American-Eurasian J. Agric. & Environ. Sci., 14 (11): 1172-1175, 2014
ISSN 1818-6769

DOI: 10.5829/idosi.aejaes.2014.14.11.86215

Efficacy of *Argemone mexicana* Linn. Leaf and Stem Extract to Restrain the Growth of Water Borne Microorganisms

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Abstract: Antimicrobial activity of petroleum ether extract of leaf and stem from *Argemone mexicana* against drinking water borne bacteria along with *Escherichia coli, Shigella spp., Staphylococcus spp. and Salmonella spp* was investigated using standard agar disc diffusion method. The petroleum ether extract of the plant showed significant antimicrobial activity against water borne microbes. The minimum inhibitory concentration (MIC) of the extract was determined to be 1.024 mg/ml. At the concentration of 2.048 mg/ml and 1.024 mg/ml, the petroleum ether extract of leaf and stem produced zone of inhibition (14 mm and 12 mm respectively) against water borne microorganisms, although no inhibition was found against *E.coli, Shigella spp., salmonella spp*. and *Staohylococcus spp*. This study suggests that natural products obtained from *A. mexicana* L. leaf and stem may have potential to be use as a new antimicrobial agent especially to eliminate the water borne microbes.

Key words: Antibacterial Activity · *Argemone mexicana* · Minimum Inhibitory Concentration (MIC) · Petroleum Ether Extract · Water Borne Microorganism

INTRODUCTION

Access to safe drinking water can prevent disease outbreaks and lower diarrheal and other disease burden. Approximately 768 million people across the world lack access to an "improved" drinking water source, approximately one in nine people [1]. Though three-fourth of total surface of the earth remaining covered with water, drinkable water registers less than three percent of the total water resources. According to the view of environment editor, Michael McCarthy, mankind's most serious challenge in 21st century might not be war or hunger or diseases or even the collapse of civic order, a UN report says, it may be the lack of fresh water [2].

Water is among the leading sources to cause microbiological hazards in humans. The effect of drinking contaminated water results in thousands of deaths every day, mostly in children under five years in developing countries. In addition, diseases caused through consumption of contaminated water and poor hygiene practices are the leading causes of death among children worldwide, after respiratory diseases [3]. Water borne microorganism mediated diseases such as diarrhea remains a major killer in children and it is estimated that 80% of all child deaths under the age of 5 years in developing countries result from diarrheal diseases [4, 5]. Globally, 1.1 billion people rely on unsafe drinking water source from lakes, rivers and open wells; the majority of these people are from Asia and sub-Saharan Africa. Furthermore, 2.4 billion people lack adequate sanitation Worldwide which also significantly contributes in causing microbial contamination in drinking water sources [6, 7]. The Millennium Development Goal target for water is to 'halve by 2015 the proportion of people without sustainable access to safe drinking water and basic sanitation'. The WHO/UNICEF [7] estimates that if these

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improvements were to be made in sub-Saharan Africa alone, 434,000 child deaths due to diarrhea would be averted annually.

For domestic water supply in developing countries, Boreholes (As low cost technology option) are generally considered as ‘safe sources’ of drinking water. When properly constructed and maintained, they provide consistent supplies of safe and wholesome water with low microbial load and little need for treatment of the drinking water. Different treatment methods are needed to eradicate or to lower the health risk caused by the use of water from unsafe sources. These methods should be easy to use, efficient, inexpensive and useful. Water is treated so that it can be used for domestic and industrial purposes as well as to reduce pathogenic microorganisms, to lower the level of turbidity, to remove taste and smell, to eradicate chemicals such as iron and manganese and to soften it. So, keeping in view the importance of water treatment for drinking purposes and existing problems, there is a great need to develop easy, useful and inexpensive methods for water treatment.

Usage of medicinal plants for the treatment of water contaminated with microorganisms could be a novel approach to improve the quality of drinking water. *Argemone mexicana,* a natural medicinal plant native to the sub-Himalayan tracts of Pakistan, India, Afghanistan and Bangladesh, has attracted immense interest due to their considerable phytochemical contents. *A. mexicana*, also known as Shialkanta in Bangladesh, is a member of the Papaveraceae family. *A. mexicana* plant is purgative, diuretic and can annihilate worms. It is also useful in the treatment of skin-diseases, leprosy, inflammations, bilious fevers, ophthalmia and opacity of cornea according to Ayurveda. Drugs prepared from this plant are used in the treatment of tape-worm caused health problems in Homoeopathic medicine system [8]. The present study was carried out to assess the antibacterial activity of crude extract of *A. mexicana* plant (Leaf and stem) and its efficacy to restrain the growth of water born microbes.

MATERIALS AND METHODS

Collection of Water Samples and Microbial Culturing: Drinking water samples were collected from various homes at the same date from the areas of Kushtia Town, Bangladesh. Samples were collected in sterilized screw cap tubes and transferred to the laboratory for analysis. During sample collection and transportation, it was the highest concern to avoid all risk of contamination. Some known water borne Gram negative bacteria including *Escherichia coli*, *Salmonela spp, Shigella spp, Staphylococcus spp* were collected from the Department of Biotechnology and Genetic Engineering, Islamic University, Kushtia, Bangladesh. From the collected water sample, 10 µL was taken into each petridish containing nutrient agar medium and was then spreader. In the same manner, species of water borne Gram negative bacteria were also cultured in plates containing nutrient agar medium. The plates were then incubated at 37°C for 20 hours.

Preparation of *Agremone mexicana* Extract: The *Agremone maxicana* plants were collected from Islamic University campus, Kushtia, Bangladesh. After collection, the leaf and stem of *Argemone mexicana* were cleaned, cut into small pieces and then dried followed by pulverized into a coarse powder. To prepare the plant extract, 5 gm of dried powder was added to 20 ml of the solvent (Petroleum ether) into a conical flask and was subjected to gentle shaking in a water bath shaker for 5-6 hours. Then the petroleum ether extract was filtered and the filtrate was air dried to concentrate and finally preserved at -20°C for the subsequent studies.

Preparation of Extract Impregnated Discs: The filter paper was punched with the punching machine to prepare the paper discs. The discs were taken in to the screw capped tube and sterilized. Then the disc papers were impregnated with each concentration of extracts and placed at room temperature for air dry for about 15 hours. The dried disc papers were then placed in oven for 1h at 37°C. After completion of the oven dry, the disc papers were labeled according to different concentration and finally the labeled disc papers were used for antibacterial activity. Sterile blank paper discs were impregnated with only sterile solvent (Petroleum ether) and used as negative control.

***In vitro* Antibacterial Activity:** The known organisms including *Escherichia coli, Salmonella spp., Staphylococcus spp., Shigella spp.* and the drinking water borne microorganisms (From the collected water samples) were tested in this study to determine the antibacterial effect of the crude extract. The *in vitro* antibacterial activity of the extract was measured by employing standard agar disc diffusion method [9, 10]. The discs (Impregnated with extract and control) were placed aseptically over the bacterial culture on nutrient agar plates and incubated at 37°C temperature for about 20 hours. After incubation, the zone of inhibition around the discs was measured by millimeter scale. The experiment was replicated three times.

Determination of MIC of Leaf and Stem Extract of *Argemone mexicana:* The lowest concentration of the extracts required to inhibit the growth of the organism *in vitro* is minimum inhibitory concentration (MIC) which was determined following the serial dilution technique according to Reiner [11]. Working solution of the plant extracts were prepared by dissolving dried extracts in solvent (Petroleum ether) in a screw-capped tube so that the final concentration was reached to 2.048 mg/ml. By using serial dilution technique, the concentration of the last test tube was made 8 µg/ml.

RESULTS

Petroleum ether extract of leaf and stem of *A. mexicana* showed significant inhibitory activity against water borne microorganisms in water samples collected from Kushtia town, Bangladesh. In this study, low concentrations of petroleum ether extract of leaf and stem of *A. mexicana* (2.048 mg/ml, 1.024 mg/ml, 512 µg/ml, 256 µg/ml, 128 µg/ml, 64 µg/ml, 32 µg/ml, 16 µg/ml, 8 µg/ml) did not show any inhibitory activity against water borne microorganisms and other microorganisms including *E.coli, Shigella spp., Salmonella app*. and *Staphylococcus spp* (Table 1). In the contrary, high concentrations (2.048 mg/ml and 1.024 mg/ml) of the extract of leaf and stem produce significant inhibitory activity against the growth of water borne microorganisms (Table 1). However, no inhibition against the growth of *E.coli, Shigella spp., salmonella app*. and *Staphylococcus spp*. was found with these highly concentrated extracts. The minimum inhibitory concentration (MIC) of the plant extract was also determined. The MIC value of petroleum ether extract of leaf and stem of *A. mexicana* was 1.024 mg/ml against water borne microorganisms and the zone of inhibition at MIC was found to be 12 mm (Fig. 1). The zone of inhibition for the high concentrated extract of 2.048 mg/ml was 14 mm (Table 1). For the comparison of the antibacterial activity of the plant extract, positive control (Different type of antibiotic disc) and negative control (Only solvent absorbed disc) were used. The negative control showed no activity while the positive control showed significant antibacterial activity against known water borne bacteria (Data not shown).

In this study, the petroleum ether extract of leaf and stem of *A. mexicana* demonstrated significant inhibitory effect against the growth of water borne microorganisms, although it was unable to restrict the growth of Gram negative bacteria such as *E. coli, Shigella spp., Salmonella app. and Staphylococcus spp.* This finding suggests that natural products can be useful to remove the water borne microbes instead of chemical disinfectants.

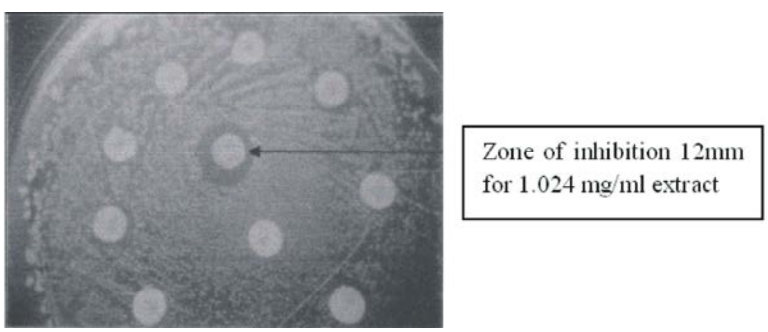

Fig. 1: Minimum inhibitory concentration (MIC) assay. The MIC of petroleum ether extract of leaf and stem of *Argemone mexicana* was determined to be 1.024 mg/ml and it produced a zone of inhibition of 12 mm against water borne microorganisms.

Table 1: Comparison of antibacterial activity of different concentration of petroleum ether extract of leaf and stem of *Argemone mexicana* on *E.coli, Shigella spp., salmonella app. and Staphylococcus spp.* and water borne microorganisms

	Petroleum ether extract of leaf and stem of *Argemone mexicana*(ig/ml)								
Microorganisms	2048	1024	512	256	128	64	32	16	8	NC
E.coli	+	+	+	+	+	+	+	+	+	+
Shigella spp	+	+	+	+	+	+	+	+	+	+
Staphylococcus spp	+	+	+	+	+	+	+	+	+	+
Salmonella spp	+	+	+	+	+	+	+	+	+	+
Water borne microorganisms	*14 mm (-)	*12 mm (-)	+	+	+	+	+	+	+	+

(-) No Growth, (+) Growth, (*) Zone of inhibition, (NC) Negative control.

DISCUSSION

In the present study, the petroleum ether extract of leaf and stem at high concentration showed significant antibacterial activity against water borne microorganisms which is supported by another study carried out by Rahman *et al*. [12]. The results of this study suggest that organic extract of *A. mexicana* may serve as an alternative to synthetic bactericides which might have significant applications in pharmaceutical or other industries for controlling water borne bacteria. Although the petroleum ether extract of leaf and stem of *A. mexicana* produced no inhibitory effect against the growth of *E.coli, Shigella spp., Salmonella spp. and Staphylococcus spp.,* a recent study reported the potentials of the antimicrobial activity of leaf, root and stem extracts of *A. mexicana* Linn. against Staphylococcus aureus, *E. coli* and *Pseudomonas aeruginosa* in case of ethyl acetate, methanol and aqueous extracts [13]. However, since the extracts of *A. mexicana* showed a marked antibiotic potential, it may explore a new era in the field of microbial control. This study also suggests the traditional use of *A. mexicana* has scientific basis. 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. Further investigation is necessary to isolate the bioactive compounds from the leaf and stem extract of *A. mexicana* and to confirm their principles of bioactivity against various microorganisms.

CONCLUSION

Petroleum ether soluble fraction of leaf and stem of *A. mexicana* showed substantial inhibitory activity against water borne microbes which can be suggestive of using natural products instead of chemical disinfectants to remove the water borne microbes.

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