Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(5): 41-46
Received: 05-07-2020
Accepted: 17-08-2020

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

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DOI: https://doi.org/10.22271/phyto.2020.v9.i5a.12420

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, 6055.5591 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 ethnomedicinal 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], antimycobacterial [6], antifungal [7, 8, 5], anti-plasmodial [9, 10], antitrypanosomal [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-ocimene, 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 divanilloylquinic 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 ℃) 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 ºC. 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 ºC.

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℃) 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 78ºC and the resultant concentrate was evaporated over a water bath at 78 ºC 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 -5ºC. The percentage yield was determined using the formula;

$$\%\, Yield = \frac{Weight\ of\ extract}{weight\ of\ plant\ material} \times 100$$

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 ºC. The proximate parameters (crude fat, crude protein, crude fiber and ash value) of the ground *Zanthoxylum zanthoxyloides* stem bark was determined using the Association of Official Analytical Chemists, 2016 [27] method while the carbohydrate content was calculated by difference using the formulae;

$$Total\ Carbohydrate\ (\%) = [100 - \%(Moisture + Fat + Protein + Fiber\ + Ash)]$$

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 1: Proximate Analysis of *Zanthoxylum zanthoxyloides* Stem Bark

S/No	Parameter	Mean Value ± SD
1	Moisture content	9.47% ± 0.60
2	Crude Fat content	6.36% ± 0.26
3	Crude Protein content	11.00% ± 0.31
4	Crude Fibre content	18.75% ± 0.11
5	Ash value	4.29% ± 0.01
6	Total Carbohydrate content	50.13% ± 0.26

* data are mean of duplicate determinations ± standard deviation

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.

Table 2: Elemental Analysis of *Zanthoxylum zanthoxyloides* Stem bark

S/No	Element	Mean Value ± SD (ppm)
1	Sodium	12946.7760 ± 0.0039
2	Potassium	1012.9924 ± 0.0002
3	Calcium	6055.5591 ± 0.0003
4	Iron	1093.8837 ± 0.0008
5	Magnesium	1478.5064 ± 0.0004
6	Zinc	216.8516 ± 0.0007

*data are mean of five determinations ± standard deviation

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 (a) Ferric chloride test	 +
Phenolics (a) Ferric chloride test	 +
Saponins (a) Foam test	 -
Flavonoids (a) Alkaline reagent test	 -
Anthocyanins (a) Hydrochloric acid/Ammonia test	-
Anthraquinones (a) Borntrager's test	 +
Alkaloids (a) Wagner's test	 +
Terpenoids (a) Salkowski's test	 +
Reducing Sugars (a) Fehling's test	 +
Carbohydrates (a) Molisch's test	 +
Phytosterols (a) Salkowski test (b) Liebermann-Burchard test	 + +
Cardiac Glycosides (a) Keller-Kiliani test	+
Phlobatannins (a) Hydrochloride 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.

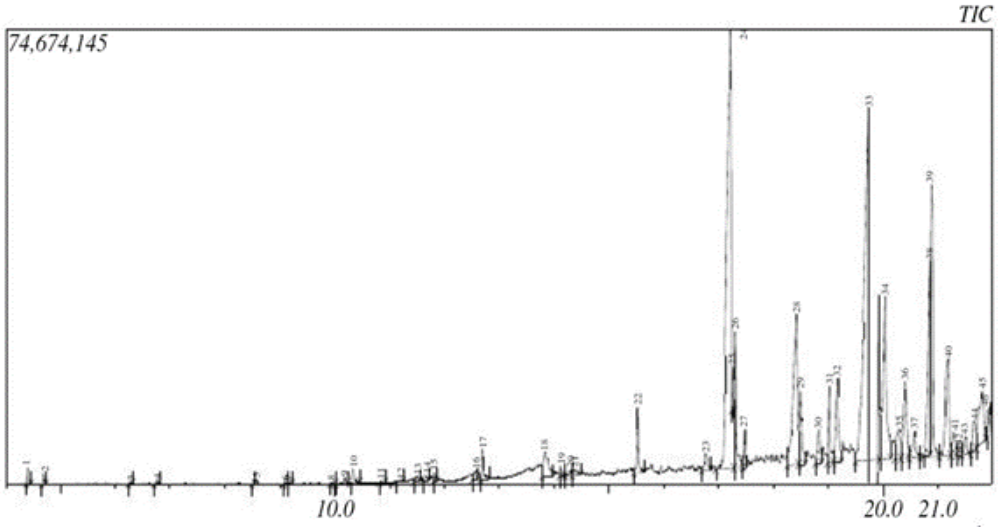

Fig 1: GC-MS total ion chromatogram (TIC) of 70% ethanolic *Zanthoxylum zanthoxyloides* extract

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-methylethyl)-
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, decahydro-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-, (16.alpha.)-
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	Stigmastan-3-ol, 5-chloro-, acetate, (3.beta.,5.alpha.)-
41	21.300	0.74	Ergost-5-en-3-ol, (3.beta.)-

42	21.403	0.27	1,4-Methanoazulen-9-ol, decahydro-1,5,5,8a-tetramethyl-, [1R-(1.alpha.,3a.beta.,4.alpha.,8a.beta.,9S*)]-
43	21.511	0.62	9,12-Octadecadienoic acid, 2-phenyl-1,3-dioxan-5-yl ester, cis-
44	21.670	0.96	10,12-Tricosadiynoic acid
45	21.824	2.88	Ergost-5-en-3-ol, (3.beta.)-
46	21.942	0.29	14-Oxatricyclo[9..2.1.0(1,10)]tetradecane, 2,6

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 (the phytosterol, 5a,5b,7a,10,10,13b-hexamethyl-3-propan-2-yl-1,2,4,5,6,7,8,9,11,11a,13,13a-dodecahydrocyclopenta[a]chrysene) with a peak area percentage of 22.46%, 7.05%, 14.80%, 6.66% and 11.50% respectively.

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 viscous translucent 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 carbohydrates 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. With the exception of (Z)-hexadec-9-enal which is majorly used in pesticides as an attractant [34] and ((E)-4-(1,3-benzodioxol-5-yl)but-3-en-2-one which has been demonstrated to possess in vitro cell growth inhibitory activity against K562 human chronic myelogenous leukemia cell line [35], there is a dearth of information on the chemical, biological and medicinal properties of the top 5 peaks. In regards to occurrence, 8,8-dimethyl-2,3,4,4a,5,6,7,8a-octahydro-1H-naphthalene and 5a,5b,7a,10,10,13b-hexamethyl-3-propan-2-yl-1,2,4,5,6,7,8,9,11,11a,13,13a-dodecahydrocyclopenta[a]chrysene has been detected in other plant materials analysed with GC-MS [36, 37, 38].

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-dimethoxychromen-2-one at peak 27, which agree with literature in terms of phytochemical constituents [4, 24]. Notably undetected are the prominent anti-sickling divanilloylquinic 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.

Funding: The authors acknowledge the Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria for the infrastructural and financial support of this study. No other funding grants were obtained elsewhere.

Author's Contributions: Abisola Ave-maria Olushola-Siedoks, Ukachi Ezinwa Igbo and Chima Cartney Igwe contributed to the study conception and design. Material preparation, data collection and analysis were performed by Abisola Ave-maria Olushola-Siedoks, Ukachi Ezinwa Igbo, Godfrey Asieba. Ibilola Adiza Damola and Chima Cartney Igwe. The first draft of the manuscript was written by Abisola Ave-maria Olushola-Siedoks and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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