# In Vitro Antibacterial Activity of *Argemone mexicana* L. (Papaveraceae)

**M. Mashiar Rahman[1], Md. Jahangir Alam[1], Shamima Akhtar Sharmin[2], M. Mizanur Rahman[1], Atiqur Rahman[3,4] and M. F. Alam[2*]**

[1]*Department of Biotechnology and Genetic Engineering, Islamic University, Kushtia 7003, Bangladesh*

[2]*Biotechnology and Microbiology Laboratory, Department of Botany, University of Rajshahi, Rajshahi 6205, Bangladesh*

[3]*Department of Biotechnology, Daegu University, Kyoungsan, Kyoungbook 712-714, Korea*

[4]*Department of Applied Chemistry and Chemical Technology, Islamic University, Kushtia 7003, Bangladesh*

**Corresponding author. Email: falambt@yahoo.com*

## ABSTRACT

***The aim of this study was to examine the efficacy of various extracts from stems of Argemone mexicana L. as antibacterial potential against a range of food-borne bacteria. The antibacterial activity of various extracts (hexane, chloroform, ethyl acetate and ethanol) of A. mexicana L. stems was determined in vitro, using agar diffusion method and MIC determination test against ten (five Gram positive and five Gram negative) food-borne pathogenic bacteria such as Staphylococcus aureus, Bacillus subtilis, Listeria monocytogenes, Clostridium botulinum, Clostridium perfringens, Escherichia coli 0157, Pseudomonas aeruginosa, Salmonella typhimurium, Campylobacter jejuni and Vibrio cholerae. The organic extracts exhibited potent antibacterial effect against B. subtilis, S. aureus, L. monocytogenes, C. Botulinum, C. perfringens, E. coli, P. aeruginosa and S. typhimurium at the concentration of 10 µl (corresponding to 300 µg/disc) of extracts. The zones of inhibition against the tested bacteria were found in the range of 10.1 to 21.4 mm, along with their respective MIC values ranging from 62.5-500 µg/ml. This study suggests that natural products derived from A. mexicana L. may contribute to the development of new antimicrobial agents.***

**Key words:** *Argemone mexicana* L., Organic extracts, Food-borne pathogens, Antibacterial activity, MIC

## INTRODUCTION

Food-borne diseases caused by the consumption of contaminated foods have a wide economic and public health impact worldwide (Mead et al., 1999). Many pathogenic microorganisms such as *Listeria monocytogenes, Staphy-*

*lococcus aureus, Bacillus subtilis, Escherichia coli, Salmonella* spp. and *Pseudomonas aeruginosa* have been reported as the causal agents of food-borne diseases (McCabe-Sellers and Samuel, 2004). A variety of different chemical and synthetic compounds have been used as antimicrobial agents to inhibit bacteria in foods. With the increase of bacterial resistance to antibiotics, there is considerable interest to investigate the antimicrobial effects of different plant extracts against a range of bacteria and to develop other classes of natural antimicrobial agents useful for the infection control (Bakri and Douglas, 2005). The Gram positive bacterium, *S. aureus* is mainly responsible for post-operative wound infections, toxic shock syndrome, endocarditis, osteomyelitis and food poisoning (Mylotte et al., 1987). *L. monocytogenes* is responsible for the severe food-borne illness, listeriosis, which has been recognized to be one of the emerging zoonotic diseases during the last two decades (Kulshrestha, 1990; Farber, 2000). The Gram negative bacterium, *E. coli*, is present in human intestines and causes urinary tract infection, coleocystitis or septicemia (Singh et al., 2000). There is, therefore, still a need for new methods of reducing or eliminating microorganisms to ensure public health. In recent years, a number of publications documented the antimicrobial activities of plant extracts (Nasar-Abbas and Halkman, 2004; Shin et al., 2004). Thus, plant extracts are promising natural antimicrobial agents with potential applications in pharmaceutical or food industries for controlling the pathogenic bacteria.

*Argemone mexicana* L. is a species of poppy found in Mexico and now has widely naturalized in the United States, Ethiopia, India and Bangladesh. In Mexico, the seeds are considered as an antidote to snake venom. 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 warts, cold sores, cutaneous infections, skin diseases, itches and also dropsy and jaundice (Chopra et al., 1986). *A. mexicana* L. is widely distributed throughout Bangladesh. The whole plant is analgesic, antispasmodic, possibly hallucinogenic and sedative (Emboden, 1979; Chevallier, 1996). It contains alkaloids similar to those in the opium poppy (*Papaver somniferum*) and so can be used as a mild pain-killer (Chevallier, 1996). Owing to its various ethnopharmacological properties, the present investigation was undertaken to evaluate the antibacterial potential of *A. mexicana* L. stem extracts against a range of food-borne pathogenic bacteria with the possible use as a natural antimicrobial agent in pharmaceutical or food industries.

## MATERIALS AND METHODS

### Plant Material

The stems of *A. mexicana* L. were collected from Kushtia area of Bangladesh in January 2006. The taxonomic identities of this plant were determined by Professor A. H. M. Mahbubur Rahman, Department of Botany, University of Rajshahi, Rajshahi 6205, Bangladesh. After collection, plant parts were cleaned with running tap water, cut into small pieces and kept under shade until drying. After proper drying, plant material was pulverized into powdered form by a grinding machine.

**Preparation of Extracts**

The air-dried powdered material (50 g) of *A. mexicana* L. was extracted with 200 ml each of hexane, chloroform, ethyl acetate and ethanol separately at room temperature, and the solvents from the combined extracts were evaporated by vacuum rotary evaporator (EYELA N-1000, Japan). The extraction process yielded in hexane (4.3 g), chloroform (6.4 g), ethyl acetate (5.4 g) and ethanol (6.3 g) extracts.

**Microorganisms**

In all, ten food-borne pathogenic bacteria including five Gram positive bacteria: *S. aureus, B. subtilis, L. monocytogenes, C. botulinum* and *C. perfringens* and five Gram negative bacteria: *E. coli* 0157, *P. aeruginosa, S. typhimurium, C. jejuni* and *V. cholerae* were used as test microorganisms. The strains were obtained from Microbiology and Biotechnology laboratory, Department of Botany, University of Rajshahi, Rajshahi 6205, Bangladesh. Cultures of each bacterial strain were maintained on Luria agar (LA) medium (Acumedia Manufacturers, Inc. Lansing, Michigan, USA) at 4°C

**Antibacterial Assay**

The agar diffusion method was used for antibacterial assay (Murray et al., 1995). Petri plates were prepared by pouring 20 ml of Luria broth (LB) medium and allowed to solidify. Plates were dried and 1 ml of standardized inoculum suspension was poured and uniformly spread. The excess inoculum was drained away and the inoculum was allowed to dry for 5 min. A Whatman No. 1 sterile filter paper disc (6 mm diameter) was impregnated with 10 µl of 30 mg/ml (300 µg/disc) of *A. mexicana* L. stem extracts of hexane, chloroform, ethyl acetate and ethanol. Negative controls were prepared using the same solvent employed to dissolve the samples. Standard reference antibiotics, streptomycin and tetracycline (10 µg/disc for each), were used as positive controls for the tested bacteria. Antibacterial activity was evaluated by measuring the diameter of the zones of inhibition against the tested bacteria. Each assay in this experiment was replicated three times.

**Determination of Minimum Inhibitory Concentrations (MICs)**

Minimum inhibitory concentrations (MICs) of various extracts of hexane, chloroform, ethyl acetate and ethanol were tested by a two-fold serial dilution method (Chandrasekaran and Venkatesalu, 2004). The test extracts were incorporated into Luria broth (LB) medium to get a concentration of 2,000 µg/ml and further, serially diluted to achieve 1,000, 500, 250, 125, 62.5 and 31.25 µg/ml. A 10 µl of standardized suspension of each tested organism ($10^6$-$10^8$ cfu/ml) was transferred to each tube. The control tubes, containing only bacterial suspension, were incubated at 37°C for 24 h. The lowest concentrations of the test samples which did not show any growth of test organisms after macroscopic evaluation were determined as MICs and were expressed in µg/ml.

## RESULTS

### Antibacterial Activity Assay

The *in vitro* antibacterial activities of various stem extracts of *A. mexicana* L. against the employed bacteria were qualitatively and quantitatively assessed by the presence or absence of inhibition zones. According to the results given in Table 1, in all, ten food- spoiling and food-borne pathogenic bacteria (five Gram positive and five Gram negative bacteria) were tested to evaluate the antibacterial potential of various stem extracts. As shown in Table 1, the chloroform, ethyl acetate and ethanol extracts significantly inhibited the growth of most of the bacteria tested whereas hexane extract showed moderate antibacterial activity in most of the cases. The chloroform, ethyl acetate and ethanol extracts exhibited strong antibacterial effects against all five Gram positive bacteria (*B. subtilis, S. aureus, L. monocytogenes, C. botulinum*, and *C. perfringens*) and three Gram negative bacteria (*E. coli, P. aeruginosa* and *S. typhimurium*) with their respective diameters of inhibition zones of 12.2 to 21.4 mm. However, no antibacterial activity was observed against two Gram negative bacteria: *C. jejuni* and *V. cholerae*, at the used concentration 10 µl (corresponding to 300 µg/disc) of plant extracts. In this study, in some cases, the polar extracts exhibited significantly higher antibacterial activity than that of standard streptomycin as regard to Gram positive bacteria while tetracycline showed higher antibacterial effect, in some other cases, than those of various extracts (Table 1).

**Table 1.** Antibacterial activity of organic extracts derived from the stems of *A. mexicana L.*

| Microorganism | Zone of inhibition | | | | | |
|---|---|---|---|---|---|---|
| | Organic extracts[a] | | | Standard[b] | | |
| | Hexane | $CHCl_3$ | EtOAc | EtOH | SM | TC |
| *S. aureus* | 14.3±0.7 | 20.2±1.2 | 16.2±1.5 | 20.2±1.1 | 14.3±0.7 | 19.3±0.7 |
| *L. monocytogenes* | 13.5±1.0 | 15.1±1.2 | 15.1±1.2 | 18.3±1.6 | 16.2±1.2 | 18.4±0.5 |
| *C. botulinum* | 12.2±0.5 | 17.1±1.2 | 15.3±1.2 | 18.2±1.5 | 14.1±0.5 | 18.1±0.6 |
| *C. perfringens,* | 11.3±1.4 | 14.0±1.2 | 14.3±1.6 | 16.2±1.1 | 15.3±0.6 | 17.3±1.6 |
| *B. subtilis* | 14.2±0.8 | 17.3±1.2 | 15.2±0.6 | 21.4±0.7 | 14.3±0.6 | 18.3±0.5 |
| *E. coli* 0157 | nd | 12.2±1.5 | 12.2±0.6 | 14.3±1.2 | 24.0±0.7 | 17.3±1.2 |
| *P. aeruginosa* | 10.1±0.6 | 15.0±1.1 | 14.2±1.1 | 15.1±1.4 | 19.0±0.5 | 20.3±1.2 |
| *S. typhimurium* | 10.3±0.7 | 13.2±0.9 | 12.2±0.6 | 13.4±0.7 | 14.0±0.5 | 20.1±1.0 |
| *C. jejuni* | nd | nd | nd | nd | 15.4±1.3 | 18.1±0.5 |
| *V. cholerae* | nd | nd | nd | nd | 15.2±0.8 | 22.1±1.2 |

[a]Diameter of inhibition zones of different organic extracts including diameter of disc 6 mm (tested volume 300 µg/disc).

[b]Standard antibiotics – SM: Streptomycin, TC: Tetracycline (10 µg/disc); nd: not detected.

Values are given as mean ± S.D. of triplicate experiment.

**Minimum Inhibitory Concentrations (MICs)**

The minimum inhibitory concentrations (MICs) defined as the lowest concentrations of various extracts of hexane, chloroform, ethyl acetate and ethanol that resulted in complete growth inhibition of the tested pathogens were found in the range of 62.5 to 500 µg/ml (Table 2). The polar extracts displayed significantly remarkable antibacterial activity against all five Gram positive bacteria, *B. subtilis, S. aureus, L. monocytogenes, C. botulinum* and *C. perfringens* and one Gram negative bacterium *P. aeruginosa*, with their MIC values ranging from 62.5 to 250 µg/ml. Chloroform and ethanol extracts showed higher antibacterial activity by having lower minimum inhibitory concentration values than hexane and ethyl acetate extracts. In this study, the Gram positive bacteria were found to be more susceptible to plant extracts than Gram negative bacteria (Table 2).

**Table 2.** Minimum inhibitory concentration of organic extracts derived from the stems of *A. mexicana L.*

| Microorganism | Minimum inhibitory concentration (MIC)[a] | | | |
|---|---|---|---|---|
| | Organic extracts[b] | | | |
| | Hexane | $CHCl_3$ | EtOAc | EtOH |
| *S. aureus* | 250 | 62.5 | 125 | 62.5 |
| *L. monocytogenes* | 250 | 125 | 125 | 125 |
| *C. botulinum* | 250 | 125 | 125 | 62.5 |
| *C. perfringens* | 500 | 250 | 250 | 250 |
| *B. subtilis* | 125 | 62.5 | 125 | 62.5 |
| *E. coli* 0157 | nd[b] | 250 | 500 | 250 |
| *P. aeruginosa* | 500 | 250 | 125 | 250 |
| *S. typhimurium* | nd | 500 | 500 | 500 |
| *C. jejuni* | nd | nd | nd | nd |
| *V. cholerae* | nd | nd | nd | nd |

[a]Minimum inhibitory concentration (MIC); [b]MIC of different organic extracts (values in µg/ml). nd: not detected.

## DISCUSSION

Plants have been model source of medicines as they are a reservoir of chemical agents with therapeutic properties. It provided a good source of anti-infective agents, for example emetine, quinine and berberine which still remain to be highly effective instruments in the fight against microbial infections. Historically, many plant extracts have been reported to have antimicrobial properties (Hoffman, 1987). Also, the renewal of interest in the food or pharmaceutical industry, and increasing consumers' demand for effective and safe natural products means that quantitative data on plant extracts are required. Various publications have documented the antimicrobial activity of plant extracts (Morris et al., 1979; Nasar-

Abbas and Halkman, 2004; Rahman et al., 2004). The results of the antibacterial screening showed that various stem extracts of hexane, chloroform, ethyl acetate and ethanol have potential antibacterial effects against most of the representative food-borne pathogens: *B. subtilis, S. aureus, L. monocytogenes, C. botulinum, C. perfringens, E. coli, P. aeruginosa* and *S. typhimurium*. This activity could be attributed to the presence of some bioactive compounds such as alkaloids, phenolics and fatty acids in *A. mexicana* L. plant and these finding are in agreement with the previous reports (Gunstone et al., 1977; Harborne and Williams, 1983; Hussain et al., 1983; Nakkady and Shamma, 1988; Chang et al., 2003). In recent years, several researchers have reported that the alkaloids, phenolics, triterpenoids, glycosides, tannins, etc. are the major bioactive molecules from plant origins which have enormous potential to inhibit microbial pathogens (Hutchings et al., 1996; Karamanoli, 2002). When considering the data obtained in different studies, most publications simply provide generalization about whether or not a plant extract possesses activity against Gram positive and Gram negative bacteria. Only few provide details about the extent or spectrum of this activity. Deans et al. (1995) observed that the susceptibility of Gram positive and Gram negative bacteria to plant-based antimicrobials had little influence on growth inhibition. It is often reported that Gram negative bacteria are more resistant to the plant-based organic extracts (Reynolds, 1996) because the hydrophilic cell wall structure of Gram negative bacteria is constituted essentially of a lipo-polysaccharide (LPS) that blocks the penetration of hydrophobic oil and avoids the accumulation of organic extracts in target cell membrane (Bezic et al., 2003). This is the reason why Gram positive bacteria were found to be more sensitive to various extracts derived from *A. mexicana* L. than were Gram negative bacteria. Moreover, the organic solvents used have varying abilities to extract bioactive substances from *A. mexicana*. These observations may be attributed to two reasons: firstly, the nature of biological active components whose activity can be enhanced in the presence of type of organic solvent; secondly, the stronger extraction capacity of organic solvent could have produced greater number of active constituents responsible for antibacterial activity (Bhattacharjee et al., 2006). In the present study, we also observed that the extracts severely inhibited the growth of all Gram positive bacteria and also of Gram negative bacteria in some cases (Table 1).

## CONCLUSION

In conclusion, the results of this study suggest that *A. mexicana* L. organic extracts may act as an alternative to synthetic bactericides which might have significant applications in pharmaceutical or other industries for controlling pathogenic bacteria. However, if plant-based antimicrobials such as crude extracts are to be used for drug or food preservation, issues on safety and toxicity will always need to be addressed.

## REFERENCES


Bakri, I.M., and C.W.I. Douglas. 2005. Inhibitory effect of garlic extract on oral bacteria. Arch. Oral. Biol. 50 (7): 645-651.

Bezic, N., M. Skocibusic, V. Dinkic, and A. Radonic. 2003. Composition and antimicrobial activity of *Achillea clavennae* L. essential oil. Phytother. Res. 17: 1037-1040.

Bhattacharjee, I., S.K. Chatterjee, S. Chatterjee, and G. Chandra. 2006. Antibacterial potentiality of *Argemone mexicana* solvent extracts against some pathogenic bacteria. Mem Inst Oswaldo Cruz, Rio de Janeiro 101(6): 645-648.

Chandrasekaran, M., and V. Venkatesalu. 2004. Antibacterial and antifungal activity of *Syzygium jambolanum* seeds. J. Ethnopharmacol. 91: 105-108.

Chang, Y.C., P.W. Hsieh, F.R. Chang, R.R.Wu, C.C. Liaw, K.H. Lee, and Y.C. Wu. 2003. New protopine and the anti-HIV alkaloid 6-acetonyldihydrocheleryth-rine from Formosan *Argemone mexicana*. Planta Med. 69: 148-152.

Chevallier, A. 1996. The Encyclopedia of Medicinal Plants. Dorling Kindersley. London ISBN 9: 780751-303148.

Chopra, R.N., S.L. Nayar, and I.C. Chopra. 1986. Glossary of Indian Medicinal Plants, p. 55. CSIR, New Delhi, India.

Deans, S.G., R.C. Noble, R. Hiltunen, W. Wuryani, and L.G. Penzes. 1995. Antimicrobial and antioxidant properties of *Syzygium aromaticum* (L) Merr Perry: impact upon bacteria, fungi and fatty acid levels in ageing mice. Flavour Frag. J. 10: 323-328.

Emboden, W. 1979. A lot of details about the history, chemistry and use of narcotic plants, including hallucinogens, stimulants, inebriants and hypnotics. Narcotic Plants Studio Vista ISBN 0-289-70864-8.

Farber, J.M. 2000. Present situation in Canada regarding *Listeria monocytogenes* and ready-to-eat seafood products. Int. J. Food Microbiol. 62: 247-251.

Gunstone, F.D., J.A. Holliday, and C.M. Scrimgeour. 1977. Fatty acids, Part 51. The long-chain oxoacids (argemonic acids) in *Argemone mexicana* seed oil. Chem. Phys. Lipids 20: 331-335.

Harborne, J.B., and C.A. Williams. 1983. Flavonoids in the seeds of *Argemone mexicana:* a reappraisal. Phytochemistry 22: 1520-1521.

Hoffman, D.L. 1987. The herb user's guide. Wellingborough, London: Thomsonûs publishing group.

Hussain, S.F., S. Nakkady, L. Khan, and M. Shamma. 1983. Oxyhydrastinine, an isoquinoline alkaloid from the Papaveraceae. Phytochemistry 22: 319-320.

Hutchings, A., A.H. Scott, G. Lewis, and A.B. Cunningham. 1996. Zulu Medicinal Plants. An Inventory. University of Natal Press, Pietermaritzburg.

Karamanoli, K. 2002. Secondary metabolites as allelochemicals in plant defence against microorganisms of the phyllosphere. p. 277-288. In: M. Reigosa and N. Pedrol, (eds.) Allelopathy from molecules to ecosystems. Science Publishers Inc., NH.

Kulshrestha, S.B. 1990. Prevalence of enteropathogenic sero groups of *E. coli* in milk products samples from Bareilly and their multiple drug resistance. Ind. Journal of Dairy Sci. 43: 337-378.

McCabe-Sellers, B.J., and E.B. Samuel. 2004. Food Safety: Emerging Trends in food-borne illness surveillance and prevention. Journal of the American Dietetic Association 104: 1708-1717.

Mead, P.S., L. Slutsker, V. Dietz, L.F. McCaig, J.S. Breese, C. Shapiro, P.M. Griffin, and R.V. Tauxe. 1999. Food related illness and dead in the United States. Emerging Infectious Diseases 5: 607-625.

Morris, J.A., A. Khettry, and E.W. Seitz. 1979. Antimicrobial activity of aroma chemicals and essential oils. J. Am. Oil Chem. Soc. 56: 595-603.

Murray P.R., E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Yolke. 1995. ASM: Manual of clinical microbiology (6th eds). Washington.

Mylotte, J.M., C. McDermott, and J.A. Spooner. 1987. Prospective study of 114 consecutive episodes of *Staphylococcus aureus* bacteremia. Rev. Infect. Dis. 9(5): 891-907.

Nakkady, S., and M. Shamma. 1988. Studies on the chemical constituents of *Argemone mexicana*. Egypt. J. Pharm. Sci. 29: 53-61.

Nasar-Abbas, S.M., and A.K. Halkman. 2004. Antimicrobial effect of water extract of sumac (*Rhus coriaria* L.) on the growth of some food borne bacteria including pathogens. Int. Journal of Food Microbiol. 97: 63-69.

Rahman, A., M.A.K. Parvez, T. Parvin, D.K. Paul, and M.A. Sattar. 2004. Antimicrobial activity of three bioactive compounds from the stream bark of Piper chaba Hunter. Bioscien. Res. 1(1): 16-20.

Reynolds, J.E.F. 1996. Martindale the extra harmacopoeia (31st eds). Royal Pharmaceutical Society of Great Britain, London.

Shin, L.S., H. Masuda, and K. Naohide. 2004. Bactericidal activity of wasabi (*Wasabia japonica*) against *Helicobacter pylori*. Int. Journal of Food Microbiol. 94: 255-261.

Singh, R., R. Chandra, M. Bose, and P.M. Luthra. 2000. Antibacterial activity of *Curcuma longa* rhizome extract on pathogenic bacteria. Current Science 83(6): 25.