Antibacterial activity of the latex of *Argemone ochroleuca* Sweet

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ABSTRACT

الأهداف: دراسة تأثير اللبن النباتي المستخلص من النبات البري أرجيمون أوشروليوكا (crude latex of *Argemone ochroleuca*) وذلك كمادة مضادة لعدد من البكتيريا المسببة للأمراض في الإنسان.

الطريقة: أُجريت هذه الدراسة في جامعة الملك خالد، أبها، المملكة العربية السعودية وذلك خلال الفترة من يناير إلى مارس 2010م. لقد تم جمع 17 مليلتر من اللبن النباتي المستخلص من النبات البري الحلو أرجيمون أوشروليوكا، ومن ثم تم دراسة تأثير كلاً من مستخلص هذا النبات الخام والمخفف باستخدام طريقة الانتشار القرصي لقياس مدى التحسس الجرثومي (agar diffusion method test) وباستخدام 1 مليلتر من مُستعلق السلالات البكتيرية التالية: العصوية الرقيقة، والأمعائية المرياحة، والمكيرة الصفراء، والأشريكية القولونية، والعنقودية الذهبية. وُضعت الأقراص بعد تحضيرها في الهواء الطلق في درجة حرارة 29 درجة مئوية ولمدة 48 ساعة، ومن ثم تم قياس أقطار مناطق التثبيط.

النتائج: أظهرت الدراسة بأن اللبن النباتي المستخلص من النبات البري أرجيمون أوشروليوكا له تأثير قوي ضد جميع السلالات البكترية، ولقد تراوح قطر مناطق التثبيط مابين 9.30 - 40.3 مليميتر، فيما ظهرت أدنى قيم التركيز المثبط مع 100 ميكرولتر / مليلتر من تركيز هذا المستخلص.

خاتمة: أشارت الدراسة إلى فعالية اللبن النباتي المستخلص من النبات البري أرجيمون أوشروليوكا ضد بعض السلالات البكتيرية المختارة، وهذا قد يجعلها من المصادر الطبيعية المضادة للبكتيريا.

Objectives: To investigate the antibacterial effect of the crude latex of *Argemone ochroleuca* (*A. ochroleuca*) as antibacterial potential against a range of human pathogenic bacteria.

Methods: This study was carried out at King Khalid University, Abha, Kingdom of Saudi Arabia from January to March 2010. Seventeen ml of fresh latex from *A. ochroleuca* Sweet was collected, and the antibacterial activity of crude and diluted latex were examined using one ml of standardized inoculum suspension, and using the agar diffusion method test against *Bacillus subtilis, Enterobacter aerogenes, Micrococcus luteus, Escherichia coli,* and *Staphylococcus aureus*. All inoculated plates were incubated aerobically at 29^{0}C for 48 hours. The diameter of the zones of inhibition was measured to the nearest mm.

Results: The crude latex of *A. ochroleuca* exhibited a potent antibacterial effect on all bacterial strains examined. The zones of inhibition against the tested bacteria were found in the range of 9.30 - 40.3 mm along with their respective minimum inhibitory concentration values 100 µl/ml.

Conclusion: The observable inhibition on selected bacteria by latex of *A. ochroleuca* makes it a promising alternative as a potential source of natural antibacterial.

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Argemone ochroleuca (*A. ochroleuca* [*Papaveraceae*]) Sweet is an annual herb, 0.2-1 meter high with yellow sap, erect stems, pithy, and covered with stiff yellow prickles. The young leaves are slightly stalked and crowded into a dense basal rosette, and stems are bluish-green and alternate. The flowers are creamy white to yellow, on a short stalk or sessile (without a stalk) at the ends of branches, and are 3-6 cm wide in diameter. The fruit is a prickly capsule 2-5 cm long. The

seeds are numerous dark brown or black and globular, and are approximately 1.5 mm in diameter.[1] In English, the common names of *A. ochroleuca* are Mexican poppy, pale Mexican poppy, prickly poppy, white thistle, and yellow poppy, and in Spanish as "chocolate". In the Kingdom of Saudi Arabia (KSA) it is a wayside weed becoming increasingly invasive in the high southern mountains of Aseer region.[2] Over the last few decades, a great interest has developed in looking for antimicrobial drugs from natural plant products. Plants, animals, and microorganisms are the main source of natural antimicrobials.[3] It was discovered that more than 10,000 biologically active compounds of microbial origin and many plant extracts are used in traditional medicine as a tonic, and remedy against constipation, fever, high blood pressure, facilitating healing of wounds, and many infectious diseases.[4-12] Since it is reported that the treatment of bacterial infections is increasingly complicated by the ability of the bacteria to develop resistance to antimicrobial agents, and acquired resistance genes may enable a bacterium to: produce enzymes that destroy the antibacterial drug; express efflux systems that prevent the drug from reaching its intracellular target; modify the drug's target site, or produce an alternative metabolic pathway that bypasses the action of the drug,[13] there is, therefore, still a need for new antibiotics from other sources for reducing or eliminating microorganisms to ensure public health. The aim of this study was to determine the antibacterial potential of crude latex of *A. ochroleuca* against 5 standard bacterial strains. Antibacterial activity of latex of *A. ochroleuca* against *Bacillus subtilis* (*B. subtilis*), *Enterobacter aerogenes* (*E. aerogenes*), *Micrococcus luteus* (*M. luteus*), *Escherichia coli* (*E. coli*), and *Staphylococcus aureus* (*S. aureus*) was carried out using agar diffusion method test.

Methods.

Latex samples. Samples of *A. ochroleuca* were collected from Abha Governorate, Aseer region, KSA. The sampling was carried out from January to March 2010. The voucher specimen was deposited in the Herbarium of Biological Department, College of Science, King Khalid University, KSA. Seventeen ml of fresh latex was collected from healthy plants by small incisions near the youngest leaves and left to flow off into Epindroff's tube.

Antibacterial testing. The antibacterial activity was examined by well-agar diffusion method.[14,15] Petri plates were prepared by pouring 10 ml of sterilized nutrient agar and allowed to solidify. Plates were dried and one ml of standardized inoculums suspension from every bacterial strain was poured and uniformly spread. Excess inoculum was drained away, and the inoculum was allowed to dry for 5 minutes. One well (10 mm in diameter) was bored in the agar using a sterile cork borer and the agar disc was removed. Aliquot of 0.1 ml of crude latex was inoculated into a well with a pipette, and the plate was held for 2 hours at room temperature for the diffusion of extract into the agar. Negative controls are inoculated with sterilized distilled water (SDW), and Kanamycin (30 µg/disc) was used as positive control to ensure the activity of standard antibiotic against the test organisms. All inoculated plates were incubated aerobically at 29^{0}C for 48 hours. After incubation, the diameter of the zones of inhibition was measured to the nearest mm. Each antimicrobial assay was performed in at least triplicate. Mean values are reported in this report. All isolates were obtained from Microbiology Laboratory, Faculty of Medicine, King Khalid University, KSA.

Determination of minimum inhibitory concentrations (MICs). Agar plate dilution test was used to determine the MIC of antimicrobial agent.[16] Crude latex was diluted using SDW as a diluents. A 0.1 ml of each dilution was introduced into one well in a nutrient agar plate already seeded with bacterial cells as described above. Incubation was aerobic at 29^{0}C for 48 hours. The minimum concentration of latex showing a zone of inhibition was taken as the MIC. All testing were carried out at the Department of Biology, College of Science, King Khalid University. The study design was approved by the Research Ethical Committee, College of Science, King Khalid University, KSA. Analysis of variance was used to compare between data. All analyses were performed at $p<0.05$ using Minitab, version 13.1.

Results.

The in vitro antibacterial activities of latex of *A. ochroleuca* Sweet was evaluated against selected bacterial strains: *B. subtilis*, *E. aerogenes*, *M. luteus*, *E. coli*, and *S. aureus*. The results of the diameters of the zones of inhibitions of crude latex, various dilutions and MIC are presented in **Table 1**. According to the results of the study (**Table 1**), crude latex of *A. ochroleuca* and various concentration up to 100 µl/ml significantly inhibited the growth of all the bacterial strains tested, whereas 500 µl/ml of crude latex showed strong antibacterial activity in all cases, with their respective diameters of inhibition zones of 25.3 - 40.3 mm. Crude latex exhibited moderate antibacterial effects against all bacterial stains, with their respective diameters of inhibition zones of 12.6 -33.6 mm (**Figure 1**). However, 100 µl/ml of latex showed lowest antibacterial effect against all bacterial strains tested, with their respective diameters of inhibition zones of 9.30 - 32.0 mm. No antibacterial activity was observed against any bacterial strain at 10 µl/ml of crude latex and for negative control. The MICs defined as the lowest concentrations of latex that resulted in a complete growth inhibition

Table 1 - In vitro antibacterial activity of latex of *Argemone ochroleuca* Sweet.

Strains tested	*Mean diameter of zone of inhibition (mm)						
	Crude latex	500 µl/ ml	300 µl/ml	100 µl/ml	10 µl/ml	SDW	Kanamycin 30 µg/disc
Bacillus subtilis	24.0	26.0	17.3	17.6	0	0	35.0
Enterobacter aerogenes	24.0	26.6	17.7	9.30	0	0	33.0
Micrococcus luteus	33.6	40.3	39.9	32.0	0	0	41.5
Escherichia coli	16.3	30.0	10.7	10.0	0	0	35.0
Staphylococcus aureus	12.6	25.3	15.4	11.0	0	0	34.0

*mean of 3 determinations, SDW - sterile distilled water, Kanamycin as a positive control

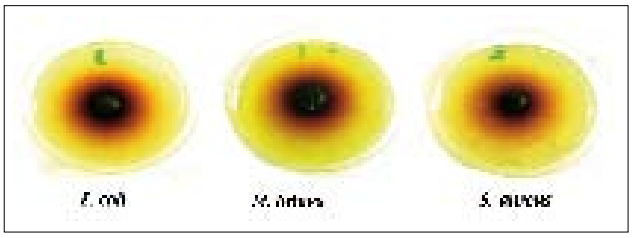

Figure 1 - Inhibition zone of *Escherichia coli (E. coli*), *Micrococcus luteus (M. luteus),* and *Staphylococcus aureus (S. aureus*) caused by crude latex of *Argemone ochroleuca* Sweet.

of the tested pathogens were found in the 100 µl/ml of crude latex of *A. ochroleuca* Sweet. As shown in **Table 1**, the efficacy of the crude latex of *A. ochroleuca* as antibacterial agent was highest against *M. luteus*, and least in *S. aureus*.

Discussion. Plants are consider as reservoirs of novel antimicrobials, and in the near future will play a crucial role to provide us with bioactive compounds, so it is very important to evaluate the natural resources in different plants to find new antimicrobial agents. It was reported that many plant extracts have antimicrobial properties.[17-19] The results of the antibacterial screening showed that the latex of *Argemone ochroleuca* has potential antibacterial effects against all of the representative human pathogenic bacteria, such as *B. subtilis, E. aerogenes, M. luteus, E. coli, and S. aureus*.

The activity of plant latex against both gram-positive and gram-negative bacteria could be attributed to the presence of broad spectrum of antibiotic compounds. Furthermore, it has been published that the chemical composition of latex is very complex, and it is difficult to pinpoint the exact responsible constituent. However, tannins and alkaloids present in latex most likely are the most effective compounds.[20] In a related study, 15 latexes from tropical plants were collected and evaluated for antibacterial activity and the results indicated that some of these extract are bioactive.[21] The latex of *A. ochroleuca* exhibited strong antibacterial effects for both gram positive bacteria (*B. subtilis, S. aureus, M. luteus*), and gram negative bacteria (*E. coli and E. aerogenes*). Although it is reported that gram negative bacteria showed less inhibition zone than gram positive bacteria due to the hydrophilic cell wall structure of gram negative bacteria, which 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.[22] Crude extract of *A. ochroleuca* was found to have less influence on tested bacteria than diluted latex (500 µl/ml). This might be due the fact that dilution will cause some bioactive material to be dissolved, and easily can diffuse through agar, in addition, the crude latex coagulates upon air exposure. Standardization for collection of latex could have been one of the limitations of the study.

In conclusion, these results are of interest since it was obtained from the crude latex of *A. ochroleuca*, which may exhibit a lower activity than the purified active compounds. Further works to discover new broad spectrum bioactive compounds are required.

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