

Characterization of Antioxidant Activity Present in Methanol Extract *Argemone mexicana* Leaf

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Abstract

Synthetic drugs are becoming less effective due to the organism resistant activities. New chemical substances with potential therapeutic effects are believed to be sourced from medicinal plants. Plant-based phytochemicals are becoming potentially more promising, there by replacing the synthetic drugs. Human health wise, Phytochemicals are reported to play a vital role in its protection at significant dietary intake, of which more than 4000 phytochemicals are cataloged. *A. mexicana* leaves were collected in the month of February 2017, from Maruda village, Gwandu and extracted using methanol as a solvent. The current study was conducted to screen for phytochemicals and antioxidant activity of methanol leaves extracts of *A. mexicana* using standard procedures of qualitative screening and total phenolic content. Total extractable of *A. mexicana* leaves showed 81.9g yield. The Qualitative phytochemical screening shows the presence of Alkaloids, Flavonoids, Saponins, Tannins, Terpenoids, Reducing sugar and absence of Steroids and Anthraquinones. Total phenolic content was 91.55 ± 0.21 mg of Gallic acid equivalent (GAE). Phenolic/antioxidant compounds are commonly present in extracts of natural product from plants these compounds may exhibit many biological effects including antioxidant activity. It is suggestive that these phenolic compounds found in the work may contribute to the anti-oxidative properties and usefulness of these in herbal medicament, because phenols have been reported to be useful in the preparation of some antimicrobial compounds such as dettol and cresol. The presence of phyto-chemical components is suggestive of being responsible for the numerous health benefits of *A. mexicana* leaves in both traditional and modern medicine.

Keywords: Antioxidants; *Argemone mexicana*; Drugs; Phytochemical

Introduction

Conventional/Synthetic drugs are becoming less effective due to the organism resistant activities. Plant-based phytochemicals are becoming potentially more effective there by replacing the synthetic drugs and also as a means of synthesizing new formulae for the discovery of new drugs that have less or no harmful side effects.

New chemical substances with potential therapeutic effects are believed to be sourced from medicinal plants. New synthetic drugs of plant derivation and new methods of producing them will continue to be an important and interesting area that should be explored for the discovery and development of new and safer drugs [1].

From the Greek Word Phytochemicals meaning plant substances, are biologically active naturally occurring chemical compounds found in plants which provide health benefit for humans further than those attributed to macronutrient and micronutrients [2]. They contribute to the plant color, aroma, flavor and protect plants from damages and diseases. Generally, plant chemicals that protect plant cells from environmental hazards are called phytochemicals [3].

Human health wise, Phytochemicals are reported to play a vital role in its protection at significant dietary intake, of which more than 4000 phytochemicals are cataloged [4]. Phytochemicals accumulate in different parts of plants such as root, stem, leaves and seeds [5], with wide-ranging dietary in fruits, vegetables, herbs, nuts, onions etc [6]. Those compounds are known as secondary metabolites and have biological properties such as antioxidants activity, antimicrobial effects, modulation of the detoxification, enzyme stimulation of immune system, decrease of platelet, aggregation of modulation of hormone metabolism and anti-cancer properties [7].

Argemone mexicana is a source of different chemical components [1], such as alkaloids as chelerythrine the glycosides, terpenoids, steroids, flavonoids, berberine, protopine, sanguinarine, optisine, reducing sugars and tannins. Long-chain aliphatic compounds and few aromatic compounds [1]. *Argemone mexicana* is regarded as one of the most significant plant species in traditional system of medicine (Brahmachari and Gorai 2013) used in different parts of the world, for management of various diseases, including inflammations, jaundice, leprosy, tumors, warts, skin diseases, rheumatism, microbial infections, malaria [1]. The yellow juice, which exudes when the plant is injured, has long been used in India as traditional medicine for emesis, as an expectorant, demulcent and diuretic dropsy, jaundice, ophthalmia, scabies and cutaneous affections [8,9], the seeds and seed oil are employed as a remedy for dysentery, ulcers, asthma and other intestinal infections [8].

Phenolic/antioxidant compounds are commonly present in extracts of natural product from plants and fungi, these compounds may exhibit many biological effects including antioxidant activity. The antioxidative effect is mainly due to the phenolic constituents, like flavonoids, phenolic diterpenes and phenolic acid [10]. The activities of such constituents are as a result of their redox properties [10].

Phenolic compounds have received an increasing attention due to their biological activities. From the pharmacological and therapeutic point of view the antioxidant properties of phenols are due to the total phenolic contents measured by folin-ciocalteu method [11]. Total phenolic content is measured by Folin-C method, adopted by Thaipong., *et al* [12]. The oxidation is due to Folin- c reagent and neutralization by sodium carbonate measured at 765 nm (Hodzic., *et al*. 2005). The principle involves electron transfer (ET) based assay and measured reducing capacity was used to express phenolic content of biological materials [13].

Materials and Methods

Plant collection

A. mexicana leaves were collected in the month of February 2017, from Maruda village, Gwandu local government, Kebbi State, Nigeria, identified by a taxonomist Dr D. Singh, Department of Biological Sciences, Kebbi State University of Science and Technology Aliero and was given (Voucher number of 152) and a specimen was deposited for future reference. The collected samples were washed using clean tap water and rinsed with distilled water and cut into small pieces with scissors and air dried at room temperature for 2 weeks. The dried sample was homogenized using mortar and pestle and stored in a glass amber bottle covered with aluminium foil paper until ready for use.

Extraction of *A. mexicana* leaves

Fifty-gram powder of *A. mexicana* leaves were weighed into five different 1000 ml beaker and each was soaked with methanol. The top of the conical flasks was covered with aluminum foil paper to prevent further evaporation of solvent and volatile constituents from the mixture. It was kept for three days with occasional stirring with a clean glass rod to ensure the maximum amounts of constituents present in the grinded plants become soluble into methanol. The mixture was filtered through Whatman filter paper No 1, England the solvent

was allowed to evaporate. The extract obtained was used for analysis such as phytochemicals, partitioning and total phenolic compound screening [14].

Phytochemical screening

Phytochemical analysis of the extracts was conducted using the qualitative test; the test was carried out to find the presence or absence of some active chemical constituents such as alkaloids, terpenoids and steroids, flavonoids, reducing sugars and tannins using the following methods.

Test for alkaloids: Hager's test

0.02g of methanol extracts of *A. mexicana* was weighed in 3 different test tubes. 10 ml of methanol was added in each test tube and test tubes were placed in vortex mixture to dissolve the extracts. The extract solutions were then filtered. 2 ml of filtrate were taken and mixed with 4 drops of 2% H_2SO_4 . To 1 ml of this mixture 6 drops of Hager's reagent was added. Yellow (turbid) color indicates presence of alkaloids [15].

Test for flavonoids: Ammonia test (modified)

0.25g of methanol extracts of *Argemone mexicana* leaf were weighed in 3 different test tubes. 5 ml of ethyl acetate was added in each test tube. The test tubes were heated at 40°C for 3 minutes in water bath. The mixtures were filtered and 2 ml of filtrate were taken and mix with 0.5 ml of 5% ammonia solution. Yellow color indicates the presence of flavonoid [16].

Test for steroids: Salkowski test

0.02g of methanol extracts of *A. mexicana* leaf were weighed in 3 different test tubes. The extracts were mixed with 2 ml of methanol and filtered. 1 ml chloroform and 1 ml concentrated H_2SO_4 were added into the filtrate. Yellow green fluorescent indicates the presence of steroids [15].

Test for Terpenoids: Salkowski test (modified)

0.004g of methanol extracts of *A. mexicana* leaf were weighed in 3 different test tubes. The extracts were treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. 3 ml concentrated H_2SO_4 were added slowly. Red violet color shows the presence of Terpenoids [17].

Test for carbohydrates: Fehling's (Reducing sugar) test (modified)

0.02g of methanol extracts of *A. mexicana* leaf were weighed in 3 different test tubes. The extracts were dissolved in 0.5 ml of methanol. Then 1 ml of water was added in it. 6 drops of Fehling solution were added. The samples were heated. Brick red precipitate indicate the presence of CHO [17].

Test for tannins: $FeCl_3$ test

0.125g of methanol extracts of *A. mexicana* leaf was weighed in 3 different test tubes. 5 ml of distilled water was added and dissolved by vortex mixture. Then samples were boiled for 3 minutes in water bath. The samples were filtered. 3 drops of 0.1% ferric chloride solution were added into the filtrate. Blue-black colouration shows the presence of tannin [16].

Test for saponins: Frothing test

0.5g of methanol extracts of *A. mexicana* leaf was weighed in 3 different test tubes. 5 ml of distilled water was added. The solutions were shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously. An emulsion formation indicates the presence of saponins [18].

Test for Anthraquinones: Chloroform layer

0.5g of methanol extracts of *A. mexicana* leaf was weighed in 3 different test tubes. 10 ml of H₂SO₄ were added in each test tube. The samples were kept in water bath for 3 min. to boil and then filtered while hot. Filtrates were shaken with 5 ml of chloroform. Chloroform layer were pipette into another test tube. 1 ml dilute ammonia was added. Resulting solutions were observed for color changes [18].

Gallic acid calibration curve

0, 1, 2, 3, 4, 5.....10 ml of prepared phenolic solution was measure in to 100 ml flask and made up to the mark with distilled water, this will have a concentration of 0, 50, 100, 150...500 mg/LGAE, from each calibration, sample and blank 20 ul was pipette in to separate curvet and to each 1.5 ml distilled water was added and 100 ul of Folin-C, then mix and its stand for 8minutes. To the solution 300 ul of sodium carbonate was added and allowed for 2hrs at 20°C and the absorbance was taken at 765 nm against blank (0 ml solution) the graph was plotted as absorbance against concentration.

Total phenolic content determination

0.02g of methanol extracts of *Argemone mexicana* leaves were weighed in 3 different centrifuge test tubes. 2 ml of methanol was added in each test tube. The extracts were dissolved using vortex mixture and kept at room temperature for 48 hours in dark. After 48 hours the test tubes were centrifuge at 5000 rpm for 5 minutes at room temperature the sample supernatant was collected and transferred in other test tubes. 300 µl sample supernatant was withdrawn from each separate test tube. 300 µl of methanol (blank) was taken as negative control to a separate test tube. 600 µl of 10% F-C reagent solution were added in each test tubes and vortex thoroughly, 2.4 ml of 700 M Na₂CO₃ solution was added in each test tube and kept at room temperature for 2h. The absorbance of total volume (3.3 ml) was taken in UV-VIS spectrophotometer at 765 nm. The total phenolic contents were determined from a standard curve prepared with gallic acid and the results were expressed as Mean ± SD [19]. The total phenolic contents of *A. mexicana* leaves was express as

TPC = CV/M mg of GAE (Gallic acid equivalent).

Where C = Concentration from calibration

V = Volume of the extract used

M = Mass of the extract used

Result

Phytoconstituents	Name of Test	Result
Alkaloids	Hager’s test	+
Flavonoids	Ammonia test (Modify)	+
Terpenoids	Salkowski test (modify)	+
Steroids	Salkowski test	-
Reducing Sugars	Fehling’s test	+
Tannins	FeCl ₃	+
Saponins	Frothing test	+
Anthraquinones	Chloroform layer test	-

Table 1: Phytochemical screening of *A. mexicana* methanol extract.

Key: + = present; - = absent.

Phytochemical screening of *A. mexicana* leaf revealed the presence of Alkaloids, Flavonoids, Terpenoids, Reducing Sugar, Tannins, Saponins and absence of Steroids and Anthraquinones.

S/NO	Sample Code	Absorbance	Concentration
1	Blank	0.000	
2	A	0.677	0.627
3	B	0.623	0.577
4	C	0.665	0.534

Table 2: Absorbance of the test sample against concentration.

S/No	Conc. of Standard (µg/ml)	Absorbance	Regression	R ²
1	0	0.012		
2	1	0.232		
3	2	0.341		
4	2	0.345		
5	4	0.405	Y= 0.088x	R= 0.0987
6	5	0.552	0.115	
7	6	0.651		
8	7	0.711		
9	8	0.801		
10	9	0.925		
11	10	1.025		

Table 3: Absorbance of Garlic acid against concentration.

Determination of concentration from standard curve

$$Y = 0.088x + 0.115$$

For A

$$0.667 = 0.088x + 0.115$$

$$0.667 - 0.115 = 0.088x$$

$$X = 0.552/0.088 = 6.28$$

For B

$$0.623 = 0.088x + 0.115$$

$$0.623 - 0.115 = 0.088x$$

$$X = 0.508/0.088 = 5.78$$

For C

$$0.665 = 0.088x + 0.115$$

$$0.665 - 0.115 = 0.088$$

$$X = 0.55/0.088 = 6.25$$

Determination of total phenolic content

$$\text{TPC} = \text{CV}/\text{M}$$

Where C = Concentration from calibration

V = Volume of the extract used

M = Mass of the extract used

For A

$$\text{TPC} = 6.28 \times 0.3/0.02 = 94.2 \text{ mg of GAE}$$

For B

$$\text{TPC} = 5.78 \times 0.3/0.02 = 86.7 \text{ mg of GAE}$$

For C

$$\text{TPC} = 6.25 \times 0.3/0.02 = 93.8 \text{ mg of GAE}$$

$$\text{Mean} = 94.2+86.7+93.8/3 = 91.55 \text{ mg of GAE}$$

$$\text{Standard deviation SD} = \pm 0.21$$

Therefore, Total phenolic content is 91.55 ± 0.21 mg of GAE.

Discussion

The plant *A. mexicana* is used for various medicinal purposes. The leaves areal part is mostly use as Anti-malaria, anti- microbial, anti-hepatitis, anti-diabetic, anti-HIV and anti-oxidants to mention but few, generally the data presented in this study provide evidence that the leaves of *A. mexicana* may contain biologically active compounds with potential values in the treatment of various disease as mention above. The leaves when extracted with methanol gave extractable values of; 15.5, 17.3, 16.8, 16.7 and 15.9g respectively yielding a total of 81.9g for the entire extractions.

Phytochemical screening of methanol extract of *A. mexicana* indicated the presence of certain secondary metabolites such as of Alkaloids, Flavonoids, Terpenoids, Reducing Sugar, Tannins, Saponins and absence of Steroids and Anthraquinones. Thus, confirming the medicinal claims of certain traditional medical practitioners that the leaf of *A. mexicana* have antimalarial, antibacterial and analgesic effect due to the presence of these secondary metabolites. And the results of this work is in agreement with the work of Al-baizyd [14], who also reported that *A. mexicana* can be used to treat several diseases, thus these metabolites might be responsible for these activities. The preliminary phytochemical screening investigation of *A. mexicana* may help in the recognition of the bioactive compounds and it may lead to the discovery and development of new drugs. These tests will also facilitate separation of pharmacologically active chemical compounds. Therefore *A. mexicana* leaves contain many secondary metabolites which are responsible for various medicinal properties and will be of great importance in phytomedicine like alkaloids which are reported to have many pharmacological activities such as Analgesic, Anti-malarial activity. Tannins are responsible for the antioxidant activities or free radical scavenging activities and heart disease prevention as reported by Mamta., *et al* [7]. These phenolic compounds found in this work may contribute to the anti-oxidative properties and usefulness of these in herbal medicament, because phenols have been reported to be useful in the preparation of some antimicrobial compounds such as dettol and cresol [20].

The presence of the above phytochemicals make the leaves pharmacologically active, these phyto-constituents are been reported to be responsible for all medicinal uses as well as the poisoning effects of *A. mexicana* leaf. The phenolic content of the methanol extract of *A. mexicana* leaves from this work were calculated to be 91.55 ± 0.21 mg of GAE, which is similar to the studies of Rajeshwari, *et al.* 2013 who reported that the maximum phenolic content of *A. mexicana* is in the range of 70 mg of GAE to 150 mg of GAE. The reported data for total phenolic content are a basis of assessment of the antioxidative and preventive role of *A. mexicana* leaves against free radical's effect, which is in agreement with the findings of Roya, *et al.* 2013, who reported that phenolic compounds are a class of antioxidant agents which act as free radical terminator and their bioactivities may be related to their abilities to chelate metals and scavenge free radicals. Thus *A. mexicana* could be used as a potential preventive intervention for free radical-mediated diseases. Hence *A. mexicana* leaves could serve as natural antioxidant supplement when administered to human system and can serve the purpose of artificial or synthetic antioxidant drugs with less harmful side effects since it has been reported to have a wide safety margin in literatures (Yusuf and Ukamaka 2018).

Conclusion

The presence of phytochemicals in the leaves extract of *A. mexicana* might be responsible for its therapeutic and antioxidant effect, and the findings of this research also supports the postulation that a single or combination of any of the identified compounds may have Anti-urolithiasis activity, Analgesic, Antimicrobial, Hepatoprotective, Anti-cancer activity, Sedatives activity, Anti-HIV, Anti-Diabetic, Anti-malaria and provide protection against free radical induced damage to biomolecules to mention but a few.

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