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Pharmacognostic evaluation, HPLC-DAD and GC-MS analysis of *Ziziphus mauritiana* (lam)

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ABSTRACT

Background & Aim: Indian jujube or ber, *Ziziphus mauritiana,* is a member of the Rhamnaceae family with elliptic paired leaves. They have historically been used to manage a variety of conditions including hypertension, diabetes, liver disease, gonorrhea, abscesses, and diarrhea. The aim of this study was to investigate and establish the pharmacognostic and physicochemical characteristics of the leaves of *Ziziphus mauritiana* (Lam) by GC-MS, and HPLC analysis.

Experimental: The dried leaves of *Ziziphus mauritiana* (Lam.) were extracted with 70% ethanol. Phytochemical screening, HPLC-DAD, and GC-MS analysis were carried out on the ethanolic leaf extract using standard method. The epidermal leaf microscopy was done on the fresh leaf, and other pharmacognostic evaluation such as chemomicroscopy, moisture content, total ash, acid insoluble ash, water-soluble ash, alcohol soluble extractive value, and water-soluble extractive values were also carried out on the powdered leaf sample.

Results: Quantitative phytochemical screening showed the presence of alkaloid and saponin at 1.86±0.23% and 1.5±0.86%, respectively. HPLC analysis revealed the presence of quercetin, rutin, catechin, ferulic acid, and gallic acid while GC-MS revealed the presence of 1,2 Benzenedicarboxylic acid and n-Decanoic acid. The pharmacognostic parameters of total ash were10.3 ±0.3% with acid-insoluble ash of 3.3 ±0.2%, water-soluble ash of 2.8 ±0.2%, moisture content of 5.5 ±0.3%, alcohol soluble extractive value of 8.1 ± 0.3% and water-soluble extractive value of 10.2 ±0.3%. Chemo-microscopic evaluation indicated the presence of lignin, cellulose, tannins, starch, calcium oxalate, oils, and protein. Microscopy, fluorescence, and organoleptic analysis were also documented.

Recommended applications/industries: The findings on the pharmacognostic characteristics, physicochemical parameters, GC-MC analysis, and HPLC analysis of *Z. mauritiana* leaf can be used as diagnostic features that are useful in identifying and monitoring of the purity of the crude plant drug as well as being useful in telling the difference between closely related species used as raw material.

1. Introduction

Since the earliest days of time, plants have been used by humans as a natural source of remedies for a wide range of ailments and illnesses. According to estimates from the World Health Organization (WHO), more than 80% of the population in developing nations relies on traditional medicine for their basic healthcare

(Nathiya *et al.,* 2012). In the past few years, there has been a remarkable surge in plant research worldwide, with various evidence pointing towards the immense potential of medicinal plants utilized in traditional systems (Mahomoodally, 2013). Due to the extensive utilization of medicinal plants in the treatment of ailments, it is important to ensure a high level of purity and safety hence, the World Health Organization states that the first stage in determining the type and level of purity of medicinal plants is to conduct a macroscopic and microscopic examination of the plant (Preeti and Tripathi, 2014).

Ziziphus mauritiana Lam, also known as jujube, is a tropical shrub native to the Indian Subcontinent, arid and semi-arid tracts of much of Asia and Africa (Figure 1). The plant is a fast-growing evergreen shrub or tree characterized by its spiny nature, ability to form thickets, small to medium size, and possession of one to several stems. It abundantly produces fruit and disperses seeds over a wide area. With a round spreading crown, it can reach a height of up to 12 meters and a diameter of 30 cm. However, its size and overall appearance can vary considerably. The branches extend and hang down, featuring simple leaves that measure 2 to 5 cm in length and 1.5 to 3 cm in width. Size discrepancies may occur, likely influenced by the quality of the site or the position of leaves on the tree (Goyal *et al.,* 2012; Gupta *et al.,* 2012).

Figure 1. *Ziziphus mauritiana* lam

Z. mauritiana Lam is used in food and medicine in a variety of ways, the leaves have historically been used as an antiseptic and anti-typhoid (Akhtar *et al.,* 2016). They are also useful for treating gonorrhea, liver illnesses, asthma, fever, and other respiratory conditions (Marwat *et al.,* 2009; Rathore *et al.,* 2012). The roots are used in treatment against skin diseases while the leaves are employed for the treatment of sores and are known to have antimicrobial, antioxidant and hepatoprotective effects (Goyal *et al.,* 2012).

Establishing pharmacognostic standards that ensures suitable identification and quality assurance are very crucial for study of medicinal plant species. Some of the crucial steps in assessing plant crude drugs include physiochemical parameters, fluorescence analysis, and macroscopic and microscopic attributes. Due to the significant medicinal importance and benefits associated with *Z. mauritiana*, there is a necessity to standardize it by presenting a comprehensive report on essential inherent characteristics. These characteristics serve as a means to ensure the quality control of the crude drug. In establishing the pharmacognostic and physicochemical standards of *Z. mauritiana*, the macroscopic, microscopic and physiochemical parameters such as extractive values, total ash as well as fluorescence characteristics were analyzed. Quantitative phytochemical screening, HPLC and GC-MS analysis were also carried out to ascertain and establish the chemical profile of the plant.

2. Materials and Methods

2.1. Collection of plant materials

The plant sample was collected in Chaza Suleja, Nigeria, in August 2022, and deposited in the NIPRD Herbarium for identification and authentication. The deposited specimen was assigned the voucher specimen number NIPRD/H/7336. The plant's powdered leaves were examined using a variety of techniques, including microscopy, quantitative phytochemical screening, organoleptic assessment, chemo-microscopy, fluorescence, HPLC, and GCMS analysis.

2.2. Extraction

A known weight (20g) of the dried powdered plant sample was macerated in 70% ethanol for 24 h, filtered and put on the water bath for evaporation. The extract was weighed and stored for subsequent use.

2.3. Pharmacognostic evaluation

2.3.1. Epidermal layer preparation for microscopy

The epidermal layer of the upper and lower layers of the leaf of *Z. mauritiana* was obtained through hand scrapping method using a razor blade. The epidermal layers were cleared in 2% sodium hypochlorite and stained in safranin O, after which they were mounted

on a glass microscope slide with dilute glycerol, then viewed under ACCUScope Binocular Microscope and photomicrograph of the epidermal layers were taken at different magnifications with a digital microscope camera attached to the microscope.

2.3.2. Chemo-microscopic evaluation

The presence of lignin, cellulose, tannin, starch, oil, and protein was examined by chemo-microscopic evaluation of the powdered plant sample using reagents and stains such as N/50 iodine, sulphuric acid (66%), concentrated hydrochloric acid, Sudan IV reagent, million's reagent, 1% picric acid, ferric chloride, and phloroglucinol (Adigwe *et al.,* 2021).

2.3.3. Physicochemical characteristics

Physicochemical parameters such as moisture content, total ash, water soluble ash, acid insoluble, extractive value and water-soluble extractive value were evaluated following African Pharmacopoeia (1986) and WHO (1998) procedure.

2.4. Fluorescence analysis

A known volume (5 mL) of 10% Aq. NaOH was added to 0.5 g of the powdered plant sample in a test tube, the color in relation to the chemical reagent (10% Aq. NaOH) added was observed and recorded under visible light, 254 nm and 365 nm. The mixture was then heated on a water bath for 10 min and color was again observed in daylight, 254 nm and 365 nm, respectively. This procedure was repeated using 5 mL of nitric acid, glacial acetic acid, 5% $FeCl_3$, concentrated H_2SO_4, concentrated hydrochloric acid, petroleum ether, iodine solution, picric acid, ethyl acetate, methanol, 5% potassium dichromate, alcoholic hydroxide, chloroform, ammonia and distilled water individually (Chase Jr and Pratt, 1949).

2.5. Quantitative phytochemical analysis

2.5.1. Total saponin content

Saponin quantitative determination was carried out using the method reported by (Koomson *et al.,* 2018). Two grams of powdered sample was put into a conical flask and 10 mL of 20% aqueous ethanol was added. The sample was heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 20 mL of 20% ethanol. The combined extract was concentrated over water bath at about 90°C. The concentrate was transferred into a 250 mL separating funnel and 10 mL of diethyl ether was added and shaken strongly. The aqueous layer was recovered while the ether layer was cast-off. The purification process was repeated. Then, 6 mL of n-butanol was added. The combined n-butanol extract was washed twice with 1 mL of 5% aqueous sodium chloride. The residual solution was heated in a water bath. After evaporation, the sample was dried in the oven to a constant weight and the saponin content was calculated. The total saponin was calculated using the equation:

$$\%\ \text{Saponin} = \frac{Weight\ of\ saponin}{Weight\ of\ sample}\ x\ 100$$

2.5.2. Total Alkaloid content

Quantitative determination of alkaloid was carried out according to the methodology by (Koomson *et al.,* 2018). Exactly 1 g of the sample was weighed into a 250 mL beaker and 40 mL of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed. The total alkaloid was calculated using the equation:

$$\%\ \text{Alkaloid} = \frac{Weightofalkaloid}{Weightofsample}\ x\ 100$$

2.6. High-performance liquid chromatography analysis

A Shimadzu HPLC system was employed for the HPLC fingerprinting analysis which included the Ultra-Fast LC-20AB prominence equipped with SIL- 20AC auto sampler, DGU-20A3 degasser, SPDM20A UV-diode array detector (UV-DAD), CTO-20AC column oven, CBM-20 Alite system controller, and Windows LC solution software (Shimadzu Corporation, Kyoto, Japan); VP-ODS column (150 × 4.6 mm, 5µm). For the fingerprint analysis of *Z. mauritiana*, the chromatographic conditions employed were as follows: A 10 µL of a 10 mg/mL solution of the ethanolic extracts was injected. A binary gradient elution system

was utilized, consisting of acetonitrile as solvent A and 0.1% formic acid in HPLC grade water as solvent B. The gradient elution protocol was as follows: 3.5 min at 18% A, 5-10 min with a gradient from 18% to 25% A, 10-30 min with a gradient from 25% to 35% A, and 30-35 min with a gradient from 20% to 10% A. The flow rate of the mobile phase was maintained at 0.6 mL/min, and the column temperature was set to 40 ˚C. The DAD detector was set at 254 nm (Fatokun *et al.,* 2020).

2.7. Gas Chromatography-Mass Spectrometry (GC-MS)

The GC-MS analysis of the crude ethanol extract of *Z. mauritiana* (leaves) was conducted using a Shimadzu QP-2010 GC equipped with a QP-2010 mass selective detector (MSD). The MSD operated in the EI mode with an electron energy of 70 eV. The scan range was set from 45 to 400 amu, and the scan rate was 3.99 scans per second. The Shimadzu GCMS solution data system was utilized for data processing.

The GC column employed was an Optima-5 ms fused silica capillary with an internal diameter of 0.25 µm and a film thickness of 0.25 µm. The stationary phase consisted of (5% phenyl)-methylpolysiloxane. Helium was used as the carrier gas at a flow rate of 1.61 mL/min. The temperature program for the GC oven included a ramp from 60 to 180 °C at a rate of 10 °C/min, followed by a hold at 180 °C for 2 minutes, and then a ramp from 180 to 280 °C at a rate of 15 °C/min. The final temperature of 280 °C was held for 4 minutes. The injection port temperature was set at 250 °C, while the detector temperature was maintained at 280 °C. In the analysis, an autosampler was employed to inject 1.0 µL of a diluted sample (1/100 in hexane, v/v) in split mode with a 10:90 split ratio. The analysis was performed in triplicate. The obtained mass spectra were compared to the mass spectra in the National Institute of Standards and Technology (NIST) collection to identify the constituents (Okhale *et al.,* 2016).

3. Results and discussion

3.1 Epidermal leaf microscopy analysis

To prevent misidentification and alterations of the crude drug of *Z. mauritiana*, it is imperative to standardize using the microscopic and macroscopic properties of the plant amongst other diagnostic characteristics. Organoleptic traits play a significant role in the detection of fake or exchange drugs, making them extremely important components in the dissimilitude of drug (Fofie *et al.,* 2018). Qualitative microscopy revealed calcium crystals, polygonal epidermal cells and abundant presence of non-glandular wavy unicellular trichome at the abaxial surface which may reduce transpiration, scatter incoming light and deter herbivory. Trichome structures according to Fatokun *et al.* (2020) are species-specific which incidentally was found to be present in this plant species. *Z. mauritiana* leaf was observed in this study to be amphistomatic with adaxial leaf surface having anisocytic and anomocytic stomata, surrounded by epidermal cells and starch granules (Figure 2). Contrarily, the abaxial surface revealed calcium crystals, polygonal epidermal cells and abundant presence of non-glandular wavy unicellular trichome as seen in Figure 3.

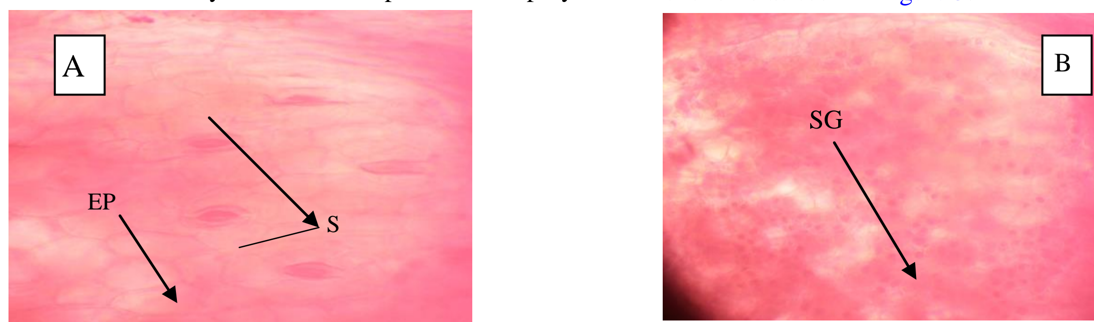

Figure 2. (A) Showing adaxial surface of the epidermal leaf of *Z. mauritiana* (x400), S: stomata and EP: epidermal cell; (B) Starch granules (SG).

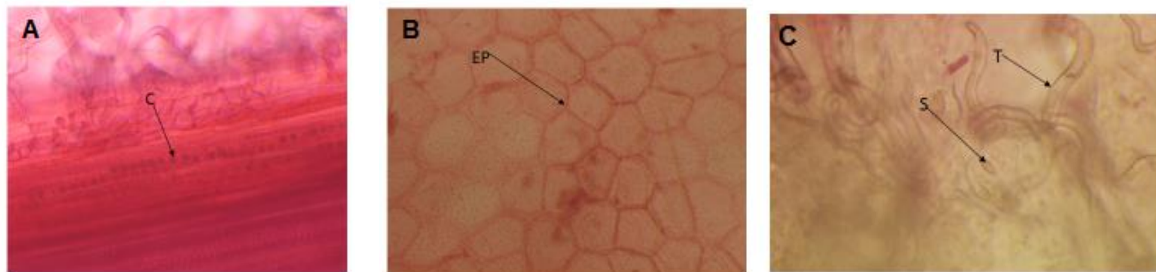

Figure 3. (A and B) Showing Abaxial surface of the epidermal leaf of *Z. mauritiana* x400, C: crystals and EP: epidermal cells (C) stomata(s) and wavy unicellular trichome (t) x400.

3.2. Physicochemical parameters

As seen in Table 1 the powder leaf of *Ziziphus mauritiana* is green, coarse, slightly characteristic choking odor and mildly bitter taste. The physicochemical parameters that were analyzed are as follows; total ash (10.3 ± 0.3); acid-insoluble ash (3.3 ± 0.2); water soluble ash (2.8 ± 0.2); moisture content (5.5 ± 0.3); alcohol soluble extractive value (8.1 ± 0.3); water soluble extractive value (10.2 ± 0.3) as seen in Table 2.

Evaluation of physicochemical parameters like moisture content and ash value is important in ascertaining the physiological and non-physiological characters, identifying the potential of microbial growth or contamination as well as the presence of impurities (Pandey *et al.,* 2015). The plant sample under study had a moisture content of 5.5 ± 0.3, which is less than 10%. This result is in accordance with the international pharmacopeia standards given that this moisture level will not support oxidation and fermentation and reduce the likelihood of microbial growth and contamination (Diallo, 2020). Total Ash value was 10.3% ± 0.3, this value gives us insight on the mineral content of the drug. The results of the moisture content and ash value are similar to the one reported by Fofie *et al.* (2018) who reported the moisture and total ash values at 7.54% and 7.41% respectively. Acid insoluble Ash (3.3 ± 0.2%), water soluble Ash (2.8 ± 0.2%), Alcohol soluble extractive value (8.1 ± 0.3%), Water–soluble extractive value (10.2 ± 0.3%) are all in accordance with the set WHO guidelines (WHO, 1998). Also, a study by Gupta *et al.* (2012) on *Z. mauritiana* revealed physicochemical parameters like total ash, acid insoluble ash, water soluble ash and moisture content values of 8.02%, 2.72%,4.11% and 13.08%, respectively, which are similar to the ones gotten in this study.

Table 1. Organoleptic evaluation of powdered leaf sample of *Z. mauritiana.*

Characters	Inference
Color	Green
Odor	Slightly characteristic choking odor
Taste	Slightly bitter
Texture	Coarse

Table 2. Pharmacognostic evaluation of powdered leaf sample of *Z. mauritiana.*

S/N	Parameters	Percentage (%)
1	Total ash value	10.3 ±0.3
2	Acid-insoluble ash	3.3 ±0.2
3	Water soluble ash	2.8 ±0.2
4	Moisture content	5.5 ±0.3
5	Alcohol soluble extractive value	8.1 ±0.3
6	Water-soluble extractive value	10.2 ±0.3

Values are mean ±SD of triplicate measurement.

3.3. Chemo-microscopic evaluation

Chemo-microscopic evaluation of *Ziziphus mauritiana* sample through a supportive test showed the presence of lignin, cellulose, tannins, starch, calcium oxalate, oils and protein as seen in Table 3. In both visible and ultraviolet light (254 and 366nm), powdered leaves of *Z. mauritiana* displayed distinctive fluorescence characteristics. Table 4 revealed the fluorescence nature of the powder leaf sample of *Ziziphus mauritiana* under UV-Visible light (254 nm and 365 nm) and daylight under different conditions.

Table 3. Chemo-microscopic evaluation of powdered leaf sample of *Z. mauritiana.*

Test	Inference
Lignin	+
Cellulose	+
Tannins	+
Starch	+
Calcium Oxalate	+
Oils	+
Proteins	+

Table 4. Fluorescence analysis of powder leaf sample of *Z. mauritiana.*

S/N	TEST	Cold			Hot		
		Daylight	254 nm	365 nm	Daylight	254 nm	365 nm
1.	Powdered sample	Green	Green	Deep green			
2.	Powdered sample+ Distilled Water	Garden green	Garden green	Deep green	Smith green	Garden green	Deep green
3.	Powdered sample+ 10% Aq. NaOH	Chestnut	Green	Emerald	Marron	Zuchini green	Purple
4.	Powdered sample+ Ammonia	Golden yellow	Green Pea color	Basil green	Olive brown	Spinach green	Dark Kelp
5.	Powdered sample+ Conc. H2SO4	Dark brown	Dark kelp	Dark green	Black	Black	Black
6.	Powdered Sample+ Conc. H_2SO_4 + water	Dark brown	Dark green	Black	Deep brown	Black	Black
7.	Powdered sample+ Conc. HCL	Green grape	Green	Green	Dark brown	Oak leaf	Rhubarb leaf
8.	Powdered sample+ Conc. HCL + water	Chartreuse green	Teal green (Chayote)	Shaded Fern green	Orange	Lemon green (Chartreuse)	Green
9.	Powdered sample+ Nitric Acid	Orange	Basil green	Spinach green	Orange	Lemon green (Chartreuse)	Green
10.	Powdered sample+ Nitric acid + water	Orange	Green	Smith green	Yellow	Lemon green (Chartreuse)	Spinach green
11.	Powdered sample+ Iodine	Orange	Olive	Brown	Orange	Sage green	Olive brown
12.	Powdered sample + 5% Ferric Chloride	Dark orange	Brown	Dark brown	Brick red	Brown	Dark brown
13.	Powdered sample + Picric zcid	Yellow	Lemon green	Green	Dark yellow	Lemon green	Green
14.	Powdered sample + Picric acid + water	Yellow	Lemon green	Green	Yellow	Lemon green	Green
15.	Powdered sample + Glacial acetic acid	Pale yellow	Green	Dark green	Light brown	Green	Dark green
16.	Powdered sample + Petroleum ether	Pale green	Pale green	Light gray	Pale green	Pale green	Light gray
17.	Powdered sample+ Chloroform						
18.	Powdered sample + Ethyl acetate	Light green	Lemon green	Grayish green	Green	Lemon green	Green
19.	Powdered sample+ Methanol	Green	Lemon green	Dark green	Green	Lemon green	Dark green
20.	Powdered sample+ 5% Potassium dichromate	Brick red	Brown	Maroon	Dark orange	Brown	Dark brown
21.	Powdered sample + Alcoholic hydroxide	Light green	Lemon green	Dark green	Brown	Lime green	Brown

3.4 Phytochemical analysis

Quantitative phytochemical screening as carried out to confirm the total alkaloid and total saponin content as highlighted in Table 5.

Table 5. Quantitative phytochemical analysis (Alkaloids and Saponins) of ethanolic leaf extract of *Z. mauritiana*.

S/N	Phytochemicals	Mean±SD* n=3
1	Total Saponins	1.5±0.86 %
2	Total Alkaloids	1.86±0.23%

*Values are mean ± standard deviation of triplicate measurement.

3.5 HPLC and GC-MS analysis

The HPLC analysis of the ethanolic powder leaf extract of *Z. mauritiana* as seen in the chromatogram (Table 6) shows the presence of ellagic acid, rutin, ferulic acid, catechin and quercetin. Highlights of chemical constituents present in the plant is shown in the GC-MS experiment as seen in Table 7. The experiment showed the abundance of compounds like 1,2-Benzenedicarboxylic acid and n-decanoic acid.

Table 6. HPLC data of major chemical compounds of *Z. mauritiana* ethanol extract.

S/N	Name of compound	Retention Time	Area
1	Ellagic acid	6.448	1088557
2	Rutin	7.356	1994760
3	Ferulic acid	8.467	1517269
4	Catechin	10.639	1016411
5	Quercetin	18.688	581510

Table 7. GC-MS data of major chemical compounds of *Z. mauritiana* ethanol extract.

S/N	Compound Name	Retention Time	Area
1	1,2- Benzenedicarboxylic acid, bis(2-ethylhexyl)	14.048	1088896
2	n-Decanoic acid	14.950	87452
3	1,2- Benzenedicarboxylic acid, diisoctyl ester	19.936	327146

The HPLC analysis of the ethanolic extracts of *Z. mauritiana* leaves revealed the presence of 35 peaks, however there are prominent five peaks detected in the sample. On comparing the prominent peaks with reference compounds, they correspond to ellagic acid, rutin, ferulic acid, catechin and quercetin respectively. Polyphenols play a key role in the defense against microbes, herbivores, and insects, and the phenolic compounds in the majority of plant extracts function as potent antifungal agents (El-Khateeb *et al.,* 2013). Tannins have general antimicrobial and antioxidant activities (Rivière *et al.,* 2009). This indicates that the leaf extract's higher concentration of gallic acid and ellagic acid may be playing an integral role as a phytochemical with antifungal properties. According to reports, the presence of tannins (gallic acid, ferulic acid, and ellagic acid) in the sampled leaf has pharmacological benefits including antioxidant, antimicrobial, and anticancer properties. The flavonoids (quercetin, catechin and rutin) contained in *Z. mauritiana* were described as having antioxidant properties (Brito *et al.,* 2015). Quercetin have also been documented to as an antifungal agent against *Candida albicans* and *Aspergillus niger* (Abd-Allah *et al.,* 2015). In addition to being used to treat serious conditions like depression, cancer, microbial infections, lipid-related diseases, etc., gallic acid has also been found to have antimutagenic, antiangiogenic, and anti-inflammatory properties (Choubey *et al.,* 2015). This is in relation to the study of Sameera and Mandakini (2015) who reported *Z. mauritiana* as possible and reliable natural source of pharmaceutical drug with antibacterial potential due to presence of important phytochemicals such as flavonoid, tannins, triterpenoids and alkaloid. Abalaka *et al.* (2011) also reported the high antioxidant activities exhibited by *Z. mauritiana* leaf extract.

GC-MS analysis identified three compounds namely 1,2-Benzenedicarboxylic acid, bis (2-ethylhexyl), 1,2-Benzenedicarboxylic acid, diisoctyl and n-Decanoic acid as shown in Table 7. The report of Kitahara *et al.* (2004) shows that decanoic acid has a high antimicrobial activities.

4. Conclusion

This study has given insight to the pharmacognostic features and chemical profile of *Z. mauritiana* which are essential features necessary for proper sourcing and identification of crude drugs. Furthermore, the findings of this study can be reported and incorporated into official documents (monographs and pharmacopeias) for guidance and regulatory purposes. Also, in the light of the biological activities attributed to *Z. maritiana*, the plant will be a good alternative for synthetic drugs - with all their unpalatable side-effects, as studies have shown it to contain phytochemicals which are good antioxidant, antimicrobial and anti-cancer agents

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6. References

Abalaka, M., Mann, A. and Adeyemo, S. 2011. Studies on in-vitro antioxidant and free radical scavenging potential and phytochemical screening of leaves of *Ziziphus mauritiana* L. and *Ziziphus spinachristi* L. compared with Ascorbic acid. *Journal of Medicinal Genetics Genomics*, 3(2): 28-34.

Abd-Allah, W.E., Awad, H.M. and AbdelMohsen, M.M. 2015. HPLC analysis of quercetin and antimicrobial activity of comparative methanol extracts of *Shinus molle* L. *International Journal of*

Current Microbiology and Applied Sciences, 4: 550-558.

Adigwe, O.P., Ibrahim, J.A., Buhari, A.H., Muhammed, K.A., Kirim, R.A., Danraka, A.M. and Egharevba, H.O. 2021. Pharmacognostic and phytochemical characterization of *Maerua angolensis* DC. *African Journal of Plant Science*, 15(4): 94-99.

Akhtar, N., Ijaz, S., Khan, H.M.S., Uzair, B., Reich, A. and Khan, B.A. 2016. *Ziziphus mauritiana* leaf extract emulsion for skin rejuvenation. *Tropical Journal of Pharmaceutical Research*, 15(5): 929-936.

Brito, S.M., Coutinho, H.D., Talvani, A., Coronel, C., Barbosa, A.G., Vega, C., Figueredo, F.G., Tintino, S.R., Lima, L.F. and Boligon, A.A. 2015. Analysis of bioactivities and chemical composition of *Ziziphus joazeiro* Mart. using HPLC–DAD. *Food chemistry*, 186: 185-191.

Chase Jr, C.R. and Pratt, R. 1949. Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. *Journal of the American Pharmaceutical Association*, 38(6): 324-331.

Choubey, S., Varughese, L.R., Kumar, V. and Beniwal, V. 2015. Medicinal importance of gallic acid and its ester derivatives: a patent review. *Pharmaceutical patent analyst*, 4(4): 305-315.

Diallo, F., *Etude phytochimique et activité antiradicalaire de trois plantes médicinales utilisées dans la prise en charge du fibrome utérin au Mali*. 2020, USTTB.

El-Khateeb, A.Y., Elsherbiny, E.A., Tadros, L.K., Ali, S.M. and Hamed, H.B. 2013. Phytochemical analysis and antifungal activity of fruit leaves extracts on the mycelial growth of fungal plant pathogens. *Journal of Plant Pathology Microbiology*, 4(9): 1-6.

Fatokun, O., Omorogbe, L., Adamu, A., Esievo, K. and Okhale, S. 2020. Pharmacognostic and physicochemical evaluation, HPLC analysis and antiproliferative properties of *Kigelia africana* (Lam.) Benth. *Journal of Phytomedicine and Therapeutics*, 19(2): 410-428.

Fofie, Y., Coulibaly, K., Traore, K., Odoh, E., Zihiri, G. and Kone-Bamba, D. 2018. Pharmacognostic study of *Ziziphus mauritiana* LAM.(Rhamnaceae). *International Journal of Pharmacognosy*, 5(6): 354-359.

Goyal, M., Nagori, B.P. and Sasmal, D. 2012. Review on ethnomedicinal uses, pharmacological activity and phytochemical constituents of *Ziziphus mauritiana* (*Z. jujuba* Lam., non Mill). *Spatula DD*, 2(2): 107-16.

Gupta, M., Bhandari, A. and Singh, R.K. 2012. Pharmacognostical evaluations of the leaves of *Ziziphus mauritiana*. *International Journal of Pharmaceutical Sciences and Research*, 3(3): 818.

Kitahara, T., Koyama, N., Matsuda, J., Aoyama, Y., Hirakata, Y., Kamihira, S., Kohno, S., Nakashima, M. and Sasaki, H. 2004. Antimicrobial activity of saturated fatty acids and fatty amines against methicillin-resistant *Staphylococcus aureus*. *Biological Pharmaceutical Bulletin*, 27(9): 1321-1326.

Koomson, D.A., Kwakye, B.D., Darkwah, W.K., Odum, B., Asante, M. and Aidoo, G. 2018. Phytochemical constituents, total saponins, alkaloids, flavonoids and vitamin C contents of ethanol extracts of five *Solanum torvum* fruits. *Pharmacognosy Journal*,10(5).

Mahomoodally, M.F. 2013. Traditional medicines in Africa: an appraisal of ten potent African medicinal plants. *Evidence-Based Complementary and Alternative Medicine*, 2013: 617459.

Marwat, S.K., Khan, M.A., Khan, M.A., Ahmad, M., Zafar, M., Rehman, F. and Sultana, S. 2009. Fruit plant species mentioned in the Holy Qura'n and Ahadith and their ethno medicinal importance. *American-Eurasian Journal of Agricultural and Environmental Science*, 5(2): 284-295.

Nathiya, S., Santhi, N. and Kalaiselvi, S. 2012. A comparative study on ontogenic expression of antioxidants and secondary metabolites in *Withania somnifera*. *International Research Journal of Pharmacy*, 3(1): 2010-2015.

Okhale, S.E., Buba, C.I., Oladosu, P., Egharevba, H.O., Ugbabe, G., Ibrahim, J.A. and Kunle, O.F. 2016. Chemical constituents and antimicrobial activity of the leaf essential oil of *Garcinia kola* Heckel (Clusiaceae) from Nigeria. *Chemical Science International Journal*, 13(5): 1-7.

Pandey, D., Joshi, A. and Hemalatha, S. 2015. Quality control standardization and I*n-Vitro* antioxidant activity of *Aganosma dichotoma* K. Schum root. *Pharmacognosy Journal*, 7(1): 74-82.

Preeti, S. and Tripathi, S. 2014. A phytopharmacological review on "*Ziziphus jujuba*". *International Journal of Research and Development in Pharmarcy and Life Science*, 3(3): 959-966.

Rathore, S.K., Bhatt, S., Dhyani, S. and Jain, A. 2012. Preliminary phytochemical screening of medicinal plant *Ziziphus mauritiana* Lam. fruits. *International Journal of Current Pharmaceutical Research*, 4(3): 160-162.

Rivière, C., Pieters, L., Dejaegher, B., Vander Heyden, Y., Van, M.C. and Quetin-Leclercq, J. 2009. Polyphenols isolated from antiradical extracts of *Mallotus metcalfianus*. *Phytochemistry*, 70(1): 86-94.

Sameera, N. and Mandakini, B. 2015. Investigations into the antibacterial activity of *Ziziphus mauritiana* Lam. and *Ziziphus xylopyra* (Retz.) Willd. *International Food Research Journal*, 22(2): 849.

WHO, *World Health Organization on Quality control methods for medicinal plant materials*. 1998: World Health Organization.