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Asian Journal of Plant Science and Research, 2011, 1 (2):43-48

ISSN : 2249 – 7412

A study of extract optimization and effect of metal ions on antibacterial properties of *Argemone mexicana*

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ABSTRACT

Argemone mexicana has influenced culture aesthetically, economically, medically & spiritually. Ethanolic and Methanolic extract of Antibacterial activity of leaf & fruit (Seed) of Argemone mexicana was evaluated against some pathogenic bacteria- Pseudomonas aeruginosa & E.coli (Gram negative) and one was Staphylococcus aureus (Gram positive). Ethanolic extract of Argemone mexicana was showing best result in seed sample with MIC value 0.00023 mg/ml. After addition of some metal ions (zinc, calcium, iron, magnesium & lead), the antibacterial activity was increased in sample along with zinc ion (10%) which was shown that some metal ion can increase the antibacterial property.

Key words: *Argemone mexicana,* leaf, fruit (seed), antibacterial activity, ethanolic & methanolic extracts, metal ions.

INTRODUCTION

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 origin[1].Some antibiotics do not have capability to treat diseases because of drug resistance capacity of pathogens [2]. The uses of herbal treatment is one of the possible way to treat diseases caused by multi drug resistant bacteria .Though Many Pharmaceuticals industries have produced a number of antibiotics from several years but in many cases it was observed that the cultures were showing resistance against the medicines [3]. The use of plant extract with their antibacterial properties is a major work which was done from last few years and become major work in therapeutic treatment [4]. To prove efficiency the plant extract used as a drugs against different types of pathogens[5-10]. According to WHO the best source of medicines are medicinal plants, therefore such plant should be studied and evaluated

properly to check there structural and functional properties as well as the particular activity of each parts of the plants [11]. Alkaloids & flavinoids have been used as antiviral, antibacterial, antiamoebial & anticancer agents. Phenolic and polyphenolic are the other group of secondary metabolites [12]. These compounds have antimicrobial activities against microorganisms. The growth of the multi drug resistant *Staphylococcus aureus* was inhibited by the extracts of leaves and seeds of *Argemone Mexicana, this* possess the analgesic, narcotic, sedative and antispasmodic properties. The fresh yellow milky seed extract contain protein dissolving substance. Which are effective in the treatment of warts, skin disease, coldsore, dropsy and also in jaundice. Soil is a major source of metal ions. Chemical elements present in the form of free ions are readily ionized and ultimately get completely absorbed. Metal ions can disrupt normal cell and tissue function through many pathways such as interaction with proteins and other biomolecules. There is only a small chance that the ion will combine with biomolecules to cause cytotoxicity, allergy and other biological influence. They can disturb important biochemical activity of plants and animals. Plants & animals absorb these elements from soil and water.

The Present study has been designed to determine the role of seed and leaf extract (Ethanolic and Methanolic extract) of *A.mexicana* for potential antibacterial activity against *Staphylococcus aureus* (Gram Positive) MTCC 2940 and *Pseudomons aeruginosa (*MTCC 2453*), Escheriachia coli* (MTCC 739), both were gram negative and also to check the activity of seed and leaf extract in the presence of metal ions.

MATERIALS AND METHODS

Leaves & seeds of *Argimone mexicana* were used as a plant sample, which were collected from residential place of Gomti Nagar, Lucknow, U.P. during spring (mid March to April, 2011). The leaves and seeds were initially rinsed with distilled water and dried on a paper towel in laboratory for 24 hours.

Preparation of Plant extract:
During the experiment, Ethanolic and Methanolic extracts of plant samples were done.

Ethanolic and Metanolic extract: After drying, the plant materials grinded with the help of mortar and pestle in the laboratory to get the powder form, then mixed with 70% ethanol and 80% Methanol in 2:15 ratio (2 gram sample grinded in 15 ml). Exposure to sunlight was avoided to prevent the loss of active compounds, the mixture were stored in sealed beaker under the dark room at room temperature for 2-days. Protected from sunlight and mixed the sample many times with the help of glass rod, then filter with whattman filter paper to get semi-solid. The semi-solid plant extract mixed with tris-buffer (pH-8.0). The plant extract kept in freezer at 4°C for further use.

Test Microorganisms:
Three bacterial strains were used during the experiment *i.e Staphylococcus aureus* (MTCC 2940) a gram positive and *Pseudomons aeruginosa* (MTCC 2453), *Escheriachia coli* (MTCC 739), both were gram negative. All the tested bacterial strains were reference strains which were taken from the microbiology laboratory of MRD LifeSciences. All the three bacterial strains were grown on nutrient broth at 37°C and maintained a nutrient agar slants at 4°C.

Screening of Bioactive compounds:
The assay was conducted by agar well diffusion method .The nutrient agar plates were prepared, then spread 50µl of two gram negative & one gram positive broth culture on nutrient agar plates. The suspension was used to inoculate 90 mm diameter Petri plates. Wells were punched in triangular form and filled with 50µl distilled water, 50µl antibiotic (tetracycline) and 50µl plant extract sample respectively. Plates were incubated at 37^0C for 24 hours. Antibacterial activity was evaluated by measuring the zone of inhibition in diameter. Distilled water was taken as a negative control for both ethanolic and methanolic extracts.

Minimum Inhibitory Concentration (MIC):
Quantitative assay was done by agar dilution method which was used to determine MIC of extract against test bacteria. The minimum inhibitory concentration is the minimum concentration of the antibacterial agent in a given culture medium below which bacterial growth is not inhibited. MIC provides an idea of effectiveness an active extract or compound against a microorganism. MIC means the lowest concentration of extract at which the test microorganism did not show any visible growth was taken as its MIC. During study of MIC 3ml nutrient broth was taken in 6 test tubes [13]. Then added 1.0 ml plant extract into first test tube and made a serial dilution, then 20µl of pathogen were added into each test tube & incubate at 120 rpm in shaker incubator for overnight. Next day least concentration of MIC test tubes was observed by taking the optical density (OD) at 600 nm by using the colorimeter.

Effect of metal ions on activity of compounds:
Antibacterial activity of plant extract was done by agar well diffusion method [14]. prepared 10% of 5ml metal ion solution (5ml distilled + 0.5gm metal ion). zinc,calcium,lead, iron and magnesium as metal ions were used.. addition of metal ions along with plant extract to know their effect. Prepared NA plates then three wells were punched on 90mm diameter petriplate and filled with 50µl autoclaved distilled water, 50µl plant extract and plant extract+ 10% metal ion (45µl + 5µlrespectively). Incubated at 37°C for overnight.

RESULTS

Antimicrobial sensitivity assay of different extract: – the study of antimicrobial activity of leaves and fruit of *Argemone mexicana* against *E.coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* had shown that the seed extract were more effective than those of the leaf extracts. The antibacterial activity of ethanolic extract of *Argemone mexicana* (leaf and seeds) had shown the more efficacy than the methanolic extract.

The ethanolic extract of *Argemone mexicana* (leaf and seed) had shown maximum activity against *Staphylococcus aureus*. Methanolic extract of *Argemone mexicana* showed highest efficacy against *Staphylococcus aureus* followed by *Pseudomonas aeruginosa* and *E.coli*.

The minimum inhibitory concentration (MIC) of plant extract against test bacteria varied from 0.25 to 0.00023 mg/ml. the lowest MIC 0.00023 mg/ml with of inhibition 10 mm, 15 mm, 15 mm were observed in *Staphylococcus aureus, E.coli* and *Pseudomonas aeruginosa* respectively. The highest MIC 0.25 mg/ml with zone of inhibition 15mm was recorded in *Staphylococcus aureus*. It was interesting to observe that the extract was effective against strain which were

sensitive to antibiotic tetracycline and also MIC values were same for sensitive strain. MIC provides an idea of effectiveness of an active extract for a compound against a microorganism.

The best result of metal ion was shown with the ethanolic plant material leaf and seed against *Staphylococcus aureus*. Plant extract of *Argemone mexicana* (leaf, seed) with the metal ions zinc, lead and iron was shown the best results against *Staphylococcus aureus* along with metal ions. (Table 1).

Table 1: Sensitivity of different bacterial strains to the various extracts of leaf of *Argemone Mexicana*

Bacterial species	Methanolic extract	Ethanolic extract
E.coli	-	+
Pseudomonas aeruginosa	-	+
Staphylococcus aureus	+	+

- = no inhibition; + = inhibition zone > 10 mm

Table 2: MIC value of ethanolic plant extract leaf, fruit (seed) against *Staphylococcus aureus*

Test tubes	Ethanolic leaf extract (OD) at 600nm	Ethanolic seed extract (OD) at 600nm	Concentration (mg/ml)
1	0.05	0.07	0.25
2	0.21	0.18	0.06
3	0.50	0.47	0.015
4	**0.09**	0.33	**0.00375**
5	0.30	0.26	0.00094
6	0.24	**0.19**	**0.00023**

Table 3: Antibacterial activity of ethanolic plant extract along with 10% metal ion against *Staphylococcus aureus*

Pathogen	10% metal ion along with leaf extract (ZOI in mm).	Leaf extract. (ZOI in mm)	10% metal ion along with seed extract (ZOI in mm).	Seed extract. (ZOI in mm).
Staphylococcus	**Zn**-25	18	**Zn**-29	20
Staphylococcus	**Pb**-25	21	**Pb**-30	18
Staphylococcus	**Fe**-15	25	**Fe**-19	18

ZOI = Zone of Inhibition in mm

Table 4: Antibacterial activity of methanolic plant extract along with 10% metal ion against *Staphylococcus aureus*

Pathogen	10% metal ion along with leaf extract (ZOI in mm)	Leaf extract. (ZOI in mm)	10% metal ion along with seed extract (ZOI in mm)	Seed extract (ZOI in mm)
Staphylococcus	**Zn**-14	12	**Zn**-15	15
Staphylococcus	**Pb**-16	14	**Pb**-14	13
Staphylococcus	**Fe**-15	13	**Fe**-15	15

ZOI = Zone of Inhibition in mm

Table showing the result of MIC in Ethanolic leaf extract 0.00375mg/ml and in Ethanolic seed extract 0.00023 mg/ml. These values showed that the metal ions can increase the antibacterial activity of plant sample.

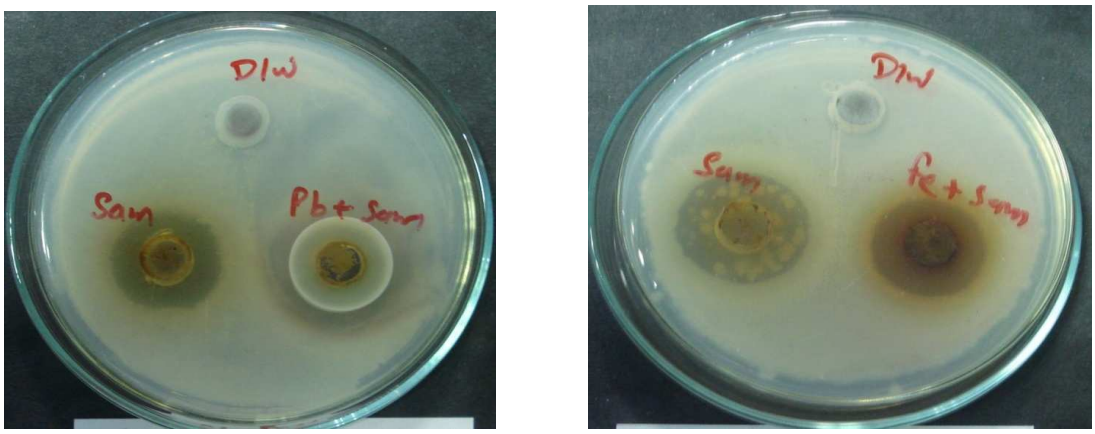

Fig 1: Effect of metal ion on ethanolic extract of leaves with the presence of lead and iron against *Staphylococcus aureus*

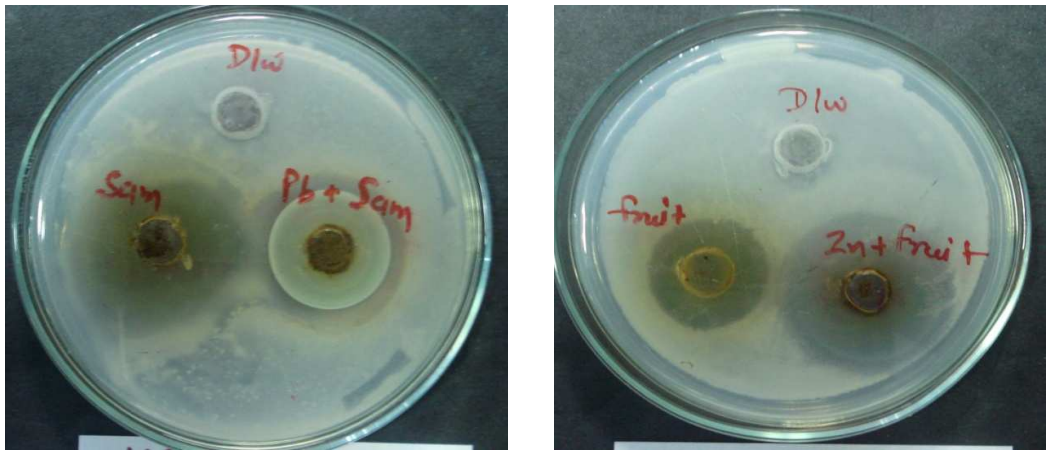

Fig 2: Effect of metal ion on ethanolic extract of seed with the presence of lead and zinc against *Pseudomonas aeruginosa*

DISCUSSION

The extraction of biologically active compounds from the plant material depends on the type of solvent used in the extraction procedure. The most commonly used solvents for investigations of antimicrobial activity in plants are methanol and ethanol [15-18].Most of the antimicrobial active compounds that have been identified were soluble in polar solvents such as methanol and ethanol instead of water [19][20]. The ethanolic extracts of the leaves and seeds of the *A. mexicana* showed greater antibacterial activity than the corresponding water extracts. This finding is interesting, because in the traditional method of treating a bacterial infection, decoction of the plant parts or boiling the plant in water is employed. Whereas, according to present study, preparing an extract with an organic solvent was shown to provide a better antibacterial activity, in accordance with the results obtained by Nair *et al*. The MIC value of Ethanolic extract of *A. mexicana* seeds were showing least concentration 0.00023 mg/ml which were very less compare to work done by Nair *et al*. [21]**.**These observations may be attributed to two reasons: firstly, the nature of biological active components whose activity can be enhanced in the presence of methanol and secondly, the stronger extraction capacity of methanol could have produced greater

number of active constituents responsible for antibacterial activity. Metal ion effect showed that some metal ions increase the antibacterial activity of *Argimone mexicana*. During the study, zinc and lead were the most effective ions which increased the antibacterial activity of ethanolic extract and showed best results.

Acknowledgement
I wish to express my immense gratitude to Mr. Manoj Verma, Director, MRD LifeSciences (P) Limited, Lucknow. I am very grateful and my heartiest thanks to Mr. R.P. Mishra (Research Scientist), Mr. Jahir Alam Khan (Research Scientist) & Ms. Chanda Sinha (Research Scientist), MRDLS, Lucknow, for there kind support throughout the research work. I am also thankful to the almighty without whose blessings nothing is possible.

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