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Bacteriostatic potential of *Argemone mexicana* Linn. against enteropathogenic bacteria

Vivek Kempraj and Sumangala K Bhat*
Biowave Resources, 519, 33rd Cross, 9th Main, IV Block, Jayanagar, Bangalore-560 011, Karnataka, India

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Bacteriostatic efficacy of 16 crude extracts derived from different parts of *Argemone mexicana* Linn. (Papaveraceae) has been analyzed on enteropathogenic bacteria such as *Klebsiella oxytoca*, *Vibrio damsella, Enterobacter aerogenes* and *Escherichia coli*. The bacteriostatic efficacy was elucidated using single disc diffusion method. The minimum inhibitory concentration (MIC) of the extracts showing higher efficacy against the test organisms was determined. The MICs of acetone extract of seed and aqueous extract of leaf on different bacteria tested were found to be between 0.005-0.02 mg/disc.

Keywords: *Argemone mexicana*, Papaveraceae, Bacteriostatic, Enteropathogens.

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Introduction

Enteric diseases pose challenge to human health throughout the world. The problem takes serious dimensions in the case of epidemic diseases. It is established that enteric bacteria infect 30-60% of the adults in developed countries and some die due to these disease[1]. Despite the availability of several antimicrobial compounds[2,3] to cure enteric diseases, search for new compounds appears still warranted. The need for new antibacterial agents attains strategic importance due to the increasing resistance being developed by enteric bacteria to the classic antimicrobial drugs[4]. It is essential to have new antimicrobial agents, preferably those that can readily be produced from simple sources such as plants[5]. It has also been opinioned that antimicrobial compounds from plants may inhibit bacterial growth by different mechanisms than those of the synthetic antibiotic compounds currently in use and may be of significant clinical value in treatment of patients infected with resistant strains[5]. In view of this fact, current study has been carried out on *Argemone mexicana* Linn. (Papaveraceae) (Plate 1) commonly known as prickly poppy or Mexican poppy and found wildly throughout India. This plant is used as medicine in several countries. In India and Mexico, the seeds are considered as an antidote to snake venom. The fresh yellow latex contains protein-dissolving substances effective in the treatment of warts, sores and skin infection. It is also used in curing dropsy and jaundice[6]. Previous studies have proved the antibacterial activity of *A. mexicana* but limited to seeds and leaves of the plant[7, 8]. However, attempt has been made in the present study to compare the bacteriostatic potential of the extracts of different parts of *Argemone mexicana* on *Klebsiella oxytoca*, *Vibrio damsella, Enterobacter aerogenes* and *Escherichia coli*.

Materials and Methods

Collection of plant material

Materials subjected for study include leaves, seeds, stem and root of *A. mexicana*. These materials were collected from the vicinity of the institute and identified by a botanist. The freshly picked parts of the plants were rinsed with running tap water and then with distilled water. They were air-dried at room temperature for 2 weeks, with no direct sunlight. Once dried, plant materials were ground and stored at 4°C before subjecting them individually to solvent extraction procedures.

Plate 1— *Argemone mexicana*

*Correspondent author
E-mail: bhat.sumangala@gmail.com

Preparation of crude extracts

Organic solvents (Acetone, Diethyl ether and Methanol)

The method of solvent extraction consisted of soaking the ground plant materials in above solvents for 24h, followed by shaking in an incubator shaker set at 200 RPM and 35°C for 4h. The standard plant material weight/solvent volume concentration (w/v) used was 1/10. The extracts were filtered using Whatman filter paper no. 1 and centrifuged at 10,000 RPM for 15 min. The supernatant was taken and concentrated on a hotplate set at 38°C until the solvents evaporate completely. The obtained solvent extracts were weighed and stored at 4°C until further use.

Water

The plant weight to water volume ratio used was 1/5. The ground dried plant material was soaked in boiling water for a period of 20 min, and then filtered through Whatman filter paper no. 1. This was followed by additional filtrations through Millipore filter with a pore size-diameter of 0.45µm. The aqueous extract was concentrated at -15°C in a freezer for 15 days and stored until further use.

Test organisms

Four different entero-pathogenic bacterial strains namely, *K. oxytoca*, *V. damsella*, *E. aerogenes* and *E. coli*, were isolated from the stool sample of a patient with gastrointestinal infection. After isolation, pure cultures of the bacteria were raised and the species identified through standard biochemical tests and confirmation was made by consulting the Berge's Manual.

Antibacterial assay by disc diffusion method

Antibacterial activity was demonstrated using single disc diffusion method[9]. A pure colony of each of the test organisms were sub-cultured into a 5 ml of nutrient broth, followed by incubation at 37°C for 24h. The test was carried out by placing each disc (5mm) impregnated with 0.03 mg of the extracts/disc on Luria Agar surface previously inoculated with 100µl suspension of test organism. Respective solvents without plant extracts served as negative control. Standard antibiotic disc of tetracycline was used (0.03 mg/disc) as positive control. Plates were incubated at 37°C for 24h to observe formation of clear zone of inhibition.

Minimal inhibition concentration (MIC)

The MIC method was applied on extracts that proved high efficacy against test organisms by the disc diffusion (Kirby-Bauer) method[9,10]. Hundred milligram of each selected extract was dissolved in 10 ml of their respective solvents from which they were extracted and serially diluted to get a least concentration of 1mg/ml of test extract. Required volume of test extract was applied accordingly to discs to get concentrations ranging from 0.005-0.03 mg/disc. The discs were dried at room temperature for 24h for removal of the solvents and stored in a sterile, dry and cool place for further use. The dried discs were used in MIC tests. A control experiment was run in parallel to study the impact of the solvent itself and tetracycline on the growth of the four test organisms. The solvents (100%) of similar volume were applied to the discs used as negative control, whereas standard Tetracycline discs with concentration ranging from 0.005-0.03 mg/disc were used for positive control trials. The inoculation and incubation of the microorganisms were done similarly as in above.

Results and Discussion

During the current study, a total of sixteen extracts of *A. mexicana* were tested against 4 bacteria mentioned in the methodology. The results of the preliminary screening of the extracts for antibacterial properties are illustrated in Table 1. From Table 1 it is

Table 1— Results of the preliminary screening of different extracts (0.03 mg/disc) of *A. mexicana* against test bacteria

Organisms	Extracts				
	Extract of leaves				
	Acetone	DW	Methanol	DEE	Tetracycline
K. o	-	13 mm	-	-	21 mm
V. d	-	11 mm	14 mm	-	15 mm
E. a	-	10 mm	-	-	26 mm
E. c	-	10 mm	6 mm	6 mm	29 mm
	Extract of seeds				
K. o	8 mm	9 mm	-	-	21 mm
V. d	10 mm	6 mm	-	-	15 mm
E. a	9 mm	14 mm	-	-	26 mm
E. c	14 mm	7 mm	6 mm	6 mm	29 mm
	Extract of stem				
K. o	9 mm	14 mm	-	6 mm	21 mm
V. d	9 mm	12 mm	-	-	15 mm
E. a	-	6 mm	6 mm	-	26 mm
E. c	11 mm	6 mm	9 mm	6 mm	29 mm
	Extract of root				
K. o	6 mm	14 mm	7 mm	-	21 mm
V. d	14 mm	6 mm	11 mm	-	15 mm
E. a	6 mm	7 mm	-	-	26 mm
E. c	9 mm	11 mm	6 mm	-	29 mm

D.W: Distilled water, DEE: Diethyl ether, K. o: *Klebsiella oxytoca*, V. d: *Vibrio damsella*, E. a: *Enterobacter aerogenes*, E. c: *Escherichia coli*. Grading of results: '-'No Inhibition; the results were rounded off for better understanding.

Table 2— Minimal inhibition concentration (MIC) of the selected extracts of *A. mexicana*

Solvent/Ext	Minimal inhibition concentration (MIC) in mg/disc			
	Bacterial species			
	K. o	V. d	E. a	E. c
D.W (Leaves)	0.01	0.01	0.005	0.01
Acetone (Seeds)	0.02	0.01	0.01	0.005
Tetracycline	0.005	0.005	0.005	0.005

Ext: extracts, D.W: Distilled water, K. o: *Klebsiella oxytoca*, V.d: *Vibrio damsella*, E. a: *Enterobacter aerogenes*, E. c: *Escherichia coli*

evident that the extent of microbial growth inhibition observed varied considerably with respect to the type of extract as well as the test organism. The acetone extract of leaf and diethyl extract of the root had no growth inhibitory activity against any of the organisms, whereas seed, stem and root extracts showed varied levels of inhibitory effects on growth of the test organisms. The methanol extracts of leaf and root showed inhibition of growth of *V. damsella* and *E. coli*. The DEE extracts showed relatively less activity against some test organisms. Aqueous extract showed relatively broad-spectrum growth inhibitory activity against the bacteria tested. None of the solvents used as control, did result in any inhibitory effect when used in the assay.

Further trials on bacteriostatic activity were carried out using distilled water extract of the leaf and acetone extract of the seed. Distilled water extract of the leaf has been selected because of the reason that it showed highest or near to highest inhibitory activity on all the bacterial species tested during the preliminary study. Acetone extract of the seed has shown highest activity against *V. damsella* and *E. coli* and second to highest (highest being distilled water extract) activity against *E. aerogenes* and *K. oxytoca*. Since the aqueous extract of the leaf has been selected as one of the component for second level trials, selecting an extract of the seeds in a different solvent appears more logical and would increase the chances of identifying new active components at later stages of the study. Table 2 illustrates the results of MIC assay of aqueous extract of the leaf and acetone extract of the seed. Discs impregnated with different concentrations of the extracts ranging from 0.005-0.03 mg/disc were tested against the organisms. The MIC (Plate 2 and 3) of the extracts ranged in between 0.005-0.02 mg/disc.

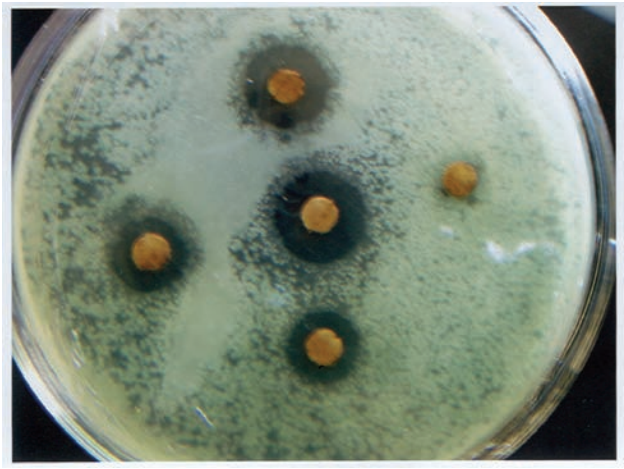

Plate 2— MIC assay of aqueous extract of leaves on *E. aerogenes*

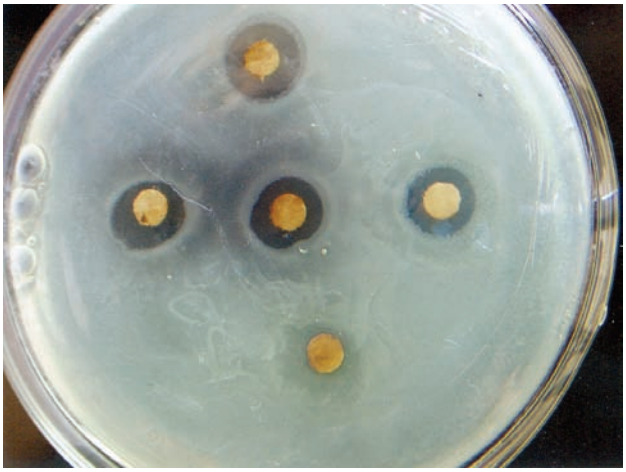

Plate 3— MIC assay of acetone extract of seed on *E. coli*

Activities of the aqueous extract of the leaf on *E. aerogenes* and activity of the acetone extract of the seeds on *E. coli* were observed to be at par with that of tetracycline (Table 2). Therefore, it is obvious that the plant contains some antibacterial components. Leaves and seeds can be used as the source material for isolation of the active ingredients. The current result has supplemented earlier report on growth inhibitory effects of solvent extracts of *A. mexicana* on few other species of pathogenic bacteria[8]. Therefore, it is evident that the plant contains active ingredients with broad-spectrum antibacterial efficacy. Studies on isolation and characterization of the active ingredients of bacteriostatic components in the above samples are on.

Conclusion

The study has confirmed broad-spectrum antibacterial property of *A. mexicana.* Leaf and seed of the plant have emerged as the principal sources of

the active ingredients with bacteriostatic potential. The active ingredients from the leaf and seed would be different because their extracts were prepared with different solvents (i.e. distilled water and acetone respectively). Chemical characterization and identification of the active ingredient(s) in the extracts are to be done for future application oriented research on this aspect. The study would be able to address the problem of tackling antibiotic resistant pathogenic bacteria in therapeutic applications to a certain extent. Meanwhile this would open new avenues in agriculture sector for the propagation of medicinal plants as alternative crops that offer better economic and social benefits to the farmers.

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