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ORIGINAL RESEARCH ARTICLE

Analysis of *Balanites aegyptiaca* wood extractives and their variation across different ecological zones

Dao Dougabka[1,*], Tikri Bianzeube[2], Wel-Doret Djonglibet[2], Jean Gérard[3], Kévin Candelier[3], GUYOT Alban[3]

[1] *Department of Fundamental Sciences, ENSTP, BP 60 N'Djamena, Chad*

[2] *Industrial technology study and research laboratory, Faculty of Applied Sciences, University of N'Djamena, BP 1117 N'Djamena, Chad*

[3] *BioWooEB, Univ Montpellier, CIRAD, Montpellier, 34398 Montpellier, France*

***Corresponding author:** Dao Dougabka, dougabkadao@yahoo.fr

ABSTRACT

This study focuses on the analysis of extractives from the wood of Balanites aegyptiaca, an endemic and iconic species of dry African regions, and the impact of climatic and geographical conditions on these extractives. The wood samples were collected from three distinct geographical zones: the Sahelian Chadian zone, the Sudanian Chadian zone, and the Sahelian Senegalese zone. A total of nine trees were analyzed in this study. The results showed that samples from the Sahelian Chadian zone, characterized by more arid conditions, had a significantly higher extractive content compared to those from the other two regions. The extractive compounds characteristic of these samples included stereoisomers of inositol, pinnitol, diosgenin, and sesquiterpenes. These compounds were found to be identical in samples from the Sahelian Senegalese and Sudanian Chadian zones, except for disaccharides, which were additionally present in the Senegalese samples. For the same species, pinnitol remained the most dominant compound in the wood from the Sahelian Chadian zone, alongside monosaccharides. This marked difference in extractive compounds is explained as a response of the tree to local constraints, particularly water stress and high temperatures, which stimulate the production of secondary metabolites such as tannins, polysaccharides, and pinnitol. These compounds are essential for the tree's resistance to unfavorable environmental conditions. The study highlights the influence of climatic factors on the biosynthesis of secondary compounds and suggests that Balanites aegyptiaca could offer promising opportunities for pharmacological and industrial applications.

Keywords: balanites aegyptiaca; chemical compounds; extractables; sahelian zone; sudanian zone

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1. Introduction

Balanites aegyptiaca, commonly known as the desert date or "soapberry tree" in Chad, is widely distributed in the arid and semi-arid zones of Africa. This thorny tree can reach a height of up to 17 meters, with trunk diameters up to 60 cm[1]. Its range is extensive, thriving in areas with annual rainfall ranging from 100 to 1000 millimeters[2] and with a lifespan of over 100 years[3]. Due to its resilience in extreme climatic conditions, *Balanites aegyptiaca* plays a crucial ecological and economic role in its natural range. Its pale yellow wood is highly valued for its mechanical properties, high calorific value, and resistance to insects, making it a preferred material for construction, agricultural tool production, and other artisanal applications[4].

Research on *Balanites aegyptiaca* wood has focused mainly on its physical and mechanical properties, but its chemical characteristics, particularly the composition of its extractives, remain largely unexplored.

Extractives are chemical compounds in plant materials that can be isolated using solvents. These compounds include a variety of substances such as tannins, resins, waxes, alkaloids, flavonoids, sterols, and lipids. They significantly influence wood properties, including its durability against biological degradation agents and dimensional stability, which are essential for construction and artisanal applications[5-6].

The production of these extractive compounds depends on the environmental growth conditions of the tree. Studies have shown that water stress, high temperatures, and humidity variations affect the biosynthesis of these compounds. For example, Bossu et al.[7] demonstrated that water deficits stimulate the production of tannins and phenols in *Bagassa guianensis*, enhancing its resistance to water stress. Similarly, Jankowska et al., and Seguin[5,8] observed that high temperatures can accelerate plant metabolism and increase the synthesis of certain compounds, although extreme temperatures may inhibit these processes or damage cells, thereby reducing the production of certain substances[9].

Studying the chemical composition of *Balanites aegyptiaca* wood extractives is therefore relevant, as this species is subject to varied environmental conditions that directly influence the biosynthesis of its secondary compounds. For this study, three distinct geographic areas were selected: the Sahelian zone of Chad, the Sudanian zone of Chad, and the Sahelian zone of Senegal. The choice of these regions is based on their environmental diversity, with each zone exhibiting variations in altitude, climate, and soil type that influence tree growth and the synthesis of its chemical compounds. For example, the Sahelian zone of Chad is more arid and has limited water availability, whereas the Sudanian zone is more humid, with higher biodiversity[10]. These contrasts provide an opportunity to study the environmental impact on the biosynthesis of secondary compounds in *Balanites aegyptiaca*, which may affect wood properties and potential uses.

In this context, this study aims to analyze and compare the chemical composition of *Balanites aegyptiaca* wood extractives from these three ecologically distinct zones. The objective is to determine the extractive compounds of this wood species to optimize its use in various industrial and artisanal sectors. This analysis of extractives could also provide insights into the influence of ecological conditions (climate, soil, altitude) on the synthesis of chemical substances and their impact on the quality and potential applications of *Balanites aegyptiaca* wood, opening new perspectives for uses in pharmaceutical, cosmetic, and wood preservation fields.

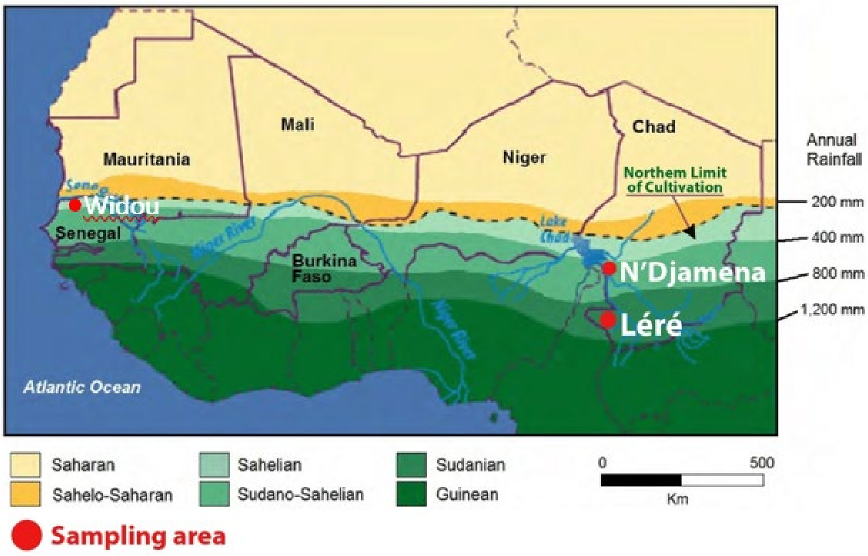
2. Materials and methods

2.1. Origin of wood

The study was conducted on wood samples of *Balanites aegyptiaca* (**Figure 1a**) from two Sahelian countries, Chad and Senegal (**Figure 1b**).

In Chad, the samples were collected from two different climatic zones, the Sahelian zone (N'Djamena region with an average annual rainfall of around 556 mm, and a temperature of 15°C for the average minimum in the coldest month and 44°C for the average maximum in the hottest month) and the Sudanian zone (Léré region with average annual rainfall of 860 mm, and an average monthly temperature ranging from 25 to 35°C, between 1980 and 2015).

In Senegal, the samples were collected from the Sahelian zone around the village of Widou Thiengoly located in the northern part of the country (average annual rainfall of 298 mm and average monthly temperature of between 20 and 38°C, between 1940 and 2015).



(a) **(b)**

Figure 1. (a): Tree *Balanites aegyptiaca*; (b): Study areas (source: FEWS, June 1997)

These three provenances were chosen to assess the influence of local growing conditions on the extractives composition of the wood. These three provenances correspond to different ecological conditions (altitude, climate…) which have an impact on tree growth.

2.2. Sampling

The sampling consisted of nine trees (three trees per provenance) collected from sandy-clay soils. The GPS coordinates of the sampling sites are presented in **Table 1**. The analyses were performed on wood powders with a particle size of 0.02 mm. To obtain the wood powders, fragments were randomly sampled from cross-sectional wood discs obtained after felling the trees at a height of 50 cm above ground level. These fragments were oven-dried at 60 °C for 24 hours and then subjected to two grinding steps. The first grinding step reduced the sample size to a maximum of 4 mm using a knife mill. The resulting particles were further processed to achieve a particle size of 0.02 mm using a hammer mill. After each grinding step, the mill was thoroughly cleaned to prevent cross-contamination between the samples.

Table 1. Location of trees.

Source	Shaft no.	Longitude (decimal degrees)	Latitude (decimal degrees)
	1	15,11338	12,05515
Chad Sahelian zone	2	15,11248	12,05421
	3	15,11191	12,05438
	1	14,08274	9,64513
Chad Sudanian zone	2	14,08517	9,64718
	3	14,08312	9,64235
	1	-15,33447	15,9028
Senegal Sahelian zone	2	-15,32110	15,91606
	3	-15,30741	16,05847

2.3. Extraction method and characterisation

To extract our wood powders, we used a mixture of ethanol and toluene (1:1, v/v) (Carlo Erba Reagents - Val-de-Reuil, France) followed by distilled water. These two solvents enabled more accurate separation and quantification of the extractables. The samples were dried in an oven at 103 ± 2°C until their mass (m_1) stabilised. The procedure followed was similar to that of Rowell et al (2005). Three replicates of the wood powders were extracted using a soxhlet (**Figure 2a**) with an ethanol-toluene mixture (12-hour extraction) and distilled water (12-hour extraction). After each extraction, the samples were dried at 103 ± 2°C for 48 h, then weighed (m_2 after ethanol/toluene extraction; m_3 after distilled water extraction). At the same time, the recovered extracts were concentrated by dry evaporation using a rotary evaporator (Rotavapor R-200 Büchi Suisse: 40°C - 9.5 MPa for ethanol/toluene extracts: 40°C - 7.2 MPa for water extracts) (**Figure 2b**).

(a) (b)

Figure 2. (a): Soxhlet extraction unit; (b): evaporation unit.

Extractible rates are determined by the following formula:

$$Ext_{Eth/tol}(\%) = \frac{m_1 - m_2}{m_1} * 100 \quad (1)$$

$$Ext_{water}(\%) = \frac{m_2 - m_3}{m_2} * 100 \quad (2)$$

- $Ext_{eth/tol}$: rate of extracts in the toluene/ethanol solution;
- Ext_{water} : water extract content in %, on an anhydrous basis;
- m_1 : initial anhydrous mass in grams ;
- m_2 : anhydrous mass after extraction with ethanol/toluene solution in grams ;
- m_3 : anhydrous mass after extraction with water in grams

2.4. Determination of extractable fractions by GC-MS analysis

The chemical compositions of the different extractable fractions were analyzed using gas chromatography-mass spectrometry (GC-MS). This method is based on the distribution of compounds between two immiscible phases: a mobile phase (carrier gas) and a stationary phase (grafted silica). Compounds exhibit different retention times in the column, allowing for their separation.

Chemical analysis was performed with a PERKIN ELMER CLARUS 680 gas chromatograph coupled with a PERKIN ELMER CLARUS SQ8 mass spectrometer, controlled by TurboMass v.6.1 software and using the NIST MS Search 2.0 database (2011) for compound identification.

Prior to injection, the extract samples were derivatized to optimize the detection of all present compounds. Specifically, 2 mg of extractable material was dissolved in 50 µL of BSTFA + 1% TMSCl (a silylation agent) in a 2 mL vial. The preparation was placed in a sealed oven at 70 °C for 120 minutes to complete the derivatization reaction. Afterward, the BSTFA was allowed to evaporate, and 1 mL of ethyl acetate was added to solubilize the silylated compounds. Finally, 1 µL of this solution was injected into the GC in splitless mode through an injector heated to 250 °C.

Chromatographic separation was carried out using a DB-5MS column. The oven temperature program started at 80 °C (held for 2 minutes), increased at 10 °C/min to 190 °C, then at 15 °C/min to 280 °C (held for 10 minutes), and finally at 10 °C/min to 300 °C, held for 14 minutes. Helium was used as the carrier gas, with a flow rate of 1 mL/min.

Following separation, the compounds were transferred to the mass spectrometer through a transfer line maintained at 250 °C and ionized at 70 eV for analysis of the ionic fragments.

3. Results

3.1. Quantification of extractables

Wood samples of *Balanites aegyptiaca* were collected from areas associated with different climatic conditions: the Sahelian zone of Chad (Léré), the Sudanian zone of Chad (N'Djamena), and the Sahelian zone of Senegal. The extractive yields for each zone are presented in **Table 2**. The table shows that the extractive yields differ across the three provenances, with mean values of 8.61%, 9.30%, and 10.43% for the Senegalese Sahelian zone, the Chadian Sudanian zone, and the Chadian Sahelian zone, respectively.

The Kruskal-Wallis test applied to these results to compare the extractive yields revealed significant differences between the Chadian Sahelian zone and the other two provenances (Chadian Sudanian and Senegalese Sahelian zones) with a p-value < 0.05. No significant difference was observed between the results for the Chadian Sudanian zone and the Senegalese Sahelian zone.

Table 2. Extractability rates according to origin.

Provenances	Extractables rate (%)
Chad Sahelian zone	10,43 ± 0,25
Chadian Sudan zone	9,30 ± 0,56
Sahelian zone of Senegal	8,61 ± 0,25

3.2. Chemical composition of extractives

Gas chromatography-mass spectrometry (GC-MS) analyses were used to characterize the chemical composition of the extractives from *Balanites aegyptiaca* wood. After separation, the compounds were transferred to the mass spectrometer via a transfer line thermostabilized at 250°C and ionized at 70 eV. This procedure enabled the identification of the structures of the main compounds. The results are summarized in **Table 3**, which lists the identified compounds and their retention times, and in **Figure 3**, which provides examples of the obtained chromatograms.

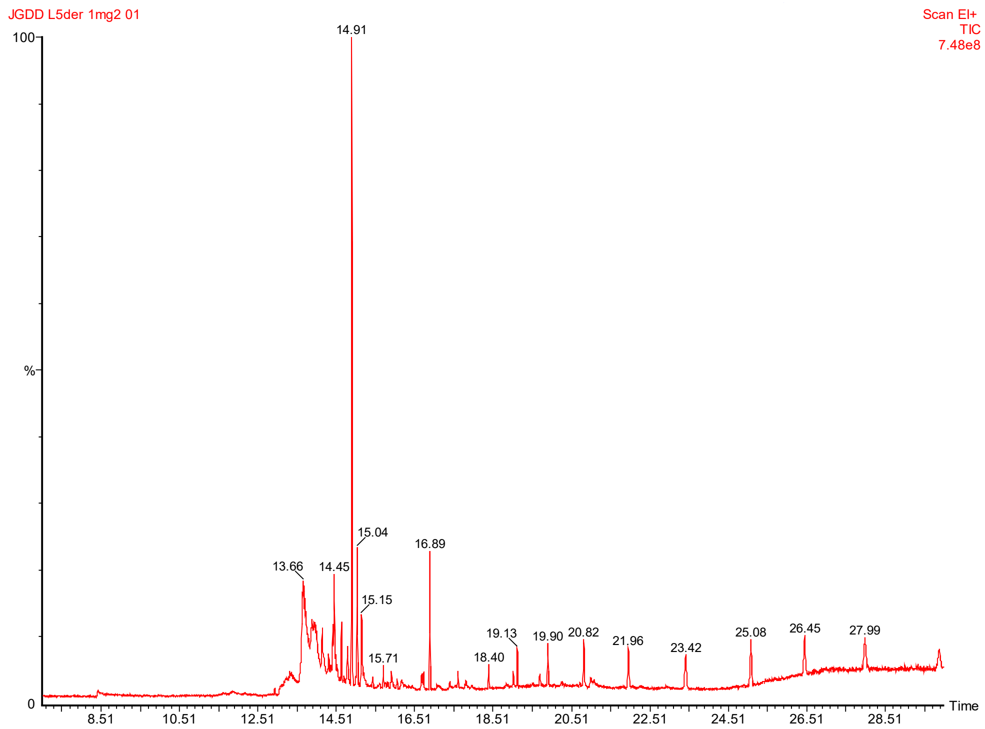
Table 3 reveals that the extractives characteristically present in *Balanites aegyptiaca* wood include stereoisomers of inositol (14–16 min), pinnitol (14.91 min), diosgenin (27.92 min), and sesquiterpenes (16.38 min, 17.13 min, 17.38 min as dominant compounds).

The extractives are largely identical between the Senegalese Sahelian and Chadian Sudanian provenances, except for the presence of disaccharides, which were detected only in the Senegalese samples. For the same species, pinnitol was the most dominant compound in the Chadian Sahelian wood, accompanied by monosaccharides. Additionally, the Chadian Sahelian samples exhibited the highest extractive content compared to the other two provenances (10.43% vs. 8.61% and 9.30%). This high extractive content in the Chadian Sahelian samples can be attributed to a chemical response to water stress conditions.

It is also worth noting that, despite the use of relatively harsh and prolonged derivatization conditions (BSTFA at 70°C for 48 hours), insoluble residues were often observed in the GC-MS vials, and a small fraction of the compounds did not evaporate. This phenomenon, characteristic of polar extracts, is likely due to the presence of polymers or macromolecules such as polysaccharides or tannins, confirming our hypothesis. This observation aligns with the findings of Bossu et al.[7], who demonstrated that water deficit stimulates the production of secondary metabolites, such as tannins and phenols, thereby helping the tree withstand water stress.

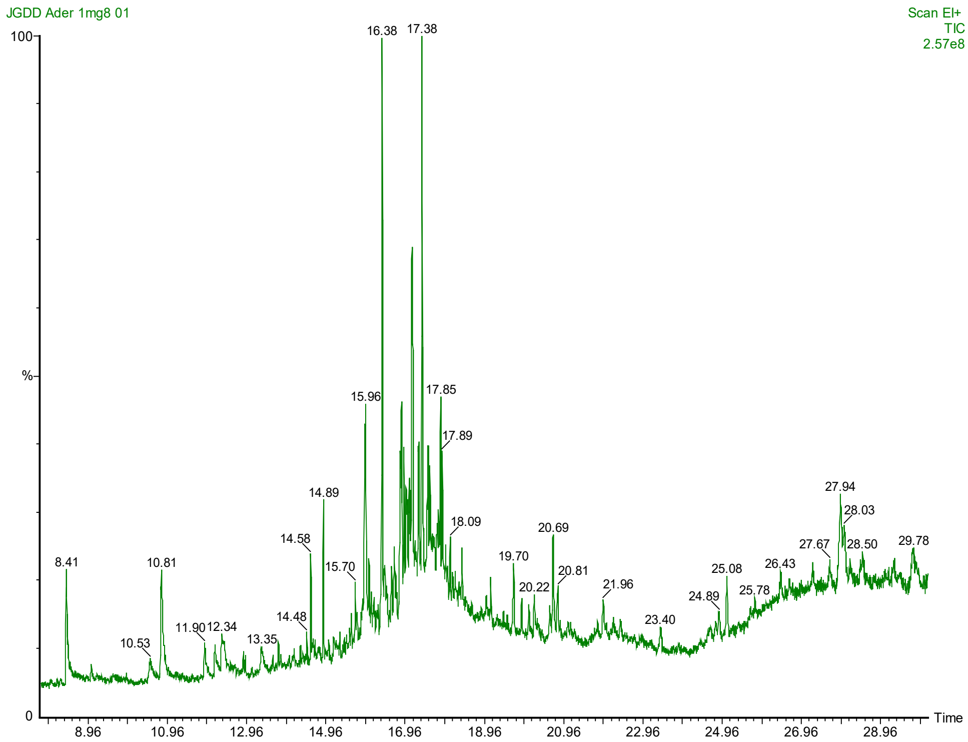
Table 3. Extractable compounds (or families of compounds) identified in *Faidherbia albida* and *Balanites aegyptiaca* wood according to origin.

Retention time (in min)	Chemical compound	Family	Specific characteristic or activity	Chad Sudanian zone	Chad Sahelian zone	Senegal Sahelian zone
13 - 17	Monosaccharides	Sugars		X	X	X
14 - 16	Other inositols	Sugars (polyols)	Surfactants	X		X
14,91	Pinnitol	Sugar (cyclitol)	Osmoprotective carbohydrate in plants	X	X	X
16,38	Representative of sesquiterpenes	Terpenes	Biological activities (e.g. antifungal and anti-termite)	X		X
17,13	Representative of sesquiterpenes	Terpenes	Biological activities (e.g. antifungal and anti-termite)	X		X
17,38	Representative of sesquiterpenes	Terpenes	Biological activities (e.g. antifungal and anti-termite)	X		X
19,8 - 22,5	Disaccharides	Sugars				X
27,92	Dyosgenin	Sterol derivative	Steroidal saponin = chemical precursor of various steroids	X		X

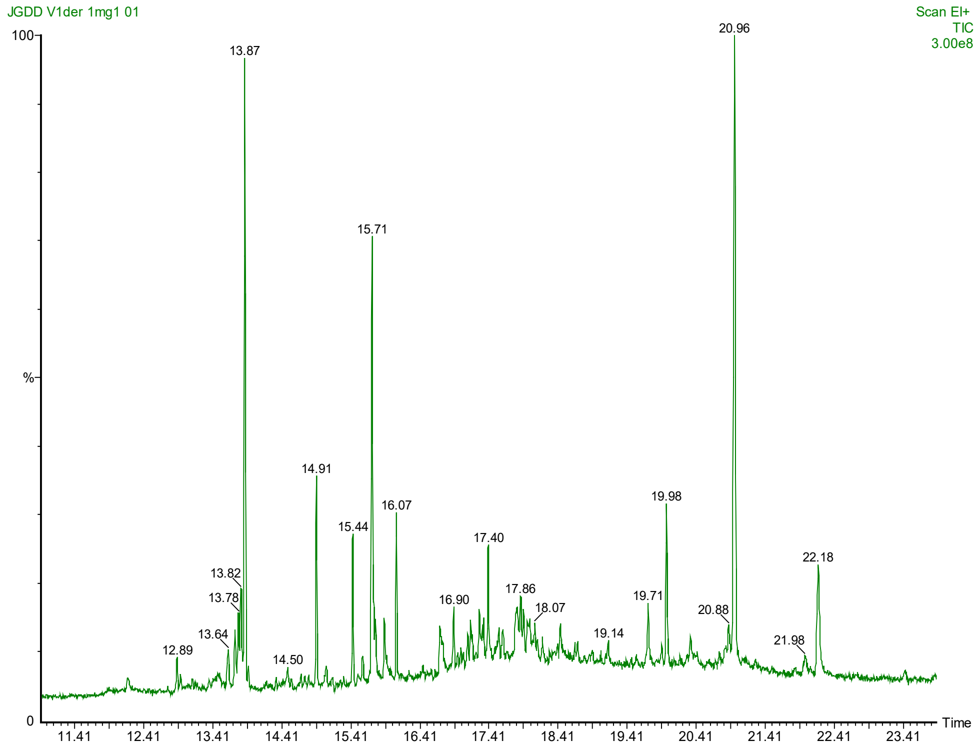


(a)

Figure 3. (*Continued*)



(b)



(c)

Figure 3. GC-MS analysis chromatograms: (a) Chadian Sahelian zone, (b) Chadian Sudanian zone, (c) Senegalese Sahelian zone.

4. Discussion

The results of this study highlight significant variations in the extractable compound content of *Balanites aegyptiaca* wood depending on its geographical origin. Wood samples from the Chadian Sahelian zone exhibited higher levels of extractables compared to samples from the other two regions. However, only two extractable compounds were identified in the extracts: pinnitols and monosaccharides. It is important to note that insoluble residues were observed in the GC-MS vials for samples from the Chadian Sahelian zone, likely due to the presence of polymers or macromolecules, such as polysaccharides or tannins, characteristic of polar extracts. These compounds suggest a biochemical adaptation to harsh climatic conditions, as such macromolecules might play a protective role against water stress by stabilizing plant tissues and enhancing wood resilience in extreme environments.

Previous research has shown that water stress can stimulate the production of secondary metabolites, such as tannins and phenols, which enhance tree resilience to unfavorable environmental conditions [1]. These defense compounds, produced in response to arid conditions, are essential for improving trees' tolerance to climatic constraints, reflecting a physiological adaptation to local ecological specifics. Thus, the predominance of pinnitol, an osmoprotectant involved in water stress tolerance, in this climatic zone is indicative of its aridity. Conversely, in the more humid Sudanian zones, a greater chemical diversity was observed, including inositol stereoisomers, diosgenin, and sesquiterpenes. These compositional differences among provenances indicate a differentiation in secondary metabolite biosynthesis influenced by environmental conditions, with each climatic zone promoting the synthesis of specific molecules.

Studies by Jankowska et al. and Seguin[5,8] emphasize that high temperatures and drought periods can stimulate the synthesis of certain protective compounds, although extreme temperatures may inhibit these processes or damage cells, thereby reducing biosynthesis. Similarly, Pantin and Blatt [11] observed that relative humidity influences transpiration and stomatal regulation, affecting photosynthesis and, consequently, the production of biomass and defense compounds. Under low humidity conditions, specific compounds increase to minimize water loss and provide protection against drought-related damage. This dynamic may explain the prevalence of certain specific compounds in the Sahelian zones, which experience drier and hotter climatic conditions.

Studies such as those by N'Guessan et al.[6] and Corna et al.[12] suggest that prolonged droughts or heatwaves can trigger stress responses in trees, leading to an increase in secondary metabolites that play a role in defense against abiotic and biotic stresses.

The specific compounds identified in this study, such as pinnitol, diosgenin, and certain sesquiterpenes, have also been reported in other parts of the tree, such as bark, roots, seeds, and pulp, according to the findings of Farid et al.[13], Pousset[14], and Chapagain et al.[9]. These observations suggest that the extractable compounds from wood may potentially reflect those from other tree organs, offering opportunities for integrated exploitation of this plant resource.

5. Conclusion

This study aimed to determine the extractive content and chemical composition of *Balanites aegyptiaca* wood from three different provenances, as well as to assess the influence of environmental growth conditions on the synthesis of these chemical compounds. The results showed that wood samples from the Chadian Sahelian zone exhibit a higher extractive content compared to those from the other two provenances; however, only two extractive compounds, pinnitol and monosaccharides, were identified in these extracts.

The extractives characteristically present in *Balanites aegyptiaca* wood include stereoisomers of inositol, pinnitol, diosgenin, and sesquiterpenes. These compounds are consistent between the Senegalese Sahelian and Chadian Sudanian samples, except for the additional presence of disaccharides in the Senegalese wood. These compositional differences between provenances appear to be linked to environmental stresses, such as water scarcity and high temperatures, which influence the biosynthesis of secondary metabolites in this tree. These findings align with existing studies demonstrating the impact of climatic conditions on chemical compound synthesis.

The variability in the extractive composition of *Balanites aegyptiaca* depending on the environment highlights the potential of this wood for diverse applications. The data obtained provide valuable insights for the sustainable use of this plant, particularly for the production of bioactive compounds and natural preservation agents.

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Conflict of interest

The authors declare no conflict of interest.

References

1. Hall J. B. (2004). Balanite aegyptiaca. Enzyklopädie der Holzgewächse: Handbuch und Atlas der Dendrologie, 1-6.
2. Le Floc'h E., Aronson J. (2013). Les arbres des déserts : enjeux et promesses. Éditions Actes Sud, 372 p.
3. Weber J. C., Montes C. S. (2010). Correlations and clines in tree growth and wood density of Balanites aegyptiaca (L.) Delile provenances in Niger. New Forests, 39 (1): 39-49. https://link.springer.com/article/10.1007/s11056-009-9153-8
4. Dougabka, D., Gérard, J., Bianzeube, T., Dendoncker, M., Vincke, C., Marchal, R., Guyot, A. (2021). Variations des caractéristiques physiques et mécaniques du bois de Balanites aegyptiaca en fonction de trois provenances. Bois et Forêts des Tropiques, 349 : 5-19. https://doi.org/10.19182/bft2021.349.a36776
5. Jankowska, A., Boruszewski, P., Drożdżek, M., Rębkowski, B., Kaczmarczyk, A., Skowrońska, A. (2018). The role of extractives and wood anatomy in the wettability and free surface energy of hardwoods. BioResources, *13*(2), 3082-3097. https://doi.org/10.15376/biores.13.2.3082-3097
6. N'Guessan, J. L. L., Niamké, B. F., Yao, N. G. J. C., Amusant, N. (2023). Wood extractives: Main families, functional properties, fields of application and interest of wood waste. Forest Products Journal, *73*(3), 194-208. https://doi.org/10.13073/FPJ-D-23-00015
7. Bossu, J., Beauchêne, J., Estevez, Y., Duplais, C., Clair, B. (2016). New insights on wood dimensional stability influenced by secondary metabolites: The case of a fast-growing tropical species Bagassa guianensis Aubl. PloS one, 11(3), e0150777. https://doi.org/10.1371/journal.pone.0150777
8. Seguin, B. (2010). Le changement climatique : conséquences pour les végétaux. Quaderni, 71(1), 27- 40. https://doi.org/10.4000/quaderni.525
9. Chapagain, B. P., Wiesman, Z. (2007). Determination of saponins in the kernel cake of Balanites aegyptiaca by HPLC-ESI/MS. Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques, *18*(4), 354-362. https://doi.org/10.1002/pca.990
10. Guihini, M. A., Diallo, M. D., Diallo, A., Saleh, M. M., Guissé, A. (2021). Distribution des ligneux sur le tracé de la grande muraille verte: cas de batha et de wadi-fira Ouest au Tchad. International Journal of Biological and Chemical Sciences, *15*(1), 144-155. http://rivieresdusud.uasz.sn/xmlui/handle/123456789/1957
11. Pantin, F., Blatt, M. R. (2018). Stomatal response to humidity: blurring the boundary between active and passive movement. Plant Physiology, 176(1), 485-488. https://doi.org/10.1104/pp.17.01699
12. Crona, B., Wutich, A., Brewis, A., Gartin, M. (2013). Perceptions of climate change: Linking local and global perceptions through a cultural knowledge approach. Climatic change, 119, 519-531. https://doi.org/10.1007/s10584-013-0708-5
13. Farid, H., Haslinger, E., Kunert, O., Wegner, C., Hamburger, M. (2002). New steroidal glycosides from Balanites aegyptiaca. Helvetica Chimica Acta, *85*(4), 1019-1026. https://doi.org/10.1002/1522-2675(200204)85:4<1019::AID-HLCA1019>3.0.CO;2-S
14. Pousset, J. L. (2004). Plantes médicinales d'Afrique : Comment les reconnaitre et les utiliser. Edisud – Secum, ISBN 2744904325