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Two Antibacterial Alkaloids from *Argemone mexicana*

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Abstract

The isolated alkaloids from *Argemone mexicana* seed showed considerable antibacterial activity against pathogenic bacteria, out of which one of them namely *Staphylococcus aureus* is Gram positive and remaining three Gram negative namely, *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Fractionation followed by TLC leds to the isolation of two known benzophenanthridine alkaloids namely and dihydroxysanguinarine and dimethylsanguinarine. The MIC was determined for each compound using a two fold serial dilution assay. The structures of these compounds are determined by $^{1}H_{1}$ and ^{13}C NMR analysis.

Keywords: *Argemone mexicana*; Alkaloids; Antibacterial activity.

Introduction

India has a rich flora that is widely distributed throughout the country. From ancient time plant and plant extracts are used for treatment and cure of various diseases (Dhar et al., 1968; Perumal Samy and Ignacimuthu, 1998, 2000; Dahanukar et al., 2000; Kumar et al., 2006).

Argemone mexicana L. (Papaveraceae), commonly known as prickly poppy, is used as a medicinal plant in several countries as antidote to snake venom, relieving tooth ache and its extract is also used to treat common colds, warts and itches, dropsy and even in curing jaundice (Chopra et al. 1986; Bhattacharjee et al. 2006).

In this study we report the antibacterial property of the main antibacterial compounds from Chloroform: Methanol (1:1) extract of *A. mexicana* seed on four pathogenic bacteria. We also identified the active compounds as dihydroxysanguinarine (1) and dimethylsanguinarine (2).

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Materials and Methods

Plant materials: Seeds of *Argemone mexicana*, collected from nearby site of Hatgobindapur College, Burdwan (23°16´N, 87°54´E), during spring (mid-March to mid-April 2017) and

herbarium sheet was prepared and Voucher specimen (voucher no. 110) submitted at Department of Botany, DBNDS Mahavidyalaya and taxonomically authenticated.

***Extraction and isolation of the antibacterial compounds*:** The air dried seeds (500 g) were extracted successively with 7 technical grade solvents (Merck) of increasing polarity, one after another on the same sample using Soxhlet apparatus following the method of Tang & Young (1982). The extract of chloroform: methanol (1:1, v/v) (21 gm) was further fractioned by column chromatography (Silica gel – G for TLC, Merck, India) for 72 h using n- hexane- ethyl acetate mixture of increasing polarity. The different fractions obtained were tested for antibacterial activity against all strains by direct bioautograhy on thin layer chromatography (TLC) plates (Lund and Lyan, 1975). The fraction was further purified by TLC on silica gel 60 F_{254} (Merck) with chloroform: methanol: water (65:35:5) as running solvent; resulting is the isolation and characterization of two active alkaloids, 1 (1.5 g) and 2 (1.2 g).

***Bacterial strains used*:** *Staphylococcus aureus, Escherischia coli, Pseudomonas aeruginosa,* and *Klebsiella pneumonia,* having strain numbers MTCC 2940, MTCC 739, MTCC 2453 and MTCC 432 were obtained from Burdwan Medical College and maintined in Nutrient Broth M002 (Himedia, India).

***Antibacterial testing*:** MIC of the isolated compounds 1 and 2 was evaluated by the broth micro dilution method in Müller – Hinton broth according to the NCCLS standard (NCCLS, 1997). Gentamycin (5 µg/ml) was used as reference standard. Observations were performed in triplicate. The lowest concentration of 1 and 2 (6.2 – 28.1µg/ml) where no visible growth was recorded. Simultaneously, TLC bioassay using Bioautography technique (Hamburguer and Cordell 1987; Didry et al. 1990) was used to determine which compound in the $ChCl_3$: methanol (1:1, v/v) extract was active.

Results

The fractionation of the seed extract of the chloroform: methanol (1:1, v/v) of *Argemone mexicana* led to the isolation of two alkaloids, dihydroxysanguinarine (1) and dimethylsanguinarine (2). Compounds were identified by direct comparison of their $^1H_1$ and ^{13}C NMR spectral data with those found and described by (Chang et al. 2003).

Table 1 shows the Compound 1 and 2 displayed significant antibacterial activities with MIC's of 6.2 and 9.3 µg/ml respectively against *Klebsiella pneumoniae*, 12.5 and 15.6 µg/ml for *Staphylococcus aureus*, 6.2 and 12.5 µg/ml for *Escherichia coli* and 15.6 and 28.1 µg/ml for *Pseudomonas aeruginosa*.

Dihydroxysanguinarine (1) was the most active compound due to its inhibition of the growth of all the test bacterial strains. This is an indication of the compound as a broad spectrum antibiotics.

Table 1: Minimum Inhibitory Concentrations (MIC) (µg/ml) of active alkaloids from *A. mexicana*

Compounds	K. pneumoniae	S. aureus	E. coli	P. aeruginosa
Dihydroxy sanguinarine	6.2 ± 0.17	12.5 ± 0.06	6.2 ± 0.21	15.6 ± 0.04
Dimethyl sanguinarine	9.3 ± 0.12	15.6 ± 0.31	12.5 ± 0.18	28.1 ± 0.14
Gentamycin	2.5 ± 0.22	2.5 ± 0.08	5.0 ± 0.20	5.0 ± 0.32

Discussion

The chloroform: methanol (1:1, v/v) fraction of seed extract of *Argemone mexicana* showed antibacterial activity against four pathogenic bacteria. This activity has been identified as alkaloids dihydroxysanguinarine (1) and dimethylsanguinarine (2) using systematic fractionation guided by antibacterial assay. These compounds have also been isolated from plants of Papaveracea family (Sloviko et al., 1985: Daskalov et al., 1988: Chang et al., 2003: Novarro and Dalgado 1999). Alkaloids of this structural type have been shown to possess antibacterial activity against bacteria that produce oral infection (Gadawsky 1989; Harkrademet et al., 1990). From this study we noted that the active alkaloids from seeds of *A. mexicana* possess an antibacterial activity. This might explain the use of this plant for the treatment of various infectious diseases in folk medicine.

Conclusion

The only alternative to antibiotic is medicinal plants. The seeds of *Argemone mexicana* is considered as one of the nature's gifted property as it shows antibacterial activity against both gram positive and gram negative bacteria. But the seed extract when adulterated with mustard oil it causes dropsy. So one should be very careful about the way of extraction, method of application, duration of application and proper dose so that no resistance variety is created in bacteria.

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