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Antimicrobial activities of the root, stem and leaf extracts of *Argemone mexicana* L.

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Abstract

The present study was designed to evaluate the antibacterial activity of the aqueous, acetone, ethanol and chloroform extracts of the leaves, stem and root of *Argemone mexicana* L. (Papaveraceae) using agar well diffusion method against four strains of bacterial species, namely, *Escherichia coli*, *Klebsiella pneumoniae, Bacillus cereus* and *Staphylococcus aureus*. Among various extracts studied, the ethanol stem extract showed greatest antibacterial activity against *Klebsiella pneumoniae* (22.86 mm); following by acetone extract (17.35 mm). The highest inhibition zone observed for ethanol extract of *A. mexicana* root against *Bacillus cereus* was 20.05 mm. The maximum activity of ethanol leaf extract against *Staphylococcus aureus* was 19.12 mm. The research recommends that the extract of the plant parts possesses new wide spectrum antibacterial activities. The stem extract showed more inhibitory effect than the root and leaf extracts. This research suggests that natural produces obtained from *A. mexicana* L. may provide to the evolution of novel antimicrobial agents.

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Introduction

Medicinal plants are of noble worthiness to mankind. They are nature's offering human beings to regulate a sickness free healthful life. They performance a necessary role in preserving our health (Bhagwati, 2003). Microorganisms have created resistance to various antibiotics and this had developed immense clinical difficulty in the curing of contagious illness (Davis, 1994). The enlarge in resistance of microbes due to indiscriminate utilize of commercial antimicrobial medicines supported scientists to investigate for modern antimicrobial substances from several sources including medicinal plants. (Karaman *et al.,* 2003). Medicinal plants are considerably serviceable and economically needed. The receive dynamic phytoconstituents that are used in the manage of various human ailments (Stary and Hans, 1998). The plant extracts have been revealed and recommended for use as antimicrobial properties (Del Campo *et al.,* 2000). Medicinal plants control effective medicinal utility that is due to the presence of a difference of phytoconstituents in the plant tissues which cast a specific physiological activity on the human body very few of these phytochemicals are toxic also (Sheeba, 2010).

Antibacterial potentialities of several plants parts such as root, stem, leaves, seeds, flowers, fruits have been well noted for some of the medicinal plants for the past two decades (Leven *et al.,* 1979). Medicinal and aromatic plants and substances are luxuriant antibacterial compounds could be an alternate manner to fight against bacterial ailments (Meera *et al.,* 1999). The plant is purgative, destroy worms, bitter cure itching leprosy, different skin disorders, bilious fever and inflammation. The seeds are used in cough, asthma, nauseant, emetic, demulcent, expectorant and laxative. The root is useful in anthelmintic (Nadkarni, 1982). It is used in treatment of antibacterial, antimicrobial, cytotoxicity, wound healing, antioxidant and antifungal properties (Mohana *et al.,* 2008; Santosh Kumar Singh *et al.,* 2009; Osho and Adetunji, 2010; Satish Kumar Verma, 2010; Shyam Prasad and Dhanapal, 2010; Perumal *et al.,* 2010; Shahedur Rahman *et al.,* 2011; Dash and Murthy, 2011). It is traditionally used as hallucinogenic, analgesic antispasmodic and antitussive (Jain and Sharma, 2001).

Phytochemically the leaves contains flavonoids, steorls, tannins, alkaloids and glycosides, (Bhalke *et al.,* 2009). Many reports have been carried out to investigate the antibacterial determines of *Argemone mexicana* extracts. Osho and Adetunji (2010) studied the antimicrobial activity of the essential oil of *Argemone mexicana*. The earlier observations on *A. mexicana* leaf and seed extracts showed considerable antimicrobial activity (Santosh Kumar Singh *et al.,* 2009; Shyam Prasad and Dhanapal, 2010).

Argemone mexicana L. (Papaveraceae), commonly known as Prickly Poppy in English and Premathandu in Tamil found in Mexico and now has widely naturalised in the United States, India, Bangladesh and Ethiopia. It occurs as wasteland weed in almost every part of India (Mukherjee and Namhata, 1990; Das and Misra, 1987). In Mexico, the seeds have been used as an antidote to snake poisoning (Bhattacharjee *et al.,* 2006). In India, the smoke of the seeds is used to relieve toothache. The fresh yellow, milky seed extract contains protein-dissolving substances effective in the treatment of diuretic, anti-inflammatory, malarial fever, leprosy, scorpion sting, warts, cold sores, wound healing, skin diseases, itches, jaundice and an antidote to various poisons (Chopra *et al.,* 1986; Prusti and Mishra, 2004; Alagesaboopathi, 2009; Dash and Murthy, 2011). The seeds are purgative and sedative (Ayurveda) (Das and Mishra, 1987), useful in skin diseases and leu coderma (Yunani) (Chaudhuri Rai *et al.,* 1985) and in Homeopathy, the tincture of the entire plant is reported to be used orally for bronchitis and whooping cough (Kala, 2005; Eldridge, 1995). The fresh juice of the leaves and the latex both are reported to be used externally as a disinfectant for open wounds

and cuts (Alagesaboopathi, 2009; Panghal *et al.,* 2010). Various isoquinoline alkaloids viz. berberine, cryptopine, coptisine, muramine, scoulerine, stylopine, cheilkanthifoline, sanguinarine, sarguinarine, chelerytherine, sanguinarine, thalifoline and protopine have been reported from the plant (Gupta *et al.,* 1990).

The present study was undertaken to evaluate the antibacterial potentials of *Argemone mexicana* leaf, stem and root extracts against some selected bacterial species with the possible use as a genuine antimicrobial agent in pharmacological industries.

Materials and methods

Collection and identification of plant materials

The fresh roots, stem and leaf were colleted in January 2012 from Shevaroy Hills of Eatern Ghats of Tamil Nadu, India and dried at 30°C for 10 days. The plant specimens were identified and confirmed with the flora of Tamilnadu and voucher specimen (No. 20.01.2012 NK) deposited in the Department of Botany, Government Arts College (Autonomous), Salem for the future reference.

Test microorganisms

In the research, we have used four bacteria such as *Bacillus cereus*, *Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumoniae* were used for bioassay. The pure strains were procured from Biomedical Engineering Research Foundation, Salem, Tamilnadu, India. The organisms were maintained on nutrient agar media at 4°C and sub cultured for 24 h before use.

Preparation of plant extracts

Fresh plant parts (leaves, stem and root) collected were cleaned individually under running tap water and then with sterile distilled water. The leaves were air dried in the laboratory at room temperature 30 ± 1°C for 8 days. While the root and stem parts were dried at 50±1°C for 48-60 h in an oven. The dried leaves, stem and root samples were ground well into a fine powder form by a grinding machine. The powder was stored in airtight bottles at room temperature before extraction. The manner of Alade and Irobi (1993) was adopted for preparation of plant extracts. A fixed weight 25 g of powdered plant materials was soaked separately in 150 ml each of aqueous, acetone, ethanol and chloroform for 48 h. Each mixture was stirred at 24 h interval using a sterile glass rod. At the end of the extraction, each extract was passed through Whatman No.1 filter paper (Whatman England), and the filterate obtained was concentrated in vaccum using evaporator. Then the extracts were used for antibacterial potentials.

Antibacterial assay

Antibacterial activity was screened by agar well diffusion method (Azoro, 2002). The agar well diffusion manner was employed for the determination of antibacterial activity of the extracts. The petriplates containing 20 ml of Muller Hinton Agar medium were seeded with 24 h culture of the microorganism. The wells (6 mm in diameter) were cut from the agar and the extract solution (5 mg/ml) was then added into it. The diameter of the inhibition zone was measured on millimeters (mm). 10 µg/ ml of ampicillin served as control. The work was done in triplicate.

Results and discussion

The results of antibacterial potentialities of aqueous, acetone, ethanol and chloroform extracts of the root, stem and leaves of *Argemone mexicana* are given in Table 1. All the extracts showed wide spectrum of screening. When the four extracts were compared with other and with that of standard antibiotic Ampicillin, the ethanol stem extract showed the highest potentiality compared to that of acetone, aqueous and chloroform extracts. The extract obtained using ethanol showed highest activity against *K. pneumoniae* (22.86 mm), *S. aureus* (13.10 mm) and *E. coli* (12.30 mm). Least inhibition zone was observed against *B. cereus* (12.05 mm). The study made on acetone extract recorded maximum activity against *K. pneumoniae* (17.35 mm), *S. aureus* (14.90 mm) and *B. cereus*

(12.38 mm) and the minimal activity was against *E. coli* (10.41 mm). The stem extracts of aqueous showed a positive significant antibacterial activity against *E. coli* (10.70 mm), *K. pneumoniae* (11.41 mm) and *S. aureus* (10.73 mm). While lower activity against *B. cereus* (10.08 mm).

Table 1. Antibacterial potentiality of the root, stem and leaf extracts of *Argemone mexicana* L. by agar well diffusion method.

Plant part	Plant extracts	Zone of inhibition (in mm)			
		Escherichia coli	*Klebsiella pneumoniae*	*Bacillus cereus*	*Staphylococcus aureus*
Root	Aqueous	10.31±0.18	16.60±0.64	17.26±0.20	16.03±0.47
	Acetone	13.40±0.65	10.45±.39	16.08±0.19	15.05±0.68
	Ethanol	18.68±0.70	14.60±0.78	20.05±0.60	10.04±0.32
	Chloroform	-	10.02±0.24	11.04±0.22	-
Stem	Aqueous	11.70±0.16	11.41±0.38	10.08±0.17	10.73±0.50
	Acetone	10.41±0.30	17.35±0.15	12.38±.40	14.90±0.83
	Ethanol	12.30±0.29	22.86±0.40	12.05±0.11	13.10±0.13
	Chloroform	10.18±0.13	10.13±0.03	10.30±0.41	10.78±0.48
Leaves	Aqueous	13.07±0.22	10.37±0.11	11.27±0.50	10.04±0.16
	Acetone	10.03±0.14	10.11±0.02	13.16±0.09	10.51±0.27
	Ethanol	10.80±0.24	19.88±0.15	11.05±0.19	19.12±0.15
	Chloroform	-	10.00±0.01	12.04±0.70	10.24±0.11
	Ampicillin 10µg/ml	21.0±0.60	28.0±0.28	26.0±0.48	24.0±0.12

Date given are mean of triplicates ± standards error. – Indicates no activity Concentration used 50 µg/ml

The chloroform extracts of the stem showed significant and maximum antibacterial activity against *S. aureus* (10.78 mm), *B. cereus* (10.30 mm) and *E. coli* (10.18 mm). While moderate activity against *K. pneumoniae* (10.13 mm) (Fig. 1). The indicative and maximum antibacterial properties of the root ethanol extracts against *B. cereus* (20.05 mm), *E. coli* (18.68 mm) and *K. pneumoniae* (14.60 mm) and less inhibition zone was recorded against *S. aureus* (10.04 mm). The research made on aqueous extract noticed maximum

activity against *B. cereus* (17.26 mm), *K. pneumoniae* (16.60 mm) and *S. aureus* (16.03 mm). Further, it recorded minimal activity against *E. coli* (10.41 mm) in root extract.

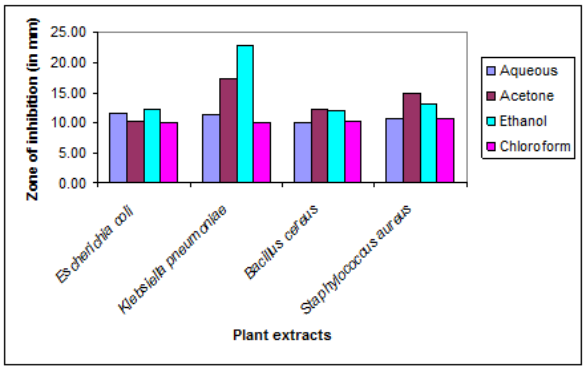

Fig. 1. Antibacterial potentiality of the stem extract of *Argemone mexicana* L. against various pathogenic bacterial stains by agar well diffusion method

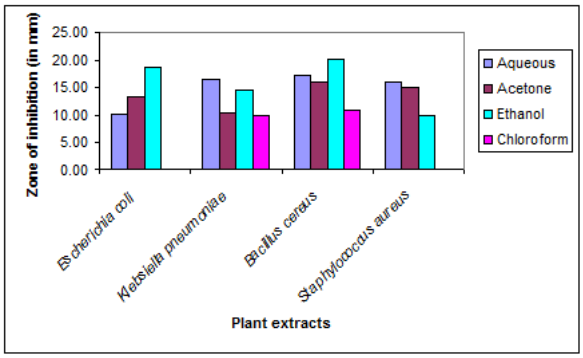

Fig. 2. Antibacterial potentiality of the root extract of Argemone mexicana L. against various pathogenic bacterial stains by agar well diffusion method.

The acetone extracts of the root showed weightly and highest antibacterial activity against *B. cereus* (16.08 mm), *S. aureus* (15.05 mm) and *E. coli* (13.40 mm) and minimal inhibition zone was observed against *K. pneumoniae* (10.45 mm). The antibacterial activities of the root chloroform extracts showed diameter of inhibition zones ranging from 11.04 to 10.02 mm, with the maximum zone was inhibition shown towards *B. cereus* (11.04 mm). Minimum inhibition zone was recorded against *K. pneumoniae* (10.02 mm). *S. aureus* and *E. coli* showed no activity (Fig. 2).

The zone of inhibition in leaf ethanol extracts showed highest activity against *S. aureus* (19.12 mm), *K. pneumoniae* (19.88 mm) and *B. cereus* (11.05 mm) and the least activity against *E. coli* (10.80 mm). The extracts using aqueous showed maximum inhibition zone observed against *E. coli* (13.07 mm), *B. cereus* (11.27 mm) and *K. pneumoniae* (10.37 mm). Minimum inhibition zone was observed against *S. aureus* (10.04 mm). Acetone extracts from leaves was activity against *B. cereus* (13.16 mm), *S. aureus* (10.51 mm) and *K. pneumoniae* (10.11 mm) and the minimal activity was against *E. coli* (10.03 mm). The leaf extracts of chloroform showed positive significant antibacterial activity against *B. cereus* (12.04 mm) and *S. aureus* (10.24 mm). While less activity against *K. pneumoniae* (10.00 mm). The activity was nil for *E. coli*. The standard drug ampicillin (10µg/m1) showed highest activity of inhibition against *B. cereus, S. aureus, K. pneumoniae* and *E. coli* (Fig. 3).

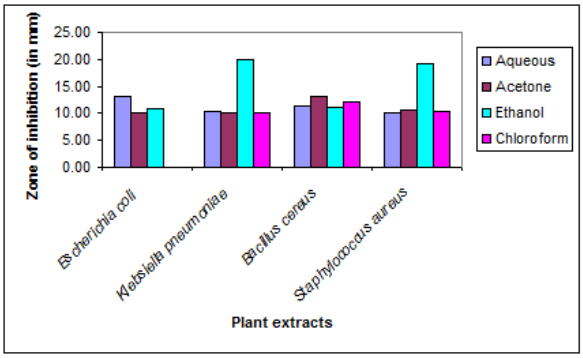

Fig. 3. Antibacterial potentiality of the leaf extract of *Argemone mexicana* L. against various pathogenic bacterial stains by agar well diffusion method.

Previous reports on *A. mexicana* leaves and seeds extracts showed considerable antibacterial activity (Bhatacharjee *et al.,* 2006; Santosh Kumar Sing *et al.,* 2009; Shyam Prasad and Dhanapal, 2010). In another research, stem and essential oil of *A. mexicana* was found to be good antimicrobial activity (Mashiar Rahman, *et al.,* 2009; Osho and Adetunji, 2010).

The phytoconstituents obtained from root, stem leaves, fruits, flowers and seeds of medicinal plants include

phenolic comapounds, essentials oils, proteins and antioxidants, together they performance as biocontrol agents (Cragg *et al.,* 1996). The inhibition activity of plants extracts against the growth of microorganisms was attributed to the presence of antioxidants (Perumal *et al.,* 2010). The result of the present work are found to be directly correlated with the observations of earlier researchers (Arshad Hussain *et al.,* 2010; Alagesaboopathi, 2011; Vijayakumar Arul Doss and Kalaichelvan, 2012; Kalaiarasan *et al.,* 2012; Garba and Okeniyi, 2012; Kahkashan Perveen *et al.,* 2012). Other details are needed to isolate and characterize the biotherapeutic potentials to evolve current antimicrobial medicines.

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