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Research Article

Comparative Pharmacognostic and Phytochemical Analysis of *Ziziphus spina-christi* (L.) Desf. and *Ziziphus abyssinica* Hochst. Ex A. Rich.

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ABSTRACT
Ziziphus spina-christi (L.) Desf. and *Ziziphus abyssinica* Hochst. ex A. Rich are very important medicinal plants of the family Rhamnaceae. The need for correct identification and authentication of powdered crude drug samples in the face of growing world demand for herbal products cannot be over-emphasized. In this study, the phytochemical and pharmacognostic profiles of the two species were established using standard methods. Quantitative leaf microscopy revealed differences in the distribution of some microstructures like stomata, trichomes and oxalate crystals. Chemo-microscopy and Phytochemical analyses showed similar profiles in both plants with Carbohydrates, tannins, cardiac glycosides, saponins, flavonoids and indole alkaloids been detected, while anthraquinones were absent. Proximate analysis reveals values for moisture content, acid insoluble, water soluble ash values, as well as water and alcohol extractive values which fall within World Health Organization (WHO) recommended standards for crude drugs from medicinal plants. The results obtained in the study could be used for identification and authentication purposes and also in the development of monograph for the medicinal plants.

Keywords: *Ziziphus spina-christi*, *Ziziphus abyssinica*, phytochemical, microscopy, chemo-microscopy, morphology, physicochemical

INTRODUCTION

The use of medicinal plants in traditional medicine is a known world-wide phenomenon. The imperative of the traditional medicine in developing countries and the increasing World Health Organization (WHO) advocacy for the deployment of herbal medicine to meet the healthcare need of most countries has necessitated the proper identification of these plants and the development of their monographs[1]. To be able to interpret or identify the morphological and anatomical descriptions of plants, their diagnostic characters have to be known. Also, as a measure against adulteration, it is necessary to evaluate all commercial varieties of crude drugs before or after being purchased. In any case, all samples of drugs should be evaluated before clinical use[2]. Evaluation is an elaborate process of establishing the correct identity, quality and purity of drugs. Proper identification is very important to avoid complete and harmful substitution. Identity is usually established by comparison of gross morphology of the drug with that of an authentic sample or by comparing its various features with a published description of the official drug. Authentication of herbal drugs can be done on the spot by evaluating its organoleptic characteristics, and in the laboratory, by analysis of its physicochemical, microscopic and biological characteristics. This is further consolidated by the collection and preservation of the identified plant in the herbarium as voucher specimen[2].

Ziziphus is a genus of spiny shrubs and small trees in the family Rhamnaceae, distributed in warm temperate, tropical and subtropical regions throughout the world. Of particular interest in this genus are the species of *Z. spina-christi* (L.) Desf. and *Z. abyssinica* Hochst. ex A. Rich due to their medicinal importance and threat to their conservation in the Jos metropolis[3,4]. *Ziziphus spina-christi* (L.) Desf., (Figure 1), known as Christ's thorn in English and *Magaryaa* in Hausa, is found in West and North Africa, the Middle East, northwest India and the eastern Mediterranean. It grows as a shrub or small tree, armed with short spines that are positioned in pairs along the branches, one of which are straight and the other curved or hooked[5]. *Z. spina-christi* is covered in whitish-brown or pale grey bark which is deeply fissured and cracked, with a twisted trunk which branches widely, drooping at the ends to form a rounded, usually umbrella-shaped gown. The simple, alternate leaves are oval, becoming more pointed at the tips with three conspicuous veins running along the length. The leaves are hairless on the upper surface, with a fine downy covering of small hairs on the underside. Christ's thorn produces small, greenish-yellow flowers, which cluster tightly in the axils of the leaves and red-brown coloured small fleshy fruits that enclose a hard stone in the center[3]. Referred to throughout history by many cultures and religions as a sacred or holy tree, Christ's thorn has also traditionally been used in medicine,

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Ziziphus spina-christi

Ziziphus abyssinica

Figure 1: Pictures of *Ziziphus spina-christi* and *Z. abyssinica*

carpentry and as a source of food[6]. The plant is used for its soothing properties and the essential oil of the leaves is used in perfumery[7, 8]. It has been used in various ethnomedical practices across the globe for the treatment of ailments such as ulcers, wounds, skin diseases, eye diseases, bronchitis, etc. It has been used as febrifuge and diuretic, and as antiseptic, antifungal and anti-inflammatory agents[9, 10]. Nevertheless, In China it has been used for birth control. Aqueous leaf extract of *Ziziphus spina-christi* may possess anti-nociceptive properties in rats with a calming effect on the central nervous system[11]. It has been described as an anti-cathartic, astringent, diuretic and tonic[12]. The plant has been shown to possess activity against bacteria and fungi[13] and also other pathogens that are quite resistant[14]. Reduced oxidative degradation and hypoglycemic effect of *Ziziphus spina-christi* has been demonstrated[15, 16]. The plant was found devoid of clastogenic activity, and teratogenicity data revealed no teratogenic or fetotoxic effects[17]. A patent is held for the use of *Ziziphus spinia-christi* in cosmetic and psoriasis[18]. In parts of Sudan, the plant is quickly coming under threat due to intensive livestock browsing and over-collection[3]. In addition, reports from some locations suggest that Christ's thorn is susceptible to invasion and parasitism by the mistletoe *Plicosepalus acacia*, which is causing premature aging and increased mortality of this species[19]. *Ziziphus abyssinica* Hochst. ex A. Rich, (Figure 1), known as Catch thorn in English, *Jujube sauvage* in French and also *Magaryaa* in Hausa, is found growing in arid or dry tropical and subtropical regions, with severe heat and slight frost. It occurs at medium to low altitudes in open woodland, open grassland and along riverbanks[20]. It is a semi evergreen spring shrub, scrambler or small tree up to 12 m tall with a straight, occasionally crooked, single bole; spreading, drooping branches forming a heavy, rounded and untidy crown. The fresh bark is creamy becoming greyish-brown, longitudinally fissured and rough in older specimens[21]. Leaves are ovate to broadly ovate, alternate along the stems, conspicuously 3-veined from the base, dark green below due to the dense, rusty and yellow to grey furry hairs. The flowers are small, star-shaped, creamy to yellowish-green with an unpleasant sharp smell, in dense, light clusters in the axils of the leaves, inconspicuous except when produced in profusion. The fruit is almost spherical, shiny red or reddish brown when mature. It is smooth, containing 1 or 2 light brown glossy seeds inside the inner stone. Catch thorn threats have not been evaluated. However, an insect whose larvae eat this species has been identified as *Tarucus sybaris sybaris*, a butterfly whose common name is 'dotted blue' [20]. The water extract of the bark of *Ziziphus abyssinica* is used to manage stomach disorders in Northern Kenya. Also, ash from the burnt leaves is mixed with salt and applied on the throat to relieve tonsillitis[20]. A fomentation of leaves soaked in boiling water is used on the chest to treat pneumonia. The root decoction is used in the treatment of mouth abscess and sore throat. It is also used as mouth wash and gargle in parts of Angola and Namibia[20]. The aqueous extract of *Ziziphus abyssinica* has been shown to possess significant activity against *Staphylococcus aureus* and *Candida albicans*[22].

This work is aimed at establishing some pharmacognostic and phytochemical parameters for these species collected from Jos metropolis that would be helpful in the identification and standardization of their powdered crude form to prevent adulteration.

MATERIALS AND METHODS

All the solvents and reagent used in the study were of Analar grade and, unless otherwise stated, were sourced from Zayo-Sigma Limited, Nigeria.

Collection and Preparation of Plant Materials

The fresh leaves of *Ziziphus spina-christi*. and *Ziziphus abyssinica*, were collected from Gwong area of Jos North Local Government Area, Plateau State, Nigeria, in August 2013, and identified at the College of Forestry, Jos, Plateau State. Some of the leaves were dried at room temperature and pulverized using a motorized blender. The dried powdered samples were used for phytochemical screening, leaf structure microscopy, chemomicroscopy and determination of proximate parameters such as extractive values, moisture content and ash values. The macroscopy, microscopy and organoleptic studies were done using the fresh leaves. Pressed specimens of the plants were deposited at the herbarium of the Department of Pharmacognosy, University of Jos with voucher numbers UJ/PCG/HSP/13R06 and UJ/PCG/HSP/13R07 for *Z. spina-christi* and *Z. abyssinica* respectively.

Table 1: Results of Organoleptic and Macroscopic Evaluation of leaves of *Ziziphus spina-christi* and *Ziziphus abyssinica*

Characters	*Ziziphus spina-christi*	*Ziziphus abyssinica*
Leaf type	Simple and alternate	Simple and alternate
Colour	Light-green	Dark-green
Texture	Papery	Leathery
Odour	Characteristic	Characteristic
Taste	Characteristic	Characteristic
Shape	Ovate	Ovate
Margin	Serrate	Serrate
Surface (Lower)	Densely covered with hairs (+++)	Densely covered with hairs (++)
Petiole	0.3 – 0.5 cm	0.5 – 0.7 cm
Apex	Mucronate	Obtuse
Venation	Reticulate	Reticulate
Leaf length	2.0 – 4.0 cm	5.0 – 8.0 cm
Leaf width	1.0 – 2.0 cm	3.0 – 5.0 cm

Table 2: The result of Microscopical analysis of leaves of *Ziziphus spina-christi* and *Z. abyssinica*

Microscopical characters	*Ziziphus spina-christi*	*Ziziphus abyssinica*
Type of epidermal wall	Straight polygonal cell wall	Straight antichral cell wall
Calcium oxalate crystals	+	+
Trichomes (unicellular covering)	+	+
Fibres	+	+
Spiral vessels	+	+
Stomata type	Anomocytic	Anomocytic
Stomata Distribution	Lower epidermis > upper epidermis Amphistomatic	On upper epidermis only Epistomatic
Parenchyma	Thin-walled	Thin-walled

KEY: + = present, - = absent, > = greater than

Organoleptic/Macroscopic Evaluation

The fresh leaves of the two plants were observed for organoleptic and macroscopic characteristics such as colour, odour, taste, leaf size, shape, petiole, venation and margin. The observations were recorded.

Microscopical Examinations

Epidermal preparations were done following standard procedure as described in Sofowora[23] and African Pharmacopeia[24]. Measurements of the epidermal cell length and width, trichome size and stomata length and width of both the upper and lower epidermis, of the two species were taken with a compound microscope (model KC-FUB) and qualitative features observed were also recorded. A small quantity of the dried leaf powder was cleared in chloral hydrate, mounted in dilute glycerol and viewed under the microscope. Drawings of the structures seen were made.

Chemo-Microscopy of Leaf Powder

Table 3: Results of Quantitative Microscopical analysis of leaves of *Ziziphus spina-christi* and *Ziziphus abyssinica*

	Z. spina-christi		*Z. abyssinica*	
Microstructures	Range (µm)	Average (µm)	Range (µm)	Average (µm)
Upper epidermis:				
Stomata length	34.00 – 37.40	35.36±1.86	32.30 – 40.80	36.38±3.31
Stomata width	27.20 – 30.60	27.88±1.52	22.10 – 27.20	24.48±1.94
Epidermis length	30.60 – 40.80	36.04±4.56	27.20 – 37.40	32.64±3.88
Epidermis width	23.80 – 34.00	28.56±5.16	27.20 – 37.40	29.92±5.59
Trichome size	102.00 – 221.00	163.00±62.00	204.00 – 612.00	421.60±174.78
Lower epidermis:				
Stomata length	27.20 – 32.30	30.60±2.69	Not visible	Not visible
Stomata width	23.80 – 27.20	25.50±1.70	Not visible	Not visible
Epidermis length	23.80 – 40.80	31.96±7.05	6.8 – 17.0	12.92±4.43
Epidermis width	13.60 – 23.80	19.38±4.09	6.8 – 13.6	10.88±2.85
Trichome size	187.00 – 408.00	267.40±84.22	272 – 663	544.00±156.27

The leaf powder was treated with the appropriate chemical reagents and observed under the microscope for the presence of substances such as starch, oil, tannins, calcium oxalate crystals, protein, and lignin as outlined in Sofowora[23] and African Pharmacopeia[24].

Phytochemical Screening

The phytochemical screenings were carried out according to standard methods outlined in Sofowora[23] and Evans[25]. The metabolites tested for were carbohydrates, saponins,

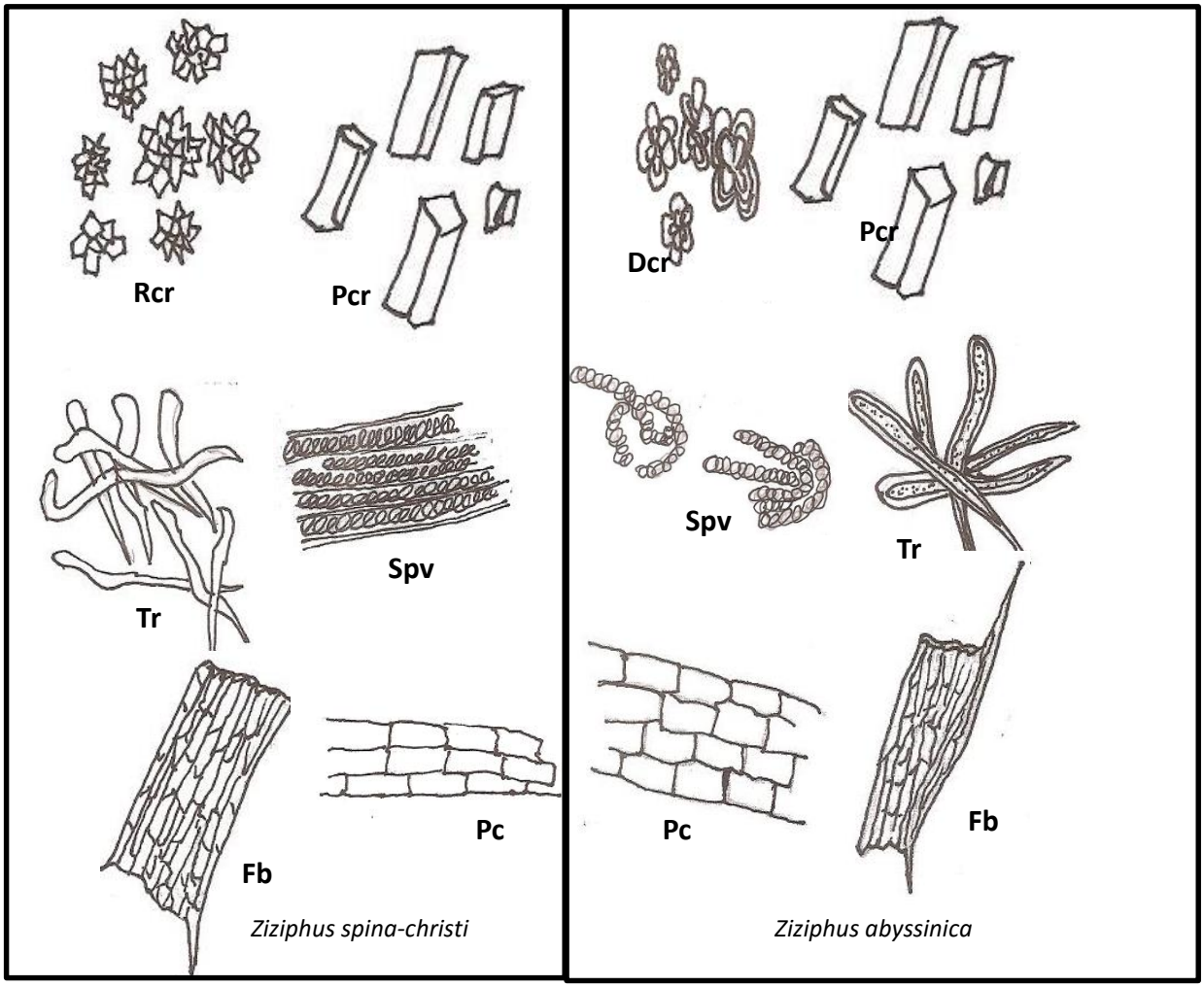

Figure 4: Microscopical features of powdered sample of leaves of *Ziziphus spina-christi* and *Ziziphus abyssinica*

Key: Rcr: Rosette crystals; Pcr: Prism shape crystals; Dcr: Druses crystals; Tr: Trichomes; Spv: Spiral vessels; Fb: Fibers; Pc: Parenchyma cells

sterols, terpenes, tannins, flavonoids, glycosides, alkaloids.

Proximate analysis

Determination of extractive values, moisture content, total ash value, acid-insoluble ash value and water soluble ash values were carried out on the powdered sample following standard method[26].

RESULTS

Morphological and Organoleptic Evaluation

The results of morphological and organoleptic studies of whole leaf of *Ziziphus spina-christi* and *Ziziphus abyssinica* are as depicted in Table 1.

Microscopic and Chemomicroscopic evaluation

The results of microscopic examination of both species are as depicted in Tables 2, and Figures 2 – 4. The quantitative measurements of the epidermal cell length and width, trichome size of both the upper and lower epidermis, and stomata length and width of the two species are as depicted in Table 3, while the results of chemomicroscopic examination of the leaf powders are as shown in Table 4.

Phytochemical Screening and Proximate analysis

Table 4: Results of chemomicroscopic examination of leaves of *Ziziphus spina-christi* and *Ziziphus abyssinica*

Metabolite	*Z. spina-christi*	*Z. abyssinica*
Starch	+++	++
Lignin	+	+
Protein	+	+
Calcium oxalate	+	+
Tannins	++	+++
Fat and oil	+	+

Key: + = Present/intensity

The results of phytochemical screenings for alkaloids, tannins, flavonoids, saponins, glycosides and carbohydrates are presented in Table 5 while the result of the proximate analysis are presented in Table 6.

DISCUSSION

The organoleptic examination of whole leaf showed some very similar features for the two species studied while characteristic differences were also observed in some.

Table 5: Results of Phytochemical screening of leaves of *Z. spina-christi* and *Z. abyssinica*

Tests	*Z. spina-christi*	*Z. abyssinica*
Carbohydrate test		
Monosaccharide	+++	++
Reducing sugars	++	++
Pentose	+++	++
Ketosis	+	+++
Combined reducing sugars	-	-
Tannins	+	++
Glycosides		
Anthraquinones	-	-
Cardiac glycosides		
Deoxy sugars	++	+++
Steroids/triterpenes	+	++
Saponin	++	+
Flavonoids	++	+++
Alkaloids	+++	+
Tropane alkaloids	-	-
Isoquinoline alkaloids	-	-
Indole alkaloids	++	+

Key: + = present/intensity; - = absent

Leaves of *Ziziphus spina-christi* are light-green in colour, papery in texture and with mucronate apex while that of *Ziziphus abyssinica* are dark-green in colour, leathery in texture and obtuse apex with the leaf size being slightly larger than that of *Z. spina-christi.* Lower leaf surface of *Z. spina-christi* are more densely hairy than that of *Z. abyssinica* (Table 1). The description of the specimens used in this study tally with the taxonomic description of the two species in Floras[27, 28].

Table 6: Result of Proximate analysis of leaves of *Ziziphus spina-christi and Z. abyssinica*

Proximate Parameters	Values (%)	
	Ziziphus spina-christi	*Ziziphus abyssinica*
Water Extractive Value	4.51±0.02	2.81±0.00
Alcohol Extractive Value	3.43±0.00	2.69±0.00
Moisture Content	7.72±0.01	5.04±0.03
Ash Value	5.78±0.05	3.84±0.03
Acid Insoluble Ash Value	5.05±0.03	3.74±0.43
Water Soluble Ash Value	1.99±0.08	1.62±0.22

Key: Values in (percentage ± SD)

The upper surface epidermis of *Z. spina-christi* shows anomocytic stomata type surrounded by straight-walled polygonal epidermal cells (Figure 2A) while the lower surface epidermis also showed straight–walled polygonal epidermal cells with numerous anomocytic stomata and numerous trichomes (Figure 2B). *Ziziphus abyssinica*

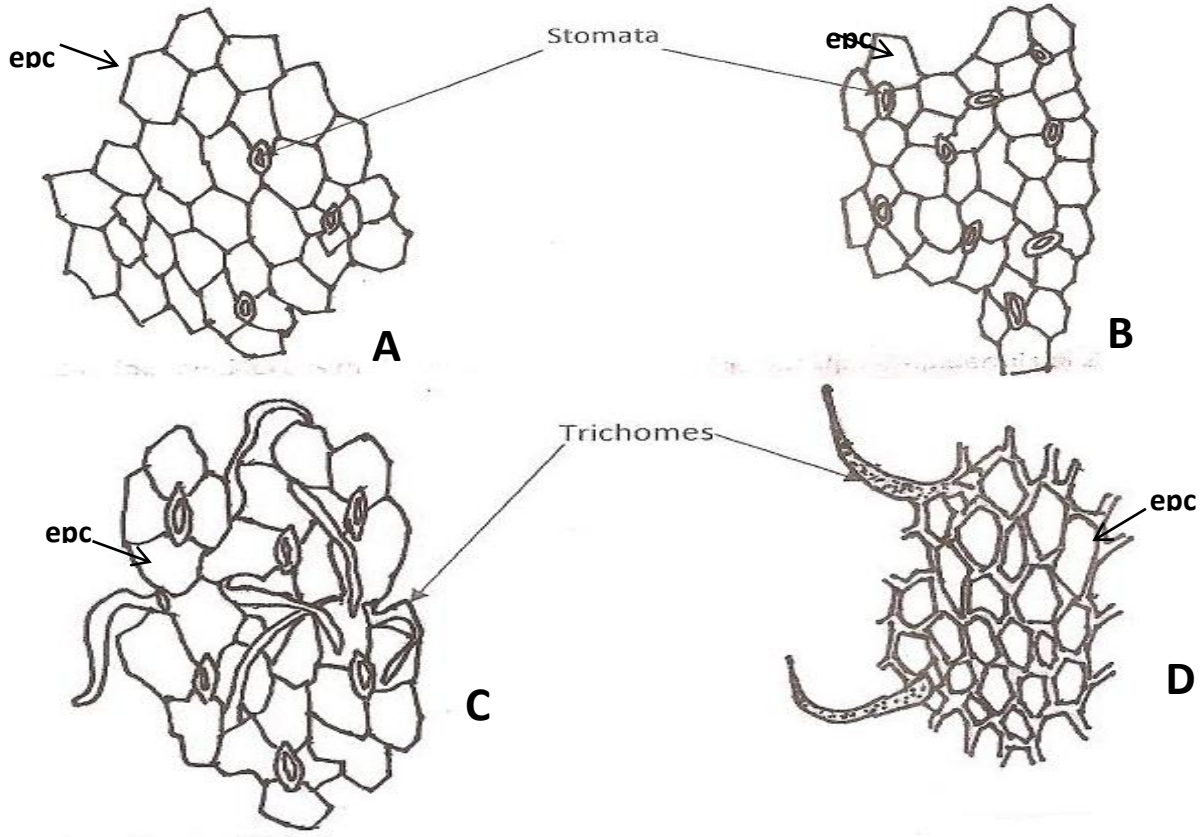

Figure 2: Drawings of epidermal layers of leaves of *Ziziphus spina-christi* and Z. *abyssinica*
Key: A: upper epidermal layer of *Z. spina-christi;* B: upper epidermal layer of *Z. abyssinica;* C: lower epidermal layer of *Z. spina-christi;* D: lower epidermal layer of *Z. abyssinica*; epc: epidermal cells

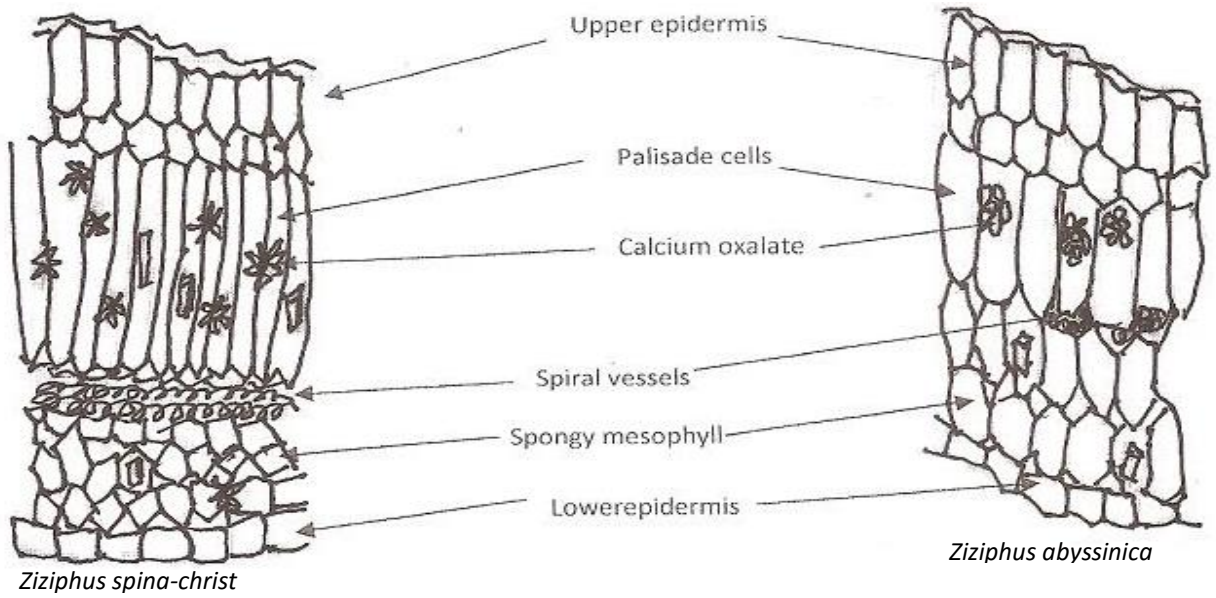

Figure 3: Transverse section of lamina of *Ziziphus spina-christi* and *Ziziphus abyssinica*

reveals similar epidermal features like that of Z. *spina-christi* except that the former is amphistomatic (stomata present on both surfaces) while the latter is epistomatic (stomata present only on the upper surface). Rosette and prism calcium oxalate crystal were seen on the palisade and spongy mesophyll of *Z. spina-christi* (Figure 3 & 4) while the druses and prism types were found on *Z. abyssinica* (Figure 3 & 4). The characteristic amphistomatic leaf type with presence of rosette calcium oxalate crystal in *Z. spina-christi,* and the epistomatic leaf type with presence of druses calcium oxalate crystal in *Z. abyssinica* can be used as diagnostic features for the species.

Quantitative microscopical analyses also revealed features that are diagnostic to the species and can be used in their separation especially when only leaf fragments are available. The diagnostic features are the lower surface epider

mal cell length of 31.96 µm ± 7.05 and 12.92 µm ±4.43 µm in *Z. spina-christi* and *Z. abyssinica* respectively; And trichome length of 163µm ± 62 µm on upper surface, 267µm ±84µm on lower surface and 421.60 µm ±174.78 on upper surface, 544.0 µm ±156.27 µm on lower surface in *Z. spina-christi* and *Z. abyssinica* respectively (Table 3).

Chemomicroscopic examination of the leaf powders of *Z. spina-christi* and *Z. abyssinica* revealed the presence of starch, oil, lignin, tannin and calcium oxalate crystals in both species. The examination also revealed that starch is more abundant in *Z. spina-chisti* than in *Z. abyssinica* while tannins were more abundant in *Z. abyssinica* than in *Z. spina-christi.* (Table 4). The presence of oils detected in the leaves of both species showed that *Z. abyssinica* can also be employed in cosmetic and perfumery uses like *Z. spina-christi.* The use of volatile constituents of *Z. spina-christi* in soothing effect and perfumery has been documented by an earlier study[7,18].

The similarity in the types of secondary metabolites found in both plants shows affinity among the two species and could also serve as a chemotaxonomic tool. These similarities might also be the reason why both species are used in ethnomedicine for treatment of almost similar ailments[9, 22, 10, 20, 4]. The presence of tannins and flavonoids in both species in high amount suggests that both species may be a good source of phenyl propane and flavone antioxidants. Tannin-containing drugs will precipitate protein and have been used traditionally as styptics and internally for the protection of inflamed surface of mouth and throat. This might support their use in ethnomedicine for treatment of tonsillitis, mouth abscesses, sore throat, ulcers etc[20, 9, 10] and they also act as anti-diarrheal and have been employed as antidotes in poisonings by heavy metals[25]. Flavonoids are known for their anti-inflammatory, anti-allergic and vaso-protective properties and many flavonoid-containing plants are diuretic or antiplasmodic while some have anti-tumour and antibacterial properties[25]. The presence of indole alkaloid (Table 5) is also suggestive of a good medicinal potential. Some indole alkaloids like vinblastine, vincristine and vindesine from *Catharanthus roseus* have been isolated and used in the treatment of various type of cancer due to their antineoplastic activity. Vinblastine (a bisindole alkaloid) is used for the treatment of Hodgkin's disease and non-Hodgkin's lymphomas[25]. Some anthraquinones have purgative effect which is dependent to their structure[25]; therefore the absence of these it might suggests the plant may not be useful as a purgative.

The water and alcohol extractive values of powdered leaves of *Z. spina-christi* were 4.51±0.02 % and 3.43±0.00 % respectively while that of *Z. abyssinica* were 2.81±0.00 % and 2.69±0.00 % respectively. The extractive values of both species in water is higher than in alcohol which might indicates that water may be a more suitable solvent than alcohol for extraction. The moisture content of *Z. spina-christi* and *Z. abyssinica* of 7.72 % and 5.04 % respectively are within the recommended range of 8 – 14% for vegetable drug[24] and this indicates that the plants can be

stored for a long period of time with less probability of microbial attack. Ash values of *Z. spina-christi* which is slightly higher than that of *Z. abyssinica* (5.78 ±0.05% and 3.84 ±% respectively) are used in determining adulterations in herbal medicinal products and they indicate the quantity of organic matter contained in the material or herbal product that the body cannot digest[29]. The acid insoluble ash and water soluble ash values of the two species were almost similar and not significantly different from one another.

CONCLUSION

This study has established microscopic, chemomicroscopic and pharmacognostic parameters for *Z. spina-christi* and *Z. abyssinica* and these would be useful in monograph development on the plants. And also, the established diagnostic features would be helpful in the authentication and standardization of the leaves of the species especially when in fragment or in powdered crude form.

REFERENCES

1. Trease GE, Evans WC. Trease and Evans Pharmacognosy, 14th edition, WB Saunders company Limited, Oval Road, London NWI 7dx, UK, 1996, 35 – 574
2. Kunle OF, Egharevba HO, Ahmadu PO. Standardization of herbal medicines - A review. *IJBC* 2012, 4(3): 101-112
3. Saied AS, Gebauer H, Hammer K, Buerkert A. *Ziziphus spina-christi* (L) Willd: a multipurpose fruit tree. *Gene Resources & Crop Evol* 2008, 55: 929 – 937
4. Burkil HM. The useful plants of West Tropical Africa. 2nd ed. Vol. 4. Families M-R. Royal Botanic Gardens. Kew, 1998, 981
5. Orwa C, Mutua A, Kindt R, Jamnadass R, Anthony S. Agroforestry Database: a tree reference and selection
6. guide version 4.0, World Agroforestry Centre, Kenya, 2009. http://www.worldagroforestry.org/resources/databases /agroforestree
7. Dafni A, Levy S, Lev E. The ethnobotany of Christ's thorn jujube (*Ziziphus spina-christi*) in Israel. *J Ethnobiol & Ethnomed* 2005, 1:8.
8. Ghannadi A, Tavakoli N, Mehri-Ardestani M. Volatile constituents of the leaves of *Ziziphus spina-christi* (L) Willd. From Busher, Iran. *J Ess Oil Res* 2003, 5(3): 1041-2905.
9. Adzu B, Amos S, Wambebe C, Gamaniel K, Dzarma S. Effect of *Ziziphus spina-christi* Willd Aqueous extract on the central nervous system in mice. *J. ethnopharmacol* 2002, 7a (1):13-16.
10. Amin G. Popular medicinal plants of Iran. Ministry of Health publications, Tehran, Vol 1, 1991, 67.
11. Nafisy AT. A review of traditional medicine in Iran. Isfahan University publications Isfahan, 1989, 133.
12. Effraim KD, Osunkwo UA, Onyeyili P, Ngulde A. Activity of aqueous leaf extract of *Ziziphus spina-christi* (Linn) Desf. *Indian J. pharmacol* 1998, 30: 271-272.
13. Duke JA, Anyesu ES. Medicinal plants of China. Reference publications, Inc, 1985
14. Shahat AA, Pieters L, Apers S, Nazeif NM, Abdel-Azim N, Berghe DV, Vlietinck AJ. Chemical and biological investigations on *Zizyphus spina-christi* L. *Phytotherapy Res* 2001, 15(7):593-597
15. Nazif NM. Pharmacognosy and chemistry of medicinal plants, National Research Center, Dokki, Cairo 12311, Egypt. *Food Chem* 2002, 79(1): 77-81.
16. Zakaria MNM, Islam R, Radhakrishnan A, Ismail M, Habibulla CK. Antidiabetic properties of *Ziziphus spina-christi* in STZ-diabetic mice. "2000 years of Natural products Research- Past, Present and future". Amsterdam, July 26-30 1999.
17. Glombitza KW, Mahran GH, Mirhom YW, Michel KG, Motawi TK. Hypoglycemic and antihyperglycemic effects of *Ziziphus spina-christi* in rats. *Planta Med* 1994, 60(3): 244-247.
18. Islam MW, Radhakrishnan R, Liu XM, Chen HB, Al-Naji MA. Safety evaluation of *Ziziphus spina-christi* L and *Teucrium stocksianum* Boiss, used in Traditional Medicine in the Arabian Gulf. International congress and 49th annual meeting of the society for medicinal plant Research (Gesellschaft for Arzneipfian zen-forschung), Erlangen, Germany, 2001.
19. Safai-Ghomi Extracts of *Ziziphus spina-chrisi* for cosmetics and psoriasis; US patent 5,849,302. Also EP 0 815 842 in Europe, 1998
20. Ward D, Shrestha MK, Musli I. Are invasive mistletoe killing *Ziziphus spina-christi*? *Isreal J Plant sci* 2006, 54(2): 113-117.
21. Bein E. Useful trees and shrubs in Eritrea. Regional Soil Conservation Unit (RSCU), Nairobi, Kenya, 1996.
22. Beentje HJ. Kenya trees, shrubs and lianas. National museums of Kenya, 1994, 722.
23. Gundidza M, Sibanda M. Antimicrobial activities of *Ziziphus abyssinica* and *Berchemia discolor*. *The Central Africa J med* 1991, 37(3) 80-83
24. Sofowora A. Medicinal plant and Traditional medicine in Africa, University of Ife press, Ile-Ife, 1982, 144-146
25. African Pharmacopoeia. General Methods for Analysis 1st Ed. Vol. (2) (OAU/STRC) Lagos, 1986, 128-139.
26. Evans WC. Trease and Evans Pharmacognosy, 16th Ed. Saunders publishers, 2009, 24-543.
27. British Pharmacopeia. Vol II, 1980, 113, 108
28. Hutchinson J, Dalziel JM. Flora of West Tropical Africa. Grown Agents for Overseas Government and Administration London Vol. 1, 1954.
29. Irvine FR. Woody plants of Ghana. Oxford University Press, London, 1961, 585-586
30. Egharevba HO, Odigwe AC, Abdullahi MS, Okwute SK, Okogun JI. Phytochemical analysis and broad-spectrum antimicrobial activity of *Cassia occidentalis* (L) (Whole plant). *NY Sci J* 2010, 3(10): 74-81.