Antimicrobial studies of the leaf extract of *Argemone mexicana* and its ointment formulation

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ABSTRACT

Background: Skin infections are rampant in children and there is a widespread resistance to common antibacterial and antifungal therapies for these infections.

Objective: This study aims to screen the extract of *Argemone mexicana leaf* for antibacterial and antifungal activity; and formulate the extract into ointment dosage forms using simple ointment B.P.C.

Methods: Preliminary phytochemical studies were performed and **t**he methanol, ethanol and n-hexane leaf extracts of *Argemone mexicana* Linn. were evaluated for antimicrobial activity against some pathogenic bacteria- *Pseudomonas aeruginosa*, *Escherichia coli, Klebsiella pneumonia*(Gram negative) and *Streptococcus spp, Staphylococcus aureus* (Gram positive) and dermatophytes such as *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Microsporum audouinii* (fungi) using well diffusion method. The most active extract was formulated into ointment and evaluated for physicochemical and antimicrobial activity.

Results: Flavonoids and alkaloids were present in all the extracts while anthraquinones and saponin were present in methanol and n hexane extract. The ranking for extract activity was methanol>n- hexane> ethanol. The methanol extract showed comparable activity with gentamicin against *Staphylococcus aureus* and higher activity against *Escherichia coli* at 250mg/ml and 500mg/ml. The order of activity of the methanol extract against the test organisms is *Staphylococcus aureus*> *Escherichia coli*> *Streptococcus spp*> *Klebsiella pneumonia*> *Trichophyton mentagrophyte*> *Trichophyton rubrums*> *Microsporum audouinii.* There was no significant variation (P> 0.05) in the zones of inhibition of bacteria with the methanol extract. The formulated ointment had a characteristic odour, smooth texture, was easy to apply on rubbing, easy to remove with soap and water and non-irritant on skin.

Conclusion: The result of this study supports the traditional uses of these plants and shows that the plant extract possesses antibacterial and antifungal activities

Key words: *Argemone mexicana*, antibacterial activity, antifungal activities, phytochemical analysis, ointment formulation

Etudes antimicrobiennes de l'extrait de la feuille d'*Argémone Mexicana* et sa formulation en pommade

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RESUME

Contexte: Les infections de la peau font des ravages chez les enfants et il existe une résistance très répandue aux thérapies antibactériennes et antifongiques courantes pour ces infections.

Objectif: Cet étude a pour objectif d'examiner l'extrait de la feuille d'*Argémone mexicana pour son activité* antibactérienne et antifongique; et formuler l'extrait en formes de dosage en pommade utilisant une simple pommade B.P.C.

Méthodes: Des recherches phytochimiques préliminaires ont été conduites et le méthanol, l'éthanol et les extraits n-hexane de la feuille d'*Argémone mexicana* Linn. ont été évalués pour l'activité antimicrobienne contre certaines bactéries pathogéniques - *Pseudomonas aeruginosa*, *Escherichia coli, Klebsiella pneumonia* (Gram négatif) et *Streptococcus spp, Staphylococcus aureus* (Gram positive) et les dermatophytes tels que *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Microsporum audouinii* (fongus) utilisant la méthode de bonne diffusion. L'extrait le plus actif fut formulé en pommade et évalué pour son activité physicochimique et antimicrobienne.

Résultats: Les flavonoïdes et les alcaloïdes étaient présents dans tous les extraits alors que les anthraquinones et saponine étaient présentes dans l'extrait de méthanol et n hexane. Le classement pour l'activité de l'extrait était méthanol>n- hexane> éthanol. L'extrait de méthanol a présenté une activité comparable avec la gentamicine contre *Staphylococcus aureus* et une activité supérieure contre Escherichia coli à 250 mg/ml et 500 mg/ml. L'ordre d'activité de l'extrait de méthanol contre les organismes de test est *Staphylococcus aureus> Escherichia coli> Streptococcus spp> Klebsiella pneumonia> Trichophyton mentagrophyte> Trichophyton rubrums> Microsporum audouinii.* Il n'y avait aucune variation importante (P> 0.05) dans les zones d'inhibition de bactérie avec l'extrait de méthanol. La pommade formulée a une odeur caractéristique, une texture lisse, était facile à appliquer dans le massage, facile à enlever avec du savon et de l'eau et non-irritant sur la peau.

Conclusion: Le résultat de cette recherche soutient les usages traditionnels de ces plantes et montre que l'extrait de la plante possède des activités antibactériennes et antifongiques

Mots-clés: *Argémone mexicana*, activité antibactérienne, activités antifongiques, analyse phytochimique, formulation de pommade

INTRODUCTION

Skin infections are a common reason for consultation in primary and in dermatological practice.[1] Patients with skin diseases constitute about 15 per cent of the total out-patients in a general hospital and most children will have a skin infection at some time. [2]

Impetigo is a contagious skin infection that most often produces blisters or sores on the face, neck, hands and also the diaper area. People who play close contact sports such as rugby, American football and wrestling are also susceptible, regardless of age.[3] It is primarily caused by *Staphylococcus aureus,* and sometimes by *Streptococcus pyogenes*. Ringworm is a fungal infection of the skin in humans. Fungi are organisms that survive by feeding on plant or animal material. Those that cause parasitic infection (*Dermatophytes)* feed on keratin, the material found in the outer layer of skin, hair, and nails. These fungi thrive best on skin that is moist, hot, and hidden from the light. It is estimated that about 20 per cent of the population is infected by ringworm or one of the other dermatophytoses at any given moment. Ringworm is caused by several different fungus organisms that all belong to a group called "Dermatophytes" of the genera *Microsporum*, and *Trichophyton* and species *Epidermophyton floccosum*.

The plant *Argemone mexicana* Linn. belongs to family of Papaveraceae which is commonly known as Mexican poppy or prickly poppy. *Argemone mexicana* is a perennial growing to 0.6 m (2ft) by 0.5 m (1ft 8in). It is in flower from June to August, and the seeds ripen from July to September. The flowers are hermaphrodite (have both male and female organs), The plant is self-fertilizing and it is known to possess antimalarial and antimicrobial activities. In Nigeria, it grows commonly in abandoned and cultivated fields of South-West, Nigeria where it is renowned for its high medicinal properties.[4] It is an herb with bright yellow flowers and yellow juice.

Traditionally, the leaves are useful in cough, wounds, ulcer, warts, cold sores, cutaneous infections, skin diseases, itches etc. The continuous evolution of bacteria resistance to currently available antibiotics has necessitated the search for novel and more effective antimicrobial agents. Historically, plants have provided a source of novel drug compounds as plant derived medicines have made large contributions to human health. The objective of this study is to screen the extract of *Argemone mexicana leave* for antibacterial and antifungal activity; and formulate the extract into ointment dosage forms using simple ointment B.P.C.

MATERIALS AND METHOD

Materials

The materials used include Methanol (MBS scientific limited, wickford, UK), Cetostearyl alcohol (BDH chemicals, Poole, England), Wool fat (BDH chemicals, Poole, England), White soft paraffin (BDH chemicals, Poole, England), Hard paraffin (BDH chemicals, Poole, England), Ethanol, *Argemone mexicana* leaf , n-Hexane, Nutrient agar (LAB M, UK), Saboraud dextrose agar (OXOID, UK), Nutrient broth (OXOID, UK). The micro organisms used were obtained from the Department of Pharmaceutical Microbiology,Faculty of Pharmacy, Sagamu, Ogun state. They include: *Staphylococcusaureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus spp*, *Klebsiella pneumonia*, *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Microsporum audouinii.*

Methods

Processing and extraction of *Argemone mexicana* leaf

The process of extraction of the chemical constituents from the leaves collected from Sagamu, Nigeria can broadly be divided into two stages; the pre-treatment and extraction stage. The pre-treatment involves the washing of the leaves (collected between 17:00-20:00 hrs) with distilled water and then air-dried at room temperature for 2 weeks. The plant was authenticated at the Forestry Research Institute of Nigeria (FRIN), Nigeria. The dried leaves were pulverized using a mechanical mill, sieved and weighed. The powdered leave (50mg) were macerated using three different (500mls) solvents (methanol, n-hexane and ethanol) in the ratio of 1:10 for 72hours. The mixture was then filtered using whatman's No 1 filter paper to remove extraneous materials. The filtrate was transferred into a clean bottle and the solvent present was allowed to evaporate using a rotatory evaporator. The remnant was then concentrated into a gel-like form using a heating mantle at 40 ^{0}C and the percentage yield was determined.

Phytochemical analysis of the Leaf Extract

The leaf extracts of *Argemone mexicana* were analyzed for the presence of tannins, saponins, flavonoids, alkaloids and reducing sugar, according to standard methods.[5-8]

Screening of *Argemone mexicana* leaf extract for antibacterial activity

Agar well diffusion technique as described by Adeniyi *et al.*[9] was adopted for the study. Different extract concentration (62.5mg/ml, 125 mg/ml, 250 mg/ml and 500 mg/ml /disc) were prepared using the solvent of

extraction (methanol, ethanol and n-hexane). About 0.2mls of an overnight culture of bacteria isolates in the nutrient broth were seeded into sterile agar plate and swirled for uniform distribution of the isolates and allowed to set. With the aid of sterile cork borer, wells of about 6mm diameter were bored on the plate and equal volume of the leaf extract (0.5ml) was transferred in the well with the aid of a micropipette. The plates were allowed to stand for one hour at room temperature to allow proper diffusion (pre-diffusion) of the extract.[10] The plates were then incubated at 37ºC for 24hours. Gentamicin was used as the standard. The zones of inhibitions were measured in millimeter (mm). The average value of the duplicate sets of the experiment and deviations were calculated.

Screening of *Argemone mexicana* leaf extract for antifungal activity

Agar well diffusion technique as described by Adeniyi *et al.*[9] was adopted for the study. Different extract concentration (62.5-500 mg/mL) were prepared using the solvent of extraction (methanol, ethanol and n-hexane), about 0.2mLs of an overnight culture of fungal strains in Sabouraud's broth were seeded into molten Sabouraud dextrose agar and poured in the sterile petri plates and swirled for uniform distribution of the isolates and allowed to set. Wells of about 6mm diameter were bored on the plate using sterile cork borer. Equal volume of the leaf extract (0.5mL) was transferred into the well with the aid of a micropipette. The plates were allowed to stand for one hour at room temperature to allow proper diffusion (pre-diffusion) of the extract.[10] The plates were then incubated at 27 °C for 48hrs. ketoconazole was used as the standard. Following an incubation period of 48 hrs, plates were removed from the incubator and antifungal activity was evaluated by measuring zones of inhibition of fungal growth.

Formulation of A*rgemone mexicana* leaf extract Ointment

Simple ointment BPC (1979) was prepared according specifications. The leaf extract was formulated into different concentration (1-20 %w/w) of 25 g ointment. Fourteen (14) ointment samples were formulated with varying concentrations of Simple Ointment.

Antibacterial and antifungal screening of *Argemone mexicana* Leaf Extract Ointment

Similar procedure used for the screening of the extracts was employed for the screening of the ointments formulations at different concentrations (0-20 %) for both antibacterial and antifungal screening.

Determination of Minimum Inhibitory Concentration (MIC)

Using the serial dilution method, various concentrations of the ointment formulations were prepared by dissolving in dimethyl sulphoxide (DMSO). MIC was performed by serial two fold dilution method at concentration ranging from 0.1-20 %. The culture plates were again seeded with test bacterial and fungal organisms and allowed to solidify and thereafter punched with a sterile cork borer (6.0 mm diameter) to cut uniform wells. The open wells were filled with 0.1 mL of the sample extract. The plates were then incubated at 37 ^{0}C for 24 h for bacteria and 27 ^{0}C for fungi organism. The lowest concentration of the extract that showed inhibition of growth of the test organisms was noted. The least concentration of dilution showing clear zone of inhibition was taken as Minimum inhibitory concentration.

Determination of Minimum Bactericidal/ Fungicidal Concentration (MBC/MFC)

The cidal concentration was carried out using the method of Doughari *et al*.[11] The ointment which does not show any visible sign of growth with the unaided eyes was removed and inoculated into a Mueller Hinton agar plates, allowed to stand for 10 minutes at room temperature, incubated at 37 ^{0}C for 24 h for bacteria and 27 ^{0}C for fungi and observed. The concentration at which there was no visible growth on the plate was recorded as the minimum cidal concentration.

Characterization of *Argemone mexicana* Ointment

Physical evaluation was carried out by observing the appearance, colour, texture, odour, ease of application, ease of removal, and feel on skin and irritation on skin. The pH of the ointment was determined and the ointment samples were observed for any instability between the drug and the type and amount of base used. The spreadability was determined by putting measured mass on the two slides having the ointment in between for a particular time, after which the upper and lower slides are separated from each other in a particular direction, the time taken for both slide to separate is taken as the spreading time. Viscosity was determined at 20 rpm, 50 rpm and 100 rpm on a the Brookfield viscometer using spindle 5 at 40 ^{0}C

RESULTS

Ethanol extract gave the highest yield (43.9%), followed by the methanol extract (35.6 %) while the n-hexane extract gave a 3.0 % yield. Table 1 shows the antibacterial activity of *Argemone mexicana* leaf extracts. As the concentration increases, the zone of inhibition increased which signifies that inhibitory effect of the test samples was concentration dependent.

The colour of the ointment containing *Argemone mexicana* extract varied from light green (1 -4%) to dark green (5-8 %) and to army green (10-20 %). The formulation had a characteristic odour, hard and smooth in texture, smooth on skin feel, easy to apply on rubbing, easy to remove with soap and water and non-irritant on skin. Table 2 shows that the pH of the formulation at all concentration was slightly acidic (4 - 6.5).

The rheological properties of *Argemone mexicana* are presented in Table 3. The formulation having 8 % of *Argemone mexicana* extract has the highest viscosity at the lowest shear rate (800.0 at 20 RPM) and at the highest shear rate (188.0 at 100RPM).

Table 4 shows that the minimum Inhibitory Concentration (MIC), which is the smallest concentration which prevents the growth of the test organism, was least against *Trichophyton mentagrophytes* (10 %w/w) while for *Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli* there were growth at all the concentrations.

Table 1: Antibacterial activity of *Argemone mexicana* Leaf extracts.

Organism	Zones of Inhibition (mm)							
	Methanol extract		Ethanol extract		N Hexane extract		Gentamycin	Ketoconazole
	500mg/ml	250mg/ml	500mg/ml	250mg/ml	500mg/ml	250mg/ml	8mg/ml	20mg/ml
Pseudomonas aeruginosa	00	00	00	00	00	00	24.0	00
Staphylococcus aureus	30.0	30.0	00	00	11.0	06.0	28.5	00
Streptococcus	23.0	20.0	00	00	11.0	06.0	16.5	00
Escherichia coli	29.0	30.0	00	30.0	06.0	05.0	17.0	00
Klebsiella spp	22.5	00.0	00	00	14.5	11.5	25.0	00
Trichophyton mentagrophyte	16.0	11.5	00	00	06.0	00	00	12.0
Trichophyton rubrum	15.5	14.0	06.0	00	11.0	04.0	00	13.5
Microsporum Audouinii	12.0	14.0	00	00	14.5	07.0	00	7.5

Table 2: Physicochemical Parameters of *Argemone mexicana* Ointment

Cream conc. (% w/w)	pH	Spread ability (sec)
0	6.42 ± 0.02	10.22 ± 0.08
1	5.77 ± 0.01	10.12 ± 0.19
2	6.45 ± 0.02	12.35 ± 0.11
3	5.96 ± 0.04	12.11 ± 0.15
4	6.35 ± 0.02	12.51 ± 0.16
5	6.40 ± 0.00	12.8 ± 0.00
6	6.32± 0.00	10.40 ± 0.20
7	5.91 ± 0.02	11.21 ± 0.00
8	6.49± 0.04	14.42 ± 0.14
10	5.71 ± 0.02	12.27 ± 0.15
11	6.37 ± 0.01	12.13 ± 0.90
12	6.10 ± 0.00	10.27 ± 0.11
15	6.49 ± 0.00	9.37 ± 0.12
20	6.22 ± 0.02	10.36 ± 0.17

TablE 3: Rheological properties of *Argemone mexicana* ointment

Sample (% w/w)	Speed of rotation					
	20 rpm		50 rpm		100 rpm	
	Viscosity	Torque	Viscosity	Torque	Viscosity	Torque
0	130.0	0.7	80.0	0.4	42.0	1.9
1	80.0	0.4	65.0	1.6	32.0	1.6
2	40.0	0.2	24.0	0.3	16.0	0.4
3	190.0	1.6	92.0	2.3	56.0	2.8
4	260.0	1.3	152.0	1.6	72.0	1.8
5	550.0	0.9	200.0	1.0	130	1.3
6	720.0	7.2	256.0	6.4	124.0	6.2
7	120.0	0.6	56.0	0.7	32.0	0.8
8	800.0	4.0	240.0	3.0	188.0	4.7
10	140.0	0.7	64.0	0.8	36.0	0.9

Table 4: Minimum Inhibitory Concentration (MIC) of *Argemone mexicana* ointment

Micro-organism	Concentration (% w/v)														
	0	1	2	3	4	5	6	7	8	10	11	12	15	20	MIC
SA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	>20
STP	+	+	+	+	+	+	+	+	+	+	+	-	-	-	12
EC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	>20
KLEB	+	+	+	+	+	+	+	+	+	+	+	+	+	-	20
PA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	>20
TR	+	+	+	+	+	+	+	+	+	+	+	+	+	-	20
TM	+	+	+	+	+	+	+	+	+	-	-	-	-	-	10
MA	+	+	+	+	+	+	+	+	+	+	+	-	-	-	12

+ = Turbidity, - = Clarity

PA – *Pseudomonas aeruginosa,*SA - *Staphylococcus aureus,* STP - *Streptococcus spp* EC- *Escherichia coli,* KLEB - *Klebsiella pneumonia,* TM -*Trichophyton mentagrophytes,* TR - *Trichophyton rubrum,* MA- *Microsporum audouinii,* MIC = Minimum inhibitory concentration,

DISCUSSION

Phytochemical compounds are known to play important role in bioactivity of medicinal plants and these help to produce definite physiological action on the human body.[12,13] Phytochemical evaluation reveals the presence of flavonoids and alkaloids in all the extracts while anthraquinones and saponin were present only in methanol and n hexane extract. Tannin was absent in n- Hexane extract. The presence of saponin is an indication that the plant is of pharmacological importance. Saponins are reported to have antibiotic activities, antifungal activities and are important in cosmetic application in addition to their emollient effects, Alkaloids too are known to have antimicrobial, anti inflammatory effect, Flavonoids are potent water soluble antioxidants which prevent oxidative cell damage suggesting antiseptic, anticancer, anti inflammatory, and mild anti hypertensive properties.

All the extracts did not show any activity against all the test organisms at 62.5 and 125 mg/ml concentrations. The activity ranking among the extracts was methanol> n-hexane> ethanol. This might be due to the fact that the active compounds are more soluble in methanol. Methanol is known to be a good solvent for extraction, dissolving large quantity of non - polar and polar compounds and thereby helps to extract the entire various chemical groups from the plant materials. The

methanol extract of the plant showed comparable activity with gentamicin against *Staphylococcus aureus* and higher activity against *Escherichia coli* at 250 mg/ml & 500 mg/ml. The order of activity of the methanol extract against the test organisms is:

Staphylococcus aureus> Escherichia coli> Streptococcus spp> Klebsiella pneumonia> Trichophyton mentagrophyte> Trichophyton rubrums> Microsporum audouinii.

The ethanol extract of the plant has no antimicrobial activities against *Staphylococcus aureus*, *Streptococcus spp, Escherichia coli,Klebsiella pneumonia, Trichophyton mentagrophytes, Microsporum audouinii* but has a little activity against *Trichophyton rubrum* at high concentration (500 mg/ml). The n- hexane extract has optimum activity against *Klebsiella pneumonia* and *Microsporum audouinii at* 500mg/ml. There was no significant variation ($p>0.05$) in the zones of inhibition of bacteria with the methanol extract. However, there were significant differences ($p<0.05$) in the zones of inhibition obtained using especially at higher concentrations in comparison with control. There was no significant variation in the zones of inhibition of the fungi under study using the methanol extract.

The pH of the formulation at all concentration was slightly acidic (4 - 6.5). This serves as a natural barrier against bacteria and fungi that are found in a more neutral or slightly alkaline environment. All the ointment formulations have accepted value since they fall within the range of 4 - 6.5 suitable for the skin. Viscosity is the measure of resistance of a fluid when it is being deformed by either shear or tensile stress; it describes a fluid's internal resistance to flow and may be thought of as a measure of fluid friction. Factors including time, temperature, spindle number and revolution per minute (RPM) affects the rheology of a product. The viscosity was carried out at 40 ^{0}C using shear rates of 20-100 RPM respectively, using spindle no 5. It was found that as the shear rate increased, the viscosity of the formulations reduced. Most semi solid dosage forms are intended to be thick when standing to prevent them from flowing from the intended area of use. The formulation having 8 % of *Argemone mexicana* extract has the highest viscosity at the lowest shear rate (800.0 at 20 RPM) and at the highest shear rate (188.0 at 100RPM). This suggests that formulations containing 8 % w/w *Argemone mexicana* is suitable for application on skin since it will not run off when applied.

Antimicrobial studies shows that the ointments containing methanol extract showed zones of inhibition against *Streptococcus spp* at concentrations 4-20 % w/w, *Klebsiella pneumoniae* at 15-20 % w/w but showed no activity on *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa.* It was also effective against *Trichophyton mentagrophytes* at all concentrations and with wider zones of inhibition than Gentamicin. The Minimum Bactericidal/Fungicidal Concentration is the concentration where there is 100 % killing of tested organisms. The Minimum Bactericidal concentration against *Streptococcus spp* was 20 % w/v while that of *Klebsiella pneumoniae* was above 20% w/v. The minimum fungicidal concentration was 15 %w/v against *Trichophyton mentagrophytes* 20% w/v against *Microsporum audouinii* but above 20 % against *Trichophyton rubrum.*

CONCLUSION

The result of this study supports the traditional uses of this plant. The plant extract possesses compounds that act on both Gram positive and Gram negative bacteria and fungal organisms which suggest that *Argemone mexicana* can be used for the production of broad spectrum drugs for the treatment of impetigo, ringworm and other microbial infections.

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