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Antibacterial Activity of Crude Extracts and Alkaloidal Fractions of *Argemone mexicana* Linn. (Papaveraceae)

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Abstract

Emergence of multi-drug resistant pathogens has necessitated the need for continuous research to discover and develop new and more effective antimicrobial drugs. Medicinal plants have always been a potential source of antimicrobial drugs and varieties of lead compounds. *Argemone mexicana,* a tropical shrub traditionally used to treat eye infections, inflammation and infertility was investigated for antibacterial activity. Crude chloroform and methanol extracts of leaves and stems, as well as fractions and crystals from chromatographic column were screened for *in-vitro* antibacterial activity against *Staphylococcus aureus* (NCTC 6571), *Bacillus subtilis* (ATCC 6633) *Escherichia coli* (NCTC 9001) and *Pseudomonas aeruginosa* (NCTC 6570) using agar-cup diffusion method. Phytochemical screening for secondary metabolites revealed the presence of alkaloids (opium and indole types) and tannins. The crude extracts showed good activity at 100mg/ml against the bacterial strains tested while at 10mg/ml only *E. coli* and *B. subtilis* were susceptible. Ten fractions were isolated from column chromatography (nine of which were alkaloidal in nature), and they all showed varying but significant degree of activity on most of the tested organisms at 100µg/ml. The needle-like crystals recovered from methanol extract showed weak antibacterial activity on some of the test organisms. The study has shown that *A. mexicana* leaves and stem bark has antibacterial activity which justified the use of this plant in traditional medicine as anti-infective agent. Further, the fact that most fractions showed antibacterial activity is an indication that *Argemone mexicana* leaf and bark has many antibacterial constituents that are mainly alkaloidal.

Introduction

The use of herbs and phytochemicals in the treatment of diseases is increasing and many studies have been conducted in different countries to prove the efficacy of medicinal plants in the treatment of infectious diseases ((Olukoya *et al.*, 1993, Idowu *et al.*, 2005). Medicinal plants have always been a major source of many important scientific drugs of the modern world (Sofowora, 1982). According to the WHO (Santos *et al.*, 1995), medicinal plants would be the best source for obtaining a variety of drugs. About 80% populations of the developed countries use traditional medicines, derived from medicinal plants. *Argemone mexicana* Linn. (Papaveraceae), commonly known as prickly poppy and called "ahon-ekun" in Yoruba land, is used as a medicinal plant in several countries. In Mexico, the seeds are considered as an antidote to

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snake venom while the smokes of the seeds are used to relieve toothache in. India. The fresh yellow, milky seed extract contains protein-dissolving substances, effective in the treatment of warts, cold sores, cutaneous infections, skin diseases, itches, and also dropsy and jaundice (Chopra *et al.*, 1986). Schlotterbeck in 1902 isolated and identified two alkaloids, berberine and protopine from *Argemone mexicana*. Chang *et al.* (2003) isolated many alkaloids from the Formosan species of which benzo[*c*]phenanthridine (±)-6-acetonyldihydrochelerythrine exhibited significant anti-HIV activity while Bhattacharjee *et al.* (2010) isolated an antibacterial agent N-demethyloxysanguinarine by chromatographic methods from the seed extract. Bhattacharjee *et al.* (2006) reported the antimicrobial activity of the seed and leaf extracts of the Indian species on four pathogens. For the Nigerian species, this study reports the preliminary phytochemistry and antimicrobial screening of the crude extracts, fractions and crystals from the leaf and stem extracts, to establish the folkloric use of *Argemone mexicana* to treat infectious diseases in Nigeria and possible isolation of the antimicrobial constituents.

Materials and Methods

Plant materials

Fresh leaves and stems of *Argemone mexicana* were collected from Olla, Irepodun LGA, Kwara State, Nigeria during the raining season. Using the leaves, fruits and flowering tops, the plant was authenticated in the Forestry Research Institute of Nigeria (FRIN), Jericho, Ibadan, Nigeria where herbarium voucher specimens were deposited. The plant samples were prepared as described by Williamson *et al.* (1996). The dried pulverized leaves and stem barks were extracted with successive quantities of chloroform and methanol using soxhlet apparatus. The extracts were concentrated in-vacuo, air dried to constant weight and were stored at 4°C for use.

Microorganisms and media

Staphylococcus aureus (NCTC 6571), *Bacillus subtilis* (ATCC 6633) *Escherichia coli* (NCTC 9001) and *Pseudomonas aeruginosa* (NCTC 6570) were obtained from University of Strachtide, Glasgow and maintained on agar slants in Pharmaceutical Microbiology Laboratory, University of Ibadan, Nigeria. All the media: Nutrient Agar (No 2), Nutrient Broth and Mueller Hilton agar were from Oxoid Laboratories, England.

Preliminary phytochemical screening

The powdered leaves and stem bark were tested for the presence of the following secondary metabolites: alkaloids (and alkaloidal types), anthraquinones, cardenolides, saponins and tannins using standard procedures (Harbone, 1984; Sofowora, 1982).

TLC and Column chromatography

Methanol extracts of *A. mexicana* was first analyzed on a precoated TLC plate, silica gel G F254 (Sandoz, Basel) using a solvent system containing dichloromethane-methanol (2:1) as mobile phase to effect a good separation yielding seven different spots whose characteristics were shown in Tables 2 and 3. The crude extracts were dissolved in dichloromethane-methanol (1:1) solvent for spotting following standard procedures (Touchstone, 1992). Column chromatography of the methanol extract on silica gel G 60 (70-230 mesh, ASTM), eluting with dichloromethane: methanol gradients of increasing polarity afforded a total of 100 fractions of 10mls each. The eluates were monitored on TLC plates and fractions with similar component were pooled together to give a final 10 different fractions which were tested for antimicrobial activity using *S. aureus* (NCTC 6571), *B. subtilis* (ATCC 6633), *E. coli* (NCTC 9001) and *P. aeruginosa* (NCTC 6570). The characteristics of each fraction labeled A–J are shown in Table 4.

Crystals recovered from the methanolic extracts of both leaves and stems were purified by recrystallisation and analysed by TLC. Melting point and antimicrobial activity were determined.

Antimicrobial screening

Using agar-cup diffusion method, a 0.1ml of a two-fold dilution of each bacteria culture was

seeded into nutrient broth. A sterile cork borer (diameter 6mm) was used to punch uniform well on the set and dried agar. Each well was filled with a 0.2ml of the crude extracts (10 and 100mg/ml), isolated fractions (100µg/ml) and crystals (100mg/ml) using methanol (40%v/v) as solvent and as a control. Ampicillin (Beecham, England) at 10mg/ml was used as control antibacterial agents. Bacterial plates were incubated at 37°C for 24 hours. All tests were performed in triplicates and the average diameter of the zones of inhibition was used as a measure of antibacterial activity (Perez *et al.*, 1990).

Results and Discussion

The pulverized leaf and stem bark yielded 13.40% and 5.76% respectively on extraction The phytochemical screening of *Argemone mexicana* for secondary metabolites showed the plant contained alkaloids and hydrolysable tannins, and majority of the components were found to be alkaloids as detected by the Dragendorff's and other reagents. The alkaloids present were found to be opium and indole types as revealed by meconic acid and indole tests respectively. Atropine alkaloid was absent when subjected to Vitali-Morin test. This is in accordance with the results obtained by Kreger (1983) and Mahesh *et al.* (2012). In this study, the total alkaloid in the plant was found to be 0.125%, an indication of high alkaloidal content which is consistent with the family Papaveraceae. The absence of cardiac glycosides in *A. mexicana* leaf and stem samples was surprising considering the marked cardiovascular action reported by Bose *et al.* (1963).

Table 1: Result of Phytochemical Screening of *Argemone mexicana* Leaves

Secondary metabolites	Test performed	Colour reaction	Result
General Alkaloids	Dragendorff's reagent	Red brown precipitate	+
	Mayer's reagent	Creamy precipitate	+
	Wagner's reagent	Dark brown precipitate	+
	Tannic acid reagent	Brownish precipitate	+
	Picric acid reagent	Intense yellow precipitate	+
Specific Alkaloids			
a) Tropane alkaloid	Vitali-Morin test	No pink colouration	-
b) Opium alkaloid	Meconic acid test	Deep red colouration	+
c) Indole alkaloid	Indole test	Yellowish green colouration	+
Anthraquinones	Borntrager's test	Colourless aqueous layer	-
Cardenolides	Keller-Kiliani test	No brown ring at interphase nor green colour in acetic acid layer	-
	Kedde's test	No brown colour	-
Saponins	Frothing test	No frothing, no haemolysis	-
	Emulsifying test	No emulsion	-
Tannins	Ferric chloride test	Blue-black precipitate	+

Key: - absent, + present

Table 2: TLC Analysis of Leaf Extract of *Argemone mexicana*

Spots	Rf	Colour (uv) 365nm	Colour (iodine tank)
A	0.04	Light yellow	-
B	0.15	Yellowish green	-
C	0.35	Yellowish green	Reddish brown
D	0.58	Yellowish green	Reddish brown
E	0.83	Green	Reddish brown
F	0.95	Yellow	-
G	1.00	Dark green	Green

Table 3: TLC Analysis of Stem Bark Extract of *Argemone mexicana*

Spots	Rf	Colour (uv) 365nm	Colour (iodine tank)
A	0.03	Blue	-
B	0.13	Yellow	Reddish brown
C	0.20	Yellowish green	-
D	0.25	Yellowish green	Reddish brown
E	0.43	Yellowish green	Reddish brown
F	0.85	Yellow	-
G	1.00	Green	Green

Mobile phase =Dichloroethane : Acetone (2:1)
Stationary phase = Silica Gel G. (precoated plate, Basel)
Temperature: 25°C, Visualization: UV-light 365nm and iodine vapour

Table 4: Column Chromatographic Separation of Methanol Extract of *A. mexicana* Leaves

Eluted fractions	Solvent system used (%)	Colour (in daylight)	Colour (in UV)	Rf	Fractions label
2	Chloroform (100)	Greenish brown	Yellow	0.98	A
3	Chloroform/acetone (90-10)	-	Light yellow	0.90	B
4	Chloroform/acetone (90-10)	-	-	0.90	C
8	Chloroform/acetone (80-20)	-	Blue	0.95	D
9	Chloroform/acetone (50-50)	-	Blue	0.98	E
11	Chloroform/acetone (20-80)	-	Light yellow	0.36	F
17	Chloroform/acetone (20-80)	Yellow	Yellowish green	0.31	G
23	Chloroform/acetone (10-90)	-	Yellow	0.18	H
26	Chloroform/acetone (10-90)	-	Blue	0.13	I
28	Acetone (100)	-	Yellowish brown	0.09	J

Table 5: Antimicrobial Screening of Extracts and Crystals from Leaf and Stem of *A. mexicana*

		Diameter Zone of Inhibition (mm)*			
Extracts	Conc. (mg/ml)	*S. aureus*	*B. subtilis*	*E. coli*	*P. aeruginosa*
Leaf	10	-	9.0±0.0	10.5±0.2	-
	100	10.5±0.4	11.0±0.6	10.5±0.3	12.0±0.3
Stem bark	100	9.3±0.6	10.5±0.4	-	-
	100	13.0±0.0	10.5±1.0	11.0±0.0	10.0±0.5
Ampicillin	10	13.0±0.0	-	-	13.0±0.0
Crystals (Leaf)	100	-	11.0±0.2	9.0±0.5	-
Crystals (stem bark)	100	9.0±0.0	9.0±0.0	9.0±0.0	-

* Means of triplicate values ± standard error; – not active

Table 6: Antimicrobial Activity of Fractions from Methanolic Extract of *A. mexicana* Leaves

	Diameter Zone of Inhibition (mm)*			
Fractions	*S. aureus*	*B. subtilis*	*E. coli*	*P. aeruginosa*
A	12.0±0.0	10.0±0.0	12.5±0.2	12.0±0.2
B	9.3±0.3	12.4±0.4	11.0±0.0	10.0±0.2
C	11.0±0.5	15.0±0.2	16.3±0.3	11.5±0.4
D	9.0±0.0	13.2±0.6	12.2±1.0	11.0±0.0
E	11.2±0.6	14.0±0.0	13.0±0.0	13.5±0.5
F	10.0±0.5	13.3±0.3	14.1±0.6	12.0±0.0
G	16.0±0.3	25.0±0.7	22.0±0.5	23.5±1.0
H	9.0±0.0	11.0±0.0	10.0±0.0	10.0±0.2
I	11.3±0.3	16.5±0.5	13.5±0.2	11.0±0.0
J	9.0±0.0	12.0±0.4	9.0±0.0	10.0±0.0
Ampicillin	13.0±0.0	-	-	13.0±0.0

* Means of triplicate values. – not active

The TLC analysis of crude extract of both leaf and stem samples showed that the plant parts contained similar, though not exactly equal constituents. Not less than seven components were detected on the analytical TLC, which was a guide to the number of fractions expected from column chromatographic isolation (Table 4). Column chromatography with dichloroethane-acetone mixture as eluent afforded ten major fractions, of which only one was found not to be alkaloidal.

Crystals recovered and purified were long needle-like, slightly soluble in acetone, chloroform and ether and melted between 115-120°C. The crystals tested positive to Dragendorff's spray with a reddish brown coloration confirming them to be alkaloids.

Antimicrobial screening of the crude extracts of leaf and stem (Table 5) showed that the extracts possess broad spectrum antibacterial activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Bacillus subtilis*. The activity of the extracts with the maximum zone of inhibition of 13mm at 100mg/ml was not as prominent as those of the fractions. The activity of the extracts at 10mg/ml was mild or negligible. Ampicillin at 10mg/ml showed more activity than the crude extracts. Gomare and Ghuget, (2012) reported that the ethanol and methanol extracts of

Argemone mexicana were more potent than the aqueous extracts. Bhattacharjee *et al.* (2006) reported that the methanol extracts of the leaves and seeds of the *A. mexicana* showed greater antibacterial activity than the corresponding water extracts.

All fractions from column chromatography showed broad spectrum of activity against Gram positive and Gram negative organisms used in the study (Table 6). Fractions labeled A, C, E, G, and I showed prominent activity. Fraction G was found to occur in abundant quantity and showed remarkable activity against both Gram-positive and Gram-negative organisms used in this study with diameter zone of inhibition that is higher than that of the reference drug, ampicillin. While fraction G showed good antibacterial activity the purified crystals from the methanolic extract showed relatively weak activity against the test organisms. Also, fraction A, though not alkaloidal but rather phenolic (tannins) possessed antimicrobial activity which is in line with reports of Scalbert (1991).

Conclusion

This study has shown that the leaf and stem extracts of *Argemone mexicana* has antibacterial property which therefore support the age long use of the plant as wound disinfectant, eye-lotion and mouth wash in tooth ache. The presence of many alkaloidal fractions that possessed antibacterial activity is interesting, necessitating further phytochemical works to characterize the active compounds for possible development of new chemotherapeutic agents.

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