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PHYTOCHEMICAL AND PROXIMATE COMPOSITION OF *ARGEMONE MEXICANA L. (Papaveraceae)* IN SOKOTO, NIGERIA

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Abstract

This study was carried out in a Biochemistry laboratory, Usmanu Danfodiyo University, Sokoto to assess the phytochemical and proximate composition of Argemone maxicana L. Sample were collected from lowland and upland location and were analyzed for flavonoid, tannins, saponin, glycosides and alkaloids and Proximate analysis for nutrient contents using oven drying (moisture), muffle furnace ashing (Ash), Soxhlet extraction (Ether Extract), distillation and ashing (crude fiber), macrokjeldahl (N and Crude protein), and difference (NFE). Data collected were analyzed using statistical package for social sciences (SPSS) version 20.0 software. Results for phytochemical revealed that tannins, saponin and glycosides were found to be significantly (P<0.05) higher in lowland than upland area. Similarly, Argemone mexicana L, at the lowland area had significantly (P<0.05) high moisture contents, dry matter, biomass, ash, crude firbre, crude protein and nitrogen free extract contents than Upland area. Therefore, it is recommended that the presence of phytochemical and proximate contents of Argemone maxicana L. is responsible for the numerous health benefits of the A .maxicana in traditional system of medicine.

Keywords: Phytochemicals, Proximate Composition, Argemone Mexicana, Human Health, Traditional Medicine.

INTRODUCTION

From the Greek word phytochemicals meaning plant substances, are biologically active naturally occurring chemicals compounds, found in plants which provide health benefit for humans further than those attributed macronutrient and micronutrients (Hasler and Bhemberg,1999). They contributed to the plant color, aroma and flavor and protect plant cells from environmental hazards. Phytochemicals accumulates in different parts of plants such as root, stem, leaves and seeds with wide ranging dietary in fruits, vegetables, herbs, nuts, onions etc (Smith,1996).

Argemone Mexicana L. is one of the multicultural species with prickly hairless, branching system with yellow flowers. It is an annual erect herb commonly known as *Mexicana Prickly* poppy belongs to the family Papaveraceae. It is approximately 1m in height with broad leaves at the base (usually measuring 5 to 11cm long with green and white spots on their surfaces, and stem is prominently lobed nous and thorny (Chang *et al*, 2013). A nature of tropical America which is distributed in tropical and sub-tropical regions of the World. In northern

Nigeria, it grows as a weed in West lands, commonly found in cultivated fields, and edge of road or vacant lots. In traditional system of medicine different parts of the plant are used for treatment of skin, inflammation, warts, rheumatism, tumor, and microbial infections (Bhattacharjee *et al*., 2006). The flower has expectorant properties and have been used in the treatment of cough. The seed are efficient in treating leprosy, dropsy and jaundice (Alagesaboopathi, C. 2013) and the latent is used against conjunctivitis, the seed oils is used as remedy for asthma, ulcers, dysentery and other intestinal conditions Rubio, P.J. and Vazattes, F.F., 2013). Juice of the plant is used as a remedy against scorpion bite (Alagesaboopathi, C. 2013) furthermore, various part of the plant has been shown to have activity against malaria, leishmeniasia, and typanosomiasis (Willcox *et al.,* 2007)

A *mexicana* is a source of different chemical compounds such as alkaloids, glycosides, amino acid, phenolics and folic acids as major phytochemical groups. A series of bioactive compounds have been reported and some of them are isolated from different part of the plant. (Chang, *et al.,* 2013)

The whole plant of *A. Mexicana* was reported to possess isoquinoline alkaloids such as berberine, cheilanthifoline, coptisine, muramine, scoulerine, stylopine, cryptopine, thalifone, sarguinarine, propopine, optisine, chelerytherine and benzylisoquinoline alkaloids (Chang et al., 2003). Seed oils otherwise called as Argemone oil reported to contain sanguinarine, and dihydrosanguinarine. It also contains palmitic, myristic oleic and linoleic acids (Singh *et al.,* 2010). Phenolic/antioxidant compounds are commonly present in extracts of natural product from plants and fungi, those compounds may exhibit many biological effects including antioxidant activity. The antioxidant effect is mainly due to the phenolic constituent like flavonoids, phenolic diterpenes and phenolic acid the activities of such constituents are as a result of their redox properties (Bhattacharjee *et al*., 2006)

Tuken *et al.,* (1998) have reported that over the last two decades, studies have revealed that wild or semi-wild plants are nutritionally important because of higher vitamin, minerals, essential fatty acids and fibre contents from the study of Smith *et al., (*1996) on wild and cultivated plants from the regions in Burkina-Faso and Niger Republic, wild plants were often higher in mineral contents particularly zinc. High level of nitrogen, sulphur and lower contents were found in the polygonacese family, and high calcium, iron and manganese in the urticaceae family. High protein content was also reported in some wild vegetable in Bostwana (Flyinan and Afolayan, 2007).

In spite of the medicinal benefit of *Argemone Mexicana* L.in traditional system of medicine, there seen to be little or no information on phytochemical properties and proximate content on the plant species. Therefore, this research was undertaken to generate baseline data on phytochemical properties as well as proximate composition of *A. maxicana* in the study area.

Materials and Methods

The Study Area

The study was conducted at the permanent site, Usmanu Danfodiyo University, Sokoto (05^{o} 10^{o}E – 05^{o} 12E, 13^{o} 040N – 13^{o} 06 40N) at 350m above sea level and the climate vary into rainy season (May – October) and dry season (November –April). The pattern of rainfall distribution ranges from 553.43 – 628.94mm with relative humidity of about 16 – 55.5% and the average

temperature range minimally from 16.3% during harmattan season but can rise up to 18.6^{o}c to maximally 44.7^{o}c (Nimet, 2024).

Sample Collection: The plant material (leaves) of *A maxicana L.* line were collected right from their stand at full stage of maturity 20 weeks after sowing (WAS). Eight (8) innermost plants were sampled from the two (2) inner rows excluding the border line rows of each of the beds, making a total of twenty-four (24) plants (per 3 beds) sampled from the plant species under study from both lowland and upland locations. The leaves sample collection was done by handpicking mature green leaves of the plant species and were put in their respective bag for phytochemical and proximate analysis.

Preparation of Sample
The samples were separately selected according to location of the plant species (lowland and upland.). The fresh leaves of *A maxicana* were thoroughly washed with tap water and was air dried, plants were crushed to small pieces using mortar are a pestle before oven drying and powered in an electric grinder (Krishnaiah *et al.,* 2009).

Proximate analysis fresh sample, weights were taken before oven dried at 70^{o}C to constant weight. The dried samples were then pulverized separately according to location/habitat (lowland and upland) using mortar with pestle and sieved in 0.5mm mean size and stored for analysis (Udo and Ugonwale,1986).

Phytochemical Analysis
The powdered plant samples were screened in Biochemistry laboratory, Usmanu Danfodiyo University, Sokoto to determine flavonoid, tannins, saponin, glycosides and alkaloids

Test for Flavonoid
Three (3) ml aliquot of the filtrated and 1ml of 10% NaOH was added. If the yellow developed, it indicated possible presence of flavonoid compound.

Test for Tannis
Five (5%) of ferric chloride solution was added drop by drop 2 – 3ml of the extract and colour produced was noted. Then condensed tannis usually gave a dark green colour and hydrolysable tannis were gives blue-black colour.

Test for Sclponin
Five (5ml) of the extract was placed in a test tube and 5ml of water was added, this was shaken strongly. The whole tube was filled froth that last for several minutes.

Test for Glycosides
Two and half (2.5ml) of 50% H_2SO_4 were added to 5cm^3 of the extract in a tube. Then mixture was heated in boiling water for 15 minutes. Then, this were cooled and neutralized with 10% NaOH, 5ml of fehlin solution was added and the mixture was boiled. A brick-red precipitate was observed, which was indicting the presence of glycosides.

Test for Alkaloids

Two (2ml) of each extract were stirred with 2ml of 10% aqueous hydrochloric acid. 1ml was treated with a few drops of Wagner's reagent and second 1ml portion were treated similarly with Mayer's reagent. Turbidity or precipitation with either of these reagents was taken as preliminary evidence for the presence of alkaloids.

Proximate Analysis

The parameters determined were Moisture by oven drying method, Biomass by moisture content, Ash by muffle furnace Ashing method, either extract by Soxhlet Extraction method using Petrolium Ether, crude fibre by Ashing method, crude protein by Macro-kjheldahl method, and nitrogen free extract (N.F.E.) by difference Udo and Ugunwale,1986).

Determination of Moisture Content

Five (sg) grams of each sample were accurately weighed and place into an empty aluminum dish. The dish was shaken until the content became evenly distributed and placed into a plus 11 Galen kamp oven, maintained at 105^{o}c. The samples were dried for 24hrs, cooled in the desiccator and dried to a constant weight.

% mixture content = $\frac{w_1 - w_2}{w_1 - w}$ x 100

Determination of Biomes

Blomes – 100 – moisture content

Determination of dry moister

Dm = $w_1 - w_2$ x 100

Determination of Crude Protein

Two (2g) grams each of the sample were accurately weighted and carefully transferred to the kjeldahl flask. Then 10grams of potassium sulphate, 0.5g of copper sulphate and 25ml of H_2SO_4 were added. The flask was then placed in an incline position and heated below the boiling point of acid until the frothing ceased. Heat was increased until the acid boiled vigorously and digested for a time and the mixture became clear. The content of the flask was then cooled and transferred quantitatively to around bottom flask with 200ml of water. A few pieces of Punic store were added in sufficient quantity that made the solution rather formed a layer below the acid layer. The contents of the flask were mixed by shaking and distilled water was added until all the ammonia had passed over the standard H_2SO_4 solution and then filtrated with a standard NaOH solution. Blank determination was carried out using all the reagents in the same quantities but without the material/samples.

% Nitrogen = $\frac{0.04(B-A)\ x100}{w(100-m)/100}$

Determination of Crude Fibre

Two (2) grams of the sample were weighed and fat extract for eight hours with petroleum either using a Soxhlet. The fat free sample was then transferred in tone (1) litre capacity spotless beaker and 200ml level was marked with a glass pencil. Boiling water was added, then 25ml at 10% sulfuric acid, mixed and the volume made to 200ml level. This liquid was boiled for 30 minutes. At the end of the boiling point, the acid solution was removed by means of suction, through the stick filter. The filtrate was then collected in a clean suction flask to control efficiency of the filtration. The residue was washed at least three (3) times with

distilled water. The distilled water (200ml) and 25ml of 10% alkali added. The beaker was heated and boiling continued for exactly 30 minutes. The stick filter was left in the beaker and another filtering procedure was repeated. The resulting residue was quantitatively transferred to a large porcelain crucible. The wash water from the beaker was drained off by the stick filled. Finally, the filter cake extracted and dried by moistening with small portion of alcohol, which was permitted to drain between additions. The glass wool was then removed from the stick by means of forceps and left with the residue in the crucible. Material sticking to the glass tube were gently brushed down into the crucible. The crucible along with the sample and glass wool were dried at 60°c to a constant weight cooled and weighed.

% Crude Fibre = $\frac{w_1 + w_2}{w}$ x 100

Determination of Ether extract

Preparation of the Thimble: A Whiteman filter paper 8 x 8 was wrapped around a wooden stick 2cm in diameter and tired with thread so as to prevent the extraction thimble from opening easily during extraction. Five grams of the sample were weighed and transferred into the already prepared thimble. The mouth of the thimble was plugged with fat free absorbent cotton. Thimble with the sample was introduced into the Soxhlet and the apparatus assembled again. The Soxhlet was filled with petroleum ether by pouring it through the condenser at the top by means of a glass funnel. The amount of solvent was 1½ times the capacity of Soxhlet. The apparatus was then placed on a water bath at 60°c, fixed by clamps to a retort stand followed by cool water circulation in the condenser. Extraction continued for 8 hours. After the extraction, the thimble with the sample was removed from the Soxhlet. The apparatus was assembled again and heated on the water bath in order to recover all the ether from the receiver flask. The flask now continued only the crude fat. The receiver flask was then disconnected and the outside was thoroughly cleaned with dry cloth to remove the film of moisture and dust. It was dried in plus 11 Galen Kamp hot air oven at 60°c for 1hr cooled in the desiccator and weighed.

Either extract = $\frac{w_1 - w}{m}$ x 100

Determination of Ash

Ten (10) grams of the sample were weighed and placed in the furnace maintained at 500°c. The residue after burning was the ash (i.e. until all the carbon had been removed).

Nitrogen Free Extract

The nitrogen-free extract (NFE) referred to as soluble carbohydrate is not determined directly, but obtained at difference between crude protein and the sum of ash, protein, either extract and crude fibre.

NFE = 100 – (% Ash + % Crude fibre + % Ether extract + % Crude Protein)

Data Analysis

Data collected from the study were analysed using statistical package from social science (SPSS), version 20.0 software.

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Results

Table 1: Phytochemical composition of *Argemone mexicana* L at Dundaye Lowland and Plant Sciences garden (UDUS) upland areas

Phytochemical screening

Site	Flavonoids	Tannins	Saponins	Glycosides	Alkaloids
Lowland	+	++	++	+	+
Upland	+	+	++	ND	+

Means follow by same latter are not significantly different at 0.05%
+ = Trace ++ = moderate

The results in table 1 revealed that lowland area had trace (+) amount of flavonoids, glycosides, alkaloids and moderate (++) amount of tannins and saponins. Similarly, upland area had trace (+) number of flavonoids, tannins and alkaloids and moderate (++) amount of saponins. The results showed that glycosides was not detected (ND) at the upland area. It could be seen from the results that lowland had significant ($p<0.05$) higher Tannis and Glycosides.

Table 2: proximate composition of Argemone *mexicana* L at Dundayr Lowland and plant Sciences garden (UDUS) upland areas

Proximate Composition

Site	% Moisture	%Dry Matter	Biomass (g)	% Ash	% Ether Extract	% Crude fiber	% Crude protein	% NFE
Lowland	59.00[a]	44.87[a]	44.57[a]	13.00[a]	2.17[a]	5.17[a]	5.66[a]	66.17[a]
Upland	55.43[b]	38.46[b]	37.16[b]	8.83[b]	2.33[b]	3.34[b]	4.32[b]	51.64[b]
SE±	0.90	1.65	1.71	0.95	0.11	0.42	0.32	3.27
Sign	*	*	*	*	NS	*	*	*

Means follow by same latter are not significantly different at 0.05%
NS = Not Significant * = Significant

The results in table 2 had shown that at lowland area, moisture content was (59.00%), % dry matter (44.87%), biomass (44.57%), % ash (13.00%), % ether extract (2.7%), % crude fibre (5.17%), % crude protein (5.60%) and % nitrogen free extract (66.17%). Moreover, at upper land area, % moisture content was (55.43%), % dry matter (38.46%), biomass (37.16g), % ash (8.83%), % ether extract (2.33%), % crude fibre (3.34%) % crude protein (4.32%) and % nitrogen free extract (51.64%). Lowland area had significant higher amount of moisture, dry matter, biomass, ash, crude fibre, crude protein and nitrogen free extract while upland area had significantly (p,0.05) higher amount ether extract.

Discussion

The Phyto chemical composition of *Argemone maxicana L.* showed that the plant species contain different phytochemicals, some of which are known to be nutritional benefit, while others have some anti-nutritional activities. The presence of flavonoids, Tannins, Saponins,

Glycosides and alkaloids at lowland area indicates that the plant species could be used as a source of medicine.

Flavonoids found in *A. maxicana L.* they are known to possess antioxidant activity. This implies that consumers of plants may be protective against oxidative cell destruction by free radicals. On the other hand, flavonoids are often reported to be toxic and are able to initiate physiological change in the body when consumed. Flavonoids are mainly water-soluble compounds which occur both in the free-state and as glycosides (Harbone, 1998).

Tannis have general anti-microbial and anti-oxidant activities. Antioxidants are compounds that inhibit oxidation. Antioxidant is mostly used for two entirely different groups of substances, industrial chemicals that are added to products to prevent oxidation, and naturally occurring compounds that are present in food and tissue. Antioxidants are used as preservatives in food and cosmetics and being oxidation inhibitors in fuels. Xu *et al.,* (1996 reported that Tannis had potential valve such as cytotoxic and antineoplastic agent, which can kill, destroy or influence the grown.

Saponins in *A maxicana L.* may be of benefit but nutritional and commercially as reported by Xu *et al.,* (1996) who reported that the plant extract containing saponins are commonly used as foaming agent for beverages such as beer and soft drinks. Saponins are also thought to have commercial application such as ore separation in industrial and mining operations. Saponins are also useful products in hydrographs, emulsion, fire extinguisher foam, tooth paste and shampoo. Saponins rich herbs also contribute to the lowering blood cholesterol and inhibition of cancer proliferation (Harbone, 1998).

Glycoside plays numerous important roles in living organisms. Many plants stored chemical in the form of inactive glycosides. These can be activated by enzyme hydrolysis which causes the sugar part to be broken off, making the commercially available for use. Many glycosides found in plants are used in medications in animal and human (Krishnaiah *et al.,*2009).

Alkaloids also have pharmacological effects; they are used in medications and as recreational drugs. Thomas *et al.,* 2004 revealed that most alkaloids have a bitter taste are poisonous while ingested. Alkaloid production in plants appeared to have evolved in response to feeding by herbaceous animals, however, some animals have evolved the ability to detoxify alkaloid. Some alkaloids can produce developmental effects in the offspring of animals that consume but cannot detoxify the alkaloid.

According to Singh, 2010 most of the known functions of alkaloids are related to protection. In addition, presence of alkaloids in the plant prevents insects and chordate animals from eating it.

Generally, the result on proximate analysis (Table 2) shows that upland area was lower in contents of organic carbon, CEL, mineral elements, biomass, dry matter, moisture content, ash, ether extract, crude fibre, crude protein and nitrogen free extract may be due to accumulation of nutrients in the lowland area which may have influence the absorption of mineral elements from the soil (Hennins, 2004).

This observation agreed with the report of Silmany *et al.,* (2004) that variation in the nutrient concentration of individual plant species may be course by differences in plant habits or environment.

Conclusion

Conclusively, it was found out that the phytochemical screening at flavonoids, tannis, saponins, glycosides and alkaloids in which tannis, saponins, glycosides have the highest values at lowland area, while at upland area saponins was the highest and glycosides was not detected. On the other hand, *Argemone mexicana L.* at lowland area had more moisture, dry matter biomass, ash, crude fibre, crude protein, and nitrogen free extract.

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