

*Full Length Research Paper*

# Antimicrobial activity of the essential oil of *Argemone mexicana* Linn.

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The increase in the number of bacterial and fungi often implicated in nosocomial and community infections as well as the gradual development of drug resistance strains of pathogenic microorganism led to the investigation of *Argemone Mexicana* Linn against *Bacillus subtilis*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, *Candida stellatoidea* and *Candida torulopsis*. Well diffusion methods and minimum inhibitory concentrations were used. *S. aureus* and *K. pneumoniae* show relative sensitivity to essential oil from aerial and root parts, while *P. aeruginosa* and *B. subtilis* were resistant. *C. albicans* and *C. stellatoidea* were susceptible to essential oil from the aerial parts, but only *C. stellatoidea* show susceptibility to essential from the root parts. However *C. torulopsis* was resistant to both essential oil from aerial and root parts of *A. mexicana*. While *C. albicans* was only resistant to the root parts essential oil. The conclusion was that the observable inhibition on selected bacteria and fungi by essential oil of *A. mexicana* makes it a promising alternative for the development of an indigenous antimicrobial agent.

**Key words:** Sensitivity, concentrations, oil extract, antimicrobial and *Argemone mexicana*.

## INTRODUCTION

*Argemone mexicana* L (Papaveraceae) is an herb with branches, which has naturalized widely in many tropical and subtropical regions although it's a native of tropical American (Siddiqui et al., 2002). It grows commonly in abandoned and cultivated fields of South-West, Nigeria where it is renowned for its high medicinal properties. *A. Mexicana* L. is known by many names in Nigeria, it is called "Kaju" in Yoruba, "Ahon ekun" in Ijebu land, "Kadinnia" among the Hausas. It is an herb with bright yellow flowers and yellow juice. *A. mexicana*'s concoction from its ethnological survey in Nigeria is used in treatment of bacterial infection. It is widely believed that the latex from this plant cures cataract, reddening and itching in the eyes. Traditional healers in Mali use *A. 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. Seeds

are purgative and sedative. In Mexico the seed is used as an antidote to snake poisoning and the fresh yellow milky seed extract contains protein-dissolving substances, effective in the treatment of warts, coldsores, cutaneous infections, skin diseases, itches and also dropsy and jaundice (Chopra et al., 1986).

The present study was to screen the essential oils obtained from the root and the aerial part of the plant, *A. mexicana*, against some selected bacteria and fungi often implicated in nosocomial and community infection.

## MATERIALS AND METHODS

### Collection of materials

Aerial and roots parts *A. mexicana* were collected from Sagamu (46 km to Lagos, Nigeria) in May, 2008 and was authenticated by Elkalif Herbarium at Plant and Applied science department of Olabisi Onabanjo University, Ago-Iwoye, Nigeria. The plant materials were washed with clean water and air-dried.

### Microbial strain

The microorganisms were supplied from the Department of medical

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Microbiology of the University and maintained on Nutrient agar (Merck, Darmstadt, Germany). The bacteria and fungi used were selected because they have been implicated with skin, oral and intestinal tract of man. Four species of bacteria *Klebsiella pneumoniae*, *Bacillus subtilis*, *Staphylococcus aureus* (ATCC 24213), and *Pseudomonas aeruginosa* (ATCC 9027) and three species of fungi *Candida albicans* (ATCC 10231), *C. stellatoidea*, and *C. torulopsis* were used in this study.

#### Preparation of inoculum

The modified method of Benkeblia (2004) was used in which Young actively growing cells were generated by growing cells in Brain-Heart Infusion broth (Merck) for 24 h at 37°C. The cell suspensions were diluted with peptone water to provide initial cell counts of about  $3 \times 10^8$  CFU/ml. While An aliquot of 1 ml was used for antimicrobial test.

#### Essential oil isolation

The air-dried plant materials (100 g) was introduced into conical flask with 100 ml of water and plugged with cotton wool. It was hydrodistilled for 3 h using a Clevenger type apparatus with a small quantity of n-Hexane (0.3 ml) which was dried over anhydrous sodium sulfate and kept in a sealed vial at 4°C until analysis and tests.

#### Antimicrobial assays

The methods of Hufford et al. (1975) were used with some modification. Agar-well diffusion assay was used to evaluate the antimicrobial activities of the essential oil. Mueller-Hinton agar (Scharlau Chemie) was used for the culturing of bacteria while Sabouraud Dextrose agar (Difco) was used for the fungi. Twenty milliliters of the specified molten agar (45°C) was aseptically mixed with 1 ml of bacterial suspension ( $3 \times 10^8$  CFU/ml) and poured into  $100 \times 15$  mm sterile Petri dishes. Once the agar has hardened, 6 mm wells were bored using a sterile cork borer. From the various concentrations of 500, 1000 and 1500 µl/ml of the essential oil, which was prepared using methanol (75%) as diluents, 0.1 ml of the oil was separately placed into each well. The plates were incubated for 24 h at 37°C for the bacteria and 24 - 72 h at room temperature for the moulds. Ampicillin (10 µg) serves as positive control for the bacteria species while Ketoconazole (10 µg) (Pfizer) serves as positive control for the *Candida* species.

The antimicrobial activity was measured as the diameter (mm) of clear zone of growth inhibition. Methanol (75%) was included in every experiment as negative controls.

#### Microplate bioassay

The modified methods of Eloff (1998) were used to determine the minimum inhibitory concentrations through the INT microplate method. Microtiter plate was setup and the initial concentration of the hydro-distilled oil at the starting concentration of 64 mg/ml was transferred into the first well. Serial dilutions were performed so that essential oil concentrations of 32, 16, 8, 4, 2, 1, 0.5 and 0.25 mg/ml were obtained. From a prepared bacteria culture yielding approximately  $1 \times 10^6$  cells an inoculum was added to all the wells and incubated at 37°C for 24 h. 0.2 mg/ml p-iodotetrazolium violet (INT) solution was added to all inoculated wells and examined to determine a colour change in relation to concentration of microbial growth after 30, 60, 120 min and 24 h.

## RESULTS

The antimicrobial activity of *A. mexicana* at different concentrations was determined by agar well diffusion method. A total of 7 microorganisms that consisted of four bacterial and three fungi were tested. Standard antibiotics (Ampicillin and Ketoconazole) were used as positive control while 75% methanol as negative control. The percentage yield of the essential oil obtained from hydrodistillation of both the fresh aerial and root parts of *A. mexicana* was 4.2% (v/w). As shown in Table 1 the results obtained from the agar well diffusion method and the measurement of the MIC values revealed that *C. albicans* was the most sensitive with the lowest MIC values of 2.0 mg/ml in the presence of essential oil while *C. torulopsis* was least sensitive to *A. mexicana* essential oil.

The inhibition zone of the essential oil (aerial parts) of *A. mexicana* on *C. albicans* was  $11.0 \pm 0.2$  mm but was resistant to essential oil isolated from the root parts. The inhibition zones recorded for the essential oil of the aerial and root parts of *A. mexicana* against *C. stellatoidea* was almost the same ( $10.0 \pm 0.1$  mm and  $10.0 \pm 0.2$  mm respectively) but *C. torulopsis* was resistant to essential oil isolated from the aerial and the root parts of *A. mexicana*.

Also in Table 2, *K. pneumoniae* was discovered to be the most sensitive recording the lowest MIC value of 2.0 mg/ml, while *B. subtilis* and *P. aeruginosa* were resistant to the essential oil obtained from the aerial and root parts of *A. mexicana*.

Except for *C. albicans* in Table 1, the performance of both the aerial part and root part followed similar pattern. The zones of inhibition were nearly the same. This may probably be due to similar compound being present in both part of the plant at almost similar concentration in the essential oils. This however needs to be confirmed by further research analysis of the constituent of the essential oils. The zones of inhibition recorded in this study were lower in size when compared with the results obtained by Indranil et al. (2006). The difference could be attributed to the methods used and the compounds on target. While this study aims at isolating the essential oil, Indranil et al. (2006) used the crude extract of *A. mexicana* with methanol and Cold and hot water as solvent. Also the variation observed in this study could be due to many factors such as agar composition as well as the volatility of oil in the open air system (Alvaro et al., 2003). Recently, Inouye et al. (2001) reported that the MIC values of essential oils were lowered two- to eightfold when evaporation was prevented.

## DISCUSSION

In this study, correlation between the two screening methods revealed that larger zones of inhibition correlated with lower MIC values (Tables 1 and 2).

**Table 1.** Antifungi activity of the essential oil of the aerial and root parts of *Argemone Mexicana* Linn.

Part of plant	Inhibition Zones (mm)					
	<i>C. albicans</i>		<i>C. stellatoidea</i>		<i>C. torulopsis</i>	
	Aerial	Root	Aerial	Root	Aerial	Root
Essential oil (10ul/ml)	11.0 ± 0.2	na	10.0 ± 0.1	10.0 ± 0.2	na	na
MIC(mg/ml)	2.0	na	4.0	4.0	na	na
Control (Ketoconazole, 10 ug/ml)	17.0 ± 0.1	12.0 ± 0.2	10.0 ± 0.3	14.0 ± 0.1	10.0 ± 0.2	11.0 ± 0.3

MIC, minimum inhibitory concentration, na, not active

**Table 2.** Antibacterial activity of the essential oil of the aerial and root parts of *Argemone mexicana* Linn.

Part of plant	Inhibition Zones (mm)							
	<i>B. subtilis</i>		<i>P. aeruginosa</i>		<i>K. pneumoniae</i>		<i>S. aureus</i>	
	Aerial	Root	Aerial	Root	Aerial	Root	Aerial	Root
Essential oil (10 ul/ml)	na	na	na	na	10.0 ± 0.1	10.0 ± 0.2	8.0 ± 0.2	8.0 ± 0.1
MIC(mg/ml)	na	na	na	na	2.0	2.0	4.0	4.0
Control (Ampicillin 10 ug/ml)	25.0 ± 0.1	25.0 ± 0.3	25.0 ± 0.2	16.0 ± 0.1	22.0 ± 0.3	21.0 ± 0.3	25.0 ± 0.1	18.0 ± 0.2

MIC, minimum inhibitory concentration, na, Not active

The demonstration of sensitivity as a result of incorporation of oil extract of *Argemone mexicana* at various levels of concentration on *C. albicans*, *C. stellatoidea*, *C. torulopsis*, *K. pneumoniae*, *B. subtilis*, *S. aureus* and *P. aeruginosa* confirmed the suggestion that the plant oil extract contain potent antimicrobial constituents (Chopra et al., 1986; Sharma and Nathawat, 1987).

Observed inhibition of the bacterial by *A. mexicana* could be of significant importance in the pharmaceutical industry, especially for treatment of diseases caused by some of the bacteria and fungi tested in this study. *A. mexicana* derived compounds could play an important role in the development of drugs to control several diseases caused by various bacterial, particularly the pathogenic *P. aeruginosa* (Siddiqui et al., 2002). The activity shown against *C. albicans*, *C. stellatoidea* and *C. torulopsis*, further gives credit to the plant, as *Candida* species are known to be resistance to most antibiotic. *Candida* species are known to be involved in several diseases such as intertrigo and Diaper rashes and chronic mucocutaneous candidiasis all of which are skin disease. This observation is perfectly in line with the assertion of Coffey (1993) who stated that *A. mexicana* can be used for the treatment of cutaneous affections, skin diseases and itches.

## Conclusion

The observable inhibition of selected bacteria and Fungi by essential oils of *A. mexicana* makes it a promising alternative compound for the development of a new indigenous antimicrobial agent against *C. albicans*, *C. stellatoidea*, *C. torulopsis*, *S. aureus*, *B. subtilis*, *P.*

*aeruginosa* and *K. pneumoniae*. The antifungi and the antibacterial activities are concentrated in both the aerial and root parts of *A. mexicana*.

Pharmaceutical and toxicological studies of the essential oils can be done in future to identify its pure compound and elucidate the components responsible for these antimicrobial activities.

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