th	5th	6th	7th	8th	Mean
10	-	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-	-
40	-	-	-	-	-	-	-	-	-
60	-	-	-	-	-	-	-	-	-
80	-	-	-	-	-	-	-	-	-
100	-	-	-	-	-	-	-	-	-

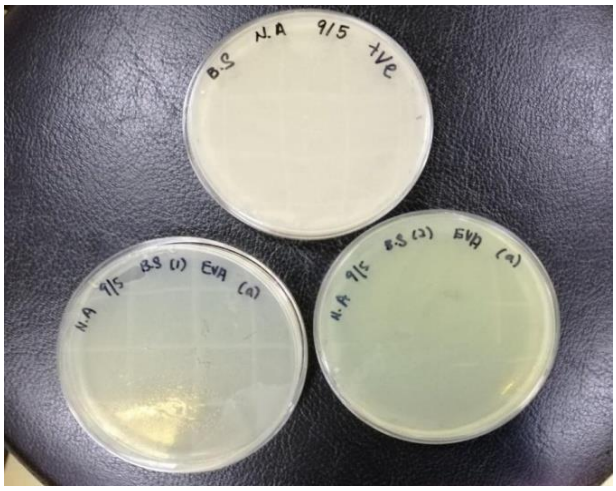
Table 3h: Zone of Inhibition for Pseudomonas aeruginosa (Set 2)

Concentration (mg/ml)	Diameter Reading of Zone of Inhibition (mm)								
	1st	2nd	3rd	4th	5th	6th	7th	8th	Mean
10	-	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-	-
40	-	-	-	-	-	-	-	-	-
60	-	-	-	-	-	-	-	-	-
80	-	-	-	-	-	-	-	-	-
100	-	-	-	-	-	-	-	-	-

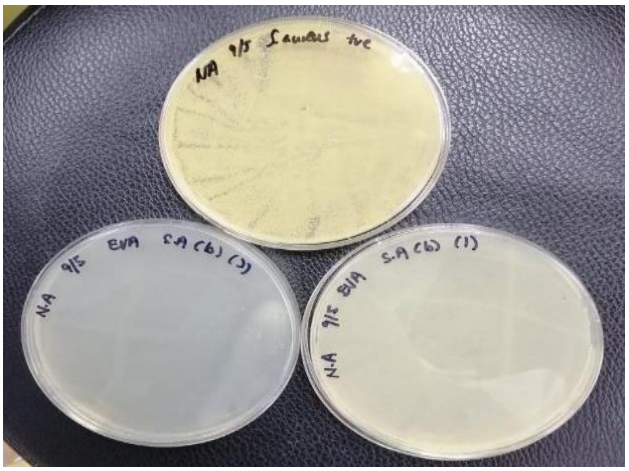
Table 3i: Minimum Inhibitory Concentration of the Plant Extract (MIC)

Sample	**Microorganism**	**Concentration**				
		1000	**500**	**250**	**125**	**62.5**
Oil Extract	Bacilus subtilis	+	+	+	+	+
	Staphylococcus aureus	+	+	+	+	+
	Escherichia coli	+	+	+	+	+
	Pseudomonas aeruginosa	+	+	+	+	+

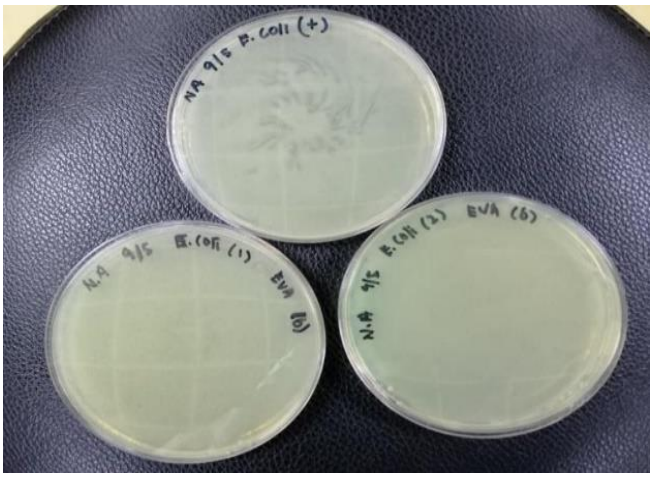
MBC



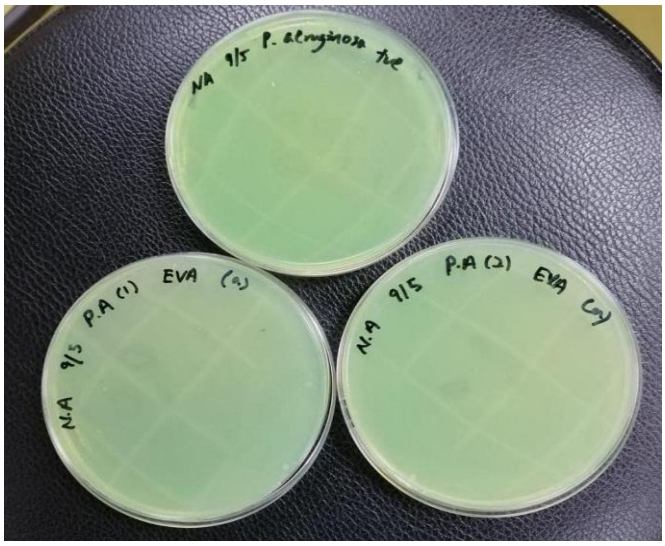
Results of Set 1 Bacillus subtilis and + control plate



Results of Set 1 S. aureus and the + control plate



Results of Set 1 E. coli and the + control plate



Results of Set 1 Pseudomonas aeruginosa and+ control

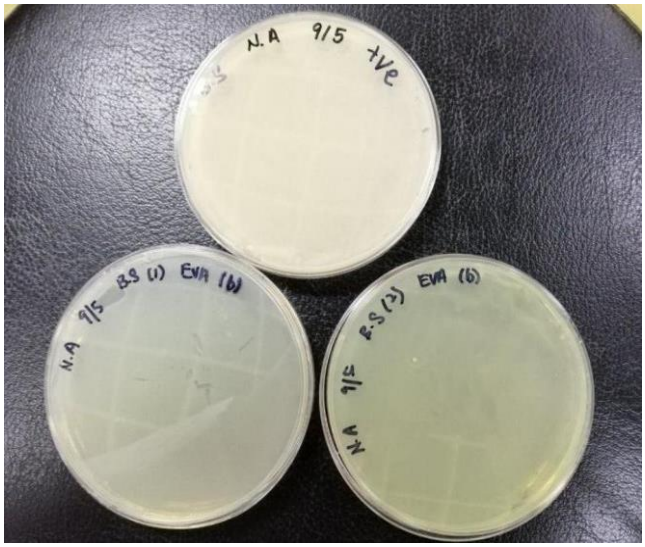
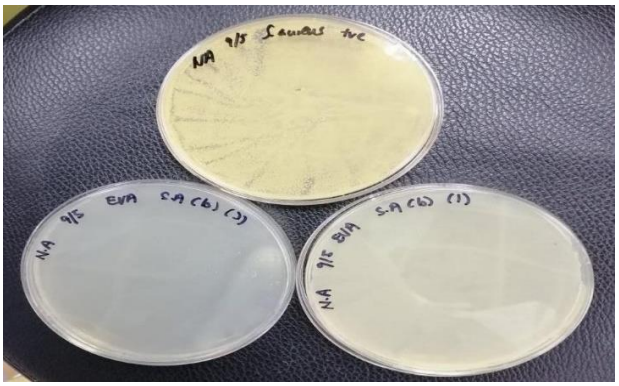
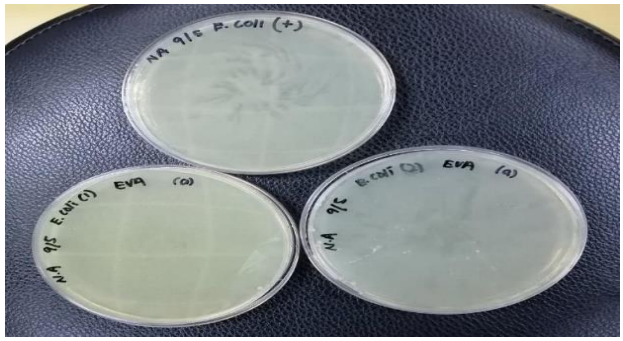


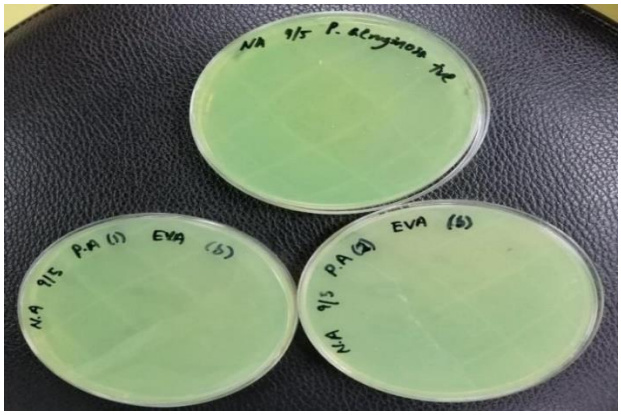
Figure 4.28 Results of Se 2 Bacillus subtilis+ control plate



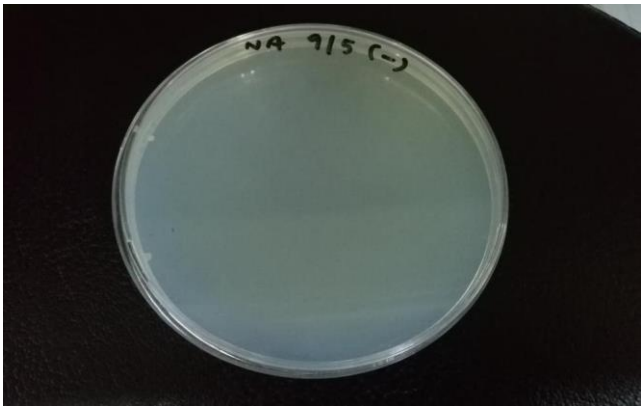
Set 2; S. aureus and the + control plate



Results of Set 2 *E. coli* and the + control plate



Results of Set 2 P. eruginosaand + control plate



Negative Control Plate

Table 4: GC PROFILE

S/N	RT	Nist Library	Molecular Weight Of Compound g/mol	Class of Compound	% Area
1.	15.0512	Copaene	204.35	Alkene	0.0374
2.	20.7191	Dodecanoic Acid	200.314	Carboxylic Acid	4.976
3.	24.2456	Tetradecanoic Acid	22.897	Carboxylic Acid	24.7498
4.	27.9728	n-Hexadecanoic Acid	256.4	Carboxylic Acid	24.7498
5.	28.6727	Methy10-Trans 12-Octadecadienoate	294.5	Methyl Ester	2.8590
6.	29.7149	Linoleic Acid Ethyl Ester	308.5	Ethyl Ester	1.5130
7.	30.9969	9,12-Octadecadienoic Acid	280	Carboxylic Acid	57.0327
8.	35.308	Isopropyl Linoleate	322.53	Ester	0.1922
9.	35.8660	Cis-11-Hexadecanal	23841	Aldehyde	0.0215
10.	36.3488	13-Octadecanal	266.46	Aldehyde	0.0600
11.	36.6348	E, E-10 12-Hexadecadie1-o1 acetate	208.4	Ester	0.0385

V. DISCUSSION OF RESULTS

The percentage yield of the fenugreek seed oil is 8.82 ±0.04% using n-Hexane as solvent. This shows that the seed is not an oil seed, according to Ren et al., 2011, the highest yield of oil was said to be 8.99%. The phytochemicals identified using standard procedure by Dragendorff's reagent is for alkaloids in plant extract via solutions of the salts of heavy metals which consists of potassium iodide bismuth nitrate. The mechanism of action is occurred through the coupling of the reagent's heavy metal atom in the reagent with nitrogen in the alkaloids to form ion pairs. These ion pairs, then form insoluble orange – red precipitates. Some may show orange-yellow, red-black or pink-purple precipitate depending on the plant genus or species. The results of this alkaloid test for the extracts of *Trigonella foenum-graecum*were negative. Saponins are chemical compounds abundant in different types of plant species. They are among the secondary metabolites and produce soap-like foam when they are shaken in aqueous solutions. Their structure is composed of one or more hydrophilic glycoside moieties held by a lipophilic triterpene derivative. This ability to foam is caused by the combination of non-polar sapogenin and water-soluble side chain. For the Frothing test of Saponins, results showed for the maceration extract of *Trigonella foenum-graecum* was positive. Frothing appearance of the creamy mist of small bubbles was formed after boiled with distilled water. This indicated that saponins were present in the maceration extract of *Trigonella foenum-graecum.*

For the Salkowski test of terpenoids or steroids, results showed for the extracts of *Trigonella foenum-graecum* were positive. Reddish brown coloration at the interface was observed after chloroform and sulphuric acid were added. When red color appears in the lower layer indicates the presence of steroids. When the concentrated sulphuric acid reacts with a steroid, for example, cholesterol, it removes two molecules of water from two molecules of cholesterol, resulting in a formation of bicholestadien, which eventually sulphonated to become the red color of bicholestadiendisulphonate complex. A reddish-brown coloration of the interface is formed to show a positive result of the presence of steroids in *Trigonella foenum-graecum.*

For the glycosides test, results showed for the ethanolic maceration extract and Soxhlet extract of *Trigonella foenum-graecum* were both positive. A red precipitate was shown after a few drops of a mixture of Fehling's reagents A and B were added. Hence, glycosides were present in the extracts of *Trigonella foenum-graecum.* The results showed in this test were contrasted to the glycosides test which is cited in another study by Alauddin. M in 2009.

The tannins test was carried out to determine the presence of the phenolic compounds. Tannins are naturally occurring complex organic compounds possessing nitrogen-free polyphenols of high molecular weight. They form a colloidal solution with water giving acid. They also precipitate proteins and alkaloids. The astringent nature of tannins is due to the fact that they can precipitate proteins and render them resistant to enzymatic attack. When applied on a wound or injury, tannins form a protective coating so as to prevent external irritation and thus promote healing. tannins produced dark green color when treated with ferric chloride solution. In this test, the maceration extract showed negative results, there was no dark green color This indicated the absence of tannins in the extract of *T. foenum-graecum*. The test for flavonoids showed that the extracts of *Trigonella foenum- graecum* were negative. No discoloration of the yellow color solution was shown after the addition of the sodium hydroxide and hydrochloric acid. Hence, flavonoids were not present in the extract of *Trigonella foenum-graecum*. However, some other literature stated that flavonoid was present in the methanolic extract of the plant. This can be due to different solvent extraction will result in different outcome of the test. Some active constituents like flavonoids may not be well extracted in n-Hexane in this case but are well extracted in methanol.

Molisch's test is a typical test for all carbohydrates. A red-violet ring at the interface between the acid and the aqueous layer was observed in this test after the addition of the sulphuric acid. To explain this phenomenon, the dehydration of the

sugars occurred due to the catalysis by concentrated sulphuric acid to form furfural and its derivatives. When carbohydrates are treated with concentrated sulphuric acid or concentrated hydrochloric acid, the hydroxyl groups of sugar are removed in the form of water, and furfural is formed from pentose sugar and hydroxymethyl furfural is formed from hexose sugar. After that, these sugars will further condense with sulphonated alpha-naphthol to yield a purple or violet-red colored complex product which is scientifically known as furfuryl-diphenyl-methane-dyes. Monosaccharides give a rapid positive test. Disaccharides and polysaccharides react slower. (32) For the carbohydrate test, results showed for the extract of *Trigonella foenum-graecum* was negative. In reference to an article proposed by Mowla in 2009 (30), showed that carbohydrates presented in the extracts of Trigonella foenum-graecum for ethanolic maceration and ethanolic Soxhlet extracts.

In the tests for phenols, no white precipitates have appeared in the macerated extract of *T. foenum-graecum*. To the test solution, a few drops of 10% lead acetate solution were added. The formation of a white precipitate indicated the presence of phenolic compounds in the extract. Hence phenol tested negative. In the tests for resins test, there was a formation of a resinous precipitate, indicating the presence of resins. In the balsam test, the absence of a dark green color showed the absence of balsam.

In the antimicrobial evaluation of the n-Hexane macerated extract was employed. *Trigonella foenum-graecum.* The extract concentrations which were used in the agar well diffusion method were 10 mg/L, 20 mg/L, 40 mg/L, 60 mg/L, 80 mg/L, and 100 mg/L respectively. Among the four pathogens, only *Bacillus subtilis* showed a zone of inhibition which means that the extract of *T. foenum- graecum* is highly selective and only inhibit the growth of *Bacillus subtilis* from this study. In the case of Set 2 agar plate of *Bacillus subtilis*, the extract started to inhibit the growth at a concentration of 20 mg/ml with a little zone of inhibition and showed the greatest activity at a concentration 100 mg/ml which obtained the highest diameter of zone of inhibition which was 27.5 mm among all the extract concentrations. This showed that higher concentrations of extract will lead to a higher zone of inhibition. (34) The antimicrobial properties of the plant extract may due to the presence of phenolic compounds in the extract. Phenol act as an active compound that can destroy the cell membrane causing cell death. There is a close relationship between antimicrobial activity and antioxidant activity since the active components which contribute to these two activities are phenolic compounds.

However, in the case of Set 1 agar plate of *Bacillus subtilis*, the extract only showed its antimicrobial activity starting from a concentration of 60 mg/L and reached its highest activity at 100 mg/L with a diameter of zone of inhibition of 8.75 mm. From the table of the diameter of zone of inhibition, as shown in the results, the diameter of zone of inhibition showed in the Set 1 agar plates is relatively small compared to Set 2 agar plates. The reasons which may be associated with this phenomenon are the insufficient concentration of the extract in the agar well, improper spreading as well as improper diffusion of extract. The irregular shape of the zone of inhibition around the wells may be due to the improper diffusion of the extract and also the improper spreading of the bacteria inoculum which may cause abundant or uneven bacterial growth on a particular part area of the agar.

For *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, there is no zone of inhibition shown in the agar plates. This may be due to the low concentration of extract and poor diffusion of the extract.

The ethanolic seed extracts was further subjected to broth dilution method to determine the MIC of *Trigonella foenum-graecum* seed extract with concentrations ranging from 62.5g/L, 125g/L, 250g/L, 500g/L, to 1000g/L. Turbidity can be seen in almost all of the assay tubes which indicated the presence of bacterial growth. Hence, the two tubes that showed the least turbidity observed which were 1000gm/L and 500gm/L were selected for testing in MBC test to further confirm the presence of bacterial growth.

In the MBC test, all the nutrient agar plates for the four bacteria strains showed the growth of the bacteria and negative results were obtained after incubating the plates for 24 hours. The negative results for both MIC and MBC tests might be due to the very low concentrations of extract as the highest concentration used in this MIC test were 1000mg/L, compared to the concentrations used in well agar diffusion test which was 100mg/L. The highly selective antibacterial nature of the n-hexane extract of *Trigonella foenum-graecum* seed oil displaying insufficient activity on the four strains of bacteria. This statement suggested that a higher concentration should be used in the determination of the MIC of the extract. The Performed GC-MS analysis of essential oil obtained from hydro-distillation of seeds powder gave 11 peaks, each for individual compound (Table 4). According to Kenny et al., in the study of solid-liquid sequential extraction (hexane, dichloromethane, methanol, and water) of *T. foenum-graecum* seeds powder. They quantified 18 phenolic compounds by using ultra-performance liquid-chromatography–mass spectrometry.

VI. CONCLUSION

Phytochemical screening of extract of *Trigonella foenum-graecum* through the maceration method showed the absence of alkaloids, Tannins, Balsam, Phenols, and flavonoids while saponins, terpenoids, resins, and cardiac glycosides were present in the seed. The antimicrobial activity of the plant extract as determined by the agar well diffusion method was observable for only *Bacillus subtilis*, while the other pathogens *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* tested in this research, were not affected. The highly selective nature of this extract makes it a poor candidate as an antimicrobial agent. Higher concentration of the plant extract may be required to inhibit the growth of these pathogens. Gas chromatography investigation detected compounds with health benefits such as Linoleic acid, 9,12-

Octadecadienoic acid, and Isopropyl Linoleatein the plant extract.

VII. RECOMMENDATION

1. In-depth search (in vivo and in vitro) into the activity of this plant on other microorganisms of public health significance should be carried
2. The bioactive principles should be isolated and evaluated both singly and in combinations to streamline and maximize their efficacy.
3. A sub-acute and chronic toxicity study should be done to determine their mid-long-term toxicity effects.
4. Isolation, purification, and investigations of the bioactive constituents of this plant are required to reveal more health benefits for the public and therapeutics uses in the medical field.

*ABBREVIATIONS MIC***:** Minimum Inhibitory

*Concentration. MBC***:** Minimum Bactericidal Concentration

T. foenum-graecum: Trigonella foenum-graecum. B. subtilis: Bacillus subtilis. S. aureus: Staphylococcus aureus., E. coli: Escherichia coli, P. aeruginosa: Pseudomonas aeruginosa

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