



Pharmacognostic, Physicochemical and Phytochemical Investigations on Aerial Parts of *Argemone mexicana* L.

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Abstract

Background and objectives: In India, *Argemone mexicana* is traditionally used against fever, wounds, worms and malaria. In Mali the efficacy and safety of “Sumafura Tiemoko Bengaly” an herbal tea based on *A. mexicana* aerial parts against malaria have been demonstrated. This study was aimed to investigate the pharmacognostic, physicochemical and phytochemical parameters of its aerial parts.

Methods: Macroscopy, microscopy, chemo-microscopy, phytochemical, mineral and physicochemical analyses were performed using standard methods. **Results:** The macroscopy showed that *A. mexicana* is an herbal plant with prickly both on its greenish stem and the pinnatlobed leaves; the flower is terminal and yellow and the fruit is a capsule with thorns. The microscopy revealed the presence of epidermal cells with actinocytic stomata, calcium oxalate prism, laticifers, palisade cells, vascular bundle, fibers and collenchyma cells in the fresh leaf and the dry aerial parts. The chemo-microscopy revealed the presence of lignins, tannins, starch, calcium oxalate, oils and proteins. Phytochemical screening revealed the presence of carbohydrates, alkaloids, tannins, flavonoids, saponins, sterols and triterpenoids. The physicochemical parameters as observed included moisture content (8.2 %); total ash value (16.7 %); acid-insoluble ash value (2.9 %); water-soluble ash value (4.8 %); ethanol - soluble extractive value (17.2 %) and water- soluble extractive value (34.3 %). Six minerals (Fe, Cu, Mn, Mg, Pb, and Cd) have been also measured. **Conclusion:** These parameters help to establish the correct identity of *A. mexicana* and check the occurrence of adulterations. Further, they are useful for the standardization and pharmacopoeia development.

Keywords: *Argemone mexicana*; minerals; pharmacognostic features; physicochemical parameters; phytochemicals

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Introduction

Recently, there has been a renewed interest in drugs of natural origin considering them as green

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medicine which has always been thought to be safe for the treatment of infections originated from microbial and non-microbial [1,2]. Pharmacognostic investigation implicates standardization and authentication of plant's crude drugs and generally this study was conducted in order to identify controversial species of plants [3]. The Pharmacognostic evaluation which includes macroscopic/organoleptic, microscopic, physicochemical and phytochemical analyses assesses the quality and purity of crude drugs in order to establish the standard pieces of information that will form the monographs to be used for their correct identification [3,4].

Argemone mexicana L. is one of the multicultural species with prickly, hairless, branching stems with yellow juice and attractive yellow flowers. Etymology of the binomial: *Argemone* comes from the Greek word "argena" meaning cataract of the eye; juice of the leaf was supposedly used to treat cataract; *mexicana* comprises Mexico with the Latin suffix 'ana', suggesting the country of origin [5]. It is an erect herb up to 5 cm in diameter. The fruit is capsule, spiny, elliptic-oblong. The seeds are brownish-black, nearly spherical, about 1.0 - 1.2 mm in diameter, covered in a fine network of veins and oils [6]. In India *Argemone mexicana* is traditionally used against fever, wounds, worms and malaria [2]. In Southern Mali *Argemone mexicana* is locally called "Bozobo", "Nienidjeni" or "Sayi Bagani" and is used in the management of malaria [7]. In Northern Nigeria this species is known as "Karanko", "Kwarkwaro" or "Kaki Ruwan Allah" in Hausa [8]. The Hausa traditional healers used the powdered leaves on swollen area of skin against inflammation [9]. This study aimed to establish the macroscopic, microscopic, chemomicroscopic, physicochemical and phytochemical standards of the aerial parts of *Argemone mexicana* that would be useful in preparing the monograph of the plant.

Materials and Methods

Ethical considerations

This study was approved by the Proposal and Health Research Ethics Committee, Plateau State Specialist Hospital of Jos (Reg. No: NHREC/05/01/2010b) dated 27th February, 2019.

Plant collection and identification

The aerial parts of *Argemone mexicana* were

collected in Blendio, Sikasso region (Mali), in September 2014 and identified by Professor D. Diallo, Department of Traditional Medicine (DMT), National Institute of Public Health, University of Sciences, Techniques and Technologies of Bamako. A voucher specimen (No 2948/DMT) was deposited at the Herbarium of the Department of Traditional Medicine for reference purpose. The plant material was pulverized into a fine powder and kept in plastic container for future use.

Morphological, organoleptic, microscopical, chemo-microscopical, physicochemical and phytochemical studies

The evaluations of the morphological, organoleptic, microscopic, chemo-microscopic, phytochemical and physicochemical parameters were determined according to standard methods [4,10-12].

Macroscopical examination

The morphological features of the fresh aerial parts of *Argemone mexicana* such as shape, size, colour, and leaf structure like margin, venation and inflorescence, fruit etc. were macroscopically observed and described using sensory organs. In addition, some organoleptic parameters of the powdered sample were also observed using the standard description of terms like color, taste, texture and odor as described [1,4].

Microscopical examination

Carefully derived tiny sections of the fresh leaf, including transverse sections of the lamina with the midrib, other portions of the whole leaf as well as the surface preparations of both epidermises and small quantities of the leaf powder were cleared using few drops of chloral hydrate solution with gentle heating using a spirit lamp. They were then mounted in dilute glycerol and observed under a compound microscope using suitable magnifications (x100 and x400) [4,13,14] and photomicrographs were taken using a mobile phone camera (Iitel A16).

Chemo-microscopic examination

The powdered aerial part sample was treated (mounted) with appropriate chemical reagents on microscope slides and observed under the microscope to ascertain the presence or absence of chemical substances as follows: phloroglucinol plus conc. hydrochloric acid for lignin, ferric chloride solution for tannins, sudan iv solution

for oil, N/50 iodine solution for starch, million's reagent for proteins and chloral hydrate plus hydrochloric acid for calcium oxalate [4,13].

Physicochemical parameters

The moisture content (loss on drying), ash values and extractive values of the dried aerial parts were determined using standard procedures [4,11,15,16].

Phytochemical screening

The powdered aerial parts were subjected to preliminary chemical tests for the presence of various primary and secondary metabolites including carbohydrates, tannins, flavonoids, alkaloids, cardiac glycosides, saponins, anthraquinones, sterols and triterpenes using standard procedures [4,10,16,17]. Elemental analysis was performed using a standard method [18].

Results and Discussion

The findings from macroscopic examination and organoleptic characters provide vital data to help in the identification and description of the plant species. The macroscopic observations revealed that *Argemone mexicana* was a prickly, glabrous, branching herb with yellow flowers (figure 1). The stem was greenish and ramified wearing right thorns or was prickly. The leaf was greenish, parted, serrate and alternate without petiole measuring 4-18 × 1.5-9 cm. The upper surface was whitish-green while the lower surface was greener. The leaf was also spiny on its edges and the dorsal pinnate with whitish veins. The flower was terminal and yellow. The fruits were capsules 1-3.5 cm in length, oblong ovoid with short and right thorns. The seeds were numerous, globosely, netted and brownish black. Traoré

reported similar results without talking about the stem and more details of other parts such as leaf, flower, and fruit [19]. The current results present more details which help to describe the plant.

The organoleptic parameters have been reported in table 1.

Table 1. Organoleptic characters of the powdered aerial parts of *Argemone mexicana*

Characters	Observations
Color	Green-brown
Odor	Slightly pleasant
Taste	Non characteristic
Texture	Smooth

According to Traoré the powdered aerial parts of *Argemone mexicana* was greenish with tobacco odor rough in touch without taste [19].

The microscopic examination of the fresh leaf showed the presence of epidermal cells with actinocytic stomata both in the upper and the lower surfaces, laticifers and xylem. The laticifers represent one common character of the Papaveraceae family. The lower epidermis seemed to contain slightly more stomata than the upper epidermis (figure 2). The transverse section of the leaf through the lamina showed the presence of cuticle, upper epidermis, palisade cells, vascular bundle, spongy mesophyll and lower epidermis (figure 3); while the transverse section of the leaf across the midrib exhibited upper epidermis, collenchymatous cells, vascular bundle and lower epidermis (figure 4). Very few microscopic investigations have been done on the fresh leaf; however, the studies of Wayal and Gurav showed that the fresh leaf was microscopically consisted by lamina and midrib [20].



Figure 1. Macroscopic features of *Argemone mexicana* aerial parts; A: habit photograph of *A. mexicana*; B: upper leaf surface; C: lower leaf surface; all pictures were captured by Adama Dénou.

The lamina (dorsiventral) showed the presence of cuticle, upper epidermis, mesophyll and lower epidermis with elongated parenchymatous cell, diacytic stomata, palisade cells and spongy parenchyma; while the midrib consisted of upper and lower epidermises, was biconvex in outline. Below to the upper epidermis and above to the lower epidermis few layers of rounded, thick-walled, cellulosic collenchymatous cells were observed.

Most parts of midrib were occupied with arc-shaped vascular bundles. The vascular bundle consisted of lignified xylem vessels (pink color

with phloroglucinol and conc. HCl) and few layers of small, rounded, non-lignified phloem parenchyma [20]. Both results confirmed that *A. mexicana* was a vascular plant or tracheobionta.

The microscopic examination of the dried aerial parts of *A. mexicana* revealed the presence of epidermal cells with actinocytic stomata, scalariform xylem, palisade parenchyma, vascular bundle, fibers and calcium oxalate prisms (figure 5). These last microscopic features were similar to the previous findings obtained by earlier worker at the department of traditional medicine in Mali [21].

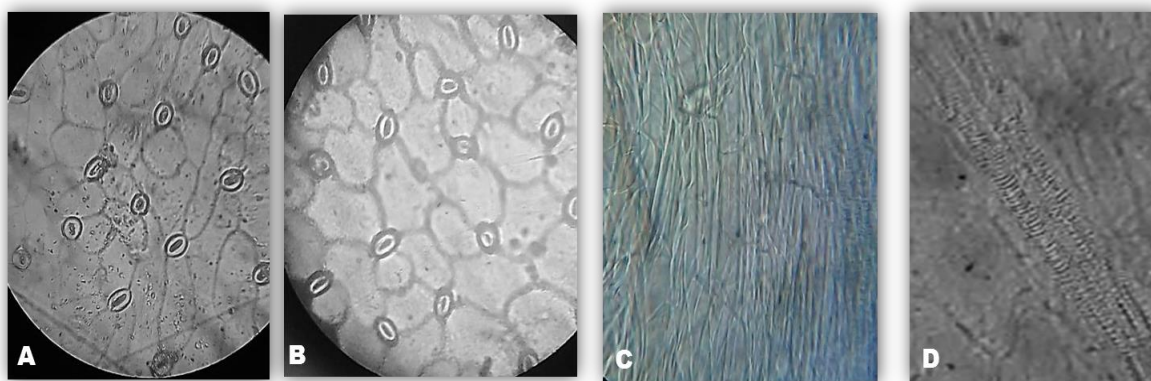


Figure 2. Micrographs of the fresh leaf of *Argemone Mexicana*; A) upper epidermal cells with actinocytic stomata; B) lower epidermal cells with actinocytic stomata; C) laticifers; D) xylem (x400)

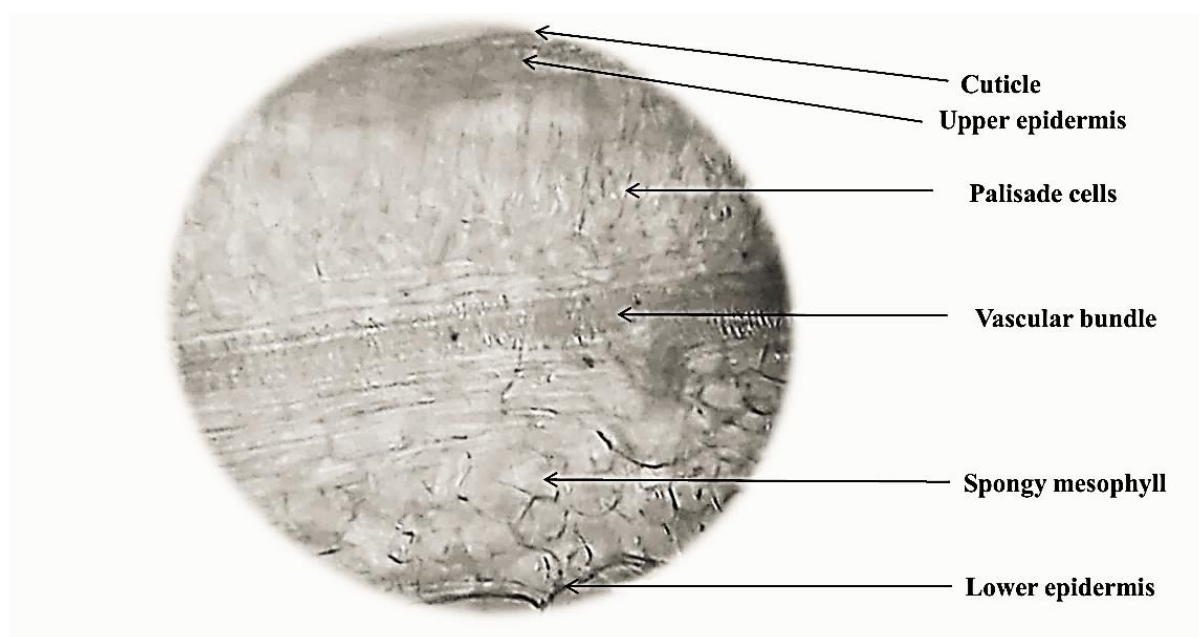


Figure 3. Transverse section across the lamina of the leaf of *Argemone mexicana* (x400)

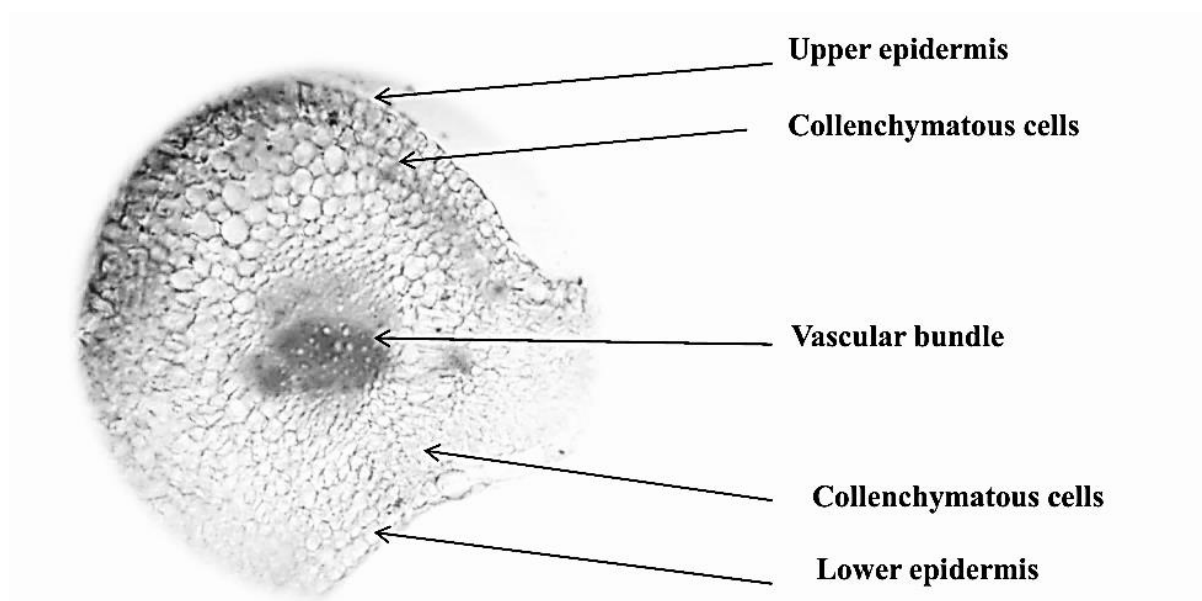


Figure 4. Transverse section across the midrib of *Argemone mexicana* leaf (x100)

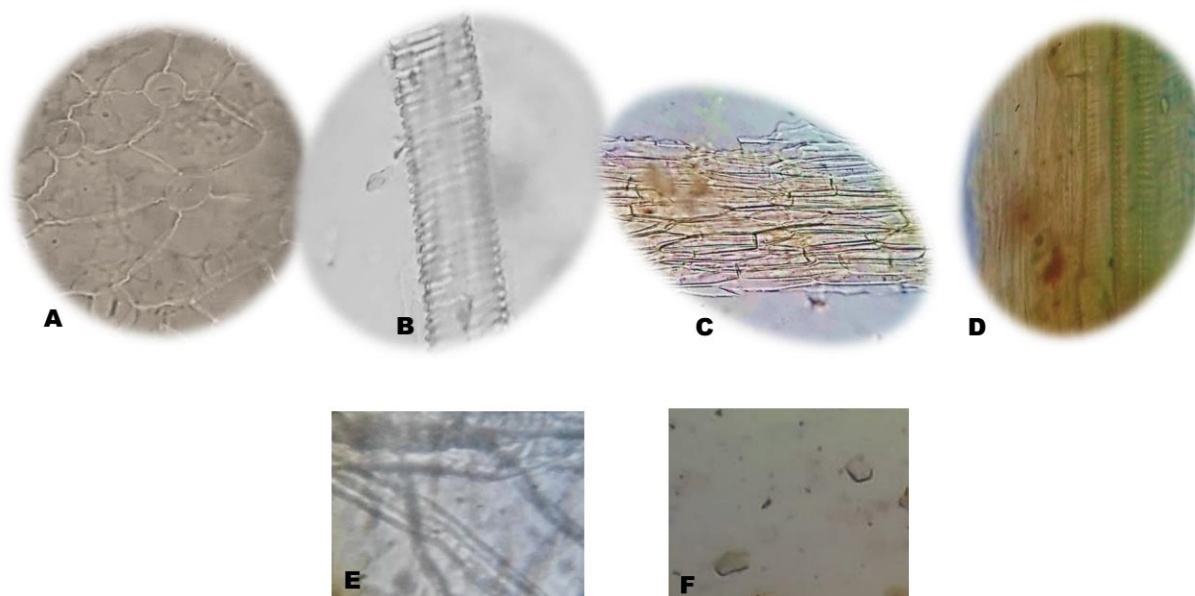


Figure 5. Micrographs from the powdered aerial parts of *Argemone mexicana*; A) epidermal cells with actinocytic stomata; B) scalariform xylem; C) palisade parenchyma; D) vascular bundle; E) fibers; F) calcium oxalate prisms (x400)

The chemo-microscopy of the aerial parts indicated the presence of some chemical substances such as lignin, tannin, starch grain, calcium oxalate prism, oil and protein. To our knowledge, it is the first time to perform the chemomicroscopy on *Argemone mexicana* aerial parts. However earlier work in Nigeria showed that the root contained tannins, protein, fat and oil [9].

Evaluation of the physicochemical parameters of the plant aerial parts has been summarized in table 2. It is to be noted that each result is a mean \pm standard deviation (SD) of three independent measurements. Moisture content (loss on drying) was determined as $8.2 \pm 0.3\%$ which is very close to the findings of the earlier works [19,21,22]. The moisture value drops within the acceptable limit range 0-14 % for moisture content of crude drugs [11]. The obtained moisture value indicated

how the plant material can be stored for longtime. When the moisture content of a drug is high, the crude drugs can easily deteriorate due to the activity of fungi and other hydrolytic micro-organisms [23].

Ash values are beneficial in determining the quality and purity of crude drugs especially in the powdered forms [13]. In this work, the total ash value was determined as 16.7 ± 2.3 % (table 2) which denotes both 'physiological ash' (ash from the plant tissue) and non-physiological ash (ash from extraneous matter) [11]. The total ash value of a crude drug reveals the degree of care taken in its preparation. The physiological ash usually involves the carbonates, phosphates, silicates, nitrates, sulphates and chlorides of metal elements taken up by the plant during its growth [24]. Elements taken up by plants from the soil include sodium, potassium, calcium, magnesium, cobalt, iron, manganese, carbon, phosphorus, nitrogen, sulfur, oxygen, chlorine and silicon [25]. The result here means that, 2 g of this plant crude drug (aerial parts), contains less than 20 % of residual substances, including impurities that do not volatilize when ignited. These findings confirmed those from previous authors [19,21,22]. The water- soluble ash value was found to be 4.8 ± 0.8 % (table 2), which is composed of physiological aspects or parts of the plant tissue where the active constituents reside [24]. The acid- insoluble ash value was determined as 2.9 ± 0.5 % (table 2), while Sanogo and coworkers mentioned 1.61% [26] and it represents silica/sand (non- physiological ash). A high acid-insoluble ash in aerial parts indicates some contaminations with earthy material (impurities). The results obtained are more or less favorable, because they show that impurities make up about 3 % in 2 g of the drug powder; which becomes very significant, especially where the drug is to be used directly in powder, as observed in traditional medicinal practice [4]. The determination of extractive values is useful in measuring the amounts of chemical constituents in drugs that are extractable by a chosen solvent (water or alcohol) under specified conditions. It helps to also tell the suggest the chemical nature of the contained constituents (their degree of polarity) and the choice of the most suitable solvent for extraction and phytochemical studies [25]. The evaluation is important as it aids to standardize the drug and secure against wrong

identification and adulteration or substitution with other drugs. The result of this work showed that, the ethanol- soluble extractive and the water- soluble extractive values were 17.2 ± 0.13 % and 34.3 ± 0.09 % (table 2), respectively which show that the aerial parts of *A. mexicana* contain more of water- soluble (very polar) constituents. Our finding is similar to the one of Sidibé who also found 34 % as water extractive value in *Argemone mexicana* aerial part from Blendio [22]. In both cases water appears to be a better solvent for the constituents. The values for moisture content is low compared to other plants, this fact showed that the plant material was well dried after its collection. These results will be useful for a standard monograph for the aerial parts of this plant, which will also aid in the detection of adulteration or substitution.

Table 2. Physiochemical evaluation of aerial parts of *Argemone mexicana*

Parameters	Values (%w/w)±SD*
Moisture content	8.2 ± 0.30
Total ash	16.7 ± 2.30
Acid-insoluble ash	2.9 ± 0.50
Water-soluble ash	4.8 ± 0.80
Ethanol-soluble extractive	17.2 ± 0.13
Water-soluble extractive	34.3 ± 0.09

*Average values of three determinations

The plant powder was subjected to preliminary phytochemical screening. It showed the presence of carbohydrates, alkaloids, mucilages, flavonoids, cardiac glycosides, sterols and triterpenes, tannins, and saponins; however, anthraquinones were absent (table 3). The presence or absence of certain important compounds in the plant powder was determined by color reactions of the compounds with specific chemicals which act as colors, precipitous or froth. This procedure is a simple preliminary prerequisite before going for detailed phytochemical investigations. Our findings were very close to those from the previous works performed in Mali [19,21,22,26] where alkaloids, flavonoids, tannins and monosaccharides were in abundance qualitatively. On the other hand, the water extract of the aerial part of this species contained carbohydrates, steroids, flavonoids, tannins and phenolic compounds, saponins, gums and mucilages, proteins, amino-acids while alkaloids, cardiac and anthraquinone glycosides, fat and oils were absent [20]. These chemical classes detected in *A. mexicana* aerial parts could made the plant useful for treating different

diseases as having a potential of providing beneficial drugs for human use.

Table 3. Phytochemical screening of aerial parts of *Argemone mexicana*

Constituents	<i>Argemone mexicana</i>
Alkaloids	+++
Tannins	+
Flavonoids	++
Saponins	+
Carbohydrates	+++
Monosaccharides	+++
Free reducing sugar	+++
Pentose	+++
Ketose	++
Combined reducing sugar	+++
Mucilages	+++
Sterols	++
Triterpenes	++
Cardiac glycosides	++
Anthraquinones	-

Key note: (+++) highly present, (++) moderately present, (+) slightly present, (-) absent

Several elemental constituents at trace levels play an effective role in the prepared medicines. The results of elemental analysis obtained by atomic absorption spectrophotometer (AAS) and expressed in mg/Kg dry weight of the samples have been shown in table 4. It is to be noted that each result is an average of two independent measurements. The iron (Fe) content (143.65 mg/Kg) was the higher followed by magnesium (Mg) (100.5 mg/Kg). Copper (Cu) and manganese (Mn) contents were low. Lead (Pb) and cadmium (Cd) were absent in our sample. Sidibe found that the aerial parts of *Argemone mexicana* collected in Blendio and Missidouyou contained Fe²⁺ at 15.79 µg/100 mg and 98.73 µg/100 mg, respectively while the Mg²⁺ values from the same two places were 7.90 µg/100 mg and 3.18 µg/100 mg, respectively [22]. In Cote d'Ivoire some authors found higher contents of iron from some medicinal plants used against anaemia such as *Tectona grandis* (266.6 mg/100g), *Amaranthus spinosus* (236.6 mg/100g), *Stylosanthes erecta* (206.6 mg/100g) and *Imperata cylindrica* (170 mg/100g) [27]. On the other hand, Zafar and coworkers mentioned that their sample contained Mg²⁺, Fe²⁺, Mn²⁺, Cu³⁺, Pb⁴⁺, Cd²⁺ in the amounts of 2023.42 mg/g, 618.6 mg/g, 62.00 mg/g, 0.00 mg/g, 86.00 mg/g and 3.4 mg/g, respectively [28]. This confirms that plants accumulate and assimilate several elements from the soil. The elements such as Fe, Mg, Mn, and Cu are essential or beneficial for human health [28,29]; while, Cd²⁺, Pb⁴⁺ and Li⁺ are non-essential [28]. The concentrations of various

elements for a given plant depend on the composition of the soil as well as permissibility, selectivity and absorbability of this plant for the uptake of these elements. Iron content in plants mainly vegetables and fruits are low, varying from 0.13 to 3.01 mg/100 g. The iron in plant is mostly present in the form of insoluble complexes of Fe³⁺ with phytic acid, phosphates, oxalates and carbonates, and its bioavailability from foods is less than 8%. The recommended intake of iron is 8-18 mg per day [29]. The main function of iron is related to the synthesis of hemoglobin and myoglobin [30-32]. It is also required for energy production. Severe iron deficiency leads to hypochromic anemia [30,31]. The toxicity of Fe in the body may be due to genetic or metabolic disorders, frequent blood transfusions or excessive intake. An excess of iron over a long period could induce liver and heart damage, diabetes, and skin changes [33]. Magnesium occurs strongly in vegetables and is very relevant in the maintenance of human health through the diet. Its content in the fresh material range from 5.5 - 191 mg/100 g; and the recommended daily intake is 200-400 mg [29]. This element acts as a calcium antagonist on vascular smooth muscle tone. Magnesium deficiency links to ageing and age-related disorders, mainly as a consequence of deficient intake in the diet. An increase in the intake of Mg helps to protect people from the incidence of chronic diseases such as diabetes, metabolic syndrome, hypertension and several cardiovascular conditions [34] where a low-Mg diet may contribute to insulin resistance, especially when the deficiency associated with a high-fructose diet. Magnesium toxic effects are not frequent [29]. Manganese is usually found in fruits and vegetables at low concentrations. Vegetables contain Mn in the range 0.01 - 0.078 mg/100 g and fruits 0.01 - 0.66 mg/100 g [34]. The recommended intake of Mn is 2 mg/day [29], and its major physiological role is being an enzyme cofactor involved in antioxidant reactions associated to the glucose metabolism [30-32]. Deficiencies in Mn are extremely rare but have shown a reduction in cholesterol, red blood cells and mucopolysaccharide abnormalities. An excess of Mn leads to toxic effects in the brain. Low concentrations of copper (Cu) have been mentioned in vegetables, ranging from 0.004 to 0.24 mg/100 g, except legumes (0.5 mg/100 g). Fruits contain small amounts of

Cu, ranging from 0.01 to 0.24 mg/100 g [35]. The recommended dietary allowance (RDA) of Cu is between 1.0 and 1.6 mg per day [29]. Copper primary functions are linked to enzyme function including Phase-I detoxifying enzymes (i.e., the cytochrome C oxidase family of enzymes) [30-32]. Further, Cu is required for the development of connective tissue and nerve coverings (myelin sheath) [31,32] and also contributes in the Fe metabolism [30,31]. The presence of essential minerals with permissible content in a given plant material contributes largely to the health benefits of this plant. Lead (Pb) and Cadmium (Cd) are known as toxic minerals or heavy metals so their absence in the analyzed sample constitutes a key safety parameter of the investigated aerial parts of *Argemone mexicana*.

Table 4. Mineral contents of *Argemone mexicana* aerial parts

Powder of <i>A. mexicana</i> aerial parts	Content (mg/Kg)					
	Cu	Fe	Mn	Mg	Pb	Cd
	19.4	143.65	23.45	100.5	0.00	0.00

The present work focused on pharmacognostical, physicochemical and preliminary phytochemical examinations of the aerial parts of *Argemone mexicana*. The outcomes of this study exhibited relevant pieces of information that could help to check the adulterations and could be used for the standardization and pharmacopoeia parameters development of *Argemone mexicana*.

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Author contributions

Adama Dénou conceptualized the study, performed the pharmacognostic, physicochemical, phytochemical and mineral investigations, wrote the manuscript; Abubakar Ahmed participated to design the study and was responsible for the microscopy analysis; Dalen G. Dafam was responsible for the mineral analysis and corrected

the first draft of the manuscript; Thomas P. Yakubu was responsible for the phytochemical analysis and participated in the microscopy analysis; Rokia Sanogo participated to conceptualize the study; Drissa Diallo was responsible for the botanical identification; Taiwo E. Alemika supervised the study. All authors read and approved the manuscript.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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Abbreviations

AAS: Atomic Absorption Spectrophotometer;
AOAC: Association of official analytical Chemists;
DMT: Department of Traditional Medicine;
RDA: Recommended Dietary Allowance