Antimicrobial and antioxidant activities of methanolic leaf extract of *Argemone mexicana* linn (papaveraceae)

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ABSTRACT

Background: Medicinal plants are well-known natural sources of remedies used in the treatment of innumerable disease since antiquity. The search for newer synthetic drugs is of immense importance due to the increasing development of resistance. Antioxidant assay is a very useful technique in identifying plants which contain antioxidants which are substances that delay or inhibits oxidative damage to a target molecule thereby preventing the occurrence of degenerative diseases.

Objectives: To authenticate the local use of *Argemone mexicana* as a wound healing agent and evaluate its antimicrobial properties against various micro-organisms in comparison with standard antimicrobial agent.

Method: Antimicrobial assay was carried out against four bacterial strains and three fungi strains using the agar well diffusion method. The formation of a clear zone of inhibition was observed and measured and compared with that of the standard. Antioxidant assay was carried out using the DPPH (1,1-diphenyl-2-picryl hydrazyl) free radical scavenging activity. The absorbance was read at 517nm.

Result: The leaf extract of A.mexicana inhibit the growth of Pseudomonas aeruginosa and Staphylococcus aureus. The plant tested positive for phenols, tannins, flavonoids, alkaloids, resins, saponins and sugars. The antioxidant assay indicates that the extract has a high percentage inhibition DPPH as compared with the standard.

Conclusion: A. mexicana has been shown to inhibit the growth of some micro-organisms and could be useful in the treatment of infections caused by these organisms including pathogenic infections. Its antibacterial potentials therefore confirm its use as wound healing. The plant possesses antioxidant activities due to the presence of flavonoids and phenolic substances which help to fight against free radicals that cause certain diseases in the body.

Key words: *Argemone mexicana*, phytochemical screening, antioxidant, antimicrobial, percentage inhibition

Activités antimicrobiennes et antioxydantes de l'extrait méthanolique de la feuille d'*Argemone mexicana* linn (papaveraceae)

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RESUME

Contexte: Les plantes médicinales sont bien connues comme sources naturelles de remèdes utilisés dans le traitement de maladies innombrables depuis l'antiquité. La recherche de nouveaux médicaments synthétiques est d'une importance immense étant donné le developpement croissant de la résistance. L'essai antioxydant est une technique très utile dans l'identification de plantes qui contiennent des antioxydants qui sont des substances qui retardent ou inhibent le dégât oxydatif à une molécule cible, empêchant ainsi l'occurence de maladies dégénératives.

Objectifs: Authentifier l'usage locale de l'*Argemone mexicana* comme un agent qui guérrit les blessures et évaluer ses propriétés antimicrobiennes contre plusieurs micro-organismes par rapport à l'agent antimicrobien ordinaire.

Méthode: Un essai antimicrobien fut réalisé contre quatre souches bactériennes et trois souches fongiques en utilisant la méthode de diffusion en gélose. La formation d'une zone claire d'inhibition fut observée et mesurée et comparée à la norme. L'essai antioxydant fut réalisé à l'aide de l'activité de libre balayage radical DPPH (1,1-diphenyl-2-picryl hydrazyl). L'absorbance était lue à 517nm.

Résultat: L'extrait de la feuille de *A.mexicana* inhibe la croissance de *Pseudomonas aeruginosa* et *Staphylococcus aureus*. La plante a testé positive aux phénols, tannins, flavonoïdes, alkaloïdes, résines, saponines et sucres. L'essai antioxydant indique que l'extrait a un pourcentage élevé d'inhibition DPPH par rapport à la norme.

Conclusion: *A. mexicana* a prouvé sa capacité à inhiber la croissance de certain micro-organismes et pourrait être utile dans le traitement des infections causées par ces organismes, y compris les infections pathogéniques. Ses potentiels antibactériens confirment ainsi son usage comme guérisseur de blessure. La plante posséde des activités antioxydantes dues à la présence des flavonoïdes et des substances phénoliques qui aident à combattre de radicaux libres qui causent certaines maladies dans le corps.

Mots-clés: *Argemone mexicana,* test phytochimique, antioxydant, antimicrobien, pourcentage inhibition

INTRODUCTION

Natural products continue to play an important role in drug discovery programs of the pharmaceutical industry and other research organizations. An important reason for the use of natural products as a source of lead compounds is the tremendous variety of species found in nature and the resulting molecular diversity of the isolated compounds.[1] In addition to the biologically active plant-derived secondary metabolites which have found direct medicinal application as drugs, many other bioactive plant compounds are useful as "leads" or model compounds (templates) for drug synthesis or semi-synthesis.[2] Research in the field of chemical and biological properties of natural products yielded drugs for the treatment of many human diseases. Additionally it gave the stimulus for the development of modern synthetic organic chemistry, and the emergence of medicinal chemistry as a major route for the discovery of novel and more effective therapeutic agents.[3]

Argemone mexicana Linn. (Family: Papaveraceae) is a prickly, glabrous, branching annual herb with yellow juice and showy yellow flowers, naturalized throughout up to an altitude of 1500 m. It occurs as wasteland weed in almost every part of India. *A. mexicana* L. (Papaveraceae), commonly known as prickly poppy, is used as a medicinal plant in several countries. In Mexico, the seeds are considered as an antidote to snake venom. In India, the smokes of the seeds are used to relieve toothache. 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.[4] *A. mexicana* is specie of poppy found in Mexico and now widely naturalized in many parts of the world. It belongs to the family of papaveraceae. Its common names include: prickly poppy, mexican poppy, blue stem, yellow thistle. As an extremely hard pioneer plant, it is tolerant of drought and poor soil.

Traditionally, the plant is reported to be used as diuretic, purgative, anti-inflammatory, analgesic and believed to destroy worms, cure itching, various skin diseases and an antidote to various poisons.[5] The seeds are purgative and sedative (Ayurveda), useful in skin diseases and leucoderma and in homeopathy. The tincture of the entire plant is reported to be used orally for bronchitis and whooping cough[6]. The fresh juice of the leaves and the latex, both are reported to be used externally as a disinfectant for open wounds and cuts.

A. mexicana is reported to possess alkaloids[7], amino acids[8] phenolics[9] and fatty acids[10] as major phytochemical groups. A series of bioactive compounds have been reported and some of them are isolated from different parts of *A. mexicana*. The whole plant of *A. mexicana* was reported to possess isoquinoline alkaloids such as berberine, cheilanthifoline and benzylisoquinoline alkaloids.[11, 12] Alkaloids such as berberine, tetrahydroberberine, protopine and benzophenanthridines have been isolated from the plant.[13] Seed oil otherwise called as *Argemone* oil reported to contain sanguinarine and dihydrosanguinarine. It also contains palmitic, myristic, oleic and linoleic acids. Previous reports on *A. mexicana* leaves and seeds extract showed considerable antibacterial activity.[14, 15] In another research, stem and essential oil of *A. mexicana* was found to be good antimicrobial activity.[16] The phytoconstituents obtained from root, stem leaves, fruits, flowers and seeds of medicinal plants include phenolic compounds, essentials oils, proteins and antioxidants[17] together they performance as biocontrol agents. The inhibition activity of plants extracts against the growth of microorganisms was attributed to the presence of antioxidants.[18]

Medicinal uses

A. mexicana Linn. (Papaveracae), also known as mexican prickly poppy[19] is a well-known weed in the agricultural and waste lands. The seed are used as emetic, demulcent, laxative[20] and antidote in snake poisoning. It has also been investigated for anticatalepsy activity, antihistamic activity[21], hepatoprotective activity, antimicrobial activity, antiallergic activity, antistress, larvicidal activity, antidiabetic activity, antioxidant, anti-inflammatory activity[22] and wound healing activity.[23] It is also used in the treatment of jaundice, leprosy, piles, dysentery and warts.[24] The whole plant is analgesic, antispasmodic, possibly hallucinogenic and sedative. It contains alkaloids similar to those in the opium poppy (P. somniferum) and so can be used as a mild pain-killer. The fresh yellow, milky, acrid sap contains protein-dissolving substances and has been used in the treatment of warts, cold sores, cutaneous affections, skin diseases, itches. The pounded seeds, mixed with mustard oil, are applied externally to treat itchy skin. The oil from the seed is demulcent and purgative. It has been used externally in the treatment of skin problems. Caution is advised in the use of this oil, prolonged ingestion produces toxic effects resembling those occurring in epidemic dropsy.

Root and leaf decoctions are applied to the skin to cure oedema, inflammation, muscle pain, ulcers, to remove warts, to kill Guinea worm and to promote wound healing. A root decoction is used as a mouthwash and eye bath to treat infections. Leaf sap is used as eardrops to cure ear inflammation. The objective of the research is to authenticate the local use of the plant as a wound healing agent, thus evaluating its antimicrobial activity as well as antioxidant properties.

METHODS

Plant identification and collection

The leaves of *Argemone mexicana* were collected from Ogosin-agbegi compound, Irewole local government, Ikire, Osun state on August 12, 2014 at about 9:00am. The sample was identified as *A. mexicana* and authenticated by Mr O. O. Oyebanji of the Department of Botany, University of Lagos with voucher number LUH 6173. The leaves were destalked and air dried at room temperature for one week until the leaves were properly dry. They were then pulverized in a mechanical grinder to obtain the sample in a powdered form. The powdered plant material was then kept for further analysis.

Preparation of plant sample

Extraction of plant material

Eighty grams of the powdered leaves of *A. mexicana* was extracted with 1 litre of absolute methanol for 72 h and then filtered. The extract solution was evaporated to dryness using a thermo-regulated water bath at 45°C to yield a crude methanolic extract. A second extraction was done by soaking the plant material residue in 1 litre of absolute methanol for 48 h and also filtered. The filtrate was evaporated to dryness and the two extracts were combined together. The weight of the extract was taken and percentage yield calculated.

Phytochemical screening

The methanolic extract of leaves of *A. mexicana* was screened for the presence of sugars, alkaloids, glycosides, saponins, steroids, flavonoids, anthraquinones, cardiac glycosides, tannins and phlobatanins by methods described in Trease and Evans Pharmacognosy text book.[25]

Evaluation of antimicrobial potential of *Argemone mexicana*

The antimicrobial assay of the extract of *A. mexicana* was carried out in the Pharmaceutical Microbiology Department of the Faculty of Pharmacy, University of Lagos, Idi-Araba campus, Lagos. The extract was screened for in-vitro antimicrobial activities against clinical isolates of the following bacteria; *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli, Salmonella typhi*, and fungi Aspergillus niger, Penicillum species, Candida albicans. Agar diffusion method was used by filling well bore using a core borer, with 0.5ml of extracts and standards of different concentration and zones of inhibition of growth of organisms were recorded.

Subculturing of bacteria

The bacteria isolates were inoculated into different prepared plates of Mueller Hinton Agar using aseptic technique to prevent contamination of any sort. The inoculated plates were then incubated at 37°C for 24hours.

Pure isolates of the organisms were then transferred into liquid medium (nutrient broth) and stored in the refrigerator until when they will be used for calibration.

Calibration of bacteria using normal saline infusion

Five universal bottles were filled with about 20ml of normal saline and sterilized by autoclaving at 121°C for 15minutes and thereafter allowed to cool.

The stored organisms in liquid media were then individually introduced into separate universal bottle containing sterile normal saline until the turbidity matches that of the McFarland standards for turbidity of bacteria suspension which is thus equivalent to 1x 10^8CFU/ml.

Subculturing of fungi

The fungi isolates were inoculated into different prepared plates of Sabouraud Dextrose Agar using aseptic technique to prevent contamination of any sort. The inoculated plates were then incubated at room temperature for 3 days (Candida albicans) and 7 days for the spore forming fungi (Aspergillus niger, Penicillium spp.) and stored on the bench until when they will be used for calibration.

Calibration of fungi using 1% Tween 80 and normal saline infusion

Two universal bottles were filled with about 20ml of 1%Tween 80 and one universal bottle filled with normal saline, sterilized by autoclaving at 121oC for 15minutes and thereafter allowed to cool.

The stored organisms on the bench were then individually introduced by washing the spores into separate universal bottle containing sterile 1% Tween 80 (Aspergillus niger, Penicillium spp.) and normal saline (Candida albicans) until the turbidity is equivalent

to 1x 108 spore forming unit.

Antioxidant assay

The antioxidant activities were determined using 1, 1, diphenyl-2-picryhydrazyl (DPPH) as a free radical. 0.0079g of DPPH was weighed and transferred into a 200ml volumetric flask. Sufficient amount of methanol was added and the crystals dissolved in it to obtain 1mM DPPH. Briefly, to a 1ml methanolic solution of the various concentrations of the extract at 0.02%, 0.04%, 0.06%. 0.08% & 0.1%, 1ml of DPPH was added. A blank solution was prepared containing 2ml of methanol and 1ml of 1mM DPPH. This procedure was also carried out for the standard drug, vitamin C also at concentrations 0.02%, 0.04%, 0.06%. 0.08% & 0.1% employed by Ayoola et al., (2008). The experiment was carried out in triplicates. The test tubes were incubated in a dark room for 20 minutes. Absolute methanol was used to zero the UV spectrophotometer and the absorbance was read at 517nm. The free radical scavenging activity was calculated using the following formula; % Inhibition of DPPH = (AB – AA) / AB * 100. Where AB is the absorption of the blank sample and AA is the absorbance of tested extract solution.

The results were expressed as percentage inhibition of DPPH.

Thin layer chromatography

Thin layer chromatography was carried out by cutting the pre-coated aluminium chromatography plates into smaller pieces. The solvents used were methanol, ethanol, n-hexane, dichloromethane and water in various ratios. The chromatography tank was filled with these solvents and allowed to saturate for 1 hour, after which the extract already dissolved in methanol was spotted on the plates with a distance of about 2cm apart by the aid of a capillary tube. Two spots were placed on each plate and the plates were placed in a tank each. Separation was successfully achieved by capillary action as the mobile phase moved over the stationary phase. Individual spots were obtained and the plates were placed in an iodine chamber and the spots were coloured brown. The solvent front was recorded for each of the plates and the distance moved by each of the spots from the origin was also recorded. These individual spots represent the different constituents present in the plant.

RESULTS

Phytochemical screening

Table 1: Phytochemical screening result

Test	Result
Alkaloid	Positive
Anthraquinone	Negative
Flavonoids	Positive
Glycosides	Positive
De-oxy Sugars	Negative
Sterols	Positive
Tannins	Positive
Phenols	Positive
Carbohydrate	Positive
Reducing sugar	Positive
Monosaccharide	Positive
Starch	Absent
Protein	Absent
Resin	Present

Antibacterial Assay

Table 2: Zones of inhibition of bacterial growth by the extract

Organism	Concentration (mg/ml)	Zone of Inhibition
P. aeruginosa	200	10.5
	100	8.0
	50	-
E. coli	200	-
	100	-
	50	-
S. thyphi	200	-
	100	-
	50	-
S. aureus	200	11.55
	100	10.5
	50	-

Table 3: Zones of inhibition of bacterial growth by the Standard

Organism	Concentration(mcg/ml)	Zone of Inhibition (mm)
P. aeruginosa	20	19.5
	10	16.5
	5	8.0
	2.5	-
E. coli	20	20.5
	10	16.0
	5	-
	2.5	-
S. typhi	20	24.0
	10	20.5
	5	15.5
	2.5	-
S.aureus	20	20.0
	10	15.5
	5	-
	2.5	-

Antifungal assay

Table 4: Zones of inhibition of fungal growth by the extract

Organism	Concentration (mg/ml)	Zone of Inhibition (mm)
A.niger	200	-
	100	-
	50	-
P. spp	200	-
	100	-
	50	-
C. albicans	200	-
	100	-
	50	-

Table 5: Zones of inhibition of fungal growth by the standard

Organism	Concentration (mcg/ml)	Zone of Inhibition (mm)
A.niger	80	-
	40	19.5
	20	7.5
	10	5.5
P. spp	80	10.5
	40	7.5
	20	5.5
	10	5.0
C. albicans	80	-
	40	-
	20	-
	10	-

Antioxidant Assay

Table 9: The concentration, absorbance, standard deviation (SD) and standard of error of mean(SEM) of DPPH with and without the extract

Concentration	Abs 1 (nm)	Abs 2 (nm)	Abs 3 (nm)	Mean abs.	SD	SEM	% inhibition
0.02%	0.343	0.378	0.351	0.357	0.018	0.011	21.353
0.04%	0.311	0.297	0.261	0.290	0.026	0.015	20.100
0.06%	0.236	0.213	0.200	0.216	0.018	0.011	53.711
0.08%	0.176	0.181	0.169	0.175	0.006	0.004	61.336
0.1%	0.161	0.154	0.147	0.154	0.007	0.004	66.529
Blank	0.458	0.462	0.443	0.454	0.010	0.006	-

Table 10: The concentration and Mean±SEM of DPPH with and without the extract

Concentration	Mean±SEM
0.02%	0.357±0.011
0.04%	0.290±0.015
0.06%	0.216±0.011
0.08%	0.175±0.006
0.1%	0.154±0.004
Blank	0.010±0.006

Table 11: The concentration and percentage inhibition of the DPPH with the extract

Concentration	% Inhibition 1	% Inhibition 2	% Inhibition 3	Mean
0.02%	25.11%	18.18%	20.77%	21.35%
0.04%	18.18%	20.77%	21.35%	20.10%
0.06%	53.90%	54.85%	52.38%	53.71%
0.08%	60.82%	61.85%	61.41%	61.36%
0.1%	66.67%	66.82%	66.10%	66.52%

Table 12: The concentration, absorbance, standard deviation(SD) and standard of error of mean(SEM) of DPPH with and without the Standard

Concentration	Abs 1	Abs 2	Abs 3	Mean abs.	SD	SEM	% inhibition
0.02%	0.387	0.342	0.338	0.356	0.027	0.016	23.55%
0.04%	0.206	0.408	0.297	0.304	0.101	0.058	28.41%
0.06%	0.119	0.115	0.113	0.116	0.004	0.002	76.25%
0.08%	0.112	0.110	0.072	0.098	0.023	0.013	80.66%
0.1%	0.067	0.098	0.105	0.090	0.020	0.012	79.78%
Blank	0.428	0.493	0.486	0.469	0.036	0.021	-

Table 13: The concentration and Mean±SEM of DPPH with and without the standard

Concentration	Mean±SEM
0.02%	0.356±0.016
0.04%	0.304±0.006
0.06%	0.116±0.002
0.08%	0.098±0.013
0.1%	0.090±0.012
Blank	0.469±0.021

Table 14: The concentration and percentage inhibition of the DPPH with the standard

Inhibition (%)	Inhibition 2 (%)	Inhibition 3 (%)	Mean (%)
9.579	30.629	30.453	23.554
30.629	30.453	24.165	28.416
76.673	76.749	75.338	76.253
77.688	85.185	79.105	80.659
80.122	78.395	80.810	79.776

Abs = absorbance
SD = standard deviation
SEM = standard error of mean

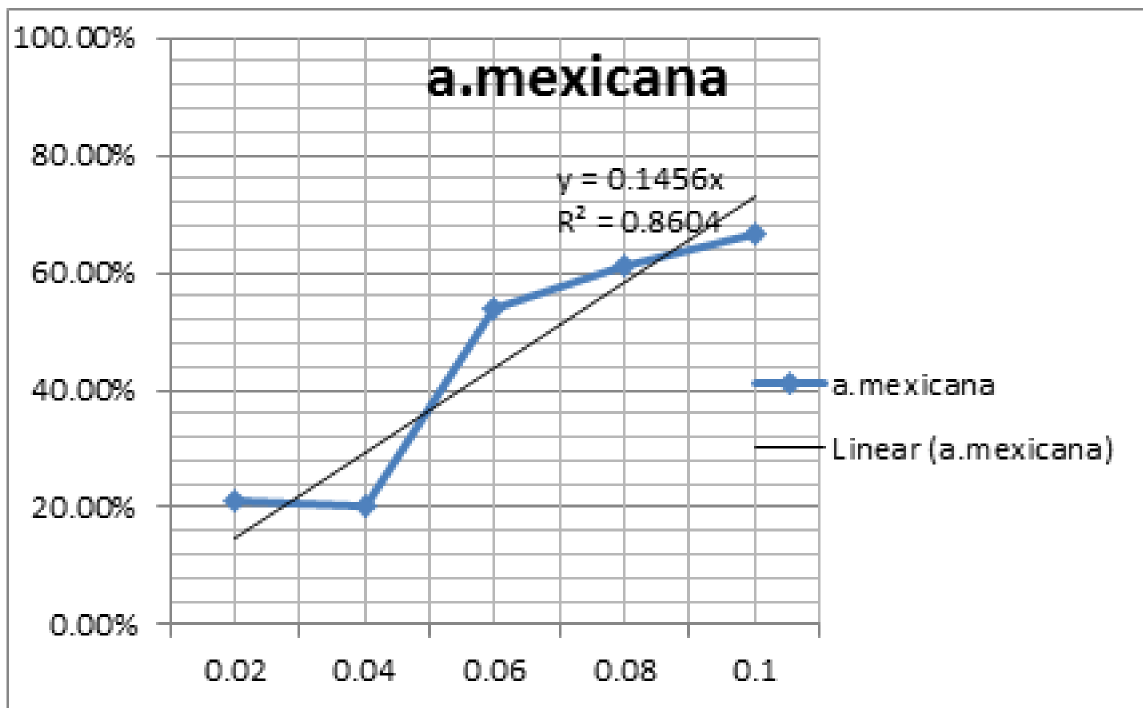

Fig 8: A graph of percentage inhibition against concentration of DPPH free-radical with the extract
IC_{50}=0.053
Note IC $_{50}$ is the inhibitory concentration that inhibit 50% Of DPPH free radical and the higher the ic50, the lower the activity

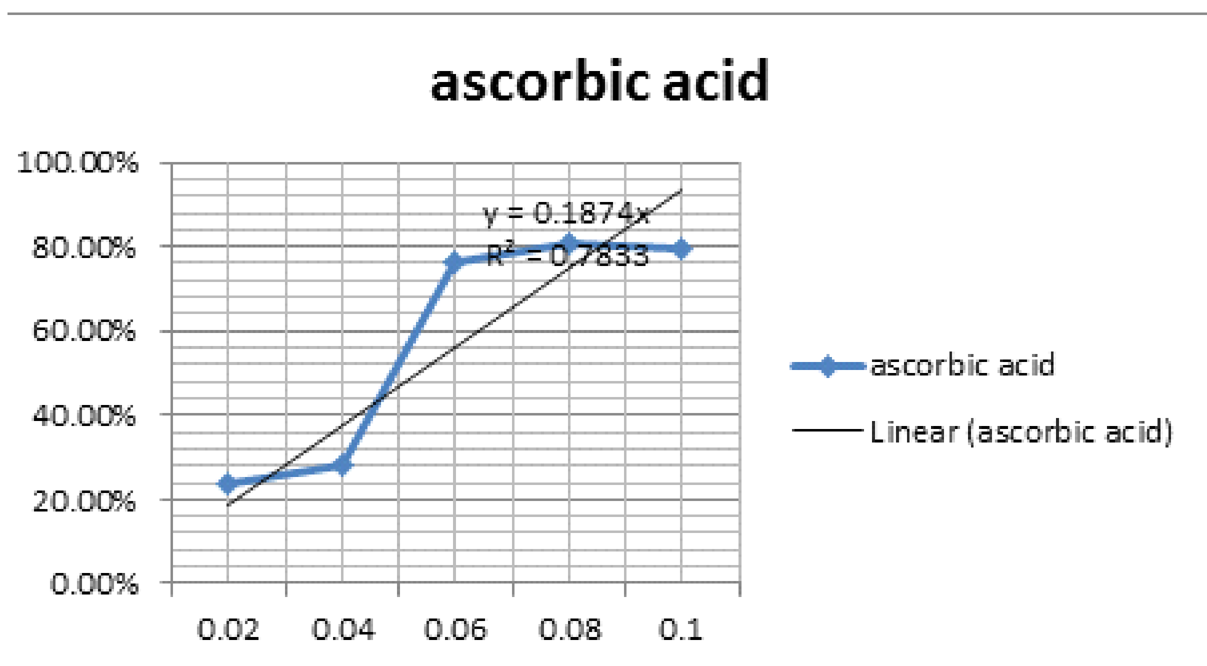

Fig 9: A graph of percentage inhibition against concentration of DPPH free-radical with the standard ic50=0.05

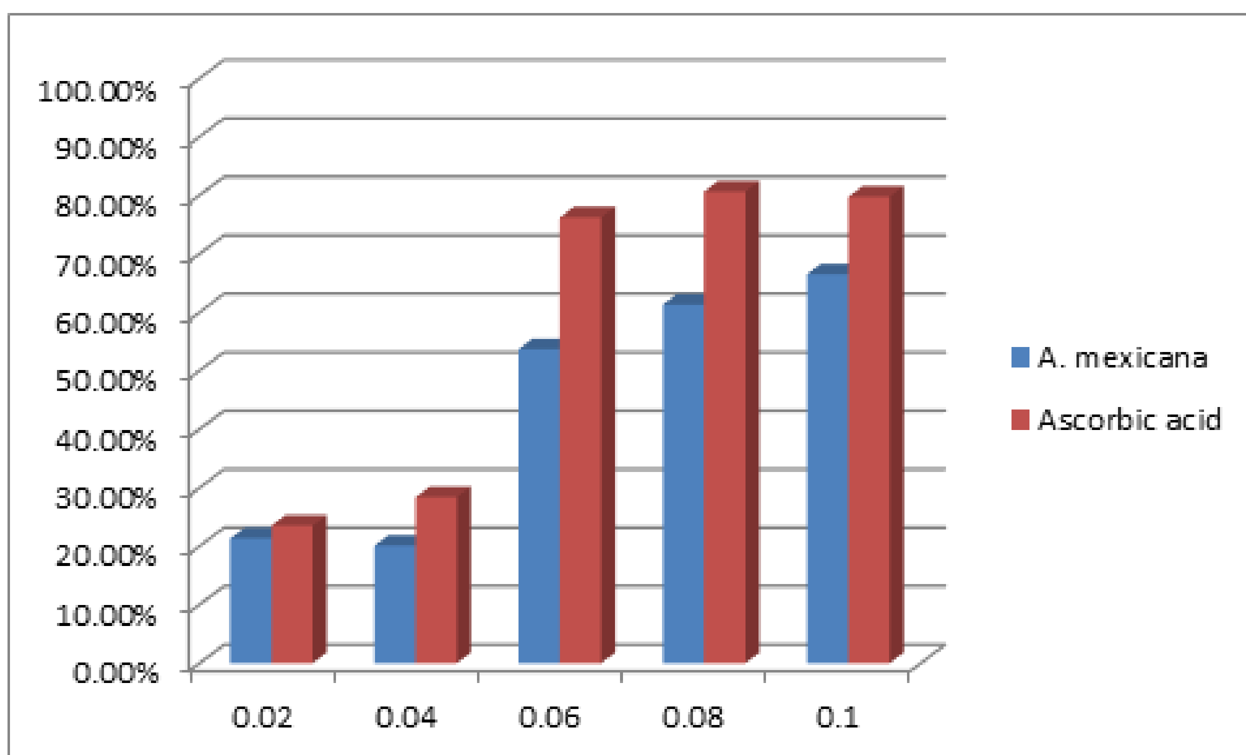

Fig 10: A bar chart showing the relationship between percentage inhibition and concentration of the extract and standard

Thin layer chromatography

Table 15: The different solvent systems used and the respective Rf values obtained for the extract

Solvent system	Number of spot		Distance moved by spot (cm)	Solvent front (cm)	Rf	HRf
Methanol: ethanol: hexane: water (4:3:2:1)	2	i. ii.	1.2 1.9	7.5	i. 0.16 ii. 0.25	16 25
Methanol: ethanol: hexane: water (1:3:3:3)	1	i.	0.8	6.2	0.13	13
Methanol: ethanol: hexane: water (5:1:2:2)	2	i. ii.	1.2 1.6	6.7	i. 0.18 ii. 0.24	18 24
Methanol:water (9:1)	4	i. ii. iii. iv.	1.9 2.1 5.3 6.1	7.0	i. 0.13 ii. 0.30 iii. 0.76 iv. 0.87	13 30 76 87
Methanol: ethanol: hexane: water (1:4:0:5)	3	i. ii. iii.	1.0 1.7 3.0	6.7	i. 0.15 ii. 0.25 iii. 0.45	15 25 45
Dichloromethane: methanol: water (5:3:2)	3	i. ii. iii.	1.5 4.1 5.6	6.0	i. 0.25 ii. 0.68 iii. 0.93	25 68 93
Dichloromethane: methanol: water (6:3:1)	4	i. ii. iii. iv.	2.8 3.5 4.8 5.3	5.8	i. 0.48 ii. 0.60 iii. 0.83 iv. 0.91	48 60 83 91
Dichloromethane: methanol: water (4:5:1)	4	i. ii. iii. iv.	0.8 2.2 3.2 5.2	5.9	i. 0.14 ii. 0.37 iii. 0.54 iv. 0.88	14 37 54 88
Dichloromethane: methanol: water (3:4:3)	3	i. ii. iii.	2.0 3.2 5.0	5.5	i. 0.36 ii. 0.58 iii. 0.91	36 58 91
Dichloromethane: methanol: water (7:1:2)	4	i. ii. iii. iv.	1.5 3.8 5.3 5.9	6.0	i. 0.25 ii. 0.63 iii. 0.88 iv. 0.98	25 63 88 98

DISCUSSION

The preliminary phytochemical screening of *Argemone mexicana* leaf extracts showed presence of alkaloids, flavonoids, saponins, triterpenoids, tannins and phenolic substances, resins, carbohydrates and cardiac glycosides.

The antibacterial activity of the methanolic leaf extract of A. mexicana Linn was tested against four (4) bacterial strains; Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus using agar well diffusion method at concentrations 200mg/ml, 100mg/ml and 50mg/ml of the plant extract and their potential activity were assessed by the presence or absence of inhibition zones. The result revealed that inhibitory effects of test samples was dose dependent, as the concentration increased the zone of inhibition was also increased. The results obtained also indicated that the extracts displayed available degree of

antimicrobial activity on the different tested strains as compared to the standard. The methanol extracts of this plant (leaves) showed maximum activity against S. aureus followed by P. aeruginosa at concentrations 200mg/ml and 100mg/ml but showed no antibacterial activity at 50mg/ml. However, there was no antibacterial activity observed in E. coli and S. thyphi at all concentrations (200, 100 & 50mg/ml). The antimicrobial activities of plant extracts were compared with standard antibiotics such as Levofloxacin, which was used as positive control. Levofloxacin was used at concentrations 20µg/ml, 10µg/ml, 5µg/ml & 2.5µg/ml and it showed maximum antibacterial activity to S. typhi at 20, 10 & 5µg/ml but was not effective at 2.5µg/ml, followed by P. aeruginosa also at concentrations 20µg/ml, 10µg/ml & 5µg/ml, followed by E. coli at 20µg/ml & 10µg/ml only and lastly S. aureus also at concentrations 20µg/ml & 10µg/ml only with no activity at 5µg/ml & 2.5µg/ml. From the results obtained, the standard (levofloxacin) showed no inhibitory activity against any of the organisms. This is due to the concentration being too low to inhibit the growth of these bacterial strains. Observed inhibition of the bacteria strains 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.

In the antifungal assay, the inhibitory activity of the methanolic leaf extract of A. mexicana was tested against three (3) fungi strains; Candida albicans, Aspergillus niger and Penicillum spp. also using agar well diffusion method at concentrations 200mg/ml, 100mg/ml and 50mg/ml of the plant extract and their potential activity were assessed by the presence or absence of inhibition zones. The results obtained also indicated that the extracts displayed no antifungal activity on the different tested strains as compared to the standard as there were no zones of inhibition observed in any of the three (3) fungal strains used. The results obtained were compared to that of the standard, Clotrimazole which was employed at concentrations 80µg/ml, 40µg/ml, 20µg/ml &10µg/ml. Clotrimazole was only active against Aspergillus niger, it exhibited zones of inhibition at all the concentration of the standard used while C. albicans and P. notatum were not inhibited at all. This may be due to the sensitivity of these organisms to clotrimazole. The extract showed no zones of inhibition, indicating its inability to inhibit the tested fungal strains probably due to the concentration not being high enough to cause inhibition.

Thin layer chromatography was carried out to identify, separate and isolate the constituents of the plant. The solvents used were methanol, ethanol, hexane, dichloromethane and water in various concentrations. After separation, different spots were obtained indicating that the constituents were successfully separated and further isolation can then be carried out.

Antioxidants through their scavenging power are useful for the management of various diseases. Reactive oxygen species are continuously formed in cells as consequence of oxidative biochemical reactions and external factors. However they become harmful when they are produced in excess under certain abnormal conditions such as inflammation, ischemia and in presence of iron ions. Under these conditions the endogenous antioxidants may be unable to counteract ROS formation. Reactive oxygen species formed may cause cellular damage and this damage may involve in etiology of diverse human diseases. Exogenous antioxidant supplement is helpful to overcome this severe problem of free radicals, which may scavenge these free radicals. The free radical scavenging activity of natural compounds can be evaluated through their ability to quench the synthetic free radicals, in which the absorbance of the reaction mixture is taken in visible range to know whether the compound possesses antioxidant activity

In this study, the scavenging capability of DPPH was determined by the decrease in its absorbance at 517 nm as well as by the degree of color change from deep violet to yellow. Both ascorbic acid and methanolic leaf extract of A.mexicana showed dose dependent activity. The free radical scavenging activity of A.mexicana was tested by its ability to bleach the stable DPPH radical. The delocalisation of DPPH molecule determines the occurrence of a purple colour, with an absorption band with a maximum around 520nm.[26] From the results obtained, both the extract and the standard displayed a decrease in absorbance as the concentration increased. While the blank solution containing only methanol and DPPH had the highest absorbance. The readings are expressed in terms of the mean, standard deviation and percentage inhibition. For the extract, the highest percentage inhibition (66.53%) was observed at a concentration of 0.1% which was the highest concentration and a mean absorbance of 0.154 ± 0.04 while the standard had the highest percentage inhibition 0f 80.66% at concentration 0.08%, with a mean absorbance of 0.098 ± 0.013. The results obtained in the present study indicate that the methanolic leaf

extract of A. mexicana exhibits free radical scavenging activity. The overall antioxidant activity might be attributed to its polyphenolic content and other phytochemical constituents. The findings of the present study suggest that A. mexicana could be a potential source of natural antioxidant that could have greater importance as therapeutic agent in preventing or slowing oxidative stress related degenerative diseases.

CONCLUSION

From the studies conducted on the methanolic leaf extract of A. mexicana, it can be concluded that various constituents such as alkaloids, saponins, tannins, phenols, flavonoids, triterpenoids, resins, carbohydrates and cardiac glycosides are present in the leaves extract which can be used in the management of various diseases. A. mexicana has been shown to inhibit the growth of some micro-organisms like Staphylococcus aureus and Pseudomonas aeruginosa therefore, the plant can be used in the treatment of infections caused by these organisms including pathogenic infections and can be used as alternative bactericide. Its antibacterial potentials therefore confirm its use as a wound healing agent. Also, the plant possesses antioxidant activities due to the presence of flavonoids and phenolic substances.

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