Report on Antimicrobial Activity and Phytochemical Screening of *Argemone Mexicana* Linn.

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Abstract

The genetic ability of pathogenic bacteria to develop resistance against commonly used antibiotics is a major medical problem and challenge worldwide, posing a big threat to human society. This has necessitated a search for novel antibacterial substances from various natural sources, including flowering plants. A wide variety of plant secondary metabolites have been identified as active principles for the treatment of various ailments. Even today plants are the almost exclusive source of drugs for the majority of the world population. People in developing countries utilize traditional medicine for their primary health care needs. The use of the plant extracts and the phytochemicals can be of great significance in therapeutic treatments and could be helpful to curb the problem of these multi-drug resistant microorganisms. The present study was conducted to evaluate the antimicrobial activity from ethanolic extract against different life threatening pathogenic microorganisms and screening for various phytochemical constituents of *Argemone mexicana* Linn. The antibacterial activity of the plant extract of *Argemone mexicana* L. was studied against gram positive and gram negative bacteria. The ethanolic extract displayed broad spectrum activity against all the test organisms. Phytochemical screening of the plant revealed the presence Tannins, Alkaloids, Flavonoids and Steroids. The results of this study support the traditional use of *Argemone mexicana* L. whole plant as an antibacterial agent.

Keywords: Medicinal plants, phytochemical constituents, *Argemone mexicana* Linn., antimicrobial activity, Phytochemical screening,

Introduction

The genetic ability of pathogenic bacteria to develop resistance against commonly used antibiotics is a major medical problem and challenge worldwide, posing a big threat to human society. Present time medicinal plants being the effective source of medicines, either it can be modern or traditional medicines, the advantage of medicines are they are useful for health. WHO had given the remark that traditional medicines are safe treatment for the infections originated from microbial and non-microbial in origin. India has a high rang of medicinal plants that are used in ancient as well as in modern pharmaceutical preparations. They have used in a preparation of drugs since centuries ago in ayuevedic, siddha and unani system. Due to the potent therapeutic value, easy avability and mode of action these medicinal plants have attended more pharmacological exploration in modern medicinal practices.

Argemone mexicana Linn belongs to family *Papaveraceae*, commonly known as Prickly Poppy in English and Premathandu in Tamil found in Mexico and now has widely naturalized in the United States, India, Bangladesh and Ethiopia. [5,8] It occurs as wasteland weed in almost every part of India. A strong branched prickly annual, 60-90 cm in height with yellow latex; leaves simple, sessile and spiny. Flowers large, bright yellow, terminal on the short leafy branches; fruits prickly capsules, oblong-ovoid, opening by 4-6 valves; seeds numerous.[4]

The plant contains alkaloids as berberine, protopine, sarguinarine, optisine, chelerytherin etc. The seed oil contains myristic, palmitic, oleic, linoleic acids etc.

2 According to Ayurveda the plant is diuretic. Purgative and destroys worms. It cures leprosy, skin-diseases, inflammations and bilious fever.

Methods and Materials

Collection and processing of plant: The plants of *Argemone mexicana* were collected from the local area and taken care for its freshness, healthy and free from any deformation. The whole plant was broke into small pieces and then blended into powder by mixture blender which then passed from the sieve to get the equal size particles. The powder should be aseptically kept in air tight container at the moisture free place.

Soxhlet extraction of plant material: For the extraction of plant material the selection of solvents is done with care to meet extrability and regulatory criteria. Depending upon the solubility of curcumin ethanol was selected for the extraction procedure, as curcumin is completely soluble in it.

100gm of powder is accurately weight and is transferred to the cup made up of 'Whateman filter paper' and placed into the extraction thimble. 500ml of ethanol was taken in round bottom flask and heated up to its boiling point, *i.e.* 65^{0}C. The ethanol gets evaporated and moved in to the condenser where it was converted in to liquid trickled in to the extraction chamber containing the plant material. The powder was extracted for 48 hrs. At the end of the extraction process, the flask containing the ethanolic extract was removed and extract was condensed at 50^{0}C in water bath for overnight. The weight of extract was measured and percentages of yield of the plant material were calculated. The extract was stored at 4^{0}C for further work. (Sahu and Padhy, 2013)

Isolation of test organisms: Pure cultures of the test organisms used for antibacterial activity were isolated from the water and soil sample by using selective media. The characterization of the test organism was done by using IMVIC test. All the test organisms were cultured on nutrient agar slant. The cultures were maintained by sub-culturing periodically and preserved at 4^{0}C prior to use. (Alzoreky, N.S. and K. Nakahara. 2003)

The gram negative bacteria includes; *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Vibrio cholerae*. While the gram positive bacteria includes; *Bacillus subtilis, Bacillus megaterium, Staphyloccus aureus,Bacillus cereus*.

Screening for antibacterial activity: All the test organisms were screened for the antibacterial activity against ethanolic extract of *Curcuma longa* by agar well diffusion method. With the introduction of variety of antimicrobials, it becomes necessary to perform the antimicrobial susceptibility test. For this the antimicrobial agent was allowed to diffuse out into the medium and interact in a plate freshly speeded with the test organism.

Stock solution of ethanolic extract of *Argemone mexicana* was prepared to carry out the antimicrobial activities against selected cultures for the further process. For the preparation of the stock solution, 1 gm of ethanolic extract was accurately weight and dissolved in 10 ml of DMSO; giving concentration of the stock solution as 100 mg/ml. this solution is then centrifuged and supernatant liquid was collected in a separate test tube, covered with paraffin wax and stored at 4^{0}C for further use.

Agar well diffusion method: The Muller-Hinton agar plates for the bacteria were prepared 0.1 ml of fresh 18 hours old broth culture was spread on the respective media. After spreading the culture, wells of 6 mm in diameter was made at the centre of the plate by using sterile cork borer. The wells were open with the help of sterile forceps. Then 100 µl of stock solution was added by using micropipette in each well.

The final concentration in the well was 10 mg/ml. The extract was allowed to diffuse; hence, the prepared plates were kept in deep fridge.

After this, plates were incubated at 37^{0}C for 24-48 hours. The zone of inhibition was measured in mm and recorded. The diameter of the zone of inhibition around each well was taken as measure of antibacterial activity. Each experiments was carried out in triplicates and mean diameter of the inhibition zone was recorded. (Rajput and Pal, 2011)

Phytochemical screening: The ethanolic extract of *Argemone mexicana* was screened for the phytochemical content by using different chemical test for each component. The ethanolic extract of the plant was used for the phytochemical test to detect the presence of alkaloids, tannins, saponins, flavonoids, cardiac, glycoside, anthraquinones and steroids according to standard method as follows. (Sawant and Godghate, 2013)

a) Alkaloids: A 5ml quantity of concentrated extract was taken into a test tube and 1 ml HCl was added the mixture was heated gently for 20 min cooled and filter, the filtrate was used for Hager's test.

b) Flavonoids: Alkaline reagent test: Extract was treated with 10 % NaOH solution; formation of intense yellow color indicates presence of Flavonoids.

c) Steroids: 1ml extract was dissolved in 10 ml of chloroform & equal volume of concentrated H_2SO_4 acid was added from the side of test tube. The upper layer turns red and H_2SO_4 layer showed yellow with green fluorescence .This indicates the presence of steroid.

d) Tannin: 4ml extract was treated with 4 ml FeCl3 formation of green color indicates that presence of condensed tannin.

e) Saponins: 5 ml extract was mixed with 20 ml of distilled water then agitated in graduated cylinder for 15 min formation of foam indicates Saponins

f) Cardial Glycosides: Plant extract treated with 2 ml glacial acetic acid containing a drop ofFeCl3 .A brown color ring indicates the presence of positive test.

g) Anthraquinones: About 0.5gram of the extract was taken into a dry test tube and 5ml of chloroform was added and shaken for 5 min. the extract was filtered and the filtrate shaken with equal volume of 100% ammonia solution. A pink violet or red color in the ammonical layer indicates the presence of free anthraquinones.

Results and Discussion

Table No.1: Phytochemical screening of ethanolic extract *Argemone mexicana* plant

Sr. No.	Phytochemical constituents	Ethanolic extract
1	Alkaloids	+
2	Saponins	-
3	Tannins	+
4	Steroids	+
5	Flavonoids	+
6	Anthraquinones	-
7	Glycosides	-

Table no.1 shows phytochemical screening of ethanolic extract of *Argemone mexicana* Linn. The presence of different phytoconstituents was determined by using various tests like Mayer's test, Killer Killani test, Salkowski test, Bortrager's test, Alkaline test and Potassium hydroxide test for Alkaloid, Glycosides, Steroids, Saponins, Anthraquinones, Flavanoids and Tannins respectively. The ethanolic extract was found to contain Tannins, Alkaloids, Flavonoids and Steroids.

Table no.2: Antimicrobial activity of ethanolic extract of *Argemone Mexicana* against gram-negative bacteria

Sr. No.	Test Organism	Zone of Inhibition (mm in diameter)
1	*Pseudomonas aerogenosa*	9.8 mm
2	*Escherichia coli*	14.9 mm
3	*Salmonella typhi*	16.4 mm
4	*Vibrio cholerae*	15.1 mm

Table no.2 shows agar well diffusion method for demonstration of antimicrobial activity of ethanolic extract of *Argemone mexicana* against gram-negative bacteria. The zone inhibition around the well observed for gram-negative bacteria varies from 9mm-16mm in diameter with highest for *Salmonella typhi* at 16.4 mm and lowest for *Pseudomonas aerogenosa* at 9.8 mm. (Fig. no.1) Results show that bacteria are sensitive to ethanolic extract of plant.

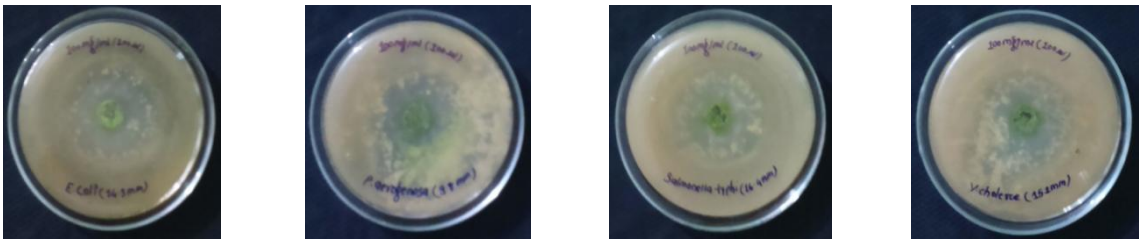

Figure no.1:- Zone of inhibition against gram negative bacteria

Table no.3: Antimicrobial Activity of Ethanolic Extract of *Argemone Mexicana* Against Gram Positive Bacteria

Sr. No.	Test Organism	Zone of Inhibition (mm in diameter)
1	*Bacillus megaterium*	15.2 mm
2	*Bacillus subtilis*	15.6 mm
3	*Bacillus cereus*	13.8 mm
4	*Staphyloccus aureus*	12.4 mm

Table no.3 shows agar well diffusion method for demonstration of antimicrobial activity of ethanolic of from *Argemone mexicana* against gram-positive bacteria. The zone of inhibition around the well observed for gram-positive bacteria varies from 12mm-15mm in diameter with highest for *Bacillus subtilis* at 15.6 mm and lowest for *Staphyloccus aureus* at 12.4mm. (Fig. no.2)

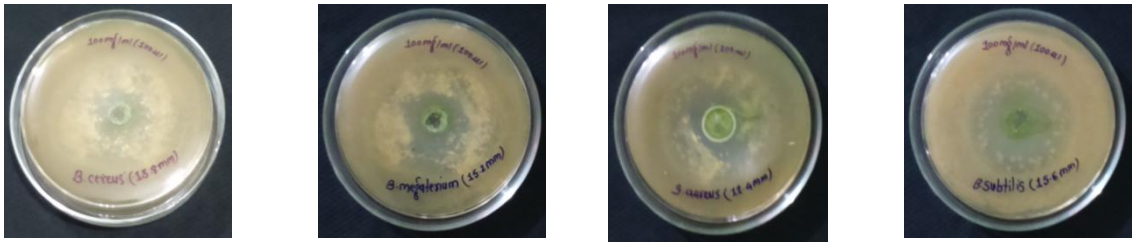

Figure no.2:- Zone of inhibition against gram positive bacteria

In current study the result showed that *Argemone mexicana* sample collected from local region of Pusad city, demonstrated antimicrobial activity against different bacterial pathogens. The ability of plant extract to inhibit the growth of all the tested organisms indicates its broad spectrum of activity. (Figure no. 2 and 3) Gram-negative bacteria were found to be more sensitive towards ethanolic extract of plant than that of gram-positive bacteria. Herbal medicines are valuable and readily available resources for primary health care and complementary health care system. These plants may prove to be antimicrobial activities, but more pharmacological investigations are necessary. Present time the emergence of multi-drug resistance in human and animal pathogenic microbes as well as undesirable side effects of certain antibiotics has triggered immense interest in the search for new antimicrobial drug of plant origin.

In similar studies Bais et al in 2013 found that methanolic extract of plant leaf shows inhibitory action against *Candida albicans* (24 mm), *Candida tropicalis* (20 mm), *Aspergilus niger* (22 mm) *and Aspergilus flavus* (18 mm) while it shows poor inhibitory effects against bacteria. Jain et al in 2012 revealed that methanolic extract of *Argemone Mexicana* fruits shows antimicrobial activity against *Streptococcus aureus* (7.5mm), *Bacillus subtilis* (7.0mm), *Pseudomonas aeroginosa* (10.0mm) and *E.coli* (9.5mm). Veni and Pushpanathan in 2014 repoted that ethanolic extrac of plant stem shows antibacterial activity against *E.coli* (13.20mm), *K.pneumoniae* (20.68mm), *S.aureus* (18.30mm), *S.typhi* (14.43mm), *B.subtilis* (13.56mm) and *P.aeuroginosa* (13.00mm). Singh et al in 2009 reported that chloroform extract of seeds at concentration of 50mg/ml shows effective inhibition activity against *E.coli* (25mm), *S.typhi* (21mm), *S.aureus* (27mm). Gomare and Ghuget in 2012 found that ethanol extract of leaves shows antibacterial activity against *S.aureus* (15mm), *B.subtilis* (15.3mm) and *P.aeuroginosa* (15.2mm). Apurba et al in 2012 found the antimicrobial activity against *S.aureus* (12mm), *Shigella desenteriae* (10.2mm) and *Candida albicans* (11.1 mm) by ethyl acetate extract of plant. Wadikar and Kadam in 2014 reported the antifungal activity of leaf extract against root rot fungi *Macrophomina phaseolina* and *Sclerotium rolfsii*.

In current investigation Tannins, alkaloids, flavonoids and steroids are found to be present in ethanolic extract of plant. Veni and Pushpanathan in 2014 investigated that phytochemical analysis of methanol, chloroform, petroleum ether, acetone, ethanol and aqueous extracts of *A.mexicana* was found that tannins, sterols/terpenes and alkaloids were present in all the 6 types of extract. The results of other tests for reducing sugar, anthraquinone, flavonoids, saponins, resins and glycosides. Singh et al in 2009 revealed the presence of flavonoids,

saponins, glycosides and alkaloids from the methanolic extract of seeds. Apurba et al in 2012 reported the presence of anthraquinone, flavonoids, saponins, steroids and alkaloids from ethyl acetate extract of leaves.

Conclusion

In vitro evaluation of plants for antimicrobial property is the first step towards achieving the goal for developing eco-friendly management of infectious diseases of humans by search for new bio-molecules of plant origin. Considering these, plant *Argemone mexicana*, screened in vitro for antibacterial as well as antifungal activity against eleven human pathogenic bacteria and yeast strain known to cause diseases in humans. The plant was selected based on traditional medicine knowledge. Based on zone of inhibition, the result of the present investigation revealed that *Argemone mexicana* organic extracts may act as an alternative to synthetic bactericides which might have significant applications in pharmaceutical or other industries for controlling pathogenic bacteria. The pharmacological activities showed by the extracts of *Argemone mexicana* leaves may be due to the presence of different chemical compounds, which works through the specific and non-specific mechanisms. However, extensive studies are needed to evaluate the precise mechanisms, active principles, and the safety profile of the plant as a remedy for different disease conditions.

Acknowledgment

Author acknowledges the valuable help rendered by Dr. S. I. Kamble, Assistant Professor, Department of Botany and Mr. P.R. Dahake, Lecturer Department of Seed Technology, Phulsing Naik Mahavidyalaya Pusad for the authentication of plant sample and in microbial studies.

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