PHYSICOCHEMISTRY AND PRELIMINARY PHYTOCHEMISTRY OF LEAVES OF SOME SUDANESE MEDICINAL PLANTS

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ABSTRACT

The leaves of *Datura innoxia, Datura stramonium, Albizzia lebbek, Albizzia zygia, Cymbopogon citratus* and *Cymbopogon schoenanchus* were selected for this study because of their public use in Sudanese folkloric medicine. Ash and moisture contents are physiochemical constants can be used as a reliable aid to check the identity, purity and strength and are of great values for characterization of the plant drugs. The extractions of any crude drug with a particular solvent yield a solution containing different phytoconstituents. In this study physicochemical parameters like the moisture and ash cntents were determined beside phytochemical study of the petroleum ether and methanolic extracts of the studied leaves. It is clear that the methanolic extracts weights are larger than those of the petroleum ether extracts and this indicates that these plants contain large quantities of polar compounds. *D. stramonium* methanolic extract is found to possess the larger weight, whereas *C. citratus* posses the least weight. The petroleum ether extract of *D. innoxia* is more than that of D. stramonium while it's methanolic extract is less than that of *D. stramonium*.The petroleum ether extract of *A. zygia* is more than that of *A. lebbek*, also the methanolic is found to be less than that of *A. lebbek*. The Rf values and the colours of the spots showed that *A. zygia* contains the largest number of spots whereas *A. lebbek* contains the least number of spots. The plates when sprayed with Vanillin/H_2SO_4 reagent, revealed larger numbers of compounds in the petroleum ether extracts, and this also indicates that this reagent is a good reagent for the separation of non-polar compounds *A. lebbek* and *A. zygia* leaves contain larger numbers of compounds than the other species.

Keywords: *Datura, Albizzia, Cymbopogon*, Physiochemistry, Phytochemistry.

1. INTRODUCTION

Herbal medicines derived from plant extracts are being increasingly utilized to treat a variety of clinical diseases**.** The pharmacognostical studies on plants give idea about identification, standardization and monograph of the plant. It is also important in long term study of plant to evaluate the medicinal and therapeutic action of the plant [1 and 2]. Physicochemical constants viz., ash and other parameters can be used as a reliable aid to check the identity, purity and strength [3]. Total ash may vary within wide limits for specimen of genuine drugs due to variable natural or physiological ash. Ashes give us an idea of the mineral matter contained in a plant. Measuring it is important, because mineral matter may be the cause of a pharmacological effect [4]. Thin Layer Chromatography is done as an important tool for the authentication of herbal drugs and formulations. The results obtained from the study could be utilized for scientific validation and formulating standards for the quality assurance of the drug. The extractions of any crude drug with a particular solvent yield a solution containing different phytoconstituents. The compositions of these phytoconstituents depend upon the nature of the drug and the solvent used. It also gives an indication whether the crude drug is

exhausted or not [5]. The aim of this study is to determine the ash (Total ash, Acid insoluble ash and Acid soluble ash) cntents , moisture cntents and some phytochemical characters of the leaves of *Datura innoxia* and *Datura stramonium* family Solanaceae, *Albizzia lebbek* and *Albizzia zygia* family Mimosaceae and *Cymbopogon citratus and Cymbopogon schoenanthus* family Poaceae.

2. Materials and methods:

2.1. Plant Materials:

The plant materials used in this study were leaves of *D. innoxia, D. stramonium, A. lebbek, A. zygia, C. citratus and C. schoenanthus*. They were cllected from different regions of the Sudan in 2016, identified and authinticated.

2.2. Methods

2.2.1.Physiochemical Analysis: The methods described by [6] were followed:

i. Determination of Moisture contents:

Moisture contents of the leaves of the studied plants were determined by accurately weighing two grams (W1) in a crucible. The samples were left in 105^{o}C oven for three hours, transferred to a desiccator for one hour to cool and finally reweigh (W2). The moisture contents (W1- W2), percentage of moisture and the percentage of the dry matter were calculated.

ii. Determination of Ash contents:

a) Total Ash:

In this method 5 grams (W1) of the dried leaves were placed in accurately weighed porcelain dishes. They were put in a muffle furnace at about 550^{o}C until light gray ash content of constant weight were obtained. The dishes were then cooled in a desiccator and reweighed (W2). The ash value (W2 – weight of the porcelain dish) and its percentage were calculated.

b) Acid insoluble ash:

The total ash from above were boiled with 25 ml of dilute hydrochloric acid, filtered through an ashless filter papers, washed with hot water until free from chlorides, the filters and their contents were dried, ignited and weighted. The final weights which represent the acid insoluble ash were recorded. The percentage of it was calculated.

2.2.2. Phytochemical Analysis:

The percentage weights of the petroleum ether and the methanol extracts:

10 grams of the dry leaves of each of the plant material studied were extracted with petroleum ether (B. P. 40-60^{o}C) in soxhlets for about four hours. The petroleum ether extracts were filtered and concentrated using the rotatory evaporator. The weights of the dried extracts and percentages were determined. The plant residues after the extraction with petroleum ether, were dried and then extracted with methanol in soxhlets for about four hours, the filtered methanolic extracts were concentrated. The dried extracts were weighed and the percentages were determined, values are the means of triplicate readings..

2.2.3. Chromatographical Finger Prints:

Thin layer Chromatographical (TLC) examinations of the petroleum ether and the methanolic extracts of the dried leaves were carried out using 10 x 10 cm thin layer plates coated with 0.25 silica gel G type 60.

Single spot from each extract were applied 1.5 cm from the base using capillaries.

The plates were developed at room temperature in standard separation tanks.

Petroleum ether: Chloroform: glacial acetic acid (55: 45: 1 v/v) was used as developing solvent for the petroleum ether extract. The solvent systems used for the methanolic extracts were; ethyl acetate: methanol: water (100: 13.5:10 v/v) and petroleum ether: chloroform: glacial acetic acid (55: 45: 1v/v). After development, the plates were examined and the spots

of the chromatoplates were visualized by the naked eye and then sprayed with the following spray reagents:

1. Vanillin / H_2SO_4 spray reagent: 1 gram vanillin was dissolved in 100 ml concentrated H_2SO_4.
2. Dragendroff's spray reagent:

a. 0.85 grams Bithmus nitrate dissolved in 10 ml acetic acid and 40 ml distilled water.
b. 8 grams Potassium iodide was dissolved in 20 ml distilled water.
Stock solution was made by mixing 1 ml of a, 1 ml of b, 4 ml acetic acid and 20 ml distilled water.

3. Ferric chloride spray reagent:

2 ml of was added to 98 ml of distilled water.

Rf-values were calculated using the following formula:

$$\text{Rf-value} = \frac{\text{Distance traveled by the substance}}{\text{Distance traveled by the solvent}}$$

3. Results:

3.1. Physicochemistry :

The leaves of *D. innoxia*, *D. stramonium*, *A. lebbek* , *A.zygia* ,*C. citratus* and *C. schoenanthus* were studied for the percentages of moisture contents, dry matter, ash contents including total ash, acid insoluble ash and acid soluble ash. The results were presented in (table 1). The percentages were calculated with reference to the dry weight of the plant. These values are the means of triplicate readings. The moisture content of the leaves studied had a rate of below 10% this water content rate, prevent oxidation reactions, fermentation and give less chance to microbial growth and contamination in drugs [7]. Moisture content of drugs should be at minimal level to discourage the growth of bacteria, yeast or fungi during storage. This result comply with the standards established by the International Pharmacopoeia, because ash values are used to determine quality and purity of crude drug. It indicates presence of various impurities like carbonate, oxalate and silicate. The water soluble ash is used to estimate the amount of inorganic compound present in drugs. The acid insoluble ash consist mainly silica and indicate contamination with earthy material. Estimation of extractive values determines the amount of the active constituents in a given amount of plant material when extracted with a particular solvent.

The ash values and the moisture contents of the leaves of *Datura innoxia*, *Datura stramonium*, *Albizzia lebbek*, *Albizzia zygia*, *Cymbopogon citratus* and *Cymbopogon schoenanchus* are of great values for characterization of the plant drugs. Their values may not be constant since they may change with different factors like the climatic conditions, the type of the soil and the age of the plant when collected [8].

Table (1): Physiochemical features of the leaves .

Plant species	Moisture contents	Total ash	Acid insoluble ash	Acid soluble ash
D. innoxia	6%	25.84%	7.7%	92.3%
D. stramonium	3.56%	38.76%	4.92%	95.08%
A. lebbek	4.4%	10.59%	1.42%	98.58%
A. zygia	6.99%	4.08%	0.575%	99.425%
C. citratus	1.14%	8.58%	2.67%	97.33%
C.schoenonthus	0.81%	9.8%	3.82%	96.18%

3.2. Percentage Weights of The Petroleum Ether and The Methanolic Extracts:

The percentage weights of the petroleum ether and the methanolic extracts of the dry leaves of *D. stramonium*, *D. innoxia*, *A. zygia*, *A. lebbek*, C. *citratus* and *C. schoenanthus* are represented in (table 2). It is clear that the methanolic extracts weights are larger (heavier) than those of the petroleum ether extracts and this indicates that these plants contain large

quantities of polar compounds [9]. *D. stramonium* methanolic extract is found to possess the larger weight. Whereas *C. citratus* posses the least weight.
The petroleum ether extract of *D. innoxia* is more than that of D. stramonium while it's methanolic extract is less than that of *D. stramonium*.
The petroleum ether extract of *A. zygia* is more than that of *A. lebbek*, also the methanolic is found to be less than that of *A. lebbek*.
C. citratus and *C. schoenanthus* have equal percentages of ether extract, but the methanolic extract of *C. schoenanthus* is more than that of *C. citratus*.

Table (2): Percentage Weights of The Petroleum Ether and The Methanolic Extracts.

Plant species	Petroleum ether extract	Methanolic extract
D. stramonium	12%	27.0%
D. innoxia	15%	20%
A. zygia	11.58%	12.63%
A. lebbek	8.33%	18.33%
C. citratus	3.33%	5%
C. schoenanthus	3.3%	8.33%

3.3. Thin Layer Chromatography:

The petroleum ether and the methanolic extract when studied chromatographically; they are separated into many compounds with different Rf-values and different colours with the spray reagents

3.3.1. The Petroleum Ether Extract:

The results of thin layer chromatography of the petroleum ether extract using the solvent system petroleum ether: chlaroform: glacial acetic acid (55: 45:1 v/v) revealed number of spots (plate 1) and they give different colours when sprayed with vanillin/H_2SO_4 and Dragendroff's spray reagents.
The Rf values and the colours of the spots (table 3) *A. zygia* is found to contain the largest number of spots whereas *A. lebbek* contain the least number of spots with the spray reagents. These plates when sprayed with Vanillin/H_2SO_4 reagent, revealed larger numbers of compounds in the petroleum ether extracts, and this also indicates that this reagent is a good reagent for the separation of non-polar compounds (Harborne,1973). *A. lebbek* and *A. zygia* leaves contain larger numbers of compounds than the other species

3.3.2. The Methanolic Extract:

The results of thin layer chromatography of the methanolic extract using the solvent systems petroleum ether: chloroform: glacial acetic acid(55:45:1 v/v) and the solvent system ethyl acetate: methanol : water (100: 13.5: 10 v/v) revealed a number of spots (table 4), these spots give different colors when sprayed with vanillin/H_2SO_4 , Dragendroff's and ferric chloride spray reagents (plates 2and 3). When the plates are sprayed with Dragendroff's spray reagent; large number of compounds appeared, the orange or yellowish green colours indicated the presence of alkaloids, Harborne (1973) stated that Dragendroffs spray reagent is a good reagent for the detection of alkaloids they are clear in *D. innoxia* and *D. stramonium*, *A. zygia* and *A. lebbek*. The brownish green colours appeared when sprayed with FeCl3 spray reagent indicated the presence of the phenolic compounds in *A.lebbek,A. zygia, C. citratus* and *C .schoenanthus* [10] stated that *Albizzia* species contain tannins which is a phenolic compound. [9] stated that the detection of phenolic compounds is by the presence of intense green, purple, blue or black colours with 1% aqueous extract or alcoholic FeCl3 solution .

Table (3): Rf-Values and Reactions With The Spray Reagents of The Petroleum Ether Extract.

	Plant species	Rf-values	Color with Vanillin/H_2SO_4	Color with Drangendraff's
1	*D. stramonium*	0.35, 0.40 0.46 , 51 , 0.81 0.90 , 0.91	Yellow, green Green, brown, yellow Purple, blue	_ , _ green- , _ _, green
2	*D. innoxia*	0.31 , 0.35 0.40 , 0.90 , 0.91	Green, green Green, purple, blue	Green , green _ , _, green
3	*A. zygia*	0.19 , 0.33 , 0.38 0.40 , 0.54 , 0.63 0.66 , 0.88, 0.91	Blue, green, green Green, purple, green Green, purple, blue	Green, bue-green Blue, _ , yellow Orange, green
4	A. l*ebbek*	0.34 , 0.88 , 0.91	Green , purple, blue	-
5	*C. citratus*	0.34 , 0.88 , 0.91	Green , purple, blue	_
6	*C. schoenanth*s	0.19 , 0.23 , 0.31 0.41 , 0.88	Purple, purple	Green -

Table 4: Rf-Values and Reactions With Different Spray Reagent of The Methanolic Extract.

Plant species	Rf-values	Colour with Vanillin/H_2SO_4	Colour with Drangendraff's	
D. Stramonium	0.06 , 0.13 0.44 , 0.63 0.88	_ , yellow Yellow, blue Green	Green, green _ , green _	 Green
D. Innoxia	0.13 , 0.5 , 0.56 0.63 , 0.88, 0.9	Purple, _, _ Blue, green, green	Green , green, y. _ , _	 Brown, green
A.. Zygia	0.13 0.19 , 0.44 0.65 , 0.88 , 0.9	_, purple , _ blue, green, green		 Brownish green
A.. Lebbek	0.06 , 0.38 , 0.44 0.54 , 0.35 , 0.88	Yellow, yellow, y. Yellow, yellow green	_ _, green green - , _	_ _ _brownish green _
C. citratus	0.85 , 0.88	Purple, brown	_	Brownish green_
C. schoenanthus	0.53 , 0.85 0.88	_ purple brown	_	Brownish green

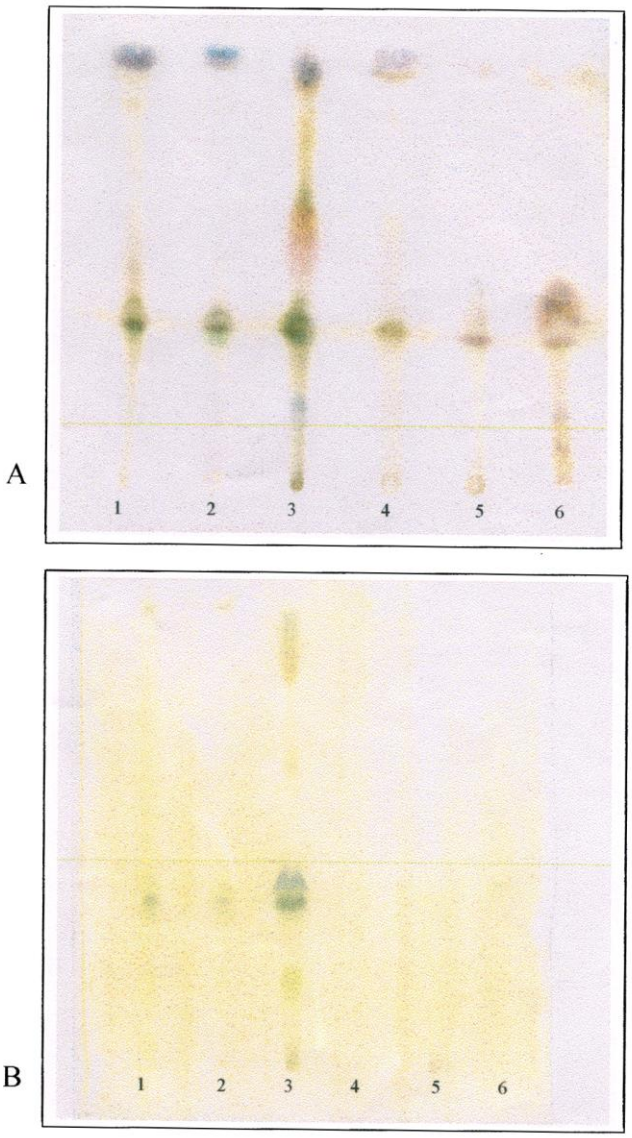

Plate (1): Thin layer chromatogram of petroleum ether extract, solvent system: petroleum ether:chloroform:glacial acetic acid (55:45.5:1v/v) sprayed with (A) vanillin/sulphuric acid spray reagent. (B) Dragendroff spray reagent.
1- *Datura innoxia* 2- *Datura stramonium* 3-*Albizzia lebbek* 4- *Albizzia zygia* 5- *Cymbopogon citratus* 6- *Cymbopogon schoenanchus*

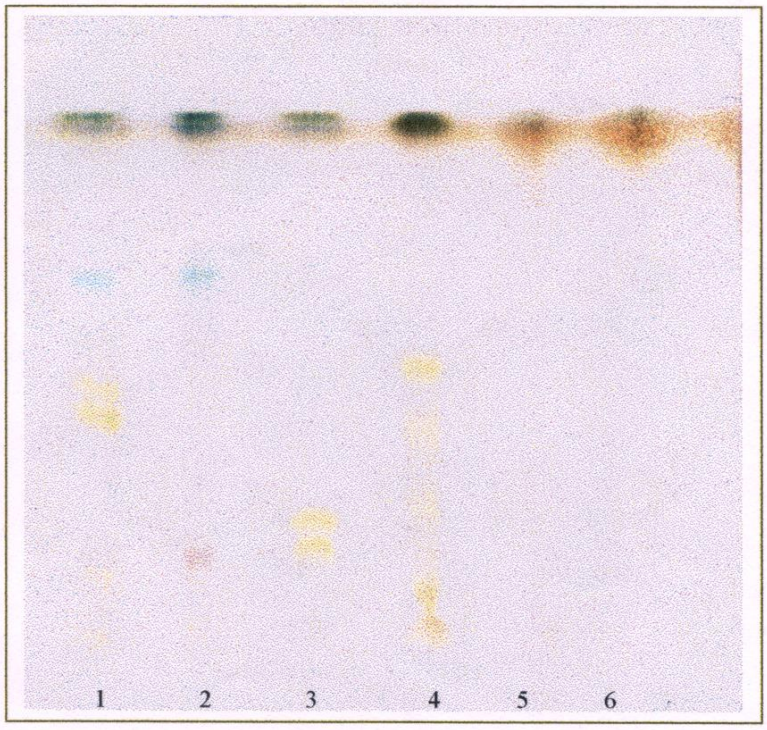

Plate (2): Thin layer chromatogram of methanolic extract, solvent system: ethylacetate:methanol:water (100:13.5:10v/v) sprayed with vanillin/sulphuric acid spray reagent.
1- *Datura innoxia* 2- *Datura stramonium* 3-*Albizzia lebbek* 4- *Albizzia zygia* 5- *Cymbopogon citratus* 6- *Cymbopogon sc*

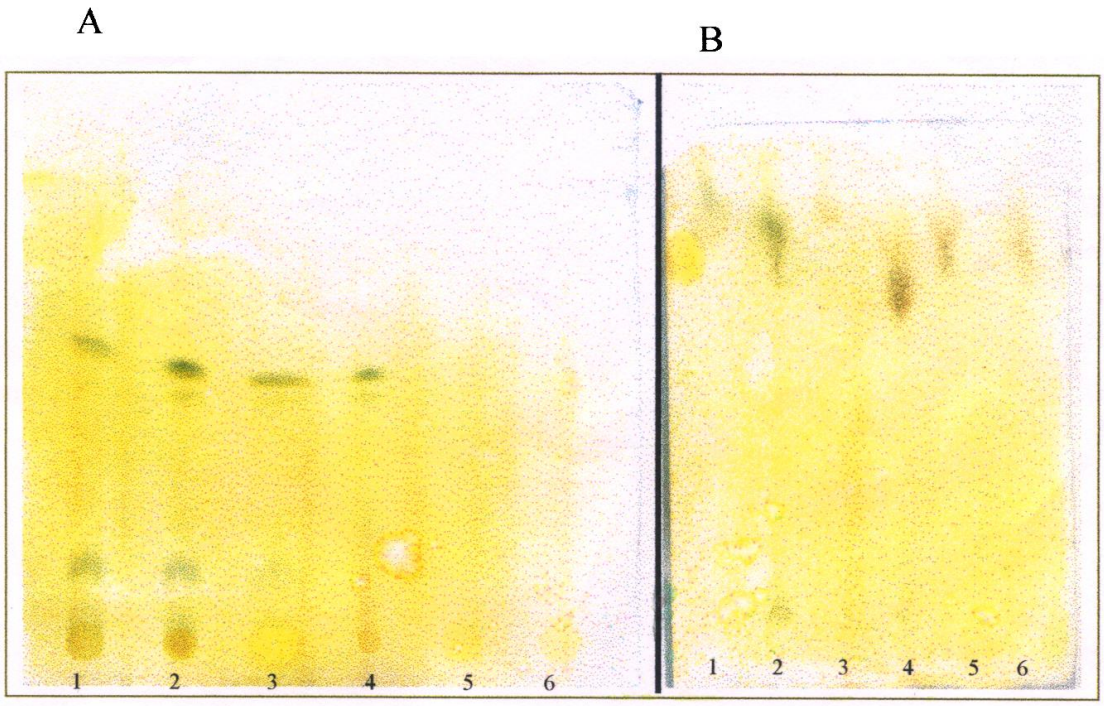

Plate (3): Thin layer chromatogram of methanolic extract, solvent system: petroleum ether:chloroform:glacial acetic acid (55:45.5:1v/v) sprayed with ferric chlride spray reagent.
1- *Datura innoxia* 2- *Datura stramonium* 3-*Albizzia lebbek* 4- *Albizzia zygia* 5- *Cymbopogon citratus* 6- *Cymbopogon schoenanchus*

CONCLUSION

These evaluations which comprise the estimation of the physicochemical and TLC profile are constant features of a plant which are highly essential for raw drugs or plant parts used for preparation of phytomedicine. Therefore, the result generated from this study would be useful in identification and standardization of the plant materials towards quality assurance and also for preparation of a monograph on the plants.

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