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STUDIES ON ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF DIFFERENT EXTRACTS OF *TRIGONELLA FOENUM-GRAECUM* LINN. AND *ANETHUM GRAVEOLENS* LINN.

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Abstract

While the use of synthetic antimicrobials and antioxidants is increasing day-by-day, people thinking about their welfare have motivated the pharmaceutical industry to discover the natural alternatives. The objective of present study was to assess antibacterial and antioxidant activity of two spices plants *i.e. Trigonella foenum-graecum* Linn. and *Anethum graveolens* Linn. Extracts of different plant parts in methanol and acetone solvent were taken from each plant and screened for antibacterial activity. *T. foenum-graecum* (leaf and seed) and *A. graveolens* (stem and seed) samples in two different solvents (methanol and acetone) were extracted and assessed for antibacterial activity against four medically important pathogens (*Escherichia coli*, *Listeria monocytogenes, Staphylococcus aureus* and *Yersinia pestis*) by Agar-well diffusion method. *T. foenum-graecum* leaf and seed extracts produced maximum zone of inhibition (ZOI) against *S. aureus* (26.4±0.09 and 28.9±0.88 respectively) in methanol extract whereas *A. graveolens* stem and seed extracts showed maximum ZOI against *L. monocytogenes* (24.9±0.75 and 25.8±1.22 respectively) in same solvent. The antioxidant activity of methanol and acetone extracts of both the plants was evaluated *in-vitro* by DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. All the plant part extracts showed potent DPPH scavenging power ranging from 21.98% to 71.82% (IC_{50} *i.e.* 36.12 µg/mL to 489.83 µg/mL). Seed extract of *T. foenum-graecum* exhibited best antioxidant activity in acetone extract (IC_{50} *i.e.* 52.29 µg/mL) whereas seed extract of *A. graveolens* in methanol displayed best antioxidant potential (IC_{50} 36.12 µg/mL). Therefore, it can be concluded that both the plants could act as a potent source of antimicrobials and antioxidants.

***Key words*:** *Trigonella foenum-graecum*, *Anethum graveolens*, Agar-well diffusion, DPPH scavenging assay.

Introduction

Plants and plant derived products are used in food, fodder, flavour and as a source of medicinal herbs from the time immemorial. Infectious diseases are one of the main causes of death, killing almost 50,000 people everyday (Anonymous, 2000). To overcome such type of loss, medicinal plants are used by 80% of the world population, especially in developing countries (Hashim *et al*., 2010). Higher plants act as a source of medicinal compounds and have continued to play a dominant role in the maintenance of human health (Farombi, 2003). In the present scenario of emergence of multiple drug resistance to various pathogenic microbial species, there has arisen a need to search for new antimicrobial substances from natural sources especially medicinal plants (Khan *et al*., 2017).

Medicinal plants and spices have full potential to produce antioxidant and antimicrobial compounds which protect the host from oxidative stress and other pathogens (Mothana and Lindequist, 2005). Most of the food-borne bacteria are sensitive to extracts from plant spices. Furthermore, gram-positive bacteria are more sensitive to antimicrobial compounds in spices as compared to gram-negative bacteria (Lawson, 1996). Spices mainly used as food additives not only add flavour or enhance fragrance but also play the role of natural antioxidants. Antioxidants protect the biomolecules from damage caused by harmful chemical species called free radicals. The overproduction of reactive oxidative species (ROS) (e.g. hydroxyl radicals, superoxide anion radical, hydrogen

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peroxide radicals) can cause the oxidative stress (Braca *et al*., 2002). These all can cause damage to different cellular components of the body. Reactive oxidative species are normal by-products of cell respiration and metabolism. Oxidative damage of proteins, DNA and lipids is associated with various diseases like cancer, cardiovascular diseases, macular degeneration, arthritis, aging, autoimmune diseases, Alzheimer's diseases, cardiovascular and neurodegenerative diseases. The antioxidants are also considered to be essential in diet and in medical therapy hence they are used for delaying aging and biological tissue deterioration (Frankel, 1996). As compared to synthetic antioxidants, naturally occurring antioxidants are more useful and are of great interest in industry as well as scientific procedures. The antimicrobial and antioxidant properties of plants are due to the presence of many active phytochemicals also called as plant secondary metabolites or natural products including flavonoids, terpenoids, carotenoids, coumarins, alkaloids, lignins, saponins etc. (Bukhari *et al*., 2008).

Trigonella foenum-graecum Linn. is also one of the medicinal herbs as well as spices or both. Its common name is Fenugreek (Hindi: methi) which belongs to family Fabaceae and has originated in Northern Africa (Altuntas *et al*., 2005). The leaves and seeds of this plant have been used in various illnesses and as health tonic. Its seed is reported to have anti-diabetic, antifertility, anti-cancerous, anti-microbial, anti-parasitic, lactation stimulant and hypo-cholesterolemic effects (Al-Habori and Raman, 2002). In Ayurveda, Fenugreek seeds and leaves are used to prepare extracts or powder for medicinal use (Basch *et al*., 2003). In addition, it is a well-known spicy agent which prevents aging, labour pain, impart immunity and add vitality to the body.

Like Fenugreek, *Anethum graveolens* Linn. (Hindi: Shatapushpa, Kadvi Saunf) is another spice which belongs to family Apiaceae and founds its distribution in many places such as India, Europe, US, China etc. It has been used for cooking and in medicine since primitive time and is supposed to possess antimicrobial and antioxidant activities (Hong Zeng *et al*., 2011). It has been reported that this plant has anti-hyperlipidaemia, diuretic, hypotensive, antispasmodic, antiemetic, laxative and anticancer activity (Koppula and Choi, 2011; Peerakam *et al*., 2014).

Lots of work has been done on different aspects (like VAM association and endophytes) of medicinal plants of Himachal Pradesh (Sagar and Thakur, 2009; Sagar *et al*., 2015; Shilpa *et al*., 2016), in the Microbiology Lab. of Department of Biosciences, H.P.U. Shimla. A brief review of this work reflects that there is hardly any report available on the antibacterial and antioxidant activity of two highly medicinal plants (*Trigonella foenum-graecum* Linn. and *Anethum graveolens* Linn.) which are being traditionally used by local people in curing different disorders (natural source of antioxidants and antimicrobials having anti-aging and antiparasitic properties) (Rana *et al*., 2016; Prakash *et al*., 2016).

Materials and Methods

Collection of plant material

Joginder Nagar area of District Mandi in Himachal Pradesh was selected for the collection of study material *i.e.* leaf, seed, and stem of spices plants. The collected plant material of both the plants was brought to laboratory for further investigations.

Processing of plant material

Leaves of *Trigonella foenum-graecum* and stem of *Anethum graveolens* were washed under tap water and then with 2% Mercuric chloride. After that leaves and stems were cut into smaller pieces for quick drying and seeds of both the plants were simply collected and dried for 15-20 days in shade. The dried plant material was crushed into fine powder with the help of pestle mortar. The fine powder was then stored in an air tight container at room temperature.

Preparation of plant extracts in different solvents

5 g dried powder of leaves and seeds of *T. foenum-graecum* and stem and seeds of *A. graveolens* were taken in separate Erlenmeyer flasks to which 50 mL of required solvents *i.e.* methanol and acetone were added. The flasks were covered with aluminium foil and allowed to stand for 3-5 days for extraction. These extracts were filtered through the Whatman filter paper no. 1 and evaporated to 40^0C using rotary evaporator. The extracts were collected and weighed and finally stock solution was prepared having concentration *i.e.* 50 mg/mL.

Procurement of bacteria

Different strains of bacteria (*Escherichia coli, Listeria monocytogenes, Staphylococcus aureus* and *Yersinia pestis)* have been procured from IGMC, Shimla and Department of Biotechnology, HPU Shimla for screening antibacterial properties of different plant extracts.

Revival of pathogen

The collected pathogens were revived in nutrient broth and stored in nutrient agar slants at 4^0C.

Screening of antibacterial activity

Different extracts (methanol and acetone) of spices plants were screened using agar-well diffusion method.

Nutrient agar medium (Beef extract 1 g, Yeast extract 2 g, Sodium Chloride 1 g, Peptone 5 g, Agar 20 g, Distilled Water 1000 mL) was used throughout the investigation. The medium was autoclaved at 121.6^{0}C for 30 minutes and poured into Petri plates. Bacteria were grown in nutrient broth for 24 hours. A 100 µL of bacterial suspension was spread on each nutrient agar plate. Agar wells of 8 mm diameter were prepared with the help of sterilized stainless steel cork borer in each Petri plate. The wells in each plate were loaded with 25, 50, 75 and 100% concentration of prepared plant extracts. The Petri plate kept as a control contained pure solvent only. The plates were incubated at 37±2^{0}C for 24 hours in the incubation chamber. The zone of growth inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in perpendicular direction in all the three replicates and the average values were tabulated. Percentage inhibition of bacterial species was calculated after subtracting control from the values of inhibition zone diameter using positive control as standard (Prakash *et al*., 2016).

$$\text{Percentage of growth inhibition (\%)} = \left(\frac{Control - Test}{Contorl}\right)\times 100$$

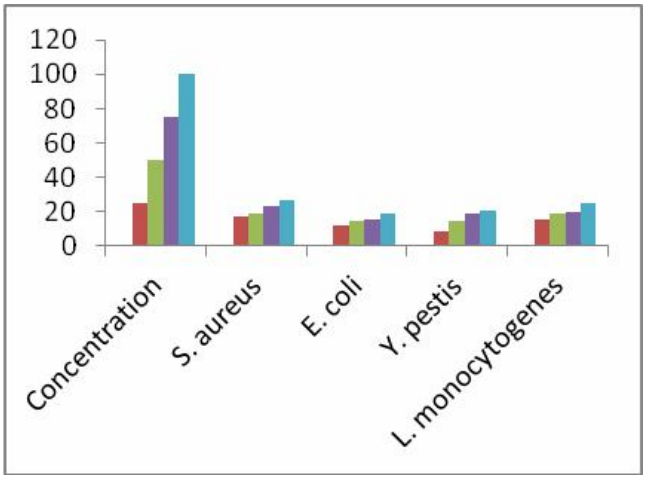
Control = average diameter of bacterial colony in control.

Test = average diameter of bacterial colony in treatment sets (Rana *et al*., 2016).

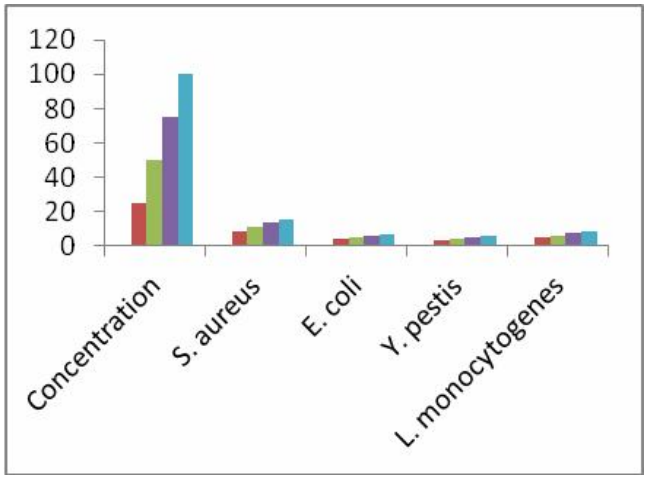
Antioxidant activity test

DPPH free radical scavenging activity assay

The free radical scavenging activity of plant extracts was measured using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay as described by Blois (1958). Briefly, to 1mL of different concentrations (20, 40, 60, 80 and 100 µg/mL) of plant or test extract, 1 mL of DPPH (0.1 mM in methanol) was added. Corresponding blank sample



(A)



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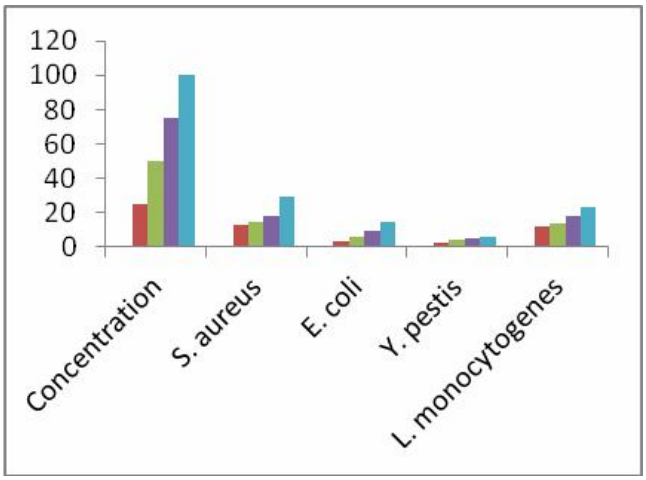

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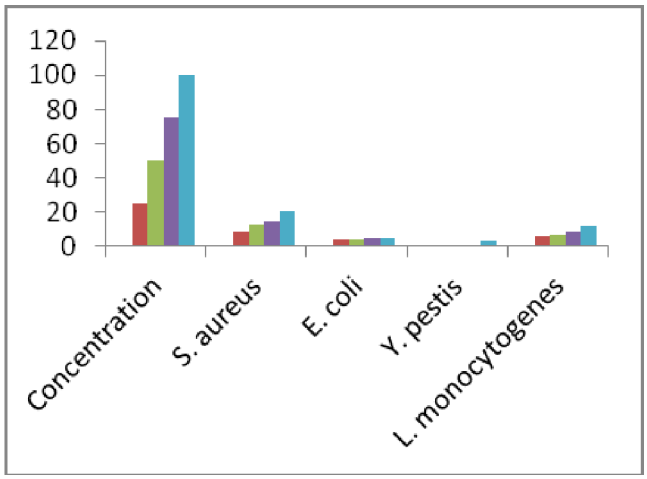

(D)

Fig. A-D: Antibacterial activity of *T. foenum-graecum* in: (A) Methanol leaf extract (B) Acetone leaf extract (C) Methanol seed extract and (D) Acetone seed extract

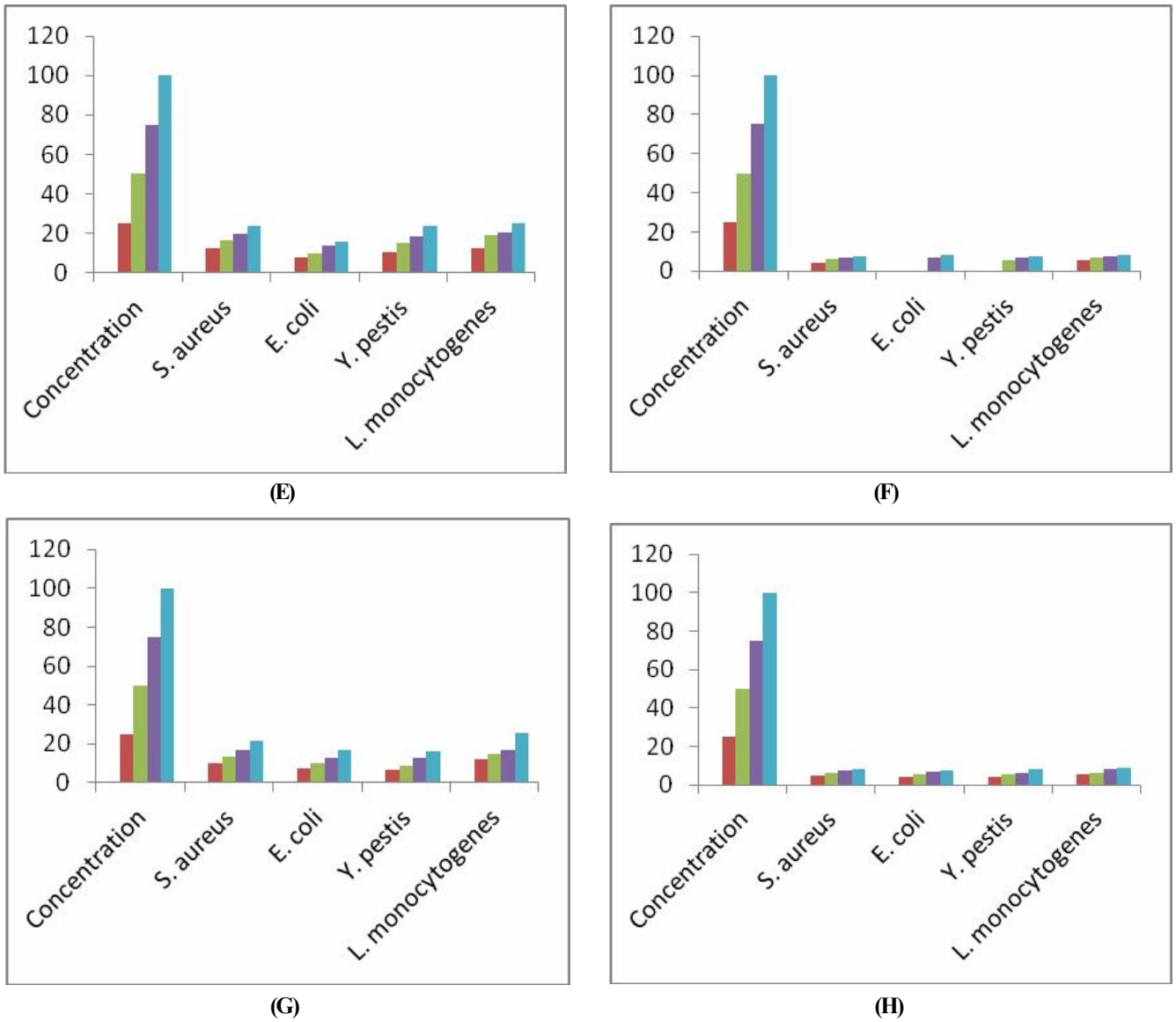

Fig. E-H: Antibacterial activity of *A. graveolens* in: (A) Methanol stem extract (B) Acetone stem extract (C) Methanol seed extract and (D) Acetone seed extract

was prepared and ascorbic acid was used as reference standard. Mixture of 1mL methanol and 1 mL DPPH solution (without plant extract) was used as control. All the tests were carried out in triplicate and the decrease in absorbance was measured at 517 nm after 30 minutes in dark using UV-VIS spectrophotometer. The percentage of inhibition was calculated using the following formula:

$$\text{DPPH scavenging effect (\%)} = \left(\frac{Acontrol - Asamlpe}{Acontrol}\right) \times 100$$

Where, $A_{control}$ is the absorbance of control; A_{sample} is the absorbance of sample

Graphs were plotted against percent inhibition v/s conc. of plant extracts and standard ascorbic acid in order to find out the values of slope and y-intercepts. IC_{50} value (the amount of antioxidant required to decrease the initial DPPH concentration by 50%) for each extract and ascorbic acid was evaluated using the following equation given below:

IC_{50} =

Results and Discussion

In present investigation, antibacterial activity of different plant part extracts in two solvents (methanol and acetone) was determined by agar-well diffusion method. By comparing different extracts in various solvents, it was found that alcoholic extract found to be a better solvent for extraction of antimicrobial active substances (Ahmed *et al*., 1998). In present study, it was observed that *Trigonella foenum-graecum* and *Anethum graveolens* exhibited different zone of inhibition (ZOI) against all four pathogenic bacteria (table 1.1 - 1.4). The

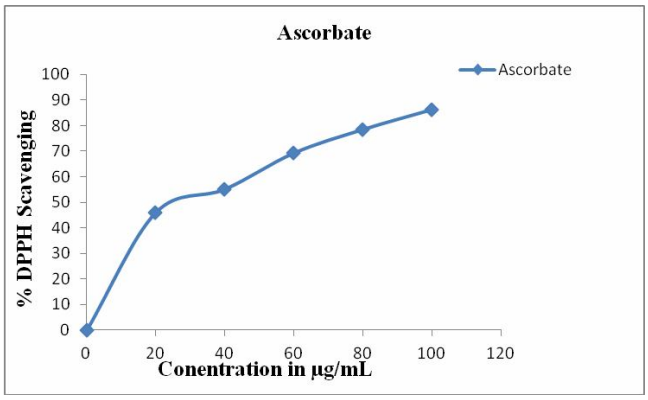

Fig. I: Scavenging activity of L- Ascorbate at different concentrations in methanol

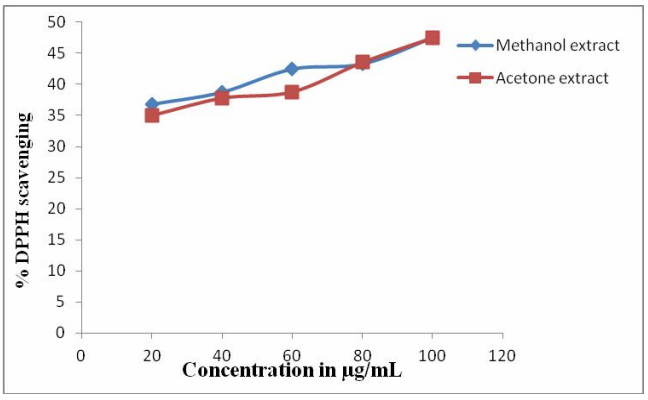

Fig. J: Scavenging activity of *T. foenum-graecum* leaf extracts at different concentrations in methanol and acetone

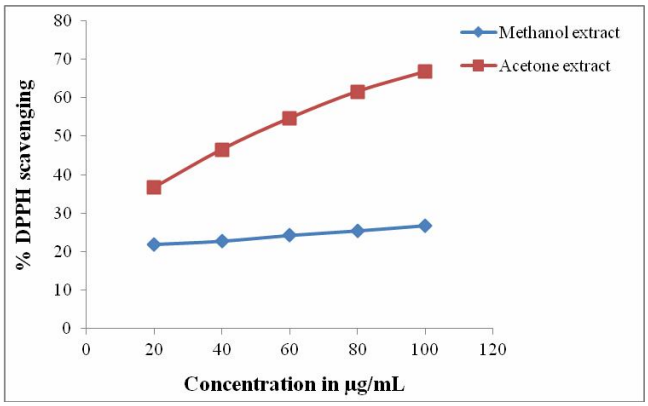

Fig. K: Scavenging activity of *T. foenum-graecum* seed extracts at different concentrations in methanol and acetone

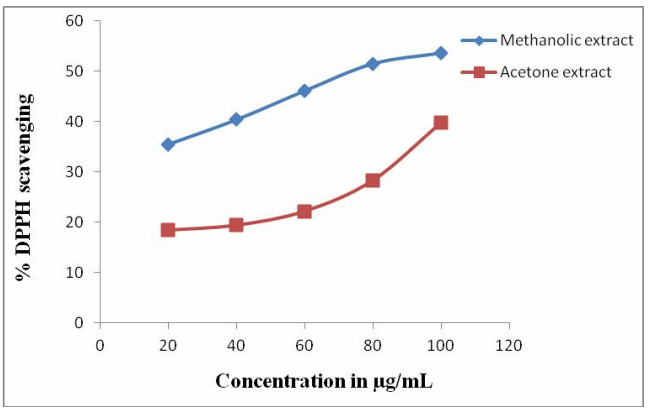

Fig. L: Scavenging activity of *A. graveolens* stem extracts at different concentrations in methanol and acetone

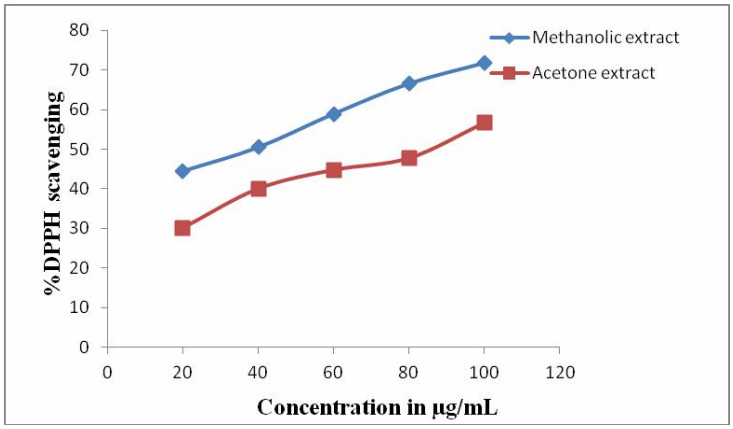

Fig. M: Scavenging activity of *A. graveolens* seed extracts at different concentrations in methanol and acetone

inhibition zone diameter increased with increasing concentration of extracts. At low concentration, *T. foenum-graecum* offered less inhibition to the growth of all the bacteria but at 100% concentration *Staphylococcus aureus* was most inhibited, having zone of inhibition (26.4 mm) followed by *L. monocytogenes* (24.8 mm), *Y. pestis* (20.6 mm) and *E. coli* (19.2 mm) in the case of leaf extract of *T. foenum-graecum* in methanol. Similar trend in the value of zone of inhibition was observed in case of acetone leaf extract of this plant. Similarly methanol seed extract of *T. foenum-graecum* gave maximum zone of inhibition in case of *S. aureus* (28.9 mm) which was followed by *L. monocytogenes* (23.2 mm), *E. coli* (14.6 mm) and *Y. pestis* (6.3 mm) at 100% concentration respectively. Same trend in the zone of inhibition was recorded in case of acetone seed extract of *T. foenum-graecum* also (table-1.1 & 1.2).

In case of *A. graveolens*, stem and seed extracts in

Table 1.1: Zone of inhibition produced by leaf extract of *Trigonella foenum-graecum* at different concentrations in acetone and methanol.

Extract	Concentrations (In %)	Inhibition zone diameter in mm (± S.E.)			
		S. aureus	*E. coli*	*Y. pestis*	*L. monocytogenes*
Methanol extract	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	17.6±0.33	12.2±0.18	8.7±0.21	15.1±0.17
	50	19.2±0.16	14.8±0.13	14.3±0.19	18.7±0.33
	75	23.7±0.15	15.6±0.20	18.9±0.08	19.9±0.12
	100	26.4±0.09	19.2±0.22	20.6±0.13	24.8±0.08
Acetone Extract	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	8.8±0.16	4.3±0.07	3.1±0.12	5.4±0.14
	50	10.9±0.08	5.1±0.12	4.2±0.20	6.0±0.20
	75	13.7±0.19	6.4±0.34	5.0±0.17	7.5±0.11
	100	15.8±0.21	7.0±0.22	6.2±0.31	8.2±0.12

Each data point represents mean of three replicates ± S.E. (Standard error)

Table 1.2: Zone of inhibition produced by seed extract of *T. foenum-graecum* at different concentrations in acetone and methanol.

Extract	Concentrations (In %)	Inhibition zone diameter in mm (± S.E.)			
		S. aureus	*E. coli*	*Y. pestis*	*L. monocytogenes*
Methanol extract	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	13.0±0.85	3.7±0.08	2.6±0.12	12.2±0.06
	50	14.6±0.19	6.4±0.12	4.3±0.09	13.9±0.13
	75	18.3±1.72	9.4±0.09	5.1±0.22	18.0±0.20
	100	28.9±0.88	14.6±0.20	6.3±0.41	23.2±0.18
Acetone Extract	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	8.9±0.16	3.9±0.29	0.00±0.00	6.3±0.90
	50	12.5±0.75	4.2±0.66	0.00±0.00	7.0±0.65
	75	14.4±0.45	4.8±0.87	0.00±0.00	8.5±0.87
	100	20.4±0.33	5.1±0.33	3.3±0.13	12.4±0.25

Each data point represents mean of three replicates ± S.E. (Standard error)

Table 1.3: Zone of inhibition produced by stem extract of *Anethum graveolens* at different concentrations in acetone and methanol.

Extract	Concentrations (In %)	Inhibition zone diameter in mm (± S.E.)			
		S. aureus	*E. coli*	*Y. pestis*	*L. monocytogenes*
Methanol extract	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	12.4±1.12	7.5±0.86	10.1±1.14	12.6±0.12
	50	16.6±0.75	9.7±1.12	14.9±0.75	18.9±1.14
	75	19.7±0.33	13.7±0.68	18.3±1.25	20.2±0.88
	100	23.5±0.20	15.9±0.22	23.4±0.25	24.9±0.75
Acetone Extract	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	4.1±0.27	0.00±0.00	0.00±0.00	5.4±0.45
	50	6.4±1.19	0.00±0.00	5.5±1.25	6.0±0.75
	75	7.0±0.46	6.9±0.88	7.2±0.88	7.5±1.50
	100	7.6±0.22	8.0±1.20	7.8±0.75	8.4±0.88

Each data point represents mean of three replicates ± S.E. (Standard error)

methanol and acetone, offered maximum hindrance to the growth of *L. monocytogenes* (24.9 mm & 8.4 mm) which was followed by *S. aureus* (23.5 mm & 7.6 mm), *Y. pestis* (23.4 mm & 7.8 mm) and *E. coli* (15.9 mm & 8.0 mm) respectively at 100% concentration of the extracts (table- 1.3 & 1.4).

Results of the present study are in agreement with the study of earlier worker (Chandra *et al*., 2011; Jha and Srivastava, 2012; Ksouri *et al*., 2015; Yadav and Chowdhury, 2017). Small variation in results can be attributed to climate conditions and different agro-climatic zones.

In this study, different extracts of *T. foenum-graecum* and *A. graveolens* in two different solvents *i.e.* methanol and acetone were tested to measure their free radical scavenging activity by DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay. Both plants showed excellent potential for scavenging free radicals. DPPH is free radical and when accepts an electron or hydrogen, it become a stable diamagnetic molecule. The reduction of DPPH radical by antioxidant extract is evaluated by decrease in absorbance in 517nm by spectrophotometrically. Change in colour from purple to light yellow can be noticed visually. The results of given extract are summarised in table 2.1 to 2.5 and represented in fig. I-M. The concentrations of tested extracts ranged between 20-100 µg/mL and increase in concentration of extracts increased the activity altogether. *T. foenum-graecum* extracts (leaf and seeds) showed best antioxidant activity in acetone extract whereas *A. graveolens* (stem and seeds) showed best activity in methanol extract. Ascorbic acid which was taken as standard for this investigation having IC_{50} value of 27.17 µg/mL. IC_{50} value for leaf and seed extracts of *T. foenum-graecum* in acetone was 121.49 µg/mL and 52.29 µg/mL respectively but in case of methanol extract the value was 123.30 µg/mL and 489.83 µg/mL respectively. It means that this plant has strong antioxidant activity in acetone as compared to methanol extract. However in case of *A. graveolens,* results were different. Here methanol extract showed best antioxidant activity. IC_{50} values for stem and seed extract of *A. graveolens* in acetone were 153.42 µg/mL and 80.29 µg/mL respectively whereas in methanol the IC_{50} was 78.20 µg/mL and 36.12 µg/mL respectively. It shows that this plant has excellent antioxidant activity especially seeds of this plant may be a good source of natural antioxidants.

Table 1.4: Zone of inhibition produced by seed extract of *A. graveolens* at different concentrations in acetone and methanol.

Extract	Concentrations (In %)	Inhibition zone diameter in mm (± S.E.)			
		S. aureus	*E. coli*	*Y. pestis*	*L. monocytogenes*
Methanol extract	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	10.4±0.33	7.2±0.65	6.4±0.42	12.4±1.20
	50	13.6±1.13	9.9±0.55	8.6±0.65	14.8±0.44
	75	16.8±0.75	12.5±1.50	12.8±0.88	16.7±0.75
	100	21.8±0.77	16.8±0.86	16.4±1.12	25.8±1.22
Acetone Extract	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	5.0±0.75	4.1±0.92	4.2±0.20	5.4±0.23
	50	6.2±0.33	5.7±1.12	5.4±0.58	6.2±0.55
	75	7.4±0.65	6.5±0.45	6.1±0.45	7.9±0.22
	100	8.3±0.88	7.2±0.15	7.8±1.10	9.0±1.20

Each data point represents mean of three replicates ± S.E. (Standard error)

Table 2.1: Free radical scavenging activity (%) of the positive control *i.e.* ascorbic acid at different concentrations.

	Concentration (µg/mL)	Methanol Extract (%)	IC_{50} Value (µg/mL)
Ascorbic acid (Control)	20	46.00±0.52	27.17
	40	55.10±1.20	
	60	69.25±0.75	
	80	78.45±0.45	
	100	86.22±1.32	

Table 2.2: Free radical scavenging activity (%) of the leaf extract of the *Trigonella foenum-graecum* at different concentrations in methanol and acetone.

Plant Name	Concentration (µg/mL)	Methanol Extract (%)	IC_{50} Value (µg/mL)	Acetone Extract (%)	IC_{50} Value (µg/mL)
T. foenum-graecum (Leaf)	20	36.80±0.66	123.30	35.00±0.25	121.49
	40	38.76±0.45		37.80±0.54	
	60	42.50±1.25		38.80±1.20	
	80	43.32±0.80		43.56±2.20	
	100	47.55±1.45		47.52±0.75	

Table 2.3: Free radical scavenging activity (%) of the seed extract of the *T. foenum-graecum* at different concentrations in methanol and acetone.

Plant Name	Concentration (µg/mL)	Methanol Extract (%)	IC_{50} Value (µg/mL)	Acetone Extract (%)	IC_{50} Value (µg/mL)
T. foenum-graecum (Seed)	20	21.98±0.28	489.83	36.63±1.50	52.29
	40	22.80±0.75		46.53±0.75	
	60	24.26±1.12		54.65±0.33	
	80	25.40±2.50		61.58±2.20	
	100	26.71±0.88		66.73±0.66	

Subhashini *et al*. (2011), Jha and Srivastava (2012) and Yadav and Chowdhury (2017) have worked upon these two medicinal plants and our results are in agreement with the results of earlier workers. However small variation in the results can be attributed to different phytochemicals present under different agro-climatic conditions in plants.

Conclusions

It is clear from the present results of our investigations that plant *Trigonella foenum-graecum* Linn. and *Anethum graveolens* Linn. extracts (in methanol and acetone solvent) inhibited the growth of almost all the pathogenic bacteria. Methanol extracts of both the plants showed considerable antibacterial activity as compared to acetone extracts. Bioactive compounds of these plants were found to be more effective in controlling the growth of gram-positive bacteria (*S. aureus* and *L. monocytogenes*) as compared to gram-negative (*E. coli* and *Y. pestis*). It also suggest that different plant extract of these plants possess potent antioxidant activity which is very much helpful in preventing or slowing the progress of various oxidative stress and stress related diseases. Further studies on the isolation and identification of compounds which are responsible for such antibacterial and antioxidant activities in these plants will lead to the availability of chemical entities with potential for various clinical uses.

Table 2.4: Free radical scavenging activity (%) of the stem extracts of the *Anethum graveolens* at different concentrations in methanol and acetone.

Plant Name	Concentration (µg/mL)	Methanol Extract (%)	IC_{50} Value (µg/mL)	Acetone Extract (%)	IC_{50} Value (µg/mL)
A. graveolens (stem)	20	35.50±1.25	78.20	18.41±0.44	153.42
	40	40.39±0.90		19.40±1.20	
	60	46.09±2.20		22.17±0.33	
	80	51.46±0.88		28.31±0.50	
	100	53.58±0.20		39.80±1.90	

Table 2.5: Free radical scavenging activity (%) of the seed extracts of the *A. graveolens* at different concentrations in methanol and acetone.

Plant Name	Concentration (µg/mL)	Methanol Extract (%)	IC_{50} Value (µg/mL)	Acetone Extract (%)	IC_{50} Value (µg/mL)
A. graveolens (seed)	20	44.46±0.50	36.12	30.09±1.80	80.29
	40	50.48±0.25		40.00±0.33	
	60	58.95±1.35		44.75±0.60	
	80	66.61±2.20		47.72±0.55	
	100	71.82±0.60		56.63±0.80	

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