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Research Article

Antiinflammatory Lupeol and Antidiabetic Compound Coexist in Ethyl Acetate and n-Hexane Extracts from Stem Bark of *Anogeissus leiocarpus* (African Birch Tree): The Therapeutic Advantages

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Abstract

Objectives: *Anogeissus leiocarpus* stem bark is endowed with numerous antidiabetic properties, such as its prognostic value, treatment of organic damages, dyslipidaemia and enhancement of haematopoiesis. In addition to its antioxidant activity on oxidative stress, it accelerated wound healing and has a wide range of safety value. From the purification of its compounds to the points of crystallization it became necessary to analyse the structures for an insight into its mechanisms of actions.

Design: Ethanolic extracts were partitioned with ethyl acetate, onto column chromatography packed with silica gel (70-230 mesh) mixed with 95% n-hexane and 5% ethyl acetate; concentrated fractions were loaded onto silica gel-coated thin layer chromatographic plate. Different compounds were observed under UV-light fluorescence and sprayed with sulfuric acid. Similar fractions were pooled, purified to crystals for Nuclear Magnetic Resonance structural analysis.

Results: Nuclear Magnetic Resonance revealed fraction A is Lupeol, a pentacyclic triterpene anti-inflammatory compound.

Conclusion: Lupeol in the crude ethanolic extract is significant in inflammatory pain of diabetes.

Keywords: Lupeol, Anti-inflammatory, *Anogeissus leiocarpus*, Purified extract, inflammatory pain, Diabetes mellitus.

INTRODUCTION

Inflammation, the vascular and cellular response in a live tissue, to injury is basically, aptly and simply defined and identified by its five cardinal manifestations of (i) hyperaemia (redness); (ii) warmth (heat), both of which result from the beneficial inflow of blood to the inflamed site; (iii) swelling, emanating from the vascular endothelium as a result of hypoxia induced by stasis of blood due to cellular and platelets aggregations at the inflammatory site; (iv) pain, which invariably leads to (v) loss of function of the affected body part(s). Irrespective of the aetiology of the injury to the live tissue, a cut, torn or burnt area or infected with microorganisms (bacteria, fungi, protozoa,

metazoan, viruses and their accompanying toxins), chemical poisons, mechanical or thermal and immune processes, increased vascular permeability and inflammatory oedema (exudates) develop, with production of inflammatory biomarkers, such as the significantly higher cytokine associated acute phase reaction and highly sensitivity C-reactive protein (hs-CRP) variously reported in type 2 diabetes mellitus (T2DM) afflicted people^{1,2,3,4,5,6}. Similar increases occurred in alloxan-induced type 1 diabetic (T1DM) dogs⁷. The increases of these inflammatory cytokine associated biomarkers were ascribed to the chronic hyperglycaemia-induced injuries, with multiple risk factors of microvascular and macrovascular damages of

T2DM and their accompanying life-threatening complications, such as cardiovascular disease, nephropathy, neuropathy and retinopathy with blindness⁸.

Indeed, the beneficial inflow of blood into the microvascular/macrovacular damaged site could provide additional increases in inflammatory cytokines, since red blood cells were reported as dynamic reservoirs of cytokines⁹.

Diabetes mellitus (DM) has remained a non-communicable, devastating heterogeneous metabolic disease afflicting over 425 million people worldwide, with enormous economic costs to governments^{10,11,12} as a result of metabolic disorders of carbohydrate, protein and lipid, hence chronic hyperglycaemia either from lack or impairment of insulin secretion, type 1 diabetes mellitus (T1DM), defective insulin action, type 2 diabetes mellitus (T2DM) or both¹³. Worldwide increases in T2DM with complications of nephropathy and retinopathy placed DM as a leading cause of death, come 2030 by WHO, with a frightening projection for 2035¹², and an unresolved health challenge for the 21st century¹⁴. The worrisome social menace from the risk factor induced by the chronic hyperglycaemia of diabetes on the mortality of COVID-19, obesity, which is a predisposing factor of diabetes and in particular, the delayed or non-healing wounds, diabetic ulcers, which led to amputations of limbs at some instance had been summarized^{7,15,16,17,18}.

Sulfonyl urea and meglitinides biguanides and thiazolidinediones^{8,19} were developed to treat DM; but were ineffective for the hyperlipidaemia associated with DM, were toxic and resistance was experienced²⁰ and costly for the developing world^{21,22}.

In developing countries with reduced economic resources, medicinal plants and phytoconstituents are currently being used, traditionally, to manage diabetes mellitus.

Recently, ethanolic extracts of *A. leiocarpus* treated DM, organic damages and restored deranged electrolytes and acid-base balance in alloxan-induced diabetic dogs, and was novel as it prevented a reversal to hyperglycaemic state¹⁵. The efficacy of *A. leiocarpus* received further credence with its antidiabetic and antioxidant properties in alloxan-induced diabetic Wistar rats¹⁶ and attenuation of dyslipidaemia in alloxan-induced diabetic dogs¹⁷.

Anogeissus leiocarpus, more importantly, exhibited a modulating effect on erythrocyte surface and serum free sialic acids, which very effectively demonstrated that elevated serum sialic acids served as a potent biomarker, predictive and prognostic of alloxan-induced diabetic dog⁷. In addition, ethanolic extract of *A. leiocarpus* accelerated wound-healing in alloxan-induced diabetic dogs¹⁸.

These antidiabetic, antidyslipidaemic, antioxidant, sialoglycoconjugate, haemopoietic and wound-healing properties confer on *A. leiocarpus* a target for further technological studies, one of which is investigating the structure of its purified product, specific for an elucidation on its mechanism of action, including its environmental soil fertility requirements.

From maximum purification, to the point of crystallization of *Anogeissus leiocarpus* stem bark extracts, Nuclear Magnetic Resonance (NMR) was applied for structural determination, for an elucidation on specific mechanisms of actions.

MATERIALS AND METHODS

Plant collections, meteorological analysis and fertility assessment of tree grown soils on *A. leiocarpus* were performed and adequately described earlier²³.

The plant was authenticated in the herbarium of the Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, Zaria, with a Voucher sample number ABU01756. The harvested stem bark was processed as described earlier²³.

Ethanol Extraction of *A. leiocarpus* stem bark

The pulverized sample was subjected to a cold maceration method using 95% v/v ethanol to obtain its ethanolic extracts as described earlier^{7,15,16,17,18}.

Qualitative and Quantitative phytochemical screening of Ethanolic Extract of *A. leiocarpus*

Standard procedures and protocols were applied to detect the different phytochemical constituents in the ethanolic extract as described earlier^{7,15,16,17,18}.

Partition of Ethanolic Extracts into Fractions.

The ethanolic extracts were partitioned with ethyl acetate²⁴ as detailed adequately earlier²³. The ethyl acetate fraction was concentrated with rotary evaporator while the aqueous fraction was concentrated on water bath before storage at 20°C.

Column Chromatography:

The ethyl acetate fraction was subjected to column chromatography packed with silica gel (70-230 mesh), 500gm of which was mixed with 95% n-hexane and 5% ethyl acetate; bubbles were removed and poured into a glass column²⁵. For each eluent system, the fractions were collected in vials and concentrated to dryness by evaporation at 40°C²⁵.

Thin layer chromatography [TLC]

A spot of each concentrated fraction collected from the column chromatography was carefully applied with capillary tube on a thin layer chromatographic plate coated with silica and left to dry, thereafter, dipped into a suitable solvent as detailed earlier²³.

The positions of different compounds were observed by fluorescence under UV-light and sprayed with sulfuric acid for the specific compounds and fractions with similar TLC profiles were pooled²⁶.

Nuclear Magnetic Resonance (NMR) and Determination of Structure of Purified Compound.

After column chromatography and TLC processes, to the point of crystallization, the purified compounds were presented to NMR (Bruker Avance III, Spectrometer frequency 400 MHz; solvents DMSO-d₆, CDCl₃, Acetone-d₆. Institute of Chemistry, University of Glasgow UK.) for determination of compound structures and analyses.

RESULTS:

Sample Dryness and Soil Texture

Samples drying process and soil fertility were normal²³.

Qualitative and Quantitative Phytochemical Constituents

The phytochemical constituents were identical²³ as previously reported¹⁵. Ethanolic extraction from the stem bark yielded 451.58gm²³.

Ethyl Acetate Fractions and Yields (Weights/Percentages) from Column Chromatography and detailed TLC

An assessment of the ethyl acetate fractions and the total yields (weights and percentages), against the purified and the ethanolic extract preparatory to NMR analyses are presented in Table 1:

Table: Ethyl Acetate Fraction and Yields (Weights/percentages) from Column Chromatography

S/No Or letter identity	Fractions	Weights (gm)	Percentage of Purified Extracts	Percentage of Ethanolic Extract
1	Interface	5.10	71.83	1.13
A	28 – 37	0.70	9.86	0.16
B	38 – 58	0.60	8.45	0.13
C	59 – 80	0.70	9.86	0.16
Total		7.10	100.0	1.58

Calculations

Percentage of Purified Extracts

$$= \frac{X \text{ (gm)}}{Y \text{ (gm)}} \times 100$$

Where X is the weight (yield) of the fraction and Y is the weight (total yields) of all the fractions

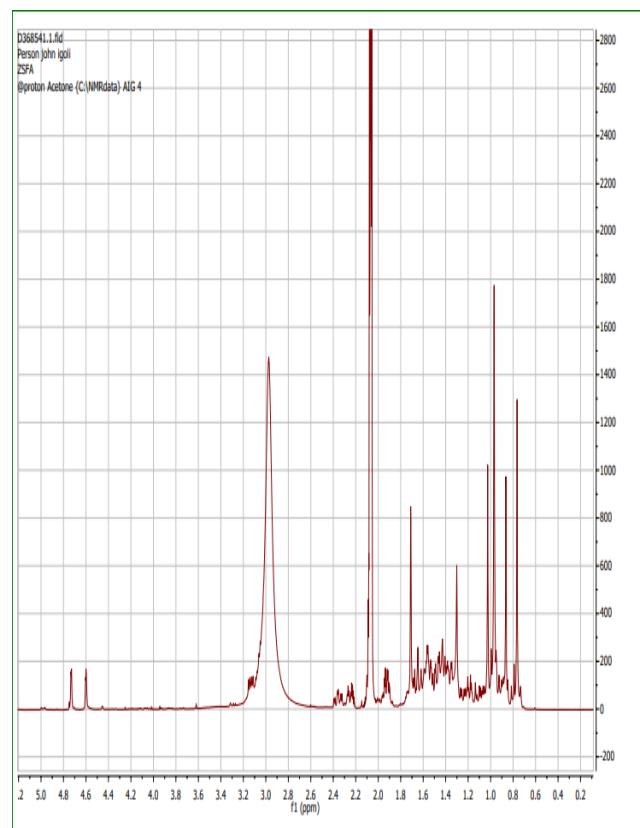
Percentage of Ethanolic Extracts

$$= \frac{X \text{ (gm)}}{Z \text{ (gm)}} \times 100$$

Where X is the weight (yield) of the fraction and Z is the weight (yield) of ethanolic extracts from stem bark.

NMR Analysis

The compound ZSFA is Lupeol a pentacyclic triterpene (Figures 1 and 2).



ZSFA 1D-Proton NMR

Figure 1: Spectra of Fraction A from NMR

ZSFA = Zaria Sample, Fraction A

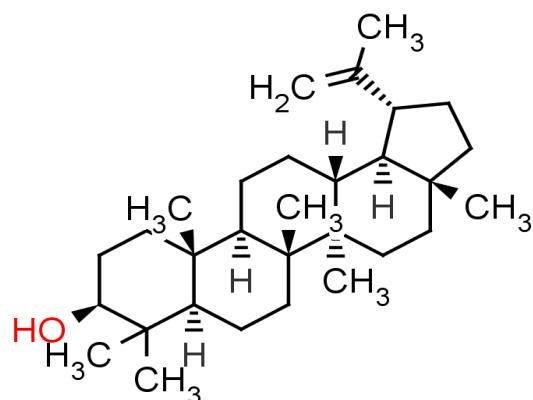


Figure 2: Structure of Lupeol.

Lupeol, a pentacyclic triterpene

DISCUSSION

The overall value of the soil fertility assessment met the recommended standards for non-deficient soils²⁷ and the phytochemical screenings of the ethanolic extracts of *A. leiocarpus* stem bark²³ agree with earlier reports¹⁵. Fraction A which was 0.70gm from the 451.58gm of the ethanolic extract of the stem bark, partitioned and totally purified with ethyl acetate, n-hexane through column chromatography and TLC, appears to be a small percentage, 9.86%, of the total purified fractions and in addition, a very small percentage, 0.16% of the ethanolic extract (Table 1).

The analysis of NMR spectra and the resultant structure in the present study, confirmed fraction A is lupeol, a pentacyclic pharmacologically active triterpenoid²⁹.

It is being speculated that only a "tincture" of lupeol is required to treat the inflammatory aspect of DM. This speculation is derived from the successes obtained from crude ethanolic extract of *A. leiocarpus* stem bark in alloxan-induced DM in dogs and rats^{7,15,16,17,18}.

Crystallization using ethyl acetate and n-hexane from the sub-fractions and interface during the process of final purifications through column chromatography and supported with thin-layer chromatography is an indication of purity of each of the four different compounds²³.

The global threat of T1DM and T2DM to the human population worldwide and their companion/pet animals, as adequately described earlier^{10,11,12,15,28}, strongly supports the research for a non-conventional drug for T1DM and T2DM, the risk factors and the unquantifiable accompanying social menace.

This is the first report that lupeol exists in *A. leiocarpus* and the latter joins other plant sources of lupeol^{29,30}. The finding of lupeol in the purified extract of *A. leiocarpus* stem bark in the

present study, is a boost to the claims of its efficacy in the treatment of the complications of diabetes mellitus, such as improvements of numerous landmarks of inflammatory response exhibited in the accelerated healing of surgically-induced diabetic wound¹⁸ and the hepato-renal damages of alloxan-induced diabetic dogs¹⁵ and rats³¹ since lupeol was reported as hepato-protective^{29,30}.

Therefore, it is being suggested and indeed conceivable that *A. leiocarpus* with its constituent lupeol can effectively treat T2DM with its accompanying organic damages as exemplified by earlier studies with crude ethanolic extracts. This is premised from its numerous advantages, such as the anti-inflammatory activity of lupeol^{29,30}, as inflammation accompanies diabetes mellitus, confirmed by the significantly higher cytokine associated acute phase reaction and a maker of inflammation, High sensitivity C-reactive Protein (hs-CRP) in T2DM diabetic people^{1,2,3,4,5,6}.

The finding of lupeol, an anti-inflammatory compound coexisting with anti-diabetic compounds, in the antidiabetic *A. leiocarpus*, in the current study, is another advantage and very significant under the context of the inflammatory pain associated with diabetes mellitus, including such cases as diabetic neuropathic pain in rats³² and diabetic human patients^{33,34}. In addition, N-acetyl neuraminic acid (sialic acid) attenuated high fat diet-induced inflammation and oxidative stress in rats³⁵. Elevated serum sialic acid, as a potent biomarker of alloxan-induced diabetes mellitus in dogs adequately described and emphasized the manifestations of sialic acids in inflammatory diabetic pain⁷.

More advantages can be inferred from report that some triterpenes exist as glycosides, saponins³⁶ and these saponins, along with terpenoids identified as phytochemical constituents in the ethanolic extracts of *A. leiocarpus* stem bark^{15,23} had been associated with anti-diabetic activities^{37,38,39,40}.

The total yield of lupeol (fraction A) at 9.86% of the ethanolic extracts of *A. leiocarpus* stem bark, in the present study was very insufficient for a bioassay investigation for its anti-diabetic activity and was equally insufficient for a study of its toxicity implication²³. However, toxicity levels of lupeol were reported to be very low as oral administration of lupeol at a dose of 2gm/kg. body weight produced no adverse effects in rats and mice, with no mortality after a 96-hour observation⁴¹. In a related development, the interface fraction of the purified extracts of *A. leiocarpus* stem bark, at a yield of 71.83% of the ethanolic extracts, orally administered to Wistar rats at a limit dose of 2000mg/kg. body weight. produced no adverse effects and no mortality over a 24-hour and a 14-day observation²³.

It is being suggested that lupeol in the crude ethanolic extracts of *A. leiocarpus* stem bark, as evidenced by the latter's maximum purification²³ might have contributed appropriately and significantly in the hepato-renal and dyslipidaemic healings^{15,17} and the antioxidant activities¹⁶, in addition to the accelerated wound-healing in diabetic dogs¹⁸. It is highly conceivable that all these fractions with lupeol, are acting synergistically.

This suggestion is advanced and received maximum supports from the reports that lupeol scavenged peroxyl radicals by bolstering the levels of antioxidants and antioxidant enzyme system in an attempt to exhibit its hepatoprotective activity⁴² in addition to decreasing lipid peroxidation levels and increasing enzymatic and non-enzymatic antioxidants, thus ameliorating the lipidemic-oxidative abnormalities in early hypercholesterolemic atherosclerosis⁴³. Indeed, lupeol influenced the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) in animals, thereby enhancing antioxidant protection⁴⁴.

CONCLUSION

In conclusion, lupeol appears to act synergistically with other fractions in *A. leiocarpus* stem bark in treating DM and its complications. The yield of lupeol in the present study is not enormous but appears therapeutically sufficient and advantageous in managing DM; this can be addressed during industrial production of a synthetic analogue, in combination with the antidiabetic, erythropoietic and thrombopoietic activities in the interface²³ and the other fractions in the purified extracts. The double bond, hydroxyl, hydrogen and methyl groups of lupeol can be of advantage in the industrial synthesis. The co-existence of lupeol with the antidiabetic compounds can allow for economic production of a synthetic analogue for the treatment of diabetes mellitus.

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Authors Contributions

Esievo, KAN: Conceptualisation; Supervision; Investigation; Writing, Editing,

Balogun, EO: Supervision; Investigation; Writing; Editing.

Esievo, KO: Investigation; Soil composition; Ethanolic Extraction and Purification, Writing.

Esievo, LO: Investigation; Ethanolic Extraction and Purification; Writing.

Esievo, EM: Investigation; Ethanolic Extraction and Purification; Writing.

Sani, D; Supervision; Investigation; Bioassay; Writing, Editing.

Wassagwa, J: Investigation; Purification; Writing; Editing.

Uyovbisere, EO: Supervision, Investigation; Soil composition; Weather conditions; Writing, Editing.

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Conflict of Interests

Authors declare no conflict of interests

Ethical Approval

No experimental animal involvement

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