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Antibacterial Activity of *Argemone mexicana* L. against Water Borne Microbes

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ABSTRACT

The aim of this study was to evaluate the potential of *Argemone mexicana* (Shialkanta) extract as antimicrobial agent against bacterial isolates originated from drinking water. Effectiveness of acetone extract, ethyl acetate extract and petroleum ether extract of *Argemone mexicana* was determined *in vitro*, using agar diffusion method and MIC determination test against four (one Gram positive and three Gram negative) water borne pathogenic bacteria *Escherichia coli, Shigella* sp. *Staphylococcus* sp. and *Salmonella* sp. The zones of inhibition against the tested bacteria were found ranging from 8 to 16 mm, along with their respective MIC values ranging from crude to 512 μg mL^{-1}. This study suggests that natural products derived from *Argemone mexicana* may contribute to the development of new antimicrobial agents.

Key words: *Argemone mexicana*, organic extracts, water-borne pathogens, antibacterial activity, minimum inhibitory concentration

INTRODUCTION

Water is the basic constituent of body mass of all living beings. Its deficiency interferes with normal physiological functions of living beings. On the other hand when contaminated water is used by living beings, it produce great health risk. The major water pollutants are pathogenic microorganisms, metals and chemicals which make water non-drinkable. Every eight second, a child dies from water related disease around the globe. While another study (Pruss *et al.*, 2002) estimated that water, sanitation and hygiene were responsible for 4.0% of all deaths and 5.7% of the total disease burden occurring worldwide (accounting for diarrheal diseases, schistosomiasis, trachoma, ascariasis, trichuriasis and hookworm disease). Worldwide in 1995, contaminated water and food caused more than 3 million deaths, of which more than 80% were among children under age five (WHO, 1996) and 2.2 million deaths in 1998. Diarrheal disease alone causes 2.2 million of the 3.4 million water related deaths per year. Many of the deaths involve children less than five years of age (Sanchez and Holmgren, 2005; Medema *et al.*, 2003). World Health Organization (WHO, 1996) estimates that globally 1.8 million people die each year from diarrheal diseases. According to another report, at least one and a half thousand million people worldwide use polluted water (Faechem, 1980) and many of them suffer water borne disease. The problem is more acute in developing countries than the developed countries where higher incidence of water-borne diseases are reported (Parveen *et al*., 2008; Luby *et al*., 2001). In these countries four-fifths of all the illness is caused by water-borne diseases, with diarrhea being the leading cause of childhood death (Luby *et al*., 2004; Qadri *et al*., 2005). In India, more than 70% of the epidemic emergencies are either water borne or are water related (Khera *et al*., 1996).

In Bangladesh water-related diseases are responsible for 24% of all deaths, 12% by diarrhea and 10% by other gastro-intestinal illness including enteric fever. Every year, gastroenteritis and diarrheal diseases kill 110,000 children below the age of five. To make matters worse a quarter of the nation's tubewells are contaminated with high concentration of natural Arsenic. Thus water plays a major role in the overall disease profile of this country. Many tubewells in Bangladesh show higher than acceptable concentrations of bacterial contaminants. In several studies (Hoque, 1998) the concentrations of fecal coliforms were measured across Bangladesh (some 2000 tubewells). Around half were found to fail the WHO (1996) guideline value (i.e., contained detectable coliform counts). Study shows that *Salmonella, Shigella, Vibrio cholera, Enterobacter, Citrobacter, Klebsiella* and *E. coli* are mainly responsible for different water related diseases (Jagals, 2000).

Bangladesh possesses a rich flora of medicinal plants. Out of 5000 species of phanerogams and pteridophytes growing in this country more than a thousand are regarded as having medicinal properties. *Argemone mexicana* (Local name: Shialkanta) is an herb with branches, which has naturalized widely in many tropical and subtropical regions (Siddiqui *et al*., 2002). It grows commonly in abandoned and cultivated fields of Mexico, United States, Ethiopia, India and Bangladesh. Traditional healers in Mali use *Argemone mexicana* to treat malaria (Wilcox *et al*., 2007). Ayurveda reported that the plant is purgative, diuretic and destroys worms. It cures skin-diseases, leprosy and inflammation bilious fevers. Roots are equally used to cure anthelmintic. Juice is used to cure opacity of cornea and ophthalmia (Osho and Adetunji, 2010).

The present investigation was undertaken to evaluate the antibacterial potential of *Argemone mexicana* extracts against water-borne bacteria with the possible use as a natural antimicrobial agent in pharmaceutical industries.

MATERIALS AND METHODS

Material: Four types of bacteria *Escherichia coli, Shigella sp., Staphylococcus sp.* and *Salmonella* sp. used in this study were isolated from drinking water of various region of Kushtia city, Bangladesh by standard methods. Pure culture of the isolates were preserved at 4°C on nutrient agar slants. Identity of the isolates were confirmed by morphological characteristics and conventional biochemical tests (Harley and Prescott, 2002). *Argemone mexicana* were collected from Kushtia city. 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 *Argemone mexicana* was extracted with 200 mL each of acetone, ethyl acetate and petroleum ether 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 acetone (6.4 g), ethyl acetate (5.4 g) and petroleum ether (6.3 g) extracts.

Antibacterial assay: The agar diffusion method was used for antibacterial assay (Murray *et al*., 1995). Sterile filter paper discs (6 mm diameter) were impregnated with 10 µL of 30 mg mL^{-1}

(300 μg disc^{-1}) of *Argemone mexicana* extracts of acetone, ethyl acetate and petroleum ether. Negative controls were prepared using the same solvents employed to dissolve the samples. Standard reference antibiotics, nalidixic acid, streptomycin and tetracycline 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 were tested by serial dilution method (Chandrasekaran and Venkatesalu, 2004). 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^{-1}.

RESULTS

Antibacterial activity assay: The *in vitro* antibacterial activities of *Argemone mexicana* extracts against the employed bacteria were assessed by the presence or absence of growth inhibition zone. As shown in Table 1, the petroleum ether soluble fraction of leaf and stem of *Argemone mexicana* showed highest inhibitory activity against water borne microorganisms compare to the other extracts. On the other hand, acetone soluble fraction of *Argemone mexicana* did not show any inhibitory activity against those microorganisms. Ethyl acetate extract shows moderate inhibitory effect against *E. coli, Staphylococcus* sp. and *Salmonella* sp.

In this study, in some cases, the petroleum ether extract exhibited significantly higher antibacterial activity than that of standard antibiotics as regard to *Staphylococcus* sp. and *E. coli* while the other extracts show low antibacterial activity than the standard antibiotics (Table 1).

Minimum Inhibitory Concentrations (MICs): The lowest concentration of acetone, ethyl acetate and petroleum ether extract that resulted in complete growth inhibition of the tested organisms were found in the range of crude to 512 μg mL^{-1} (Table 2).

Petroleum ether extract showed higher antibacterial activity by having lower minimum inhibitory concentration value than acetone extract and ethyl acetate extract. In this study, the Gram-positive bacteria were found to be more susceptible to plant extracts than Gram-negative bacteria (Table 2).

Table 1: Antibacterial activity of *Argemone mexicana* extracts against water borne microbes

	Zone of inhibition (mm)					
	Organic Extracts of *Argemone mexicana*			Standard		
Microorganisms	Acetone extract	Ethyl acetate extract	Petroleum ether extract	TC	SM	NA
Escherichia coli	-	9	15	12	23	14
Shigella spp.	-	-	12	13	16	22
Staphylococcus sp.	-	8	16	14	14	11
Salmonella sp.	-	10	10	14	15	16

Diameter of inhibition zones of different organic extracts include 6 mm disc (tested volume 300 μg disc^{-1}). SM: Streptomycin, TC: Tetracycline, NA: Nalidixic acid; -: Not detected

Table 2: Determination of Minimum Inhibition Concentration (MIC) of *Argemone mexicana* in different solvents (μg mL^{-1})

	Minimum inhibitory concentration (MIC)		
	Organic extracts		
Microorganisms	Acetone extract	Ethyl acetate extract	Petroleum ether extract
Escherichia coli	-	Crude	Crude
Shigella sp.	-	-	Crude
Staphylococcus sp.	-	Crude	512
Salmonella sp.	-	Crude	Crude

MIC of different organic extracts (values in μg mL^{-1}). -: Not detected

DISCUSSION

Plants have been model source of medicines as they are a reservoir of chemical agents with therapeutic properties. They provide 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. Various publications have documented the antimicrobial activity of plant extracts (Hoffman, 1987; Nasar-Abbas and Halkman, 2004; Rahman *et al.*, 2004). In the present study, the antibiotic potential of the extracts of the leaf and stem of *Argemone mexicana* has been determined against the water borne bacteria. For the comparison of the plant extracts activity control (different type of antibiotic disc) and negative control (only solvent absorbing disc) was used. The negative control showed no activity against all tested bacteria. The positive control showed significant antibacterial activity against water borne bacteria. The findings of this study agree with the result of other research (Rahman *et al.*, 2009; Bhattacharjee *et al.*, 2006; Nakkady and Shamma, 1988; Chang *et al.*, 2003). This activity could be attributed to the presence of some bioactive compounds such as alkaloids, phenolics and fatty acids in *Argemone mexicana* plant. Although the findings of this study agree with the other workers findings but the diameter of formed zone showed in this study varies from other study result. The sources of the microbes used in this study may be the reason for this difference.

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 *Argemone mexicana* than were Gram negative bacteria (Rahman *et al.*, 2009).

CONCLUSION

In conclusion, the results of this study suggest 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. 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.

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