Elemental analysis and phytochemical characterisation of Zanthoxylum zanthoxyloides (Lam.) Zepern. and Timler stem bark

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Abstract

Zanthoxylum zanthoxyloides is being used in African traditional medicine to treat an array of pathological conditions. Most notably is Sickle cell anaemia, one of the most important genetic haematological conditions affecting individuals of African descent. The stem bark of Zanthoxylum zanthoxyloides (Lam.) Zepern. and Timler was analyzed for its proximate, elemental and phytochemical content. The proximate analysis parameters were determined to be 9.47%, 6.36%, 11.00%, 18.75%, 4.29% and 50.13% for the moisture, crude fat, crude protein, crude fiber, ash and total carbohydrate content respectively. Results show the sample contains: 12946.7760 ppm Na, 1012.9924 ppm K, 6055.5911 ppm Ca, 1093.8837 ppm Fe, 1478.5064 ppm Mg and 216.8516 ppm Zn. Gas chromatography-mass spectrometry of the 70% ethanolic extract identified 46 chemical constituents representing various phytochemical classes including phytosterols, terpenes, coumarins and fatty aldehydes. The findings provide information on the elemental and phytochemical content of Zanthoxylum zanthoxyloides stem bark and a basis for identifying the bioactive constituents responsible for the attributed pharmacological actions of Z. zanthoxyloides.

Keywords: Zanthoxylum zanthoxyloides, GC-MS, Elemental analysis, Phytochemicals

1. Introduction

Zanthoxylum zanthoxyloides (Lam.) Zepern. and Timler, also known as Fagara, Orin ata, Senegal prickly-ash is a member of the family Rutaceae. The plant is native to West Tropical Africa, particularly coastal countries like Benin, Gambia, Nigeria, Ghana, Senegal, Togo and Cote D’Ivoire [1]. It grows abundantly in the savannah, dry forest vegetation and in coastal areas [2]. The Zanthoxylum zanthoxyloides plant is a prickly low branching shrub, forest liana or prickly tree armed with large woody thorns [3]. A major characteristic of this plant is that the trunks, branches, branchlets, leaf stalks and inflorescence axes are covered by prickles or spines [4].

An array of ethnomedical literature detail the medicinal use of various parts of the plant in the prevention, treatment, cure and management of diverse ailments. Zanthoxylum zanthoxyloides has been demonstrated to possess antibacterial [5], antifungal [7], antiparasitic [11, 12], antiproliferative [13, 14], antileishmanial [15], anti-inflammatory [16, 17], antioxidant [18], anthelmintic [19,22] and anti-sickling activity [23, 24].

Several studies have analysed the chemical constituents of various parts of the Z. zanthoxyloides plant. The essential oil from the Z. zanthoxyloides has been extensively studied and demonstrated to contain fatty acids, the monoterpene hydrocarbons; α-pinene, myrcene, (E)-β-ocimen, oxygenated terpenoids [25] and coumarins [5]. Hexadecanoic acid, germacrene D and decanal have been detected in the leaves and bark while pellitorine has been found in abundance in the root and stem bark. The coumarin content of Z. zanthoxyloides has, also, been studied and profiled [26]. With regards to the pharmacological actions of the chemical constituents, a series of phenolic acids including vanillic acid, hydroxybenzoic acid, 2-hydroxymethyl benzoic acid, para-fluorobenzoic acid as well as three divanillyloquinic acid isomers have been postulated to be responsible for the pharmacological properties of Z. zanthoxyloides in sickle cell anemia [23, 24, 4].

The present study is designed to identify and characterize the elements and bioactives found in Zanthoxylum zanthoxyloides stem bark utilizing qualitative and quantitative tools.
2. Materials and methods

2.1: Plant material: The dried stem of *Zanthoxylum zanthoxyloides* was purchased from an herbalist store (coordinate: 6.507060, 3.369277) in Lagos, Nigeria and authenticated at the Lagos University Herbarium, Department of Botany, University of Lagos, Akoka, Nigeria by Dr. G. I. Nodza. A voucher specimen with voucher number 8383 was deposited for reference purposes.

2.2: Sample pre-treatment: Dirt and other unwanted debris were removed from the plant material. The stem was further allowed to air dry for 48 hrs at room temperature (approximately 29°C) to achieve a percentage moisture content of less than 10% which was ascertained using an A&D MS-70 Moisture Analyzer (A & D Store, Illinois, USA) at 105°C. The outer bark was stripped from the stem and homogenized using a Solitaire mixer grinder (VTCL, India). The resultant homogenized stem bark was stored in an airtight amber glass container at -5°C.

2.3: Sample extraction: 500g of the homogenized stem bark was weighed on an Explorer analytical balance (Ohaus, Switzerland) and its polar components was extracted with 70% ethanol via cold macerated at room temperature (approximately 29°C) for 72 hrs. The sample was filtered with Whatman no 1 filter paper (Sigma-Aldrich, Germany). The filtrate was concentrated using a Rotavapor (Buchi, Switzerland) at 78°C and the resultant concentrate was evaporated over a water bath at 78°C until a constant weight was achieved. The semi-solid extract was weighed, packaged in an air-tight amber glass container and stored in a freezer at -5°C. The percentage yield was determined using the formula:

\[
\% \text{Yield} = \frac{\text{Weight of extract}}{\text{weight of plant material}} \times 100
\]

2.4: Proximate analysis: The ground stem bark was analysed for its moisture, crude fat, crude fiber, crude protein, carbohydrate and ash content. The moisture content was taken using an A&D MS-70 Moisture Analyzer at 105°C. The proximate parameters (crude fat, crude protein, crude fiber and ash value) of the ground *Zanthoxylum zanthoxyloides* stem bark were determined using the Association of Official Analytical Chemists, 2016 [27] method while the carbohydrate content was calculated by difference using the formulae;

\[
\text{Total Carbohydrate} = \left[ 100 - \% \text{Moisture} - \% \text{Fat} - \% \text{Protein} - \% \text{Fiber} - \% \text{Ash} \right]
\]

2.5: Elemental analysis

The AOAC official methods for elemental determination in food after dry ashing 999.11 [27] with slight modification was adopted for the digestion and elemental analysis of the sample. The prepared sample solution was analysed with the AA-7000 Atomic absorption spectrophotometer (Shimadzu, Japan) for K, Na, Ca, Fe, Zn and Mg.

2.6: Phytochemical screening

Preliminary phytochemical analysis was carried out on the 70% ethanolic extract of *Zanthoxylum zanthoxyloides* stem bark using standard qualitative methods [28-30] with slight modification.

2.7: GC-MS Analysis- The volatile constituents of the 70% ethanolic extract of *Zanthoxylum zanthoxyloides* stem bark was analysed using a QP2010SE Ultia Gas chromatography-mass spectrometer (Shimadzu, Japan).

2.8: Statistical Analysis: The results for the proximate and elemental analysis are presented as the mean ± SD of duplicate and pentaplicate determinations respectively. The relative standard deviation, RSD, values of all measurements were smaller than 10%.

3. Results

| Table 2: Elemental Analysis of *Zanthoxylum zanthoxyloides* Stem Bark |
|---|---|---|
| S/No | Element | Mean Value ± SD (ppm) |
| 1 | Sodium | 5046.7760 ± 0.0039 |
| 2 | Potassium | 1012.9924 ± 0.0002 |
| 3 | Calcium | 6055.5591 ± 0.0003 |
| 4 | Iron | 1039.8387 ± 0.0008 |
| 5 | Magnesium | 1478.5064 ± 0.0004 |
| 6 | Zinc | 216.8516 ± 0.0007 |

Results show the sample contains; 12946.7760 ppm Na, 1012.9924 ppm K, 6055.5591 ppm Ca, 1093.8837 ppm Fe, 1478.5064 ppm Mg and 216.8516 ppm Zn.

| Table 3: Phytochemical Screening of ethanolic *Zanthoxylum zanthoxyloides* extract |
|---|---|
| Phytochemical | Result |
| Tannins | + |
| Phenolics | + |
| Saponins | - |
| Flavonoids | - |
| Alkaloids | + |
| Terpenoids | - |
| Reducing Sugars | + |
| Carbohydrates | - |
| Alkaline reagent test | + |
| Ferric chloride test | - |
| Ferric chloride test | - |
| Borntrager’s test | + |
| Wagner’s test | - |
| Salkowski’s test | + |
| Fehling’s test | - |
| Molisch’s test | + |
| Salkowski’s test | + |
| Keller-Kiliani test | - |

- = negative test; + = positive test.
Phytochemical screening carried out on its ethanolic extract demonstrated the presence of tannins, phenolics, anthraquinones, alkaloids, terpenes, phytosterols, cardiac glycosides, reducing sugars and carbohydrates.

Fig 1: GC-MS total ion chromatogram (TIC) of 70% ethanolic Zanthoxylum zanthoxyloides extract

Table 4: Peak Report TIC of ethanolic Zanthoxylum zanthoxyloides extract

| Peak Number | Retention Time | Area %  | Compound Name                                      |
|-------------|----------------|---------|---------------------------------------------------|
| 1           | 4.408          | 0.14    | 1-Propanol, 2-ethoxy-                             |
| 2           | 4.694          | 0.13    | Furfural                                          |
| 3           | 6.274          | 0.02    | 2-Furancarboxaldehyde, 5-methyl-                  |
| 4           | 6.763          | 0.03    | Triethylenediamine                                |
| 5           | 8.533          | 0.07    | Levoglucosenone                                   |
| 6           | 9.094          | 0.02    | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-|
| 7           | 9.165          | 0.03    | Octanoyl chloride                                 |
| 8           | 9.953          | 0.03    | 2-Nitrohept-2-en-1-ol                             |
| 9           | 10.182         | 0.12    | 1,4:3,6-Dianhydro-.alpha.-d-glucopyranose         |
| 10          | 10.338         | 0.32    | 5-Hydroxymethylfurfural                           |
| 11          | 10.859         | 0.04    | N-[2-Hydroxyethyl]succinimide                     |
| 12          | 11.201         | 0.05    | 2,3,2H-Benzofuran-2-one, 3,3,4,6-tetramethyl-      |
| 13          | 11.512         | 0.22    | 3,4-Methylenedioxybenzylidene acetone             |
| 14          | 11.657         | 0.23    | 3,4-Methylenedioxybenzylidene acetone             |
| 15          | 11.827         | 0.15    | Piperonal                                         |
| 16          | 12.588         | 0.40    | 3,4-Methylenedioxybenzylidene acetone             |
| 17          | 12.701         | 0.58    | Benzofuran-2-one, 3-methyl-3-aza-2,3-dihydro-     |
| 18          | 13.842         | 1.41    | Cyclopentanecarboxylic acid, 3-ethenyl-2-methylene-|
| 19          | 14.134         | 0.19    | Dodecanoic acid                                   |
| 20          | 14.317         | 0.17    | 2-Cyclohexen-1-one, 2-hydroxy-6-methyl-3-(1-methyl)-|
| 21          | 14.380         | 0.29    | Benzoic acid, 4-hydroxy-3-methoxy-                |
| 22          | 15.538         | 1.27    | N-Isobutyl-(2E,4Z)-octadienamide                  |
| 23          | 16.773         | 0.63    | 1-Oxaspiro[2.5]octane, 2,4,4-trimethyl-8-methylene-|
| 24          | 17.232         | 22.46   | Naphthalene, decalhydro-1,1-dimethyl-             |
| 25          | 17.270         | 0.42    | n-Hexadecanoic acid                               |
| 26          | 17.316         | 2.73    | 3-Buten-2-one, 4-[4-(dimethylamino)phenyl]-       |
| 27          | 17.487         | 0.71    | Scoparone                                         |
| 28          | 18.430         | 7.05    | cis-9-Hexadecenal                                 |
| 29          | 18.500         | 2.00    | Cyclopentanone, 2-(2-nitro-2-heptenyl)-           |
| 30          | 18.844         | 1.02    | Cholest-5-en-3-ol (3.beta.-), tetradecanoate      |
| 31          | 19.032         | 1.41    | Cyclopenta[c][1]benzopyran-4(1H)-one, 7-dimethylamino)-2,3-dihydro-|
| 32          | 19.173         | 2.61    | Squalene                                          |
| 33          | 19.738         | 14.80   | 3,4-Methylenedioxybenzylidene acetone             |
| 34          | 20.053         | 6.66    | Furan, 2,5-ditricyclo[3.3.1.1(3,7)]dec-1-yl-     |
| 35          | 20.293         | 1.34    | Pregn-1,4,6-triene-3,20-dione, 6,16-dimethyl-    |
| 36          | 20.415         | 2.85    | Cholest-5-en-3-ol (3.beta.-), carbonochloridate   |
| 37          | 20.582         | 0.92    | Z,Z,Z-4,6,9-Nonadecatriene                        |
| 38          | 20.875         | 5.41    | Ergosta-5,22-dien-3-ol, acetate, (3.beta.,22E)-   |
| 39          | 20.907         | 11.50   | A-Neooleana-3(5),12-diene                         |
| 40          | 21.195         | 3.81    | Stigmaster-3-ol, 5-chloro-, acetate, (3.beta.,5.alpha.-) |
| 41          | 21.300         | 0.74    | Ergoster-5-en-3-ol, (3.beta.-)                    |
The top five peaks detected are peak 24 (the hydrocarbon terpene, 8,8-dimethyl-2,3,4,4a,5,6,7,8a-octahydro-1H-naphthalene), peak 28 (the irritant fatty aldehyde, (Z)-hexadec-9-enal), peak 33 ((E)-4-(1,3-benzodioxol-5-yl)but-3-en-2-one), peak 34 (2,5-bis(1-adamantyl)furan) and peak 39 (2,6-di(1-adamantyl)tetradecane, 2,6-

4. Discussion

Proximate and Elemental analysis of Zanthoxylum zanthoxyloides stem bark

The proximate analysis of Zanthoxylum zanthoxyloides stem bark described the nature of its chemical constituents and quantified its closely related components while the elemental analysis quantified selected minerals. As expected with plant barks which confer rigidity and protection to plants, the Z. zanthoxyloides stem bark was observed to contain a significant content of crude fibre, the indigestible component comprising of cellulose, hemicellulose and lignin, and total carbohydrates, the nitrogen-free extracts comprising of soluble simple and complex carbohydrates and organic acids, with values of 18.75% and 50.13%, respectively. Of interest with regards to bioactives present in Z. zanthoxyloides is the crude fat and crude protein contents. Several studies [4, 5, 7] have detailed the volatile and non-volatile oils of the Z. zanthoxyloides plant with particular attention to the peppery pellitorine and other olefinic isobutylamides. The crude protein content quantifies the nitrogenous components, inclusive of both protein and non-protein components. The relatively high crude protein of 11.00% of the stem bark is partly due to the diverse range of alkaloids and other nitrogenous moieties that the plant has been reported to contain [4].

Quantification of the elemental constituents of the Z. zanthoxyloides stem bark provides information on its micro-nutritional characteristics. Although it varies widely particularly in plant samples due to its strong relationship with cultivational factors, particularly soil, water and fertilizers used, elemental analysis is still a useful tool. The results show that the elements; sodium, potassium, calcium, magnesium, iron and zinc are in abundance. Though the herb is not ingested in its crude form, but rather in the form of aqueous/ alcoholic/ hydroalcoholic extracts/ decoctions/ infusions when used medicinally, it is imperative to consider the effect of dissolved minerals which are likely to be proportional to the content in the crude form depending on the extraction technique. Sodium, potassium, calcium, magnesium, iron and zinc are physiologically beneficial to the body system. However, depending on the amount of Z. zanthoxyloides stem bark used and the extraction process, the possibility of attaining toxic quantities of such minerals in herbal products containing Z. zanthoxyloides or its extract is the significant. The daily recommended value for individuals of 4 years and above for Na, K, Ca, Mg, Fe and Zn levels is 2000mg Na, 3510mg K, 1000mg Ca, 400mg Mg, 18mg Fe and 15mg Zn [31-33] and these values can possibly be surpassed by supplementation with the herbal preparations. Of specific concern, is the relatively high sodium and low potassium content in the stem bark. Evidence demonstrates that a reduced sodium intake positively influences cardiovascular, neurovascular and renal function [33] while the reverse association is supported for potassium intake [32].

Phytochemical screening and GC-MS analysis of the ethanolic Zanthoxylum zanthoxyloides extract

Extraction of the Z. Zanthoxyloides stem bark via cold maceration with 70% ethanol produced a bright yellow viscus transaluent extract with a yield of 10.77%. The phytochemical screening of the Z. zanthoxyloides stem bark extract was used to determine the major phytochemical groups present in the extract. The findings of the preliminary qualitative analysis of the extract is shown in Table 3 and it revealed the presence of tannins, phenolics, anthraquinones, alkaloids, terpenoids, phytosterols, cardiac glycosides and the absence of saponins, flavonoids, anthocyanins and phlobatannins. It is important to note that the absence of flavonoids was contrary to previous studies [18]. Possible explanations include differences in geographical location, sample collection time, strain variation, flavonoid content below detection limits or inappropriate detection method. The GC-MS data characterized the volatile compounds in the extract and confirmed the presence of the phytochemical groups; phenolics, alkaloids, terpenes, terpenoids, carbohydrates, phytosterols and coumarins amongst others.

Also detected in the extract was the anti-sickling phenolic acid, 4-hydroxy-3-methoxybenzoic acid at peak 21, (2E,4Z)-N-(2-methylpropyl)octa-2,4-dienamide at peak 22 and 6,7-dimethoxycarbonyl-2-one at peak 27, which agree with literature in terms of phytochemical constituents [4, 24]. Notably undetected are the prominent anti-sickling divanilloxyquinic acid A, B and C. A reason for this could be the method of analysis; the authors used LC/MS/NMR for the identification and detection of these acids [23]. As with the preliminary qualitative phytochemical screening, flavonoids were undetected. It is important to note that as GC-MS was used in our work, the scope is limited to bioactives that are detected using this analytical tool. Further studies utilizing other analytical methods will provide more information on undetected phytochemicals.
5. Conclusion
The findings presented from the preliminary qualitative and quantitative evaluation of the *Zanthoxylum zanthoxyloides* stem bark provides information on the nature of phytochemicals present and quantifies the micro-nutrients.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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