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Taxonomic Identification of *Boswellia dalzielii* Hutch Based on Chemical composition

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Abstract

The family *Burseraceae* comprises a total of 18 genera and 540 species. *Boswellia dalzielii* is a botanical species commonly employed in tropical and subtropical regions for its therapeutic properties, serving as a remedy for a diverse range of health conditions. The taxonomic identification and chemical composition of *B. dalzielii* in northern Nigeria have not been extensively documented in scientific literature, despite the recognized medicinal efficacy of this plant species. The soxhlet extraction method was employed to extract ethanol, methanol, and aqueous crude from the leaves. Subsequently, the extracted samples were subjected to analysis using gas chromatography combined with mass spectrometry. Fourier Transform Infrared spectroscopy (FTIR) was employed for the purpose of functional group identification in the crude extract. There was clear and noticeable distinction between the species under consideration on the X axis of principal component (PC) 1. The methanol and aqueous chemicals that were extracted from *B. dalzielii* showed a higher degree of similarity and were found to cluster together on the left side of the PC1 axis. PC2 did not indicate a clear distinction of the extract. The ethanol extract included 15 different chemicals, the methanol extract contained 12 different chemicals, and the aqueous extract contained 11 different chemicals.

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Introduction

The significance of plants for human survival lies in their ability to generate oxygen, provide sustenance, and offer medicinal properties (Usman et al., 2022). The discipline of Plant Identification serves as the fundamental scientific basis upon which the fields of modern medicine, cuisine, farming, and botany have been established and advanced (Abdulrahman et al., 2018). Taxonomic instruments utilized for the purpose of identification. The study of

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plant taxonomy emerged because of the imperative to precisely classify and designate plant species (Abdulrahman et al., 2018; Abdulrahman et al., 2021). The formal description and naming of plant species are believed to have been completed for only approximately one-third of the total number of plant species in the globe(Fatiha et al., 2014). Hence, the task of identifying all plant species that now exist is of utmost importance, yet it presents significant challenges. Morpho-anatomical traits are commonly regarded as classical indicators for comprehending the evolutionary relationship among plants (Mahmoud et al., 2019). Chemical identification is also necessary to accurately classify plants within a specific taxonomic group (Mahmoud et al., 2019). Chemometrics is a scientific discipline employed for the comprehensive and simultaneous analysis of plants (Idris et al., 2023). The utilization of innovative chemometric techniques has significantly contributed to the progress of plant identification investigations in the context of herbal and medicine development (Idris et al., 2023). Chemometrics is a very efficacious methodology employed in the field of plant science to analyse and identify diverse plant species and their constituent components (Umar et al., 2023). The genus Burseraceae encompasses a diverse assemblage of over 150 species of trees and shrubs, primarily distributed over the African continent. *Boswellia dalzielii* is extensively utilised within the traditional medical system of Nigeria for the management of many diseases (Dogara et al., 2022). Nevertheless, the categorization of plants in terms of taxonomic identification remains a subject of extensive discussion due to the substantial diversity in the chemical and molecular composition of plants, even within closely related species (Abdulrahman, 2022). Hence, the results obtained from the chemical analyses conducted on the plants will serve as vital empirical data to substantiate the classification and recognition of these botanical specimens (Abdulrahman, 2022). In the context of conducting research on the creation of pharmaceutical and herbal medicines, it is crucial to emphasize the significance of providing comprehensive taxonomic identification that is based on the chemical makeup of the substances involved. This study provides solid evidence for further biological investigation of the species, which may then be used to develop both traditional medicinal remedies and cutting-edge pharmaceuticals. Adulteration detection in the country is another potential application. The authors of the report suggest more research into numerical analysis and de novo species sequencing. Similarly, it offers a comprehensive chemical analysis of the leaf through the utilization of FTIR and GCMS analysis techniques. Hence, the present work contributes to the existing taxonomic knowledge and provides valuable insights for application in the field of natural product creation.

Materials and Methods

Plant Collection and Herbarium Deposition

During a field survey conducted in Zaria, Kaduna State, Nigeria, plant materials of the species B. *dalzielii* were collected. The identification of the obtained plant material was conducted by referencing the available sample in the herbarium at Ahmadu Bello University Zaria, as well as through a comparison with relevant literature. The specimens were placed in the herbarium of A.B.U and assigned the voucher number ABU0070340.

Extraction

The dried samples were ground into a coarse powder in the grinding machine. Weighing the powdered forms (500 g). Soxhlet was used for both ethanolic, methanol and aqueous extraction. Whatman no. 2 filter paper was utilized to filter the extraction's byproduct. The E-Z-2-Elite evaporation equipment was utilized to extract crude extract from

the ethanolic and aqueous portions of plants. At 40°C, the vacuum was set, and the pressure of the solvent for the methanol, ethanolic, and aqueous extracts at 300 and 72, correspondingly. The resulting crude extracts were allowed to continue drying in the dryer at 40°C (Abdulrahman et al., 2018).

FTIR Spectra Measurement

The mid-infrared spectra were acquired by scanning the spectral range of 4000-400 cm-1 using the Perkin Elmer Spectrum 400 Infrared spectroscopy instrument. The spectra were obtained from five leaf samples of Serai Kayu and Serai Kayu Hutan, utilising an air cooled Deuterated Triglycine Sulphate (DTGS) detector (Dogara, 2023). The Attenuated Total Reflectance (ATR) technique was employed to directly scan all samples, with a total of 16 scans and a resolution of 4 cm-1. Following the exportation of the data to an ASCII file, a baseline correction procedure was applied to each FTIR spectrum in order to mitigate the spectral discrepancies caused by the baseline (Dogara, 2023). To distinguish between the ethanol, methanol and aqueous extract of *B.dalzielii*, a multivariate analysis was conducted using the SIMCA-P (V.14.1 Umetrics Sweden) software. This analysis incorporated principal component analysis (PCA) and hierarchical cluster analysis (HCA) (Abdulrahman et al., 2021).

Gas chromatography Mass spectrometry (GC-MS) Conditions

Gas chromatography/coupled mass spectrometer was used for the GC/MS analysis. In a Temperature programme, the chemical mixtures were separated on a Column: HP-5MS 30 m x 0.25 mm, 0.25 mm film thickness. 10 minutes at 60°Celsius, then up to 230°Celsius in 3 minutes with a 1-minute hold (Mahmoud et al., 2019). The injector temperature was 245°Celsius, and the carrier helium gas flow rate was 1 millilitre per minute. The ion source and analyzer temperature for the MS was 260°C (Rahim et al., 2018), and the voltage was 70 e V(Mahmoud et al., 2019).

Results and Discussion

The integration of Fourier Transform Infrared Spectroscopy (FTIR) with chemometrics has demonstrated promising capabilities as expedient techniques for distinguishing between plants belonging to the same cultivar or distinct species (Abdulrahman et al., 2018; Abdulrahman 2022). The data set produced from the combination of ethanolic, methanolic, and aqueous samples resulted in a spectrum filter model. This model was derived using the X matrix and exhibited the largest variation (R2X (cum) = 0.999) as well as the highest predictive power (fitness of the model) (Q2 (cum) = 0.998). The R2 score quantifies the proportion of variability in the training data set that can be explained by the principal component analysis (PCA) technique (Abdulrahman, 2022). The R2 metric is a measure of goodness-of-fit that quantifies the degree to which the model aligns with the observed data. A high value of R2, approaching 1, is necessary to demonstrate the degree of fit between the dataset and the model (Abdulrahman, 2022). As per the concept of cross-validation, Q2 represents the proportion of the variance in the dataset that is accurately predicted by the model. The predictive performance of the model on new data is evaluated in the second quarter. A high Q2 value indicates a heightened degree of predictability (Abdulrahman et al., 2021). Hence, the model is suitable for conducting additional study on the Principal Component study (PCA) and Hierarchical Cluster Analysis (HCA) models. Principal Component Analysis (PCA) provides a diverse range of techniques to enhance the representation of data classification and facilitate the grouping of instances into clusters based on shared characteristics. In order for

two groups to be effectively differentiated, it is important that they possess specific commonalities within the same dimensions that establish their distinctiveness (Abdulrahman et al., 2021). The observed fingerprints found on the positive loading line of the score plot for the ethanolic, methanolic, and aqueous extracts of *B. dalzielii* have provided valuable insights into the established relationship, including similarities and discrimination (Figure 1). On the X axis of PC 1, there was evident and notable differentiation among the species under investigation (Figure 1). The compounds extracted from *B. dalzielii*, namely the methanolic and aqueous compounds, exhibited a higher degree of similarity and were shown to cluster together on the left side of the PC1 axis. Conversely, the ethanolic compounds of *B. dalzielii* were positioned on the right side of the PC1 axis. This variation accounted for 0.596% of the total observed variation, as depicted in Figure 1. The Y-axis, namely PC2, does not exhibit distinct distinction of the extract in Figure 1. Nevertheless, there was observed variance within the same species in the chemicals present in the ethanolic extract, as evidenced by the distribution along both the negative and positive axis in Figures 1. The second principal component (PC 2) explains 0.389% of the variation (Figure 1). The utilization of multivariate statistical analysis can greatly enhance traditional methods of examining the chemical composition by revealing relationships among compounds and organizing geochemical data into meaningful and comprehensible clusters (Abdulrahman, 2022; Abdulrahman et al., 2019; Abdulrahman et al., 2021). Variables tend to be high in species that are physically close to them, while variables on the other end of the spectrum tend to be low in these species. The development of plot scores is independent of variables close to the plot origin (Figure 2).

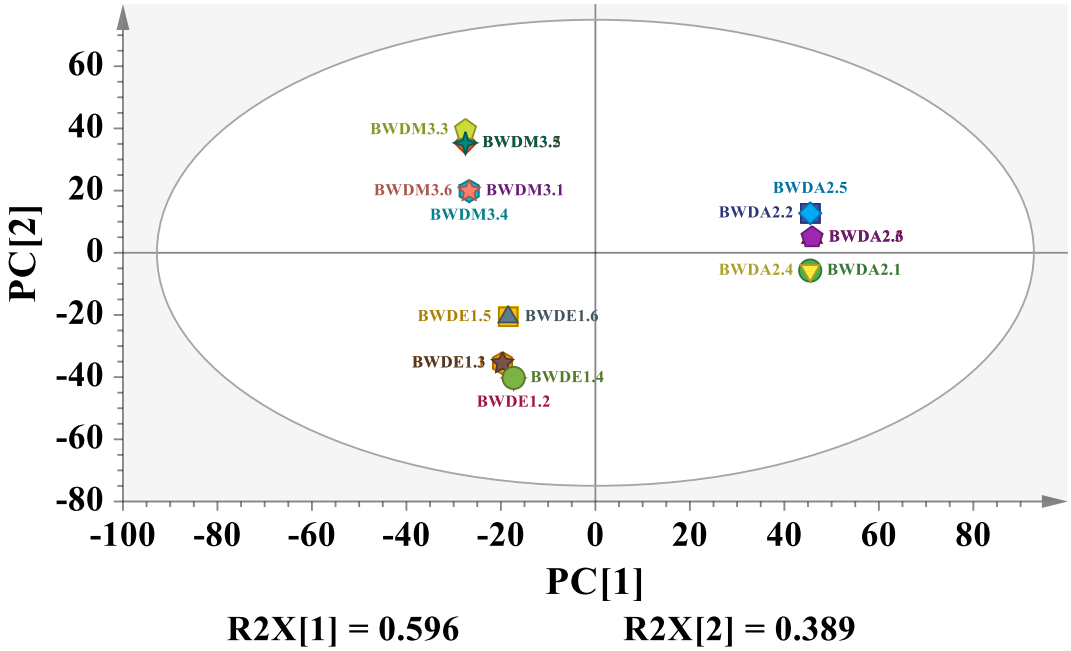

Figure 1: PCA Score Plot of FTIR combine data set of *Boswellia dalzielii*; BWDM= *Boswellia dalzielii*methanol,BWDE= *Boswellia dalzielii*ethanol, BWDA= *Boswellia dalzielii*aqueous.

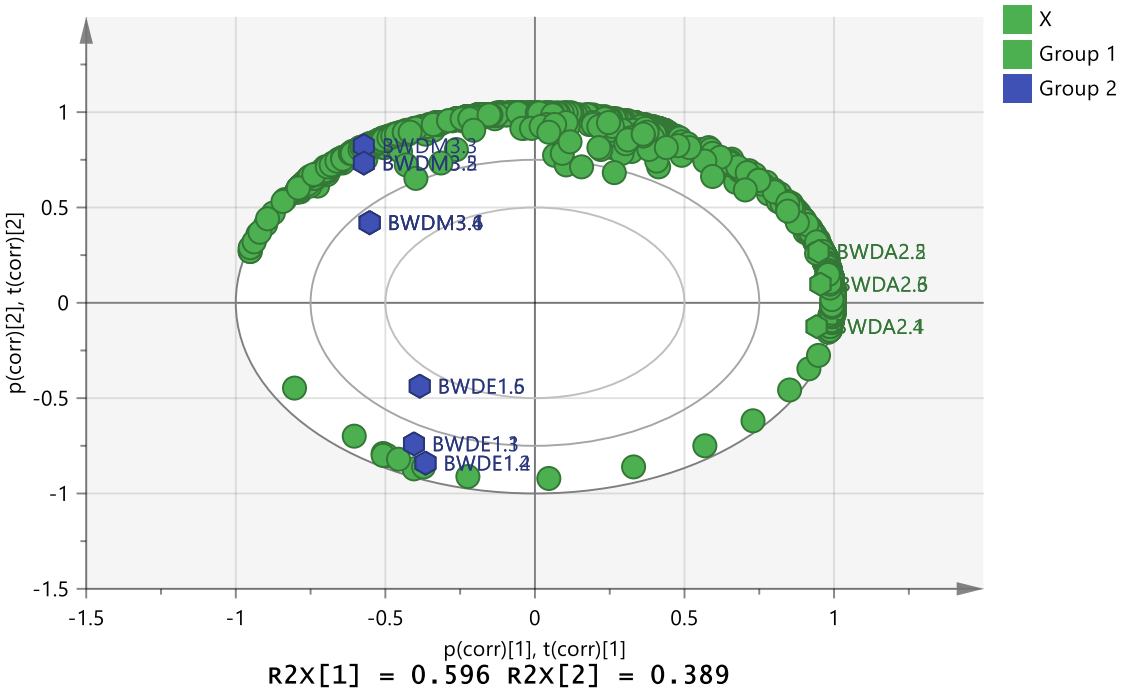

Figure 2: PCA Score Plot of FTIR combine data set of *Boswellia dalzielii*

To further identify similarities and differences, and to group them into distinct categories, the HCA was developed (Fatihah et al., 2012). Hierarchical Cluster Analysis (HCA) was developed using the model to further categorize the data. When it comes to HCA data visualization, the Dendrogram (Tree plot) is your best bet. The tree diagram (Figure 2) displays the total number of groups. There are two main clusters of tree plots shown in Figure 3; the first cluster contains solely the *B. dalzielii* aqueous extract component. The compounds found in ethanol extracts of B. *dalzielii* and methanol extracts of *B. dalzielii* are found in the first main group on the left side of Figure 3.

The hierarchical clustering analysis (HCA) dendrogram provided additional validation of the link between variables, specifically highlighting their similarities and differences, which had already been established by the principal component analysis (PCA). The hierarchical cluster analysis (HCA) model exhibited a comparable trend in the score plot. The dendrogram was partitioned into distinct clusters according on the characteristics of the solvents. Plant extracts can vary widely depending on factors such as the kind of extraction used, the solvents used, the chemicals present, and the polarity of the metabolites (Abdulrahman et al., 2019). The utilization of Fourier Transform Infrared (FTIR) analysis in conjunction with chemometrics has yielded a highly effective approach for distinguishing between plant species that have been extracted using various solvents.

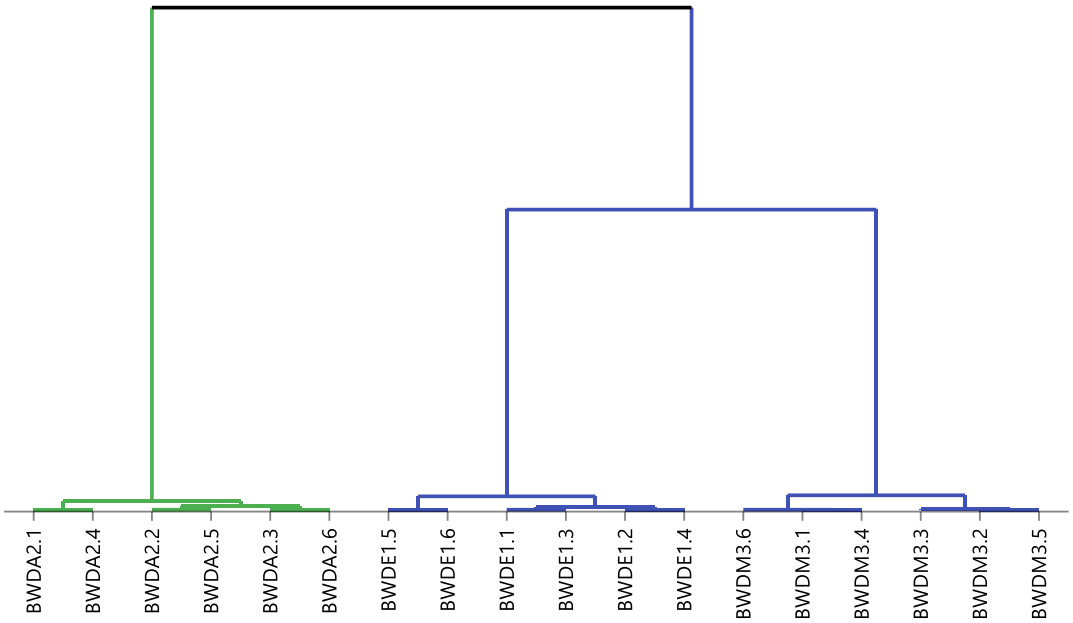

Figure 3: HCA FTIR data set of *Boswellia* dalzielii display relationshipof different compounds from ethanol, methanol and aqueous; BWDM= *Boswellia dalzielii*methanol,BWDE= *Boswellia dalzielii* ethanol, BWDA= *Boswellia dalzielii*aqueous.

Phytochemicals as inherent plant compounds with the ability to attenuate and prevent a variety of illnesses (Moneruzzaman et al., 2015). The development of plant resources involves the observance of authenticity and safety norms(Abdulrahman et al., 2018). Herbal therapies or modern pharmaceuticals? Quality control is critical and cannot be stressed. The assessment of quality and authenticity is critical in the study of ethno pharmacology. The use of several plant species in the production of modern pharma ceuticals or herbal treatments. Supplements for the diet. Botanical research and analysis is a laborious procedure. The scientific understanding of an entity's chemical composition. Scientists employ Gas Chromatography-Mass Spectrometry (GC-MS), a hybrid analytical method that combines the separation capabilities of gas-liquid chromatography with the detection power of mass spectrometry, to assess the presence of many chemicals in each sample (Uka & Akwo, 2022). Thus, it is important to chemically identify each therapeutic plant. Results from a dry examination of *B. dalzielii* leaves revealed the presence of 15 different chemicals in the ethanol extract, 12 different compounds in the methanol extract, and 11 different compounds in the aqueous extract (Table 1). Only compounds with a similarity of 75% or higher were considered. The ethanol extract contained the following major compounds: octadecanoic acid (13.42 percent), pentane (12.64 percent), sulfurous acid (11.15 percent), and phthalic acid (10.36 percent), while the methanol extract contained hydrofol acid (25.89 percent), lineoleoyl chloride (17.63 percent), and 2,3,3-trimethyl- (9.75 percent) and the aqueous extract; 9-Octadecenoic acid (21.89percent), Lineoleoyl chloride (18.21percent), Isooctanol (10.67 percent) and Isooctyl alcohol (9.14percent)as shown in Table 1. The

variations in the compositional constituents were attributed to the characteristics and methods employed in the extraction (Abdulrahman et al., 2019).

Table 1: Ethanolic, methanolic and Aqueous Chemical Composition of *Boswellia dalzielii*

Ethanol				Methanol			Aqueous		
S/N	RT	Compounds	Area	RT	Compounds	Area	RT	Compounds	Area
1	8.046	Decane	7.43	10.032	Undecane	3.12	9.045	3-Methylpyridazine	7.89
2	9.342	Pentane	12.64	11.042	2,3,3-trimethyl-	9.75	10.342	Benzoic acid	3.64
3	10.567	Phthalic acid	10.36	12.446	monoethyl ester	2.43	11.332	Isooctanol	10.67
4	11.211	2,8-dimethyl	4.63	13.331	4,6-Dimethyldodecane	7.65	12.232	methyl ester	8.56
5	12.043	Octadecanoic acid	13.42	15.03	n-Heptadecylic acid	2.14	13.432	Undecanone	7.65
6	14.056	9,12-Octadecadienoyl chloride	8.74	16.111	Lineoleoyl chloride	17.63	14.231	Cyclohexanol	1.89
7	15.321	1,2-Cyclohexanedimethanol	5.79	17.523	10-Undecynoic acid	1.78	15.212	9-Octadecenoic acid	21.89
8	17.038	:8-Heptadecyne	3.95	18.038	5-Decen-1-ol	4.67	16.381	Isooctyl alcohol	9.14
9	18.00	Octadecanoic acid	4.78	19.356	Hydrofol Acid	25.89	17.231	Lineoleoyl chloride	18.21
10	18.567	Sulfurous acid,	11.15	20.435	-7-hydroxymethyl, (cis)	3.17	18.222	Carbolic acid	3.33
11	19.005	Octa5,8,11,14-Eicosatetraenoic acid	2.17	22.000	Heptadecanoic acid	1.89	19.321	Benzene	5.95
12	20.357	Decane	8.19	23.879	dodecyl 2-propyl ester	4.38			
13	22.043	Squalene	8.12						
14	23.076	Supraene	5.36						
15	24.432	Hydrofol Acid	1.52						
		Total	**100 %**			**84.5%**			**98.82%**

Conclusion

Hence, based on the available information, it appears that there is a lack of scholarly literature pertaining to the taxonomic identification of *B. dalzielii* in northern Nigeria, specifically in relation to its chemical composition. The present investigation was prompted by a dearth of knowledge regarding the conventional classification of the plant, given its historical usage in the treatment and control of cancer, malaria, and typhoid fever. The study encompasses a comprehensive examination of the chemical composition of the leaf extract utilizing ethanol, methanol, and aqueous solvents. Hence, this work establishes a fundamental basis for subsequent investigations into the potential development of herbal pharmaceuticals.

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