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STUDY ON ANTIBACTERIAL ACTIVITY OF AERIAL PART OF *ARGEMONE MEXICANA* LINN

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ABSTRACT
On the contrary in India, herbal drugs are an integral part of the Indian system of medicine (Ayurveda) which is an ancient and main stream system. The antimicrobial activity of aerial part of *Argemone mexicana* was investigated and it is found that ethyl acetate extract (EAE) of *Argemone mexicana* is most active. The two Gram-positive, two Gram-negative bacterial strains and yeast were used. Ethanolic and aqueous extracts of Argemone mexicana samples showed antibacterial activity against *S. aureus, B. subtilis, S. aeruginosa* and *E.coli.* From these results it may be stated that Gram-positives bacteria are more susceptible to EAEAM antibacterial activity than Gram-negatives bacteria. Antibacterial activity of EAEAM may be due to the flavonoids, aromatic acids, and its esters. Phytochemical analysis showed the presence of glycosides, tannins, alkaloids, saponins and flavonoids. Therefore results of this study indicate the ethanolic extract of aerial part of *Argemone mexicana* possesses antibacterial activity.

KEYWORDS: Antimicrobial activity, *Argemone mexicana*, Aerial part, Ethanolic extract.

INTRODUCTION
As global temperatures are rising steadily, the duration of the summer season is also increasing in many countries currently. Therefore, the optimal conditions for living of pathogenic bacteria is also increasing. Various side effects, such as food poisoning, infection, spoiled food, and more, are expected with the increase of harmful microbial activities. It is appreciable that now Union Health Ministry is working on a proposal to inculcate the Indian System of Medicine into modern medical education. Thus, antimicrobial substances are being developed very rapidly.[1]

Plants normally produce various secondary metabolites.These metabolites are polyphenols, flavonoids, terpenoids, steroids, quinones, alkaloids, polysaccharides and so on. Studies have shown that from 9% to 16% of patients with gastrointestinal disorders use alternative remedies, with the highest rates in patients with irritable bowel syndrome, often considered to have a component of functional etiology.[2]

The *Argemone mexicana* plant is commonly grown plant and used as medicinal preparation. *Argemone mexicana* belongs to Kingdom- Planate, Division- Magnoliophyta, Order- Ranunculales, Family- Papaveraceae, Genus- *Argemone*, Species- *Argemone mexicana*. It is erect prickly herb abounding throughout, in areas up to 1,500m elevation on road side and waste places.[3]

Argemone mexicana has number of health benefits like hepatoprotective activity[4], antioxidant activity[5], *in vitro* antitrichomonal activity[6], peripheral analgesic activity[7], antibacterial and antifungal activity[8], anthelmintic activity[9], *in vitro* anti-cancer activity[10], antidiabetic activity[11], and wound healing activity.[12] The whole plant is good tonic, depurative. The flowers are bitter, digestive, astringent, and stomachic. The root are useful in guinea-worm infestation, skin diseases, leprosy, pruritus.[13] In the traditional ayurvedic books it is reported that it possesses antimicrobial activity and used in the treatment of microbial infection. Hence the present study was undertaken with the aim of exploring the antimicrobial activity of *Argemone mexicana* linn.

MATERIAL AND METHODS
Plant material
The plant was identified on the basis of its vernacular name Mexican poppy and its morphological characteristics. The aerial part of identified *Argemone mexicana* linn was collected from near CDS College, Bhind, MP.

The plant was identified and authenticated by Dr. Zia ul Hasan, Prof. Botany Saifia Science College, (Barkatulla University) Bhopal (M.P.). Voucher specimen number is 317/Bot/Saifia/2012.

Preliminary screening for Antibacterial activity

The preliminary screening for antibacterial activity was carried out on the basis of procedure described by Hegazi. The method is based upon the diffusion of antibacterial principle in extract from a vertical hole into solidified seeded nutrient agar layer of a petridish to such an extent that growth of the added microorganism is prevented entirely in a circular zone.

The 20 extracts of various propolis samples were subjected to antibacterial activity by using disk plate diffusion method. For the present study, two gram positive and two gram negative organisms were selected viz. *Staphhylococcus aureus, Bacillus subtilius, Pseudomonas aerogenosa and Escherichia coli.*

Preparation of Extracts

Grange has reported that antibacterial activity of *Argemone mexicana linn* how's the maximum zone of inhibition at 200 mg/ml.[14] So 100 mg, 150 mg and 200 mg of each extract was weighed and dissolved in 1ml of dimethyl sulfoxide (DMSO) to obtain the concentration of 100 mg/ml, 150 mg/ml and 200 mg/ml. 0.1 ml of this solution was used for measurement of zone of inhibition.[15]

Preparation of Standard

50 mg of the Ampicillin was dissolved in the 100 ml of sterile water.[16]

Microorganisms used

Cultures of *Staphhylococcus aureus* (MTCC 389), *Bacillus subtilius* (MTCC 1924), *Pseudomonas aeruginosa* (MTCC2 242) and *Escherichia coli* (MTCC 40) were obtained from Microbial Type Culture Collection and Gene Bank, Chandigarh, India. The microorganisms were identified by various staining techniques and bio-chemical reactions. The microorganisms were maintained by sub-culturing and used at regular intervals in nutrient agar medium.

Preparation of standard inoculum

McFarland Constants

A chemically induced precipitation reaction can be used to approximate the turbidity of a bacterial suspension which is produced by the interaction of barium chloride with sulfuric acid.[17]

Procedure

- Ten test tubes of equal size were set up.
- 1% chemically pure sulphuric acid solution was prepared.
- 1.175 % aqueous solution of barium chloride ($BaCl_2$) was prepared.
- Slowly with constant agitation the designated amount of the two solutions were added to the tubes as given in the table to make a total of 10 ml per tube.
- The tubes were sealed and the suspended barium sulfate ($BaSO_4$) precipitate corresponds approximately to homogenous cells densities per ml.
- The McFarland standard tubes were stored in the dark at room temperatures, as they are stable for six months.

Table 1 McFarland Concentration.

Tube no.	0.5	1	2	3	4	5	6	7	8	9	10
$Bacl_2$ (ml)	0.05	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
H_2SO_4 (ml)	9.97	9.91	9.81	9.71	9.61	9.51	9.41	9.31	9.21	9.1	9.0
Cell density (1×10^8/ ml)	1.8	4	5	10	13	16	19	22	25	28	31

Table 2: Preparation of Assay Media.

Sl. No.	Ingredients	Weight (g)
1.	Beef extract	5.0
2.	Peptone	7.0
3.	Agar	23.0
4.	Distilled water	q. s. 1000 ml

8*pH 7.4 was maintained for the assay media.

The above mentioned quantities of different ingredients were accurately weighed and dissolved in appropriate amount of distilled water. The prepared media was sterilized by autoclaving at 121^0C for 15 minutes.

Procedure

The petridishes were thoroughly washed and sterilized in hot air oven at 160^0C for one hr. Inoculum was added to 30 ml of sterile nutrient agar medium and was poured into sterile petridishes for solidifying. Bores were made on the medium using sterile borer.

0.1ml of test solution was added to the respective bores, 0.1ml of the Ampicillin at a concentration of 100 µg/ 0.1 ml was taken as standard reference. A control having only DMSO in the cup was maintained in each plate.

The petridishes were kept in the refrigerator at 4^0C for 45 minutes for diffusion to take place. After diffusion, the petridishes were incubated at 37^0C for 24 hr and zones of inhibition were observed and measured using a scale.

Antibacterial activity of all the compounds was carried out against all four microorganisms. The same media was used both for subculturing and for estimating antibacterial activity. All the reading was taken in

triplicate and is reported in Standard Error Mean (± SEM). The results are tabulated in the Table No. 3.

RESULTS

Table 3: Evaluation of antibacterial activity.

Plant	Treatment	Dose (mg/ml)	Zone of inhibition(mm) (Mean±SEM)			
			Gram negative bacteria		Gram positive bacteria	
			E.coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*
Argemone mexicana	Ethyl acetate extract	100	14.3±0.34**	16.1±0.34**	15.5±0.32**	12.9±0.67**
		150	16.1±0.35**	18.9±0.67**	18.7±0.65*	16.2±0.34**
		200	19.1±0.58*	19.7±0.68**	20.3±0.59**	18.7±1.12**
	Ethanol Extract	100	10.1±0.56**	14.1±0.31*	14.2±0.36**	10.7±0.51**
		150	13.1±0.23**	15.1±0.35**	17.9±0.56**	11.9±0.59**
		200	16.1±0.33**	16.1±0.34**	23±1.11**	14.1±0.34**
	Aqueous Extract	100	9.97±0.36**	12.3±0.65*	12.2±0.23**	10.9±0.31**
		150	11.1±0.42*	13.2±0.16*	13.0±0.50*	9.75±0.59**
		200	14.1±0.52**	14.21±0.31**	16.01±0.46**	13.1±0.31*
	Standard (Ampicillin)	25µg/ml	24.3±0.31	26.1±0.21	25.1±0.32	25.7±0.19

Each value represent Mean±SEM, n=5. One-way ANOVA followed by Dunnet test through Instat software, compare all vs. standard applied. Statistically significant at **P<0.01, *P<0.05.

DISCUSSION

Argemone mexicana showed significant results (P<0.01) by applying one way ANOVA (Dunnet test). Ethyl acetate extract of *Argemone mexicana* was more effective than ethanolic extract of *Argemone mexicana* gainst *E. coli* (Gram –ve). Least active extract was ethanolic extract of *Argemone mexicana* gainst *E. coli* (Gram –ve). In case of antibacterial activity against (Gram –ve) *Pseudomonas aeruginosa*, ethyl acetate extract of *Argemone mexicana* was more active followed by ethyl acetate extract and least active was ethanolic extract. . Ethyl acetate extract of *Argemone mexicana* showed significant results against *Candida albicans* which was further followed by ethanolic extract.

CONCLUSION

The two Gram-positive, two Gram-negative bacterial strains and yeast were used. According to the results in the Table, only ethyl acetate, ethanolic and aqueous extracts of Argemone mexicana samples showed antibacterial activity against *S. aureus, B. subtilis, S.aeruginosa, E.coli, A. fumigates and C. albicans*. From these results it may be concluded that Gram-positives bacteria are more susceptible to EAEAM antibacterial activity than Gram-negatives bacteria. Antibacterial activity of EAEAM may be due to the flavonoids, aromatic acids, and its esters. The mechanism of this activity is attributed to a synergism between phenolic and other compounds in the resin. The strong antibacterial activity of Argemone mexicana may be due to high total phenolic and flavonoid contents. There are numerous questions yet to be answered concerning chemical compositions and antibacterial properties of Indian *Argemone mexicana* and further research is required for clarification.

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REFERENCES

1. New tonic for medical education. The Tribune, 2005, dated March 29.
2. Sutherland LR and Verhoef MJ- Why do patients seek a second opinion or alternative medicine Clinical Gastroenterology, 1994; 19: 194–197.
3. Chatterjee A and Pakrashi SC. The treatise of Indian medicinal plant. Publication and information directorate New Delhi, 1997; Vol-1: 139-140.
4. Das PK, Panda P, Pani SR and Sethi R- Hepatoprotective activity of plant *Argemone mexicana* (linn) against carbon tetrachloride (CCl4) induced hepatoxicity in rats: *International journal of pharmaceutical research and development*, 2009; 8(2): 002.
5. Perumal P, Sekar V, Rajesh V, Sampathkumar R. and Shuja NKH- Invitro antioxidant activity of *Argemone mexicana* roots: *International journal of pharmtech research coden (USA)*, 2010; 2(2): 1477-1482.
6. Dahab MM, Koko WS, and Osman EE- In vitro antitrichomonal activity of *Xanthium brasilicum* and *Argemone mexicana* l different extracts: *Journal of medicinal plants research*, 2011; 5(2): 151-155.
7. Sharma MC, Kohli DV and Sharma S- Pharmacological screening effect of ethanolic and methanolic extract of fruits of medicinally leaves: *Digest journal of nanomaterials and Biostructures*, 2010; 5(1): 229–232.
8. Shyam prasad G. and Dhanupal R- Antibacterial and antifungal activity of methanolic extract of

Argemone mexicana leaves: *International journal of Phytopharmacology*, 2010; 1(2): 64-67.

9. Jaliwala YA, Panda PK, Chourasia N, Bhatt NK, Pandit A. and Mohanti PK- In vitro anthelmintic activity of aerial parts of *Argemone mexicana* linn: *Journal of pharmacy research*, 2011; 4(9): 3173-3174.
10. Kiranmayi G, Ramkishan G, Kothai R and Jaykar B- In vitro anti-cancer activity of methanolic extract of leaves of *Argemone mexicana* linn: *International journal of pharm tech research*, 2011; 3(3): 1329-1333.
11. Nayak P, Kar DM and Maharana L- Antidiabetic activity of aerial parts of *Argemone mexicana* linn in alloxan induced hyperglycaemic rats: *Pharmacology online*, 2011; 1: 889-903.
12. Dash GK and Murthy PN- Evaluation of *Argemone mexicana* linn leaves for wound healing activity: Available online at www.scholarsresearchlibrary.com, *J. nat. prod. plant resource*, 2011; 1(1): 46-56.
13. Prajapati, Purohit, Kumar and Sharma. A handbook of medicinal plants a complete source book. CRC press, 1st edition, 2007; New Delhi, 59-60.
14. Grange JM, Davey RW- Antibacterial properties of propolis: *Journal of the Royal Society of Medicine*, 1990; 83: 159-160.
15. Pereira ADS, Bicalho B, De Aquino Neto FR- Composition of propolis from *Apis mellifera* and *Tetragonisca angustula*: *Apidologie*, 2003; 34: 291-8.
16. Mellion E, Stratis E, Chinon I- Volatile constituents of propolis from various regions of Greece-antimicrobial activity: *Food Chem*, 2007; 103: 375-380.
17. Bailey, Scott's. Diagonistic microbiology, St. Louis, CV. Mosby Company, 8th Edition, 1990; 171-194.
18. Shyam Prasad G, Dhanapal R- Antibacterial and antifungal activity of methanolic Extract of *argemone mexicana* leaves; *International Journal of Phytopharmacology*, 2010; 1(2): 64-67.