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# Nutra-pharmaceutical potential evaluation of *Trignollafoenum graecum* seeds extracts in different solvents

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**ABSTRACT**

This research work has been designed to evaluate the antioxidant and antimicrobial potential of *Trigonellafoenum graecum* seeds. The phytochemical, antifungal and antioxidant components of *Trigonellafoenum graecum* seed was extracted by using four solvent systems (80% methanol, 80% ethanol, 100% methanol and 100% ethanol). *Trigonellafoenum graecum* seeds showed maximum extract yield (42.9 g/100g DW) in 80% methanolic solvent system. Antioxidant analysis of *Trigonellafoenum graecum* seeds extracts was performed in terms of total phenolic and total flavonoid contents, showed that 80% methanolic extract presented highest total phenolic contents (70.2 mg GAE/g DW), Also 80% methanolic extract showed maximum total flavonoid contents (93.2 mg CE/g DW). Antioxidant activity was determined by DPPH radical scavenging activity and measure of reducing power. Results revealed that 80% methanolic extract showed highest radical scavenging activity and reducing potential. Antimicrobial activity of *Trigonellafoenum graecum* seeds was investigated by Disc Diffusion Method. Results showed that 80% methanolic extract exhibited highest antibacterial and antifungal potential against *E. coli* (30 mm DIZ) and *A. Paraciticus* (29 mm DIZ), respectively. Overall, the present investigation showed that 80% methanolic *Trigonellafoenum graecum* seeds extract can be used effectively to make antimicrobial, antioxidant agents which can be utilized in different nutra-pharmaceutical industries.



**Capsule Summary:** The antioxidant and antimicrobial potential of *Trigonellafoenum graecum* seeds extracts in different solvents was evaluated and results revealed that the *Trigonellafoenum graecum* has promising bioactivity and could have possible applications in Nutra-pharmaceutical industries.

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## INTRODUCTION

*Trigonellafoenum graecum* is a perennial forage legume as well a typical spice crop cultivated all over the Sub-continent for centuries. Aside from South Asia, the crop is cultivated in areas of Northern Africa, the Middle East, Mediterranean Europe, China, South East (SE) Asia, Australia, the United States, Argentina, including Canada. India is the world's biggest *T. foenum graecum* manufacturer, however due to increased domestic demand it does not have a significant percent of the international *T. foenum graecum* market (Basu

et al., 2014; Basu and Agoramoorthy, 2014; Basu, 2006). The crop has been proposed for Asia, Africa, and Latin America's tropical and semiarid areas (Zandi et al., 2015). For centuries, the species has been used in Ayurvedic medicine in India, as well as traditional Tibetan and Chinese medicine. Modern research has also shown that *T. foenum graecum* seed and can be used to treat a variety of diseases, and effectively lowering blood glucose as well as cholesterol levels in both animal and human specimens in clinical trials (Acharya et al., 2006a). The presence of a diverse range of important phytochemicals (diosgenin, trigonelline, *T. foenum graecum ine*, galactomannan) is responsible for *T. foenum graecum* 's medicinal properties (Zandi et al., 2015).As a result, the crop is in high demand on a global scale in the medicinal, nutraceutical, and functional food industries. *T. foenum graecum*, also known as a chemurgic grain, is commonly used in industrial sectors. Its seeds are a good source of the steroid diosgenin, which is used as a substitute in the drug companies (Mehrafarin et al., 2010).

Besides that, as a forage legume as well as a natural nitrogen fixer, it can be easily integrated into localized crop cycles (short-term rotation) for natural soil sustainment, nitrogen fixation, and animal feed as hay including silage. Since the crop develops well under water stress, the cost of manufacture is less in comparison to many other commercial crops.*T. foenum graecum* seems to be well known as an international spice crop, with parts cultivated in North Africa, Mediterranean Europe, Russia, the Middle East, China, India, Pakistan, Iran, Afghanistan, areas of Far East and SE Asia, Australia, the United States, Canada, and Argentina (Acharya et al., 2007; Acharya et al., 2006b). India once had and currently has the world 's biggest *T. foenum graecum* harvesting area (Acharya et al., 2006a).

That plant has been proposed for agricultural development in Asia Africa, and Latin America's drought as well as in semiarid regions (Acharya et al., 2008; Basu and Agoramoorthy, 2014; Solorio-Sánchez et al., 2014; Zandi et al., 2015). For millennia, the crop has already been used regularly as a typical forage crop in many old civilizations throughout Eurasia. *T. foenum graecum* has been stated to be an essential herbal medicine in Indian Ayurvedic medicinal practices, as well as in traditional Chinese medicine and Tibetan medicines, for the treatment of a variety of diseases in humans and animals. In historical writings and scriptures, ancient Muslim scholars and practitioners documented the use of *T. foenum graecum* in traditional Islamic medicinal practices (Basu and Agoramoorthy, 2014). Modern clinical trials have also shown the medicinal herb's effectiveness in the treatment of a variety of human and animal diseases (Acharya et al., 2007; Acharya et al., 2006a; Acharya et al., 2008; Zandi et al., 2011).

Based on above mentioned facts, this research was designed to evaluate the antioxidant and antimicrobial potential of *Trigonellafoenum graecum* seeds. The phytochemical, antifungal and antioxidant components of *Trigonellafoenum graecum* seed was extracted by using four solvent systems.

## MATERIAL AND METHODS

### Chemicals, sample collection and preparation

Different chemicals were purchased from the Sigma Chemicals Co. (St, Louis, MO, USA). All standard antibiotic discs and culture media were purchased from Oxoid Ltd. (Hampshire, UK). Different parts of *Trigonellafoenum graecum*i.e. seeds were obtained from the market of Lahore, Pakistan. The seeds of *Trigonellafoenum graecum*were washed with tap water and then dried at 40 $^{o}$C in an oven to maintain constant weight. By using a commercial blender, dried seeds were grounded into a fine powder. Then the ground material was passed through 80-mesh sieve. The passed material was used for extraction purpose. Polythene bags were used to store the ground samples at 4 $^{o}$C until forstudy.

### Extraction of bioactive compounds

For extraction of bioactive compounds, four solvent systems (80% methanol, 100% methanol, 80% ethanol and 100% ethanol) were being used. In this regard powdered seeds and (20g) were extracted with 200mL in an orbital shaker for 8 hours at room temperature (Gallenkamp, UK). In order to separate the extract from residue whatman No. 1 filter paper was used. The resulting residues were extracted two times with the same solvent system. The drying of extracts was done at temperature of 45˚C and their yield was calculated by weighing extracts. The extracts were kept in a refrigerator at 4˚C for studying further(Hassan, 2019).

### Antioxidant activity

#### *Determination of total phenolic contents (TPC)*

The method which was used to determine the total phenolic contents of medicinal plant was based on the procedure of (Hussain et al., 2018). Folin-Ciocalteu, a reagent that was used to to determine the phenolic contents. 0.5 mL of Folin-Ciocalteu reagent was mixed with 50 mg of crude extract and than 7.5 mL deionized water was added in it. The mixture was retained at room temperature for 10 min and then 1.5 mL of 20% sodium carbonate (w/v) was added in it. The resulting mixture was kept in water bath for 20 min at temperature of 40˚C and then cooling of this mixture was done in an ice bath. By adjusting the spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan) at 755nm, absorbance was measured. Amount of TP was calculated by using gallic acid calibration curve. The results were expressed as mg GAE/g DW. Each sample was analyzed thrice to get average results of phenolics.

#### *Determination of total flavonoid contents (TFC)*

The procedure of (Dewanto et al., 2002) was followed in order to determine the total flavonoid contents with minor

modification. Aqueous extract of one milliliter that comprised of 0.01 g/mL of dry matter was placed in a volumetric flask having capacity of about 10 mL liquid. After that 5 mL of distilled water was added followed by 0.3 mL of 5% $NaNO_2$. After 5 min, 0.6 mL of 10% $AlCl_3$ was added in it. The solution was stayed for 5 min and then 2 mL of 1 M solution of NaOH was added and required volume was made up with distilled water. At 510 nm, absorbance was measured. Results of total flavonoids amounts were expressed as mg Catechin equivalent (CE)/g DW. Absorbance for each sample was taken three times in order to get the average results of total flavonoids.

***DPPH radical scavenging assay***

DDPH radical scavenging assay was applied to determine the free radical scavenging activity of medicinal plants. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical was used to determine the scavenging activity as described by Suleman et al., (2018) with little ammendments. Methanol solvent was used to determine the DPPH solution (33mg/L). Absorbance of the resulting solution was taken at 0 min. Then extract solutions (250µg/mL) were prepared. After that 5mL of methanolic solution of DPPH was added in 1mL of extract solution. The mixture was placed for 30 minutes in the dark place. Then by using a spectrophotometer, absorbance was measured at wavelength of 517nm with methanol was taken as a blank solution. Free radical scavenging activity was expressed as percentage inhibition and calculation was done by using Eq. 1 (Where, Ac and As are the absorbance values of control and sample, respectively).

$$\text{Inhibition } (\%) = \left[\frac{A_c - A_s}{A_c}\right] * 100 \quad (1)$$

***Determination of reducing power***

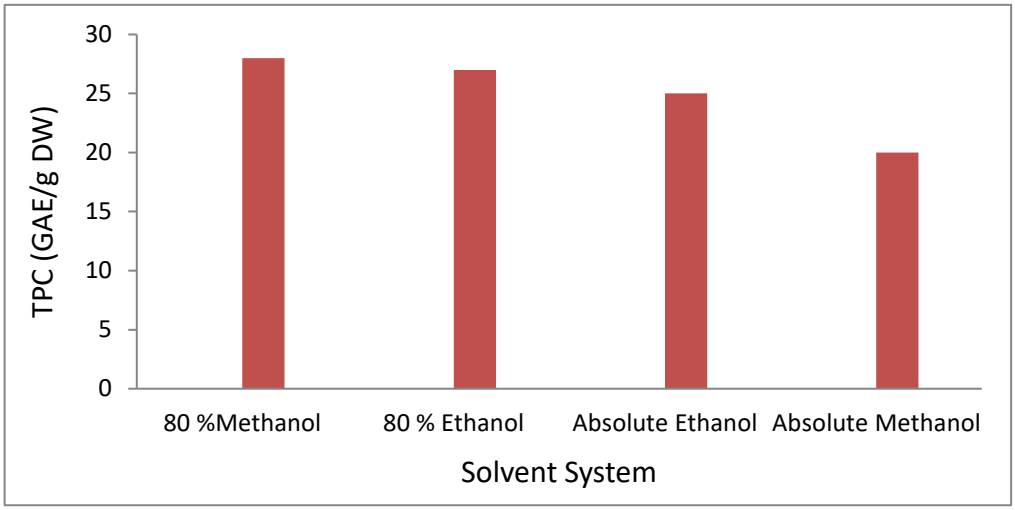


**Fig. 1:** Total phenolic contents (mg GAE/g DW) in *Trigonellafoenum graecum* seed extracts

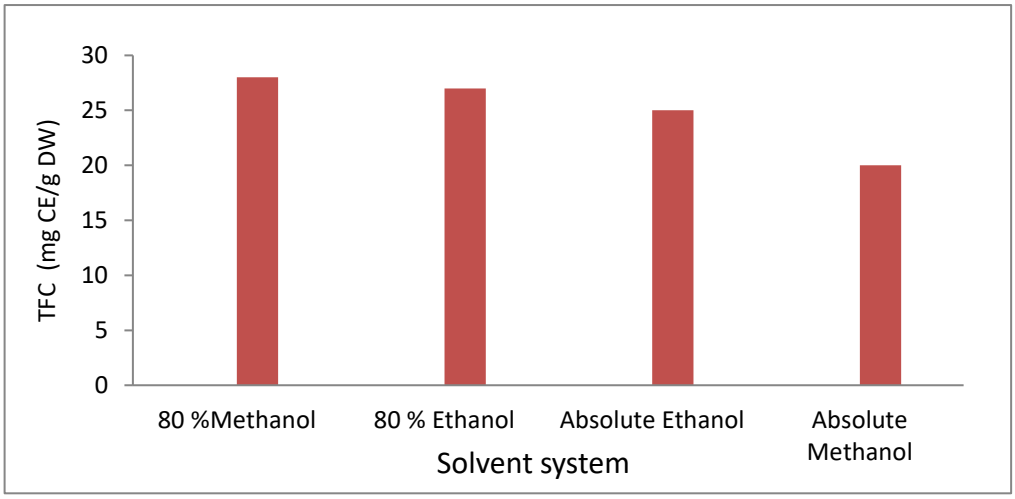


**Fig. 2:** Total flavonoid contents (mg CE/g DW) in *Trigonellafoenum graecum* seed extracts

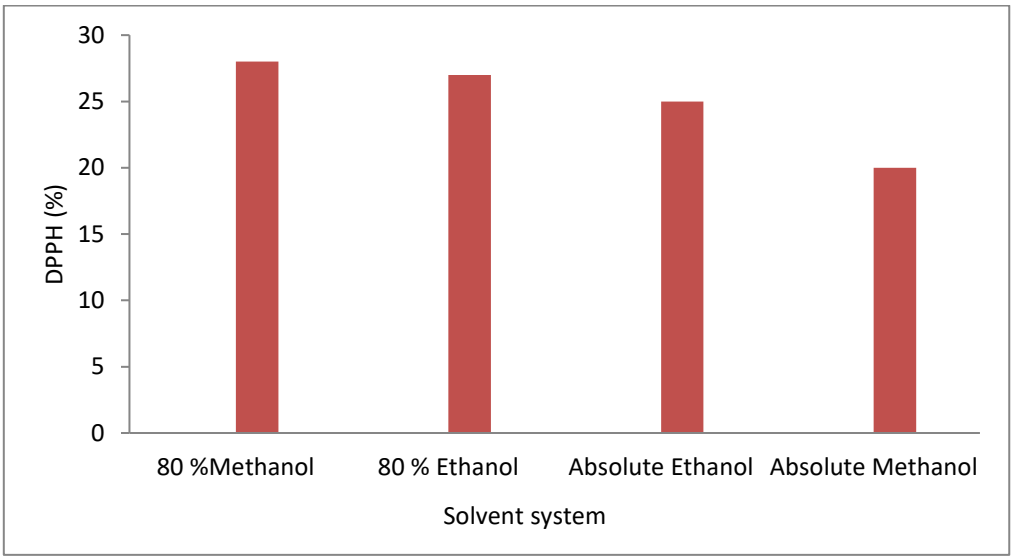


**Fig. 3:** DPPH radical scavenging activity of *Trigonellafoenum graecum* seed extracts

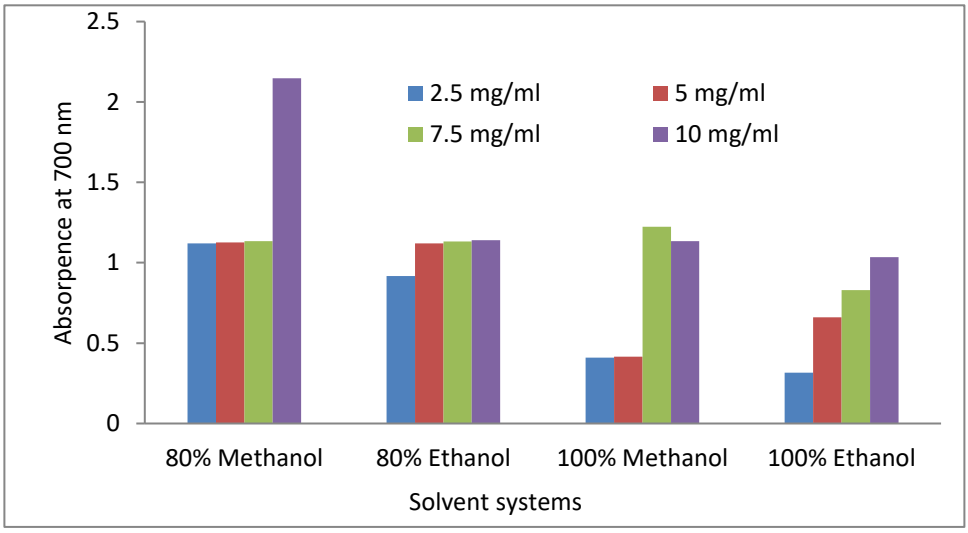


**Fig. 4:** Reducing Power of *Trigonellafoenum graecum seed extracts*

The reducing power of the plants seeds extracts was determined according to the procedure described by (Hassan, 2019) by doing slight changings. Different extracts having Concentration range (2.5-10.0 mg) were mixed with the buffer of sodium phosphate solution (5.0 mL, 0.2 M, pH 6.6) and potassium ferricyanide (5.0 mL, 1.0%) solution. The incubation of resulting mixture was done at 50 ˚C for 20 min. Then 5 mL of 10% trichloroacetic acid was added in the mixture and was centrifuged for 10 min at 980 *g* at 5 °C in a refrigerated centrifuge (CHM-17; Kokusan Denki, Tokyo, Japan). Two layers were formed. The 5 mL of upper layer of the solution was poured in beaker and diluted it with 5.0 mL of distilled water and ferric chloride (1.0 mL, 0.1%) solution, and absorbance was measured at 700 nm using spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan). Analysis was done thrice for each sample and results were averaged.

**Antimicrobial analysis**

***Microbial agents***

The extracts of seeds were tested individually against a panel of microorganisms which may include five fungal strains (*Aspergillusparasiticus, Aspergillusflavus, Fusariumoryzae, Fusariumtritichum, Aspergillusoryzae*) and three bacterial strains (*Escherichia coli, Pasturellamultocida* and *Staphylococcus aureus*) obtained from the Fungal Bank, University of Punjab, Lahore. Fungal strains were cultured overnight at 28°C in Potato Dextrose agar (Oxoid Hampshire, UK), however the bacterial strain was cultured at 37 °C in nutrient agar (OxoidHampshire, UK). The slants of microbial strains were stored at 4°C. Antimicrobial potential of plants extracts was determined by using the disc diffusion assay.

**Table 1:** Percentage yield of *Trigonellafoenum graecum* seeds extract

| S. No | Solvent system | Percentage yield (g/100g DW) |
|---|---|---|
| 1 | 80%Methanol | 42.9±0.52[a] |
| 2 | 80% Ethanol | 37.7±0.43[b] |
| 3 | 100% Methanol | 29.2±0.35[c] |
| 4 | 100% Ethanol | 20.2±0.35[d] |

Values are mean±SD of three samples analyzed individually in triplicate at $p$ <0.05. Superscripts alphabets within the column depicted significant difference among different solvent system.

### *Disc diffusion assay*

Antimicrobial activity of seeds of *Trigonellafoenum graecum was* tested against fungal strains (*Aspergillusparasiticus, Aspergillusflavus, Fusariumoryzae, Fusariumtritichum, Aspergillusoryzae*) and bacterial strains (*Escherichia coli, Pasturellamultocida* and *Staphylococcus aureus*) by previously adopted method (NCCLS, 2004) with little modifications. Potato dextrose agar (PDA) solution was prepared and autoclaved. About 20mL PDA solution was poured in sterilized petri plate under laminar air flow. Sterilized discs (6mm) of wicks sheet impregnated with 50µL of particular plant extract were placed on the agar plates. To compare the activity with standard antibiotics, Fluconazol (30 µg/disc) (Oxoid) and Rifampicin (30 µg/disc) (Oxoid) were used as positive reference for fungal and bacterial strains respectively. Disc without samples were used as a negative control. Test discs and standard disc were placed in separate petri dishes. The plates were incubated at $28^0$C for 48h for fungal growth and $37^0$C for 24h for bacterial growth. Antimicrobial activity was evaluated by measuring the diameter of inhibition zones (mm) by zone reader.

### Statistical analysis

All experiments were performed in triplicate (n=3) and the data was reported as mean ± SD. Data were analyzed using Minitab 2000 Version 13.2 statistical software (Minitab Inc.Pennsylvania, U.S.A) at 5% significant level. Antifungal activity data was presented as mean values at 95% confidence interval. Significant differences of mean were calculated by using LSD.

## RESULTS AND DISCUSSION

### Percentage yield

The percentage yield of plant extracts is determined by various factors such as the volume of solvent used, the condition of the plant, and the process of extraction (Kaur and Kaur, 2018)The essence of the extraction solvent, on the other hand, is most essential for extract yield and the antioxidant abilities of the plant materials. As a consequence, the presence of antioxidant compounds with varying chemical properties and polarities, which may or may not be soluble in a given solvent, is highly dependent on the existence of the solvent system used. Polar solvents are widely used to extract polyphenols from plant matrices.Aqueous mixtures of ethanol, methanol, acetone, and ethyl acetate are the most appropriate of these solvents. Methanol and ethanol have long been used to derive antioxidant compounds from plants and plant-based foods (Ashraf et al., 2018) Methanol is a stronger and more commonly used solvent for removing antimicrobial and antioxidative components from plants (Hafeez et al., 2020).

Table 3.1 indicates the extraction yields from *Trigonellafoenum graecum*'sseeds in various solvent systems. The highest extraction yields was obtained from 80% methanol (42.9 g/100 g DW). The extraction capacity of various solvent systems for recovering extractable components from seedswas evaluated in the following order: 80% methanol > 80% ethanol > absolute methanol > absolute ethanol Yields of extract from seeds were obtained in the following order: 80% methanol>absolute methanol>80% ethanol>absolute ethanol.

The higher extract yields obtained in this analysis with 80% methanol agreed well with the previous results of (Shabir et al., 2011) who recorded the highest extract yield from Moringaoleifera using 80% methanol. Similarly, using 80% methanol yielded the largest extract yields from various parts of the medicinal plant (BAO et al., 2021). Furthermore, (Burgal et al.) stated that strongly polar solvents such as methanol have a high capacity to evaluate bioactive constituents.

### Antioxidant potential

### *Total phenolic contents*

Plants' phenolic compounds are thought to be the most effective antioxidants (Sakihama et al., 2002). They are important in scavenging free radical activity (Agbo et al., 2015) Because of the presence of their hydroxyl groups, phenolic compounds have potent free radical scavenging activity, which directly leads to their antioxidant capacity (Cheurfa et al., 2021). Fruits of *Trigonellafoenum graecum* are preferable owing to the presence of phenolic compounds. Total phenolic levels in *Trigonellafoenum graecum* increase dramatically with fruit size (Zhang et al., 2020).To calculate the overall phenolic content of natural goods, the Folin-Ciocalteu Method is used. It works on the basis of oxidation and reduction reactions. The oxidation in this reaction is caused by the Folin-Ciocalteu reagent (phosphomolybdic-phosphotungstic acid), which produces a colored product with a maximum yield at 765 nm . The gallic acid equivalent is used to express the complete phenolics results (Novakov et al., 2021).

Table 3.2.1 shows the phenolic content effects of the medicinal plant. The overall phenolic content of *Trigonellafoenum graecum* seeds and varied significantly (p 0.05) from 52.3-70.2 (mg GAE/g DW), respectively. It was discovered that the highest levels of TPC were obtained from seedsin an 80%methanolic extract, followed by

absolute methanol>80% ethanol>absolute ethanol. Our findings are confirmed by the previous analysis of (Nakilcioğlu-Taş and Ötleş, 2021) who stated that higher phenolic contents were obtained from 80% methanol, than 80% ethanol for plant extracts.

***Total flavonoid contents***

The majority of their functions are due to their powerful antioxidative properties(Soares et al., 2020). One of the most significant natural components present in flavonoids are found in plants. These components are polyphenolic compounds with several properties such as oxidative enzyme inhibition, free radical scavenging activity, anti-inflammatory action, and hydrolytic enzyme inhibition (Atanassova et al., 2011).

The total flavonoid contents of the currently studied medicinal plant sections are represented as Catechin equivalents (CE) in table 3.2.2. The flavonoid concentrations in the seeds of the medicinal plant ranged from 76.3-93.2 (mg GAE/DW). TPCs were found higher in the 80% methanolic extract, followed by 80% ethanol > absolute methanol > absolute ethanol.

The current findings are compatible with the approach described by(Ahiakpa et al., 2013) who found that total flavonoids in pods of *Trigonellafoenum graecum* were higher in methanolic extracts than in aqueous and other extracts, suggesting that the ethanolic extraction system was more effective. It was proposed that *Trigonellafoenum graecum* pods contain flavonoids that are more soluble in organic solvents than in aqueous solvents. Furthermore, (Sajid et al., 2012) examined the overall flavonoids contents (24.6 mg/100 g quercetin equivalent) in an aqueous methanolic extract of *P. pinnata*.

***DPPH radical scavenging activity***

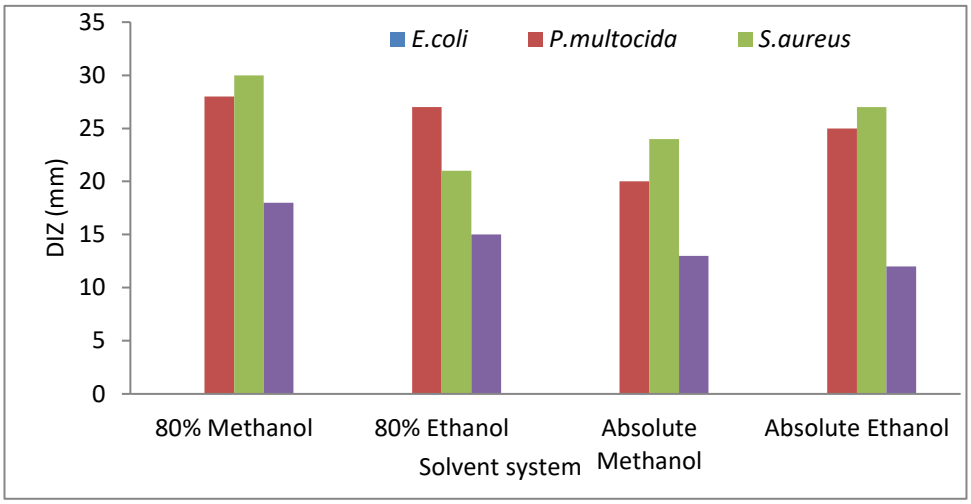


**Fig. 5:** Antibacterial activity of *Trigonellafoenum graecum* seed extracts

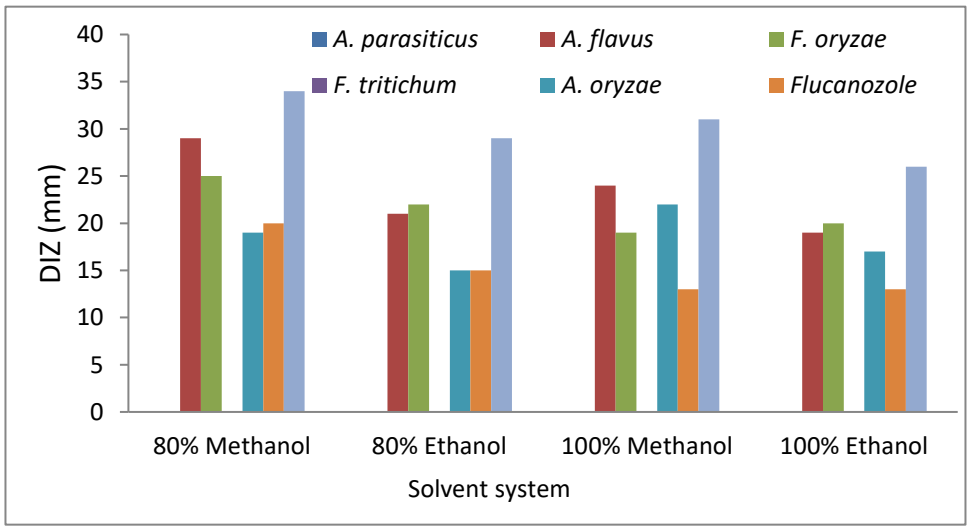


**Fig. 6:** Antifungal activity of *Trigonellafoenum graecum seed extracts*

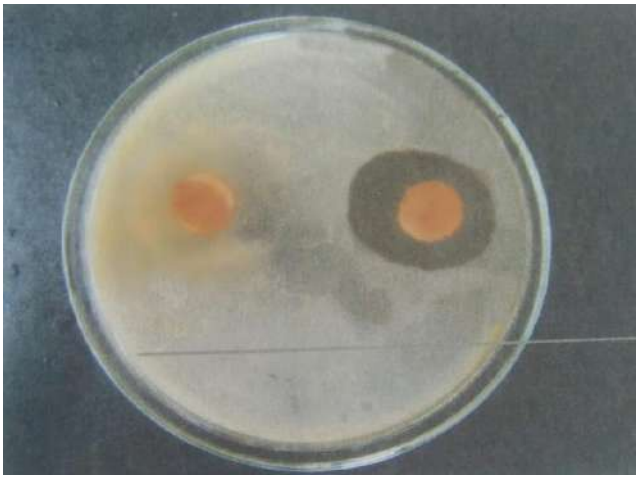

**Fig. 7:** Inhibition zones produced by 80% and absolute ethanolic seeds extract against *A. flavus*

Polyphenols, flavonoids, phenolics, terpenes, and tannins are natural antioxidants that can scavenge free radicals (Hassan et al., 2009). The DPPH radical scavenging assay may be used to assess the antioxidant efficacy of these items. This assay is commonly used to evaluate the scavenging potential of compounds that serve as free radical or hydrogen donors to DDPH (Choudhary et al., 2011). DPPH is a stable nitrogen-centered free radical compound. Its color varies from violet to yellow when reduced due to hydrogen or electron donation. Substances that can conduct such reactions are known as stronger antioxidants and strong radical scavengers. It has also been discovered that as the extract concentration increases, so does the potential of DDPH to scavenge free radicals (Ebrahimzadeh et al., 2010).

It is a critical mechanism that describes the oxidation phase of a proton radical scavenger. The reduction capability of plant extract was evaluated by decreasing the absorbance of DPPH solution to 517 nm (Singh et al.). The antioxidant molecule incorporates a hydrogen-donating electron, which leads to its ability to scavenge free radicals, which is an essential feature of antioxidants (Njayou et al., 2013).The DPPH radical assay was used because it is a fast, effective, simple, and fast method for investigating the overall antioxidant activity of plant extracts as well as pure compounds. This procedure is often used for screening several samples for radical scavenging potential and is not affected by sample polarity (Aliyu et al., 2009). The DDPH free radical scavenging assay was used to assess the antioxidant capacity of the medicinal plant *Trigonellafoenum graecum.* This assay also looked at new possible natural antioxidant outlets. The DPPH concentrations of medicinal plant seeds were determined to be lower due to scavenging ability.

Table 3.3.1 demonstrated a substantial variation in the DPPH radical scavenging behaviors of *Trigonellafoenum graecum* extracts through solvent systems. The aquoues alcoholic extracts of *Trigonellafoenum graecum* seeds demonstrated adequate DDPH radical scavenging capacity. The 80%methanolic extract of *Trigonellafoenum graecum* seeds had the largest DDPH radical scavenging efficiency (p 0.05), followed by: 80% ethanol> absolute methanol> absolute ethanol.

***Reducing power***

The tendency of plant extracts to minimize may be an indicator of their capacity for antioxidant activity (Zhang et al., 2011). The tendency of phenolic compounds to be antioxidants is usually due to their redox properties. Because of these properties, they can serve as a reducing agent, such as an oxygen quencher and electron donor. Plants are a rich source of antioxidants, according to studies on medicinal plants and vegetables. These plants have the ability to guard against such oxidative stresses in biological environments (Sylvie et al., 2014).Electrons are donated to reactive radical species as a result of the existence of antioxidant compounds, which are neutralized into stable and nonreactive species (Amante et al., 2020).

Table 3.3.2 shows the reducing ability of *Trigonellafoenum graecum* seeds extract. The reducing potential values of the extracts studied were determined at various concentration levels ranging from 2.5 to 10.0 mg/mL. However, the highest reducing strength was shown by an 80%methanolic seeds extract. Since reducing power is concentration dependent, the findings in Fig. 3.2.2 show that the reducing power of *Trigonellafoenum graecum* increases with concentration.

**Antimicrobial activity**

The antimicrobial bio-active components of medicinal plants are rich sources. A wide variety of medicinal plants can treat many infections as they have potential antimicrobial activity (Javid et al., 2015). The phytochemical study of *Trigonella foenum graecum* methanol extract revealed that certain phytoconstituents including alkaloids, saponins, cardenolides, anthraquinones and tannis exist in the methanol extract. The antimicrobial ability of these plant metabolites has been defined. The presence of these secondary metabolites may thus attribute antimicrobial properties of the medicinal plants (Olorunnipa et al., 2013)

***Antibacterial activity***

Antibacterial components can be found in all plant sections, including , fruits, roots, flowers, pods, seeds, and stems (Joshi et al., 2012). The disc diffusion procedure was used in this study to assess the antibacterial activity of *Trigonellafoenum graecum* seeds extracts against three bacterial strains: *P. Multocida, E. coli, and S. aureus*. Each extract was added to a particular bacterial strain, and the diameter of the inhibition zone (DIZ) was determined with a zone reader. According to the findings, methanolic seed

extract had the highest inhibitory effect against *P. Multocida* (p 0.05) of all the solvents tested (30 mm DIZ).

***Antifungal activity***

The disc diffusion system was used to test the antifungal properties of *Trigonellafoenum graecum*seeds against a panel of five fungal strains, including *Aspergillusparasiticus, Aspergillusflavus, Fusariumoryzae, Fusariumtritichum, and Aspergillusparasiticus*. Table 3.4.2 indicates the diameter of the inhibition regions. The findings revealed that methanolic extracts of *Trigonellafoenum graecum* seeds had the highest inhibitory activity (29 mm DIZ) against *A. parasiticus* (p = 0.05). While ethanolic extracts had a mild antifungal effect. The findings have showed that seed extract Have no any antifungal action against *Fusariumoryzae*. The current results are consistent with the findings of (Dahham et al., 2010), who discovered that methanolic extracts of *Punicagranatum*had higher efficacies against fungal strains, which could be attributed to the existence of active phytoconstituents such as phenols, flavonoids, and tannins.

**CONCLUSIONS**

Traditional medicinal plants have always made the way for development of new types of therapeutics. *Trigonellafoenum gracum* is an important medicinal plant belongs to family Fabaceae. The plant has recently attracted great interest in the pharmaceutical, nutraceutical and functional food industries due to its rich medicinal properties. This present research work was conducted to investigate the biological activities of *Trigonellafoenum gracum* seeds. The active phytoconstituents were determined. A considerable quantity of TFC, TPC were found to be present in examined extract of *Trigonellafoenum gracumm*. It was revealed that extract of *Trigonellafoenum gracum* exhibited excellent antioxidant activity. Moreover, extracts of *Trigonellafoenum gracum* exhibited effective microbial activity. Phytoconstituents of plants can be used in food and cosmetics industries in order to stop the process of oxidation. Important pharmacological components present in the extracts of *Trigonellafoenum graceum* can be effective against various microorganisms. Hence, *Trigonellafoenum graceum* might have potential application in Nutra-pharmaceutical industries.

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