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Int. J. Drug Dev. & Res.
| July-September 2013 | Vol. 5 | Issue 3 | ISSN 0975-9344 |
www.ijddr.in

Full Length Original Research Paper

Evaluation of antibacterial activity of Methanol and Acetone extracts of *Trigonella foenum, Coriandrum sativum* and Brassica nigra

Malik Seema[1]*

Sawhney Ankur[2]

Vyas Manjari[3]

Kakroo Akanshi[4]

Aggarwal Parish[5]

Department of Biotechnology, Ambala College of Engineering and Applied Research, Devsthali, (Near Mithapur), Ambala, Haryana, India

Corresponding Authors:
Malik Seema*
Email id:
seema.seemamtech@gmail.com

Abstract: The aim of the study was to screen antibacterial activities of methanol and acetone extracts of three spices *Trigonella foenum*, *Coriandrum sativum* and *Brassica nigra* available in India. Seeds extract of the spices with methanol and acetone were screened for antibacterial activity against two Gram negative and two Gram positive bacteria i.e. *Escherichia coli, Pseudomonas aeruginosa, Lactococcus lactis* and *Bacillus amyloliquifaciens* respectively. The *in vitro* antibacterial activity was performed by agar well diffusion method. *Pseudomonas aeruginosa* and *Escherichia coli* were found most sensitive against acetone extract of *Trigonella foenum*. Methanol extract of *Coriandrum sativum* were found most effective against *Escherichia coli* and *Lactococcus lactis*. Methanol extract of *Brassica nigra* found effective against *Pseudomonas aeruginosa, Bacillus amyloliquifaciens* and *Escherichia coli*. Acetone extract of *Brassica nigra* was found effective against *Bacillus amyloliquifaciens, Lactococcus lactis* and *Escherichia coli*. The results obtained in the present study suggest that *Trigonella foenum, Brassica nigra* and *Coriandrum sativum* revealed a significant scope to develop a novel broad spectrum of antibacterial herbal formulation.

Keywords: Agar well diffusion, Antibacterial activity, *Trigonella foenum, Coriandrum sativum, Brassica nigra.*

Introduction

Plants are used medicinally in different countries and are a source of many potent and powerful drugs. Medicinal plants are used by 80% of the world population as the only available medicines especially in developing countries [1]. Among ancient civilizations, India has been known to be rich repository of medicinal plants [2]. In recent years, drug resistance to human pathogenic bacteria and fungi has been commonly reported from all over the world [3]. Therefore, the increasing prevalence of multidrug resistant strains of microorganisms and the recent appearance of strains with reduced susceptibility to antibiotics raises an urgent need to search for new sources of antimicrobial agents. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases [4].

Clinical microbiologists have great interest in screening of medicinal plants for therapeutics. The active principles of many drugs found in plants are secondary metabolites [5]. The antimicrobial activities of plant extracts may reside in a variety of different components, including aldehydes and phenolic compounds. Screening of medicinal plants for antimicrobial activities is important for finding potential new compounds for therapeutic use [6].

Spices and herbs have been used for thousands of centuries by many cultures and scientific experiments have documented the antimicrobial

properties of spices. *Trigonella foenum* is used traditionally as a demulcent, laxative, lactation stimulant and exhibits hypocholesterolemic, hypolipidemic and hypoglycemic activity in healthy and diabetic animals and humans. The defatted seeds material of fenugreek may reduce gastrointestinal absorption of glucose and cholesterol and increase bile acid secretion [7]. *Coriander sativum* contains antioxidant, which can delay or prevent the spoilage of food seasoned with this spice. Chemicals derived from *Coriander sativum* leaves were found to have antibacterial activity against *Salmonella choleraesuis* [8]. Whereas *Brassica nigra* baths are recommended for treating headaches, colds and cough. The consumption of one spoonful of mustard seeds 2 or 3 times a day could have laxative effects. Also, the mustard seeds, whether white or black, are used for treating respiratory problems [9].

Materials and Methods

Preparation of plant extracts

Seeds of *Trigonella foenum, Coriandrum sativum* and *Brassica nigra* were cleaned with deionized water, air dried in the shade and pulverized into a fine powder in a blender. The powdered plant materials were soaked in acetone and methanol (plant material to solvent ratio was 1:2, m/v) in conical flasks and closed by foil paper and kept in dark place for 7 days. The crude methanol and acetone extracts were filtered by passing the extracts through Whatmann No. 1 filter paper and then concentrated under vacuum at 40^0C by using a rotary evaporator. The residual extracts were stored in refrigerator at 4^0C in small and sterile plastic bottles.

Test Organisms

Antibacterial activity of spices of the powder extracts was investigated against two gram positive and two gram negative bacterial isolates, which were procured from MTCC, IMTECH, Chandigarh. These include Gram negative *Pseudomonas aeruginosa, E. coli* and Gram positive *Lactococcus lactis, Bacillus amyloliquifaciens*. The tested bacteria were cultured on Nutrient agar (HiMedia, Mumbai) and incubated at 37^0 C for 24 h and maintained at 4°C.

Inoculum preparation

10 ml of Nutrient broth was taken into 4 test tubes and pure colony of freshly cultured bacteria was added to the tubes. The optical density of each tube was measured with the spectrophotometer and microbial population was confirmed to be within 10^6 ml^{-1} to 10^8 mL^{-1}. These suspensions were used as inoculums.

Screening of antimicrobial activity of plant extracts

The *in vitro* antibacterial activities of the test samples were carried out by Agar well diffusion method [10, 11]. Nutrient agar was used as culture media and the test microorganisms were seeded into respective medium by spread plate method with the 24 hrs of bacterial cultures growth. Well of 6mm size were made with sterile borer into agar plates containing bacterial inoculums. 100 µl volumes of the plant extracts were poured into the well of the inoculated plates. Standard Streptomycin and Ampicillin were used as positive control. Methanol and acetone solvent were used as negative control. The plates thus prepared were left at room temperature for ten minutes allowing the diffusion of the extract into the agar. After incubation for 24 hrs at 37˚C, the plates were observed for zone of inhibition surrounding the

well containing the plant extracts. The diameter of the inhibition zone as indicated by clear area which was devoid of growth of microbes was measured and expressed in mm.

Results

Plant based antimicrobial compounds have enormous therapeutic potentials as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials. There are a lot of antimicrobial drugs of which some are discovered or established and some are hidden in the nature. Hence, the last decade witnessed an increase in the investigations on plants as a source of human disease management [12, 13] and more natural antimicrobials have driven scientists to investigate the effectiveness of inhibitory compounds such as extracts from plants [14].

In the present study two solvents namely acetone and methanol were selected for the plant extraction. According to Table 1 *Pseudomonas aeruginosa, Bacillus amyloliquifaciens* and *Lactococcus lactis* were found sensitive to methanol extracts of *Trigonella foenum* which offered inhibition zone of 8mm, 9mm and 11mm respectively. *E. coli* was not found sensitive to the methanol extract of *Trigonella foenum*. Acetone extract of *Trigonella foenum* was effective against only two tested bacteria which recorded inhibition zone of 12mm against *E. coli* and 13mm against *Pseudomonas aeruginosa*. No activity of acetone extract of *Trigonella foenum* was observed against *E. coli* and *Lactococcus lactis*.

The methanol extract of *Coriandrum sativum* recorded inhibition zone of 11mm against *E. coli,* 8mm against *Bacillus amyloliquifaciens* and 10mm against *Lactococcus lactis* whereas acetone extract of *Coriandrum sativum* offered inhibition zone of 9mm against *Pseudomonas aeruginosa* and 10mm against *E. coli*. The activity against *Bacillus amyloliquifaciens* and *Lactococcus lactis* was not observed in acetone extract.

The Methanol extract of *Brassica nigra* was effective against *Pseudomonas aeruginosa., E. coli* and *Bacillus amyloliquifaciens* and offered inhibition zone of 10mm, 9mm, and 11mm. respectively. *Lactococcus lactis* was found resistant to methanol extracts of *Brassica nigra*. On the other hand acetone extract of *Brassica nigra* recorded zone of inhibition of 8mm. against *Lactococcus lactis,* 11mm against *E. coli* and 14mm against *Bacillus amyloliquifaciens*. *Pseudomonas aeruginosa* was found resistant to acetone extract of *Brassica nigra*. Negative control disc (containing only methanol and acetone) showed no zone against any tested bacteria. All the positive controls showed antibacterial activity against tested bacteria.

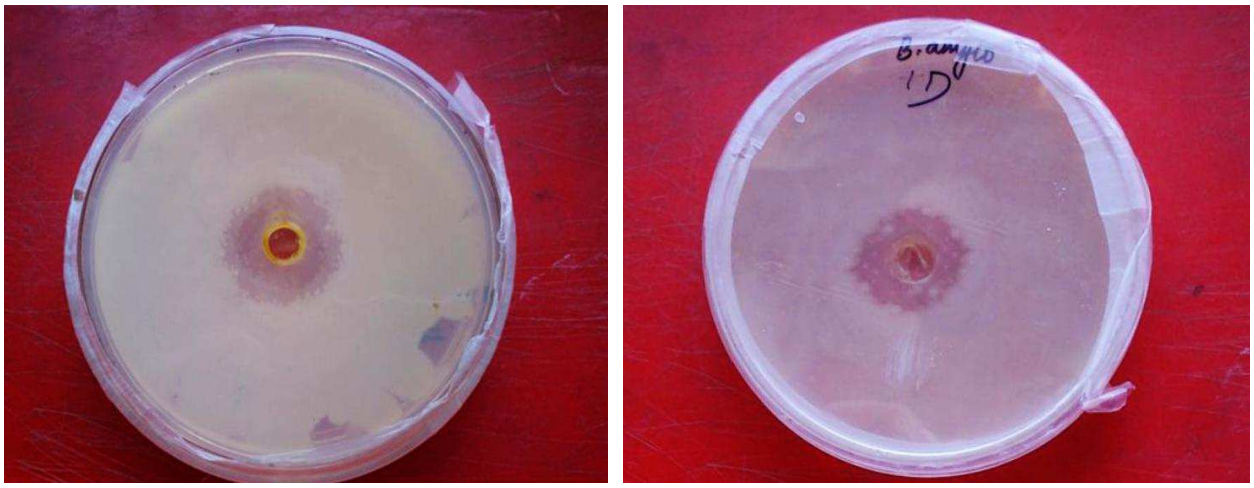
Table 1: Antimicrobial Activity of methanol and acetone extract of *Trigonella foenum, Coriandrum sativum* and *Brassica nigra* against tested bacteria

Name of Plant	Extract	Diameter of zone of inhibition in mm. (mean±SD)			
		Pseudomonas aeruginosa	*E. coli*	*Bacillus amyloliquifaciens*	*Lactococcus lactis*
Trigonella foenum	Methanol	8±0.61	**NA**	9±0.25	11±0.12
	Acetone	13±0.30	12±0.38	**NA**	**NA**
Coriandrum sativum	Methanol	**NA**	11±0.75	8±0.59	10±0.32
	Acetone	9±0.24	10±0.61	**NA**	**NA**
Brassica nigra	Methanol	10±0.32	9±0.81	11±0.37	**NA**
	Acetone	**NA**	11±0.70	14±0.29	8±0.41
Negative Control	Methanol	**NA**	**NA**	**NA**	**NA**
	Acetone	**NA**	**NA**	**NA**	**NA**
Positive control	Streptomycin	12	10	13	11
	Ampicillin	17	13	14	13

NA: No Activity

Figure 1: Antibacterial activity of acetone extract of *Trigonella foenum* recorded zone of inhibition of 12 mm against *E. coli* and 13mm against *Pseudomonas aeruginosa*

Figure 2: Antibacterial activity of methanol extract of Coriander offered zone of inhibition of 11 mm against *E.coli* and 8mm against *Bacillus amyloliquifaciens*



Discussion

The main objective of this work was to increase the utilization of biomass from spices in order to isolate new biologically active compounds. Spices were recognized as having food preserving possibilities by the Egyptians some 3,000 years ago. The antibacterial factors were found in the essential oils of the spices. Gram negative bacteria were more resistant to the seeds of the spices than gram positive bacteria [15].

This study deals with two Gram positive and two Gram negative bacterial strains. In the present work, the antibiotic potential of the methanol and acetone extracts of the *Trigonella foenum, Coriandrum sativum* and *Brassica nigra* has been determined against *Bacillus amyloliquifaciens, Lactococcus lactis* and *Pseudomonas aeruginosa, Escherichia coli*. In this study, methanol extract of *Trigonella foenum* were found effective in inhibiting the growth of *Bacillus amyloliquifaciens, Lactococcus lactis* and *Pseudomonas aeruginosa*. On the other hand, acetone extract of *Trigonella foenum* showed antimicrobial activities against *Pseudomonas aeruginosa* and *Escherichia coli*. Methanol extract of *Coriandrum sativum* showed inhibitory activity against *Escherichia coli, Bacillus amyloliquifacien* and *Lactococcus lactis* whereas

acetone extracts of the *Coriandrum sativum* showed activity against *Pseudomonas aeruginosa* and *Escherichia coli*. Methanol extract of *Brassica nigra* found effective against *Pseudomonas aeruginosa, Bacillus amyloliquifaciens* and *Escherichia coli*. Acetone extract of *Brassica nigra* was found effective against *Bacillus amyloliquifaciens, Lactococcus lactis* and *Escherichia coli*. Negative control disc produced no zone of inhibition of against any tested bacteria indicating that the solvents (methanol and acetone) did not possess any antimicrobial effect on the tested bacteria.

Conclusion

From the present study it can be concluded that the extracts of *Trigonella foenum*, *Coriandrum sativum* and *Brassica nigra* were found to be effective antibacterial agents against bacteria**.** This study paves the way for further attention and research to identify the active compounds responsible for the plant biological activity. Further studies should be undertaken to elucidate the exact mechanism of action by which extracts exert their antimicrobial effect.

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Article History:----------

Date of Submission: 04-06-2013

Date of Acceptance: 19-06-2013

Conflict of Interest: NIL

Source of Support: NONE