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Quantitative and Qualitative Phytochemical Screening and Anti-Microbial Activities of *Argemone mexicana Linn*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Argemone mexicana L is one of the medicinal plants used traditionally for its curative properties. The observed activities could be due to the presence of phytochemicals present in the plant. The purpose of this study is to carry out the quantitative and qualitative analysis of the constituent phytochemicals of Ethanol and aqueous extracts of the leaves of the plant as well as determine its microbial activities against some selected bacteria and fungi. Results showed that alkaloid is present in both ethanol and aqueous samples. Terpenoid is present in aqueous sample but abundantly present in ethanol sample. Phenol is present in aqueous sample but abundantly present in ethanol sample. Steroids are present in ethanol sample but absent in aqueous sample. While anthraquinones are present in the aqueous sample, the quantity is not much compared with what was obtained from the ethanol sample. Cardiac glycoside is absent in both samples but flavonoid is present in both samples. It was observed that saponins and tannins were abundantly

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present in the ethanol extract but not much in the aqueous extract. The results of the quantitative analysis showed that for aqueous extract, alkaloid is 9.5 % w/w, flavonoid (0.36% w/w), saponin (4.7%w/w), tannin (0.1% w/w) terpenoid (0.45 w/w) and total phenol (0.65% w/w). For ethanol extract it was alkaloid (9.7 %w/w), flavonoid (1.59% w/w), saponin (6.3% w/w), tannin (1.06% w/w), terpenoid (0.75% w/w) and total phenol (0.74% w/w). The MIC and MBC measured in µg/mL of ethanol and aqueous samples against *Staphylococcus aureus* were (3.125:6.25) and (12.5:12.5) respectively. *Bacillus subtilis* (25:25) and (25;25), *Escherichia coil* (6.25: 25) and (12.5: 50), *Salmonella typhi* (>50: 25) and (6.25: 12.5), *Klebsiella pneumonia* (12.5:25) and (1.56:6.25), *Pseudomonas aeruginosal* (50:50) and (3.125:6.25), *Tricophytum rubrum* (25:25) and (3.125: 3.125), *Candida albican* (25:25) and (6.25:12.5) respectively. All Our study showed that the plant has high potential as an antifungal and antibacterial agent.

Keywords: Argemone mexicana; phytochemicals; quantitative analysis; antimicrobial; ethanol extract.

1. INTRODUCTION

Plants have been known to be very important to all living organisms. They provide different types of products such as fruits, bark, leaves and medicines. Nearly 80% of plant species are used as medicine [1]. Medicinal plants have two types of metabolites namely primary and another secondary [2]. There has been ongoing research for discovery of new therapeutic ways of managing diseases. Drug development processes are currently focused on natural sources of plant origin [2]. Using plant materials for prevention and treatment of infectious diseases successfully over the years has attracted the attention of scientists worldwide [3]. There have been reports that in an effort to manage diseases traditionally, quite a number of medicinal plants have been used [4]. Local people who use medicinal plants for their therapeutic properties often claim that there are no negative side effects which has sparked a surge in interest in them. Some medicinal plants have been found to be have antioxidant properties [5,6,7]. Some others are reported to possess antibacterial properties [8], while other have antihypertensive properties [9]. The therapeutic principles of medicinal plants is being further investigated by scientists who now conduct comprehensive research on different plant species all over the world [10]. Phytochemicals found in plants have a wide range of bioactivity, including antioxidant, anti-inflammatory, and anti-cancer properties. Approximately 25% of the active ingredient has been identified from plants used as prescription medicines at this time [11].

Salmonella typhi, Klebsiella pneumonia, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, and *Escherichia coli* are bacteria which are known to cause several human infections [12]. *Salmonella typhi* is a gram-negative bacterium which attacks the intestinal tract. It also attacks the blood and is known to be responsible for typhoid fever which is still a health burden on developing nations. There are significant efforts in research and medical advancements the world over, typhoid fever still remains a major worldwide public health concern [13]. *Klebsiella pneumonia* is a gram-negative bacteria that typically cause nosocomial infections and shows a great deal of antibiotic resistance. Klebsiella species cause a wide range of diseases including pneumonia, urinary tract infections (UTIs), bloodstream infections and sepsis [14,15]. *Staphylococcus aureus* remains a versatile and dangerous pathogen in humans. It is a gram-positive bacterium. The natural habitat of *Staphylococcus aureus* in humans is the skin and nasopharynx. It can cause a wide variety of infections involving skin and soft tissues, endovascular sites and internal organs [16]. *Bacillus subtilis* also known as the hay bacillus or grass bacillus, is a Gram-positive bacterium. It is found in soil and the gastrointestinal tract of ruminants, humans and marine sponges. It is widely used for the production of heterologous proteins and also for microbial production of chemicals, enzymes, and antimicrobial materials for industry, agriculture, and medicine [17]. *E. coli* is a Gram-negative, rod-shaped non-spore-forming bacteria. It's commonly linked to urinary tract infections (UTIs) [18] and post-operative wound infection. *S. aureus* is a Gram positive bacteria that causes a variety of infections, including skin infections, bacteremia, endocarditis, pneumonia, and food poisoning. Trichophyton rubrum, though classified as a dermatophyte, is known to be a major causative agent for superficial dermatomycoses like onychomycosis and tinea pedis and is known to account for as many as 69.5% of all dermatophyte infections [19].

Candida albicans is a dimorphic fungus as it proliferates in either a yeast form or a hypha form. The switch between these forms is the result of a complex interplay of external and internal factors and is coordinated in part by polarity-regulating proteins that are conserved among eukaryotic cells [20].

One of the most important medicinal plants is *Argemone mexicana* Linn which grows in the dry field areas [21,22]. It belongs to the family of Papaveracea and commonly found on road-sides. It was reported to grow throughout the subtropical and tropical regions. It has yellow juice and yellow flower with height varing between 0.3 to 0.12m long [23]. Some parts of the plant have medicinal effect and also possess potent emetic and narcotic activities [24]. The plant was reported to also have antihelmintic, antiinflammatory, wound healing, anti-bacterial and antifungal properties [25]. It is locally used in the management of dropsy and jaundice diseases [25]. The local people in India has been reported to use the root for the treatment of chronic skin diseases [25]. The petroleum ether, chloroform, methanol and aqueous extracts of the leaves of *Argemone mexicana* Linn were evaluated for their wound healing activity in rats using excision, incision and dead space wound models respectively. The results showed that the animals treated with methanol and aqueous extracts of the plant showed faster rate of wound healing compared to other extracts. It was also concluded that the activity may be attributed to the presence of phytoconstituents like alkaloids, triterpenoids, tannins and flavonoids in the extracts [26]. Osho & Afetunji (2010) [27] investigated in vitro antimicrobial study with essential oil of the plant against some common bacterial and fungal pathogenic microbes and found promising results. Investigation carried out on the methanolic extract of the leaves of the plant was found to exhibit cytotoxic activity against healthy mouse fibroblasts (NIH3T3) and three human cancer-cell lines (AGS, HT-29 and MDA-MB-435S) using the MTT [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide assay as reported by [28].

2. MATERIALS AND METHODS

2.1 Plant Material and Extraction

Young fresh leaves of the plant were collected and authenticated by the taxonomists of the Nigeria Natural Medicine Development Agency. The plant materials were washed to remove adhering dirt followed by rinsing with distilled water. It was then shade dried and pulverized in a mechanical grinder followed by sieving (sieve no. 40) to obtain coarse powder. The powdered leaves (500 g) was successively extracted with Ethanol and water for 48 h in a soxhlet extractor. Following extraction, the liquid extracts were concentrated under vacuum to yield dry extracts. The test micro-organisms namely *Staphylococcus aureus, Bacillus subtilis, Escherichia coil, Salmonella typhi, Klebsiella pneumonia, Pseudomonas aeruginosa, Tricophytum rubrum* and *Candida albican* were all collected from Spectralab Laboratories in Sagamu, South-West Nigeria.

2.2 Preliminary Screening of Secondary Metabolites

Quantitative and qualitative phytochemical screening for alkaloids, flavonoids, glycosides, phenols, cardiac glycosides, saponins, sterolss, tannins and anthraquinones were determined using various established methods.

2.2.1 Test for alkaloids

A few drops of dilute iodine solution were added into 3 ml test solution added. Blue colour appeared; and disappeared on boiling and reappeared on cooling [Khandewal K.R., 2008].

2.2.2 Test for flavonoids

2-3 ml. of extract and few drops of sodium hydroxide solution were added into a test tube. Formation of intense yellow colour that became colourless on addition of few drops of dilute HCl indicates the presence of flavonoids [29].

2.2.3 Test for phenols

0.5 ml of FeCl3 (w/v) solution was added into 2 ml of test solution, formation of an intense colour indicates the presence of phenols [30].

2.2.4 Test for saponins

0.5g extract was diluted with 20 ml of distilled water and was shaken in a graduated cylinder for 15 minutes. A 1 cm. layer of foam, indicates the presence of saponins [31].

2.2.5 Test for tannins

Few drops of 10% lead acetate solution were added into 5 ml of extract. Formation of yellow or red precipitate indicates the presence of tannins [32].

2.3 Quantitative Determination of Chemical Constituents

2.3.1 Determination of alkaloid content [33]

Plant sample (5 g) was weighed into 250 mL beaker and 200 mL of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hrs. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was removed and washed with 1% ammonium hydroxide and then filtered. The residue is the alkaloid and this was oven dried for 30 mins at 60^{o}C and reweighed. The alkaloid content of the samples was determined by difference using the equation:

$$\text{Percentage alkaloid} = \frac{W_2 - W_1}{W} \times 100$$

Where,

W = weight of sample
W_1 = weight of empty filter paper
W_2 weight of paper + precipitate

2.3.2 Determination of saponin content [34]

Plant samples (20 g) each were put into conical flask and 100 mL of 20% aqueous ethanol was added. The samples were heated over a hot water bath for 4 hours with continuous stirring at about 55^{o}C. The mixture was filtered and the re-extracted with another 200 mL 20% ethanol. The combined extracts were reduced to 40 mL over water bath at about 90^{o}C. The concentrate was transferred into a 250 mL separatory funnel and 20 mL of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 mL n-butanol was then added. The combined n-butanol extracts were washed twice with 10 mL of 5% aqueous sodium chloride. The remaining solution was heated on a water bath. After evaporation, the samples were dried in the oven to a constant weight. The saponins content was calculated thus:

% Saponin = Weight of Saponin/ Weight of Sample X 100

2.3.3 Determination of flavonoid content [35]

Plant samples weighing 10 g were extracted repeatedly with 100 mL of 80% aqueous methanol at room temperature. The whole solution was filtered through what man filter paper no. 2. The filtrates was later transferred into a crucible and evaporated into dryness over a water bath and weighed to constant weight.

% Flavonoid = Weight of Flavonoid/ Weight of Sample X 100

2.3.4 Determination of tannin content [33]

Plant sample (500 mg) was weighed into a 50 mL plastic bottle. 50 mL of distilled water was added and shaken for 1 hour in a mechanical shaker. This was filtered into a 50 mL volumetric flask and made up to the mark. Then 5 mL of the filtrate was pipetted out into a test tube and mixed with 2 mL of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M Potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min (Van-Burden and Robinson, 1981).

2.3.5 Determination of MIC (Minimum Inhibitory Concentration)

The MIC was done using broth micro dilution method and 96 well plates were used. The oil samples were dissolved in double strength Tryptone soya Broth to obtain a solution of 50ug/mL. This was then diluted serially in sterile 96 well plate to obtain concentration range of 25 ug/mL 12.5 ug/mL, 6.25 ug/mL,3.125 ug/mL,1.563 ug/mL,0.781 ug/mL,0.396 ug/mL,0.195 ug/mL. The drug used as the reference were gentamycin [10ug/ml] and ketoconazole [1%] for the anti-bacterial and anti-fungal assay respectively. The reference drugs were also diluted to obtain 10ug/ml,5ug/ml,2.5ug/ml,1.25ug/ml,0.625ug/ml and 0.1325ug/ml for gentamicin (Bacteria) and 1%, 0.5%, 0.25%, 0.125%, 0.0625%, and 0.031255% for ketoconazole (fungi).

Each of the micro plate wells were inoculated with 10uL of the micro-organism and incubated at 37^{0}C and 25^{o}C for 24 hours and 48hours for bacteria and fungi respectively. The least concentrations which showed no growth or turbidity after hours of incubations were streaked on N.A. The least concentration with no trace of growth was taken as the minimum inhibitory concentration (MIC).

2.3.6 MMC (Minimum Microbicidal Concentration)

After checking for growth or turbidity in the test plates (MIC determination). 10ul of 0.2mg/ml of

p-INT solution (piodonnitrotetrazolium violet) was added to the wells. The plates were further incubated at 37^0C for 30 mins. Wells with colour change from yellow to pinkish red was an indication of bacterial /microbial growth .

The least concentration which showed no trace of growth or colour change was taken as the MBC/MFC (MMC).

3. RESULTS AND DISCUSSION

In this study, the phytochemicals occurring in the ethanol and aqueous solvent extracts were analysed qualitatively and quantitatively. The major phytochemicals found were alkaloids, flavonoids, tannins, phenols, saponins, glycosides, cardiac glycosides and anthraquinones (Table 1). Several studies have reported antibacterial activity of alkaloids. For example, Alkaloids have different types of activities as pain-killers, antimicrobial, stimulants, muscle relaxants, anaesthetics, antimicrobial, anti-diabetic, anti-cancerous, anti-HIV, antioxidants etc. [36,37,38]. It is found to be present both in the ethanol and aqueous extracts of the plant. However the quantitative analysis indicated that in ethanol sample it was 9.7% while in aqueous sample it was 9.5% (Table 2). This result agrees with the findings of [39] and [40]. Terpenoids are known to possess activity against bacterial and viruses. It has also been used as anti-malaria, anti-inflammatory and to treat cardiovascular diseases [41]. It was abundantly present in the ethanol extract more than the aqueous extract (Table 1). Also in the quantitative determination, there was 0.75% of the phytochemical in ethanol extract compared to 0.45% found in the aqueous extract (Table 3). Saponins are reported to be used in hypercholesterolemia. It was also used as an antioxidant, anticancer, anti-inflammatory and for hyperglycemia [42,43]. Saponins have antifungal properties [44]. In this present study, saponin was found to be abundantly present in the ethanol extract compared to the aqueous extract. The quantitative analysis revealed that there was 6.3% of saponin in the ethanol extract compared to 4.7% in aqueous extract (Table 4). Rievere, et al. [45] reported that tannins have antimicrobial and antioxidant activities. There were also reports that tannins may be used as cytotoxic and antineoplastic agents [46]. The qualitative analysis determined indicated abundance of tannins in ethanol extract as compared to aqueous extract. While quantitative determination indicated that there was 1.06 compared to 0.1% of tannins (Table 5 – Table 9).

Table 1. List of photochemicals and their activity in Ethanol and Aqueous extract

Phytochemical	Ethanol Extract	Aqueous
Alkaloids	+	+
Flavonoids	+	+
Tannins	++	+
Saponins	++	+
Terpenoids	++	+
Phenols	++	+

+ : present, ++ : abundantly present, - : absent

Flavonoids have shown to have the ability to modify the body's reaction to allergen, carcinogens and virus. They also show antimicrobial and anticancer activity. It is present both in the ethanol and aqueous extracts of the plant but the quantity found in ethanol sample (1.59%) is more than what was found in the aqueous sample (0.36%). (Table 10 – 13) with the calibration curve for the standard (Fig. 2). These contents show different types of activities against different pathogens. Therefore, it can be used in the treatment of diseases. Phenolic compounds have anti-oxidative, antidiabetic, anticarcinogenic, antimutagenic and anti-inflammatory properties [47,48]. The amount found in the aqueous sample (0.65%) is smaller compared to what was found in ethanol sample (0.74%) Table 14 – 17. The calibration curve for the standard is shown in Fig. 3.

3.1 Total Phenolic Content (TPC) Determination

The summary of results for the quantitative analysis of both aqueous and ethanol samples of the plant is indicated in Table 18 and Fig. 4.

Table 2. Quantitative determination of alkaloids

Sample	Amount used (g)	Yield (g)	Avg yield (g)	% Yield (%w/w)
Ethanol extract	1.0	0.094	0.0965	9.7
	1.0	0.099		
Aqueous extract	1.0	0.095	0.0945	9.5
	1.0	0.094		

Table 3. Quantitative determination of terpenoids

Sample	Amount used (g)	Yield (g)	Avg yield (g)	% Yield (%w/w)
Ethanol extract	1.0	0.007	0.0075	0.75
	1.0	0.008		
Aqueous extract	1.0	0.004	0.0045	0.45
	1.0	0.005		

Table 4. Quantitative determination of saponins

Sample	Amount used (g)	Yield (g)	Avg yield (g)	% Yield (%w/w)
Ethanol extract	1.0	0.009	0.0075	6.3
	1.0	0.006		
Aqueous extract	1.0	0.048	0.047	4.7
	1.0	0.046		

Table 5. Quantitative determination of *Tannin (using spectrophotometric method)*

Absorbance values for the ethanol extract					
Concentration	**abs 1**	**abs 2**	**abs 3**	**Average abs**	**Avg abs-blank**
200ug/ml	0.246	0.247	0.246	0.246	0.119
400ug/ml	0.251	0.251	0.250	0.251	0.124
600ug/ml	0.266	0.267	0.264	0.266	0.139
800ug/ml	0.285	0.285	0.285	0.285	0.158
1000ug/ml	0.309	0.308	0.309	0.309	0.182
Blank	0.126	0.126	0.128	0.127	***

Table 6. Absorbance values for the aqueous extract

Concentration	abs 1	abs 2	abs 3	Average abs	Avg abs-blank
200ug/ml	0.201	0.200	0.204	0.202	0.075
400ug/ml	0.233	0.233	0.233	0.233	0.106
600ug/ml	0.252	0.252	0.256	0.253	0.126
800ug/ml	0.287	0.287	0.287	0.287	0.160
1000ug/ml	0.316	0.315	0.315	0.315	0.188
Blank	0.126	0.126	0.128	0.127	***

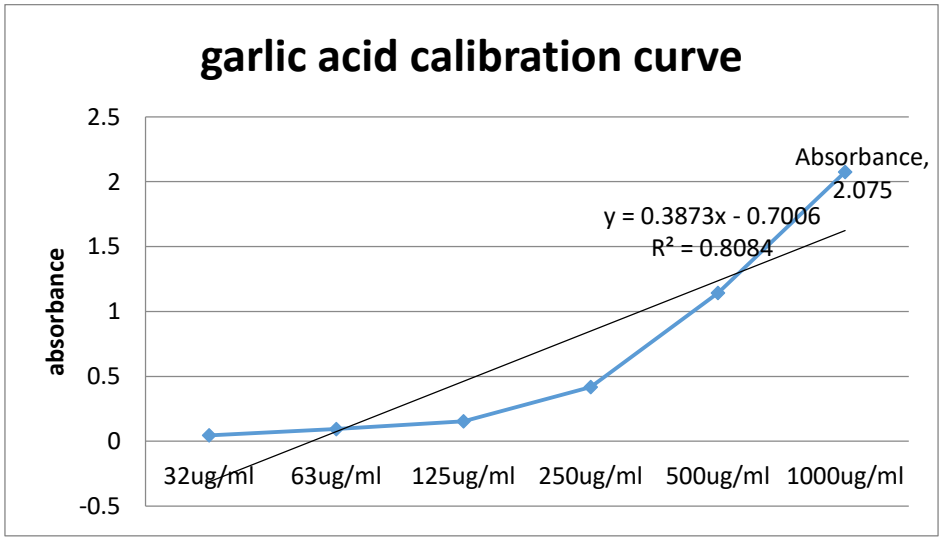

Fig. 1. Calibration curve for the standard

Table 7. Absorbance values for the standard (GARLIC ACID)

Concentration	abs 1	abs 2	abs 3	Average	abs-blank
1000ug/ml	2.514	2.514	2.155	2.154	2.075
500ug/ml	1.222	1.223	1.222	1.222	1.143
250ug/ml	0.498	0.496	0.497	0.497	0.418
125ug/ml	0.233	0.233	0.233	0.233	0.154
63ug/ml	0.173	0.174	0.173	0.173	0.094
32ug/ml	0.122	0.127	0.126	0.125	0.046
Blank	0.078	0.079	0.079	0.079	**

Calibration curve

Table 8. Absorbance values

Concentration	Absorbance
32ug/ml	0.046
63ug/ml	0.094
125ug/ml	0.154
250ug/ml	0.418
500ug/ml	1.143
1000ug/ml	2.075

Table 9. Concentration of tannin (in ug) in the different extracts at garlic acid equivalence

Sample concentration	Ethanol fraction	Aqueous extract
200ug/ml	2.12	2.00
400ug/ml	2.13	2.08
600ug/ml	2.17	2.13
800ug/ml	2.22	2.22
1000ug/ml	2.27	2.29

Table 10. Determination of total flavonoid content absorbance values for the ethanol extract

Concentration	abs 1	abs 2	abs 3	Average abs	Avg abs-blank
200ug/ml	0.254	0.255	0.253	0.254	0.166
400ug/ml	0.285	0.283	0.283	0.284	0.196
600ug/ml	0.309	0.309	0.309	0.309	0.221
800ug/ml	0.344	0.342	0.345	0.344	0.256
1000ug/ml	0.398	0.397	0.395	0.397	0.309
Blank	0.088	0.088	0.088	0.088	***

Table 11. Absorbance values for aqueous extract

Concentration	abs 1	abs 2	abs 3	Average abs	Avg abs-blank
200ug/ml	0.194	0.196	0.194	0.195	0.107
400ug/ml	0.197	0.197	0.196	0.197	0.109
600ug/ml	0.202	0.204	0.202	0.203	0.115
800ug/ml	0.273	0.271	0.277	0.274	0.186
1000ug/ml	0.288	0.289	0.282	0.286	0.198
Blank	0.088	0.088	0.088	0.088	***

Table 12. Absorbance values for the standard (quercetin)

Concentration	Abs 1	Abs 2	Abs 3	Average abs	Abs- blank
20ug/ml	0.409	0.411	0.410	0.410	0.109
40ug/ml	0.445	0.447	0.448	0.447	0.146
60ug/ml	0.461	0.460	0.460	0.460	0.159
80ug/ml	0.487	0.487	0.487	0.487	0.186
100ug/ml	0.510	0.510	0.510	0.510	0.210
Blank	0.302	0.301	0.301	0.301	**

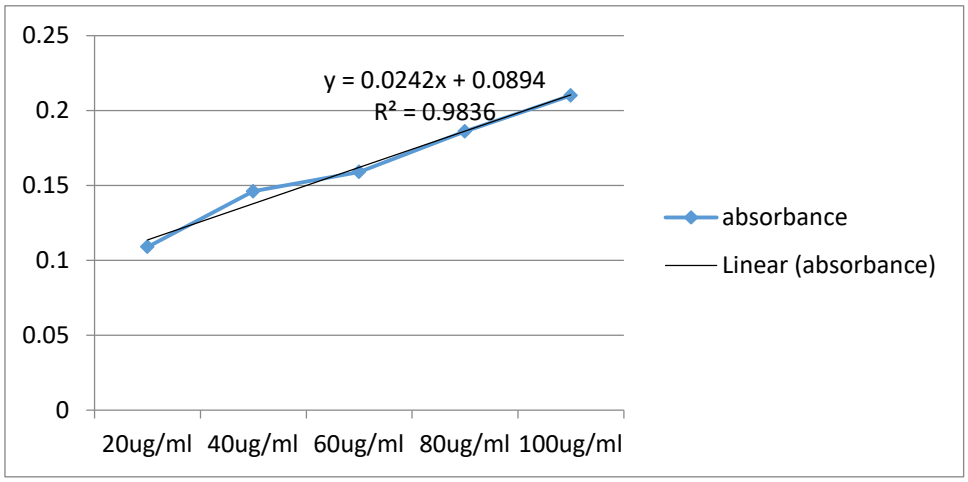

Fig. 2. Calibration curve for the standard

Table 13. Concentration of flavonoid content (in ug) in the different extracts at quercetin (standard) equivalence

Sample concentration	Ethanol fraction	Aqueous fraction
200ug/ml	3.17	0.72
400ug/ml	4.4	0.81
600ug/ml	5.43	1.06
800ug/ml	6.88	3.99
1000ug/ml	9.07	4.49

Table 14. TPC determination absorbance for the ethanol fraction

Concentration	abs 1	abs 2	abs 3	Average abs	Avg abs-blank
200ug/ml	0.364	0.366	0.363	0.364	0.146
400ug/ml	0.416	0.415	0.416	0.416	0.198
600ug/ml	0.510	0.511	0.510	0.510	0.292
800ug/ml	0.573	0.573	0.573	0.573	0.355
1000ug/ml	0.718	0.715	0.714	0.716	0.498
Blank	0.211	0.211	0.218	0.218	***

Table 15. TPC determination absorbance for aqueous extract

Concentration	Abs 1	Abs 2	Abs 3	Average Abs	Abs- blank
200ug/ml	0.276	0.275	0.277	0.276	0.058
400ug/ml	0.355	0.356	0.354	0.355	0.137
600ug/ml	0.427	0.429	0.429	0.428	0.210
800ug/ml	0.445	0.441	0.440	0.442	0.224
1000ug/ml	0.607	0.607	0.608	0.607	0.389
Blank	0.211	0.211	0.218	0.218	***

Table 16. Total phenolic content determination for the standard (garlic acid)

Concentration	Abs 1	Abs 2	Abs 3	Average	Abss- Blank
50ug/ml	0.099	0.100	0.098	0.099	0.004
80ug/ml	0.100	0.101	0.101	0.101	0.006
120ug/ml	0.435	0.436	0.436	0.436	0.341
160ug/ml	0.845	0.847	0.847	0846	0.751
200ug/ml	1.621	1.620	1.620	1.620	1.525
Blank	0.095	0.095	0.095	0.095	**
Calibration curve					

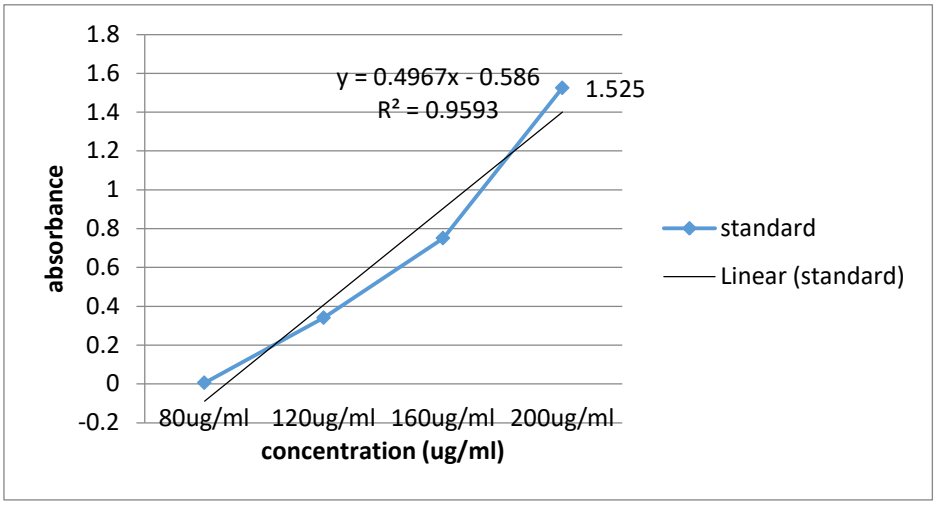

Fig. 3. Calibration curve using garlic acid as standard

Table 17. TPC of the different extracts (in ug) at garlic acid equivalence

Sample concentration	Ethanol extract	Aqueous extract
200ug/ml	1.47	1.3
400ug/ml	1.58	1.46
600ug/ml	1.77	1.6
800ug/ml	1.89	1.63
1000ug/ml	2.18	1.96

Table 18. Summary of results of quantitative phytochemical screening for the different samples

Sample	% alkaloids content (%w/w)	% flavonoid content (%w/w)	% saponin content (%w/w)	% tannin content (%w/w)	% terpenoid content (%w/w)	% total phenol (%w/w)
Ethanol extract	9.7	1.59	6.3	1.06	0.75	0.74
Aqueous extract	9.5	0.36	4.7	0.1	0.45	0.65

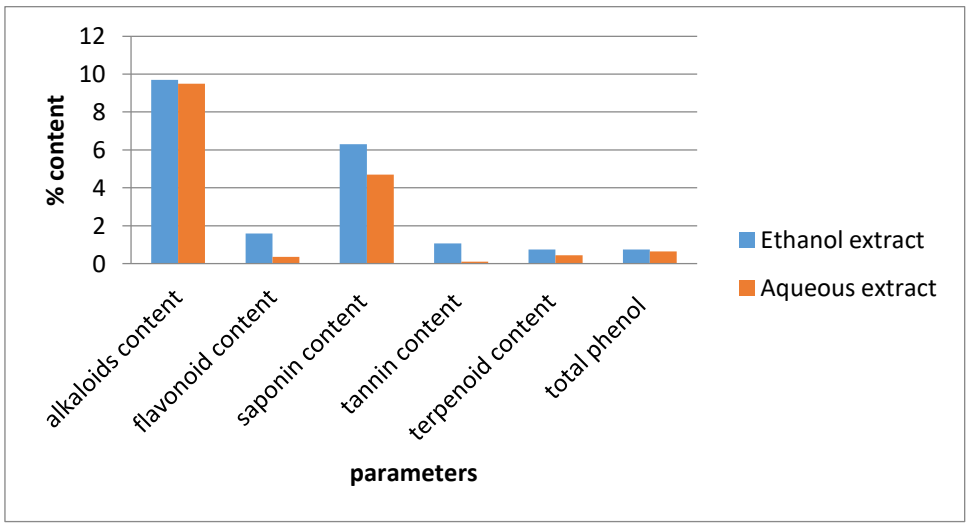

Fig. 4. Summary of quantitative phytochemical analysis

Table 19. Anti-microbial activities of ethanol and aqueous extracts against tests micro-organisms

0rg.	Ethanol (ug)		H2O (ug)		BO	B&E	B&O	GENT (ug)		KET (%)	
	MIC	MBC	MIC	MBC				MIC	MBC	MIC	MBC
1.	3.125	6.25	12.5	12.5	-	-	+	5	5	NA	NA
2.	25	25	25	25	-	-	+	5	10	NA	NA
3.	6.25	25	12.5	50	-	-	+	10	10	NA	NA
4.	>50	25	6.25	12.5	-	-	+	>10	>10	NA	NA
5.	12.5	25	1.56	6.25	-	-	+	10	10	NA	NA
6.	50	50	3.125	6.25	-	-	+	>10	>10	NA	NA
7.	25	25	3.125	3.125	-	-	+	NA	NA	1%	1%
8.	25	25	6.25	12.5	-	-	+	NA	NA	0.25	0.5%

KEY-BO=Broth only, B&E =Broth & Extract, B&O=Broth & Organism, GENT=Gentamicin. KET=Ketoconazole,NA- Not applicable

Key: 1-Staphylococcus aureus, 2- Bacillus subtilis, 3-Escherichia coil, 4-Salmonella typhi,5-Klebsiella pneumonia,6-Pseudomonas aeruginosa, 7-Tricophytum rubrum, 8-Candida albican

3.2 Antimicrobial Activity

The results of the antimicrobial activities are shown in above Table 19. The extracts of the plant were tested against five bacteria and two fungi. Against staphylococcus aureus, the result for the ethanol extract was MIC -3.125 while that of aqueous was 12.5 (µg) while the MBC was 6.25 and 12.5 (µg) respectively. This result is in agreement with [36] and [37]. In their work, they reported zones of inhibition of the extracts to be in the range of 10.1 to 21.4 mm with MIC values ranging from 62.5-500 µg/mL [48] also demonstrated that the ethanolic extract of the seeds possesses significant antibacterial activity against the pathogenic bacteria, P. aeruginosa, E. coli and S. aureus with MIC value 230 µg/L. According to [40], both ethanolic and aqueous extracts of A. mexicana were found to have antibacterial potential against Streptococcus mutans and Porphyromonas gingivalis responsible for oral cavity infection. They concluded that the alcoholic extract showed greater potency compared with the aqueous extract. A. mexicana was found to possess activity against multidrug resistant P. aeruginosa isolated from clinical samples [39]. The leaf extracts (acetone, methanol, ethanol and aqueous) of Twenty-seven strains were used for the study. They concluded that the aqueous extract was more effective when compared to the other extracts. Comparing the results we obtained, it is observed that our results agree with their findings. The activity of the extracts against *Bacillus subtilis, Escherichia coil, Salmonella typhi* and *Klebsiella pneumonia* was carried out by [49] measuring zones of inhibition. In their work, they found out that ethanol stem extract was of greater activity against K. pneumoniae (22.86 mm). Doss, et al. [50] equally worked on the aqueous and ethanol leaf extracts of *A. mexicana* against the above mentioned bacteria. However, the results they obtained is at variance with what we obtained. While the ethanol and aqueous extracts both have equal activity on *Bacillus subtilis,* the ethanol extract has more activity against *Escherichia coil* but the aqueous extract is more potent against *Salmonella typhi, Klebsiella pneumonia, Pseudomonas aeruginosa, Tricophytum rubrum,* and *Candida albican* above Table 19.

4. CONCLUSION

Argemone mexicana L. is used in different parts of the world for the treatment of several diseases which includes skin diseases, tumors, inflammations, warts, rheumatism, malaria, leprosy and microbial infections. The phytochemicals present in the plant confers the pharmaceutical efficacies which will be helpful in guiding researchers undertake further investigations of the plant as an anti-fungi and anti-bacterial agent. In conclusion, while the ethanol extract was potent against *Staphylococcus aureus* and *Escherichia coil,* aqueous extract was more active against *Salmonella typhi,Klebsiella pneumonia, Pseudomonas aeruginosa, Tricophytum rubrum*, and *Candida albican.* There is need for systematic research of this medicinal plant and more in-depth and extensive studies in all relevant aspects are still more warranted.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our

area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It's not applicable.

ETHICAL APPROVAL

It's not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. WHO. Regional Office for Western Pacific, research guidelines for evaluating the safety and efficacy of herbal medicines. Manila; 1993.
2. Kunle OF, Egharevba HO. Preliminary studies on vernonia ambigua: Prhytochemistry and Antimicrobial screening of the whole plant. Ethnobotanical Leaflets. 2009;13:1216-21.
3. Roopashre TS, Raman D, Shobha RRH, Narendra C. Antibacterail activity of antipsorriatic herbs: Cassia tora, Momordica charantia and Calendula officialis. International Journal of Research in National Products. 2008;3:20-28.
4. Eze VC, Ezeja MI, Onoja SO, Obi OI. Antibacterial, phytochemical and antioxidant properties of the leaf and root bark extract of *Baphia nitida* on bacteria associated with wound and enteric infections. World Journal Pharmaceutical Research. 2015;4(3):1111–1122.
5. Manurung H, Kustiawan W, Kusuma I, Marjenah W. Total flavonoid content and antioxidant activity of tabat Barito (Ficus deltoidea Jack) on different plant organs and ages. Journal of Medicinal Plants Sudies. 2017;5(6):120-125.
6. Neagu E, Radu GL, Albu C, Paun G. Antioxidant activity, acetylcholinesterase and tyrosinase inhibitory potential of *Pulmonaria officinalis* and *Centarium umbellatum* extracts. Saudi Journal of Biological Sciences. 2018;25:578–585.
7. Bogucka-Kocka AC, Zidorn M, Kasprzycka G. Szymczak - phenolic acid content, antioxidant and cytotoxic activities of four Kalanchoe species. Saudi Journal of Biological Sciences. 2018;25:662–630.
8. Agra MF, Silva KN, Basílio IJLD, de Freitas PF, BarbosaFilho JM. Survey of medicinal plants used in the region Northeast of Brazil. Rev Bras Farmacogn. 2008;18:472-508
9. Albuquerque UP, Monteiro JM, Ramosa MA, Amorim ELC. Medicinal and magic plants from a public market in Northeastern Brazil. J Ethnopharmacol. 2007;110:76-91.
10. Oyenihi AB, Smith C. Are polyphenol antioxidants at the root of medicinal plant anti-cancer success. Journal of Ethnopharmacology. 2019;229(30): 54-72.
11. Gill NS, Bajwa J, Sharma P, Dhiman K, Sood S. Evaluation of antioxidant and antiulcer activity of traditionally consumed Cucumis melo seeds. J Pharmacol Toxicol. 2011;6:82–89.
12. Pitout JDD. Multiresistant Enterobacteriaceae: New Threat of an Old Problem. Expert Review of Anti-infective Therapy. 2008;6(5):657–669.
13. Crump JA, Sjölund-Karlsson M, Gordon MA, Parry CM. Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance, and antimicrobial management of invasive salmonella infections. Clin Microbiol Rev. 2015;28(4): 901-37.
14. Martin RM, Cao J, Brisse S et al. Molecular epidemiology of colonizing and infecting isolates of Klebsiella pneumoniae. mSphere. 2016;1:e00261–16.
15. Gorrie CL, Mirceta M, Wick RR et al. Gastrointestinal carriage is a major reservoir of klebsiella pneumoniae infection in intensive care patients. Clin Infect Dis. 2017;65:208–15.
16. Bhavsar MM, Devarajan TV, Nembi PS, Ramakrishnan N, Mani AK. Metastatic endogenous endophthalmitis: A rare presentation with methicillin-resistant *Staphylococcus aureus* prostatic

abscess. Indian J Crit. Care Med. 2017;21(3):172–175.
17. Errington J, Aart LT. "Microbe Profile: *Bacillus subtilis*: Model organism for cellular development, and industrial workhorse". Microbiology. 2020;166 (5):42 5–427.
DOI: 10.1099/mic.0.000922
18. Jorgensen J, Ferraro M. Antimicrobial susceptibility testing: A review of general principles and contemporary practices. Clin. Infect. Dis. 2009;49:1749–1755.
19. Dogra S, Uprety S. The menace of chronic and recurrent dermatophytosis in India: Is the problem deeper than we perceive? Indian Dermatol Online J. 2016; 7:73–76.
20. Kibbler CC, et al. Management and outcome of bloodstream infections due to *Candida* species in England and Wales. J. Hosp. Infect. 2003;54:18–24.
21. Bhattacharjee I, Chatterjee SK, Chatterjee S, Chandra G. Antibacterial potentiality of *argemone mexicana* solvent extract against some pathogenic bacteria. Mem. Inst. Oswaldo Cruz. 2006;101(6): 645-8.
22. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants (Including the Supplement).Council of Scientific and Industrial Research, New Delhi; 1986.
23. Krishnamurthy A. The wealth of India. Vol. VIII, Publication and Information Directorate, CSIR, New Delhi. 1969;15 :415-417.
24. Willcox ML, Graz B, Falquet J, et al. "*Argemone mexicana* decoction for the treatment of uncomplicated falciparum malaria". Trop R Soc Trop Med Hyg. 2007;101:1190-1198.
25. Chevallier A. The encyclopedia of medicinal plants dorling kindersley. London an excellent guide to over 500 of the more well known medicinal herbs from around the world; 1996.
26. Dash GK, Murthy PN. Evaluation of *Argemone mexicana* Linn. Leaves for wound healing activity. J.Nat. Prod. Plant Resour., 2011 1 (1): 46-56.
27. Osho A, Afetunji T 2010. Antimicrobial activity of the essential oil of *Argemone mexicana* Linn. J Med Plant Res 4: 19-22.
28. Uddin SJ, Grice D, Tiralongo E 2011. Cytotoxic effects of Bangladeshi medicinal plant extracts. Evid-Based Comp Alt, Article ID 578092,
DOI: 10.1093/ecam/ nep111.
29. Khandewal K.R., (2008) Practical Pharmacognocy. Nirali Prakashan, Pune, edition: 19.
30. Gibbs R.D., (1974) Chemotaxonomy of Flowering Plants. Vol.1, McGill Queen's University Press, Montreal and London.
31. Kokate C K, Purohit A P and Gokhale SB. (2001) Carbohydrate and derived Products, drugs containing glycosides, drugs containing tannins, lipids and protein alkaloids. Text book of Pharmacognosy, 7, edition: 133 -166, 167- 254, 255-2 69, 272-310, 428-52 3.
32. Treare GE, Evans WC. (1985) Pharmacognosy 17th edn., Bahiv Tinal, London. P 149.
33. Harborne JB. 1998. Phenolic compounds. In Phytochemical Methods—A Guide to Modern Techniques of Plant Analysis, ed. JB Harborne, pp. 66–74. New York: Chapman & Hall. 3rd ed
34. Obdoni, B.O., and Ochuko, P.O. (2001). Phytochemical studies and comparative efficacy of the crude extracts of some Homostatic plants in Edo and Delta States of Nigeria. *Global Journal of Pure and Applied Sciences*. 8b:203-208.
35. Boham, B.A. and Kocipal-Abyazan, R. (1994) Flavonoids and Condensed Tannins from Leaves of Hawaiian vaccinium. vaticulatum and V. calycinlum. Pacific Science, 48, 458-463.
36. Saranya MS, Arun T, Iyappan P 2012. In vitro antibacterial activity and preliminary phytochemical analysis of leaf extracts of *Argemone mexicana* L. - A medicinal plant. Int J Curr Pharm Res 4: 85-87
37. Rahman MM, Alam MJ, Sharmin SA, Rahman MM, Rahman A, Alam MF 2009. In vitro antibacterial activity of *Argemone mexicana* L (Papaveraceae). CMU J Nat Sci 8: 77-84
38. Pandey A, Karanwal V. A study of extract optimization and effect of metal ions on antibacterial properties of *Argemone mexicana*. Asian J Plant Sci Res. 2011;1: 43-48.
39. Sahu MC, Debata NK, Padhy RN 2012. Antibacterial activity of *Argemone mexicana* L. against multidrug resistant Pseudomonas aeruginosa, isolated from clinical samples. Asian Pac J Trop Biomed: S800-S807.

40. Rosas-Pinon Y, Mejia A, Diaz-Ruiz G, Aguilara MI, SanchezNietoc S, Rivero-Cruza JF 2012. Ethnobotanical survey and antibacterial activity of plants used in the altiplane region of Mexico for the treatment of oral cavity infections. J Ethnopharmacol 141: 860-865.
41. Gali K, Ramakrishnan G, Kothai R, Jaykar B 2011. In-vitro anti-cancer activity of methanolic extract of leaves of *Argemone mexicana* Linn. Int J PharmTech Res 3: 1329-1333.
42. Mandal P, Sinha Babu SP, Mandal NC. Antimicrobial activity of Saponins from Acacia auriculiformis. Fitoterapia., 2005;76(5): 462-565.
43. Manjunatha BK. Antibacterial activity of Pterocarpus santalinus. Ind. J. Pharm. Sci. 2006;68(1):115-116.
44. Aboada OO, Efuwape BM. Antibacterial properties of some Nigerian species. Biol. Res. Comm. 2001;13:183-188.
45. Rievere C, Van Nguyen JH, Pieters L, Dejaegher B, Heyden YV, Minh CV, Quetin-Leclercq J. Polyphenols isolated from antiradical extracts of Mallotus metcalfianus. Phytochemistry. 2009;70: 86-94.
46. Aguinaldo AM, El-Espeso BQ, Guovara, MG. Nanoto. Phytochemistry. In: Guevara B.Q (ed) A. Guide book to plant screening phytochemical and biological. University of Santo Tomas, Manila, Philippines; 2005.
47. Arts IC, Hollman PC. Polyhenols are disease risk in epidemiological studies. Amer. J. Clin. Nut. 2005;81:317-325.
48. Scalbert A, Manach C, Morand C, Remesy C, Jimenez L. Dietary olyhenols and the prevention of diseases. Cri. Rev. Food. Sci. Nutr. 2005;45:287-306.
49. Alagesaboopathi C, Kalaiselvi N 2012. Antimicrobial activities of the root, stem and leaf extracts of *Argemone mexicana* L. Int. J Biosci 2: 61-68.
50. Doss A, Mubaracki HM, Vijayasanthi M, Venkataswamy R. In-vitro antibacterial activity of certain wild medicinal plants against bovine mastitis isolated contagious pathogens. Asian J Pharmaceut Clin Res 2012;5: 90-93.

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