Enzyme inhibition, Antioxidant and Insecticidal activities of flavonoids and fixed oil from *Albizia zygia* (J. F. Macbr)

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Abstract: *Albizia zygia* (Leguminosae) is a gum producing tree and wide spread in tropical Africa. It has secondary metabolites of medicinal importance. This study investigates the enzyme (urease and α-glucosidase) inhibition, antioxidant and insecticidal activities of the crude extract and isolates. Terpenoids (lupeol and Betulin) and flavonoids (4',7-dihydroxyflavanone, 3,4',7-Trihydroxyflavone, 4',5,7-trihydroxy-3'-methoxyflavone and 5,7,4'-trihydroxy-3',5'-dimethoxyflavone) were isolated from *A. zygia* and were shown to possess significant enzyme inhibition (urease and α-glucosidase) and antioxidant activities, when activities were compared with thiourea, 1-deoxynojiromycin hydrochloride (DNJ) and butylated hydroxylanisole used as standards in the three assays. 4',7-dihydroxyflavanone (21.5 ±0.32), 5,7,4'-trihydroxy-3',5'-dimethoxyflavone (31.2 ± 0.10) and the fixed oil (32.9 ± 0.56) were the most active in the antioxidant assay. The fixed oil obtained from *A. zygia* was dominated with hexahydrofarnesyl acetone, palmitic acid, 2-hydroxyethyl ester, oleic acid, and oleic acid, methyl ester. This study therefore justifies the use of *A. zygia* in the treatment of tumour and oxidation related diseases.
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Introduction

Albizia zygia (DC.) J.F. Macbr. is a deciduous tree belonging to the family Leguminosae. It is a gum producing tree and wide spread in tropical Africa from the east, to the west, north and south in countries like Kenya, Angola, Tanzania, Nigeria, Senegal etc. Its local names are nongo/ mkenge (Swahili), nyie avu (Igbo), okuro (Ghana), ayin rela (Yoruba) and West African Albizia (English) [1,2]. Its young leaves are used as vegetable in soups, the leaves and shoot are used as feed for livestock. It produces timber used in construction and gum from its bark is used as stabilizer in ice-cream. The tree is used as shade for crops like cocoa and coffee in agroforestry [3,4]. Decoction of the bark can be used to treat fever and sterility in women, also used as laxative, to aid digestion (stomachic), as aphrodisiac, as anthelminthic and antidote. The bark when pounded can be applied to wounds, toothaches, yaws and sore while the bark sap can be used to treat pink eye. Leaf decoction can be used to treat fever and diarrhoea. It serves as expectorant and cough medicine when the root is ground. In modern day medicine, the gum obtained from the bark is used in coating drugs [5,6]. The genus *Albizia* consists of over 150 species. Phytochemical screening, antioxidant, antimicrobial and pharmacognostic activity of methanol and hexane extract of *A. zygia* stem revealed the presence of alkaloid, saponin glycoside, steroids, reducing sugar, resin, and flavonoid and cardio active glycoside [7-8]. The essential oil according to Oloyede and Ogunlade [9] was dominated with linalool and thymol and possessed free radical scavenging activity. Phytochemical studies of *A. zygia*, resulted in the isolation of lupen-20(30)-en-3β-ol and its glycoside, stigmast-5-en-3β-ol, and 5α-stigmasta-7,22-dien-3β-ol albiziaprenol and phytol [2,5]. Albiziasaponins A, B, C, macrocyclic alkaloids budmunchiamines L4, L5, and L6, albizinin, kaempferol, quercetin, eucaediflavone, albiproflavone, prenylated flavonoids such as sophoflavescenol and kurarinone have been reported from *A. lebbeck*. *A. julibrissin, A. procera, A. amara* and *A. adianthifolia* [5, 10-14]. Abdalla and Laatsch [2], reported the antimalarial activity of three flavonoids viz 4',7-dihydroxyflavanone; 3',4',7-trihydroxyflavone and 3',4',7-trihydroxy-3-methoxyflavone (3-O-methylfisetin) isolated from *A. zygia* stem using *Plasmodium falciparum*, only one 3',4',7-trihydroxyflavone showed significant antimal arial activity. Prior to these, lupen-20(30)-en-3β-ol and its glycoside, 14α-stigmast-5-en-3β-ol, and 5β-stigmasta-7, 22-dien-3β-ol, albiziaprenol and phytol were isolated from *A. zygia* stem [2].

In continuation of our research activities on *A. zygia* [8-9], we embark on isolation of secondary metabolites and investigation of enzyme inhibition,

free radical scavenging and insecticidal activities of crude extracts and isolates from this plant. This is because scientists in recent times focus attention on the discovery of enzyme inhibitors and antioxidants from plants. Enzyme inhibition studies aim to correct pathological condition like gastric tract syndromes and urinary tract infection [15,16]. Free radicals on the other hand have been claimed to cause cardiovascular diseases, like cancer, brain dysfunction, and aging process in humans. Antioxidants have the ability to counter the effect of excess free radicals thereby stopping chain reactions [17] which could lead to cancer, Parkinson's disease etc. This plant was also screened for insecticidal activity using maize weevil (*Sitophilus zeamais*) at lethal doses 50% and 100% concentration since the plant was reported to possess this activity. Hence, this present study is aimed at determining the enzyme inhibition, antioxidant and insecticidal activities of *Albizia zygia*.

Experimental

Materials and Method

Plant Material

Fresh stem bark of *Albizia zygia* was collected from The Botanical Gardens, University of Ibadan, Nigeria in January 2016 and identified at the Botany Herbarium. These were air-dried under a shade for a period of three weeks and ground into smaller pieces at the Wood Extraction Laboratory, Department of Chemistry, University of Ibadan, Nigeria.

Chemical and Reagents

The following BDH chemicals and reagents were used: chloroform, dichloromethane, ethyl acetate, n-hexane, methanol, hydrochloric acid, ammonia solution, conc. tetraoxosulphate (VI) acid, conc. hydrochloric acid, ammonia solution, sodium potassium tartarate, potassium chloride, glacial acetic acid. General purpose chemicals obtained were distilled prior to use. Dimethylsulphoxide (M & B, England), P-nitro-phenyl-α-D-glucopyranoside (p-NPG), sodium carbonate, sodium dihydrogen phosphate and di-sodium hydrogen phosphate, sodium nitroprusside, urease (Jack bean), α-glucosidase (*Saccharomyces cerevisiae*), thiourea, 1-Deoxynojiromycin hydrochloride, di-nitro salicylic acid (DNS) and silica gel 30 - 260 microns and 2,2-diphenyl-1-picrylhydrazyl (DPPH), and butylatedhydroxylanisole (BHA) were obtained from Sigma Chemical Co (St Louis, MO). Ultra-pure water (HPLC grade, Duksan, Korea) was used throughout the experiments. Potassium phosphate buffer (100 mM), pH=7.4, was prepared in distilled water. The absorbance spectra of the solutions were obtained employing Synergy H1 Hybrid multi-mode microplate reader.

Isolation of compounds from ethyl acetate fraction of stem bark of *A. zygia*.

The an air-dried sample of *A. zygia* (1.0 kg) was powdered and extracted (2×) with 5L MeOH using cold extraction method. The extracts were pooled and evaporated under reduced pressure to give a dark brown residue (102.3 g). This extract was partitioned between hexane, ethyl acetate and water, Gradient elution column chromatography of ethyl acetate fraction (50 g) of *A. zygia*. gave 487 fractions (20 mL each) which was pooled together to give 28 fractions based on retardation factor (R_f) obtained from Thin Layer Chromatography (TLC). Silica gel (230–400 mm mesh size) was used as adsorbent. Fractions were collected accordingly Hexane 100% (1-44), hexane: ethyl acetate 90:10 (45-52), 85:15 (53-67), 80:20 (68-71), 75:25 (72-75), 70:30 (76-78), 60:40 (79-82), 50:50 (79-82), 40:60 (85-87), 30:70 (88-157), 25:75 (158-164), 20:80 (165-179), 15:75 (180-186), 10:90 (187-192), 5:95 (193-195) ethyl acetate 100% (196-199), ethyl acetate: methanol 95:5 (200-206), 90:10 (207-268), 85:15 (269-302), 80:20 (303-311), 75:25 (312-322), 70:30 (333-363), 65:35 (364-371), 60:40 (372-378), 50:50 (379-387), 45:55 (388-397), 40:60 (398-430), 35:65 (431-438), 30:70 to methanol 100% (440-487). AZ1 was obtained as colourless crystalline substance from hexane: ethyl acetate 90-80%, while AZ2 also colourless needle like crystals was obtained from fractions 70-60% hexane: ethyl acetate. Fraction at ethyl acetate in hexane 40-50%, 55-70%, 75-80% and 85- 100% were labelled AZ3-AZ6 respectively and are yellow powder. They were washed with ethanol and recrystallized with DCM. Fractions obtained as yellow oil in the ethyl acetate: methanol fractions were pooled together after Thin layer chromatography (TLC) showed similar profile. The compound was labeled AZ7.

General Experimental Procedures and Analysis

Silica gel (230–400 mesh) was used for column chromatography (CC) using gradient elution technique. Thin layer chromatographic analysis of the samples was carried out at room temperature using Precoated TLC Aluminium sheets (Silica gel 60 F_{254} (20 cm × 20 cm, 0.2 mm thick; Merck, Germany), Buchi Rotary Evaporator fitted with Vacuum pump V-700 and B-490 heating bath was used to concentrate samples and fractions. The retention factor (Rf) was calculated in each case. TLC plates were visualized under UV at 254 and 366 nm and by spraying with ceric sulfate reagent solution under heating or vanillin sulphuric acid. The compounds were further characterized by spectroscopic analysis, UV-Visible, Infra-red and Nuclear magnetic Resonance (NMR). Ultra violet (UV)/Visible absorption spectra of 0.01% w/v of the samples were obtained in methanol on Evoltion 300 Thermo Scientific UV – visible

spectrophotometer. The Infra red (IR) spectra were recorded in KBr disc on FT-IR-8900 Fourier Transform IR Spectrophotometer Shimadzu IR spectrometer in the range 4000-400 cm^{-1}. ^{1}H NMR was recorded on AVANCE AV-400 spectrometer operating at 400 MHz for ^{1}H. The chemical shift values (δ) are reported in ppm. Data from the spectrometry and comparison with literature confirmed the structures of the compounds. This oil mixture was subjected to Gas chromatography and Gas Chromatography-Mass spectrometry (GC and GC-MS).

Enzyme Inhibition Assays

Determination of urease activity

Urea (850 µL), the sample (0 -100 µL), phosphate buffer (100 mM, pH 7.4) were mixed, made up to volume of 985 µL and incubated at 30 °C. The ammonia concentration in the enzymatic reaction was determined after 60 minutes at 37 °C when 15 µL of urease enzyme, 500 µL of solution A (0.5 g phenol and 2.5 mg of sodium nitroprusside in 50 mL of distilled water) and 500 µL of solution B (250 mg sodium hydroxide and 820 µL of sodium hypochlorite (5%) in 50 mL of distilled water) were added together. Activity of uninhibited urease was used as control activity of 100%. Modified Berthelot spectrophotometric method was used to evaluate initial urease inhibitory activity of samples and standard at 1 mg/mL. Thiourea was used as the standard. Increase in absorbance at 630 nm was measured after 50 min, using a microplate reader (Spectramax plus 384 Molecular Device, USA). All assays were performed in triplicate in a final volume of 200 µL at pH 8.2 (0.01 M $K_2HPO_4.3H_2O$, 1 mM EDTA and 0.01 M $LiCl_2$). The results (change in absorbance per min) were processed by using SoftMax Pro software (Molecular Device, USA). IC_{50} (Percentage Inhibitory activity and the concentration that induces inhibition halfway between the minimum and maximum response of each compound) was also calculated [15, 18].

α-Glucosidase inhibition assay

α-Glucosidase (Sigma, type 111, from yeast), 0.1 units/mL. *p*-Nitrophenyl- α-D-glucopyranoside at 6 mmol/L was used as substrate and dissolved in buffer A prepared by mixing 0.1 mol/L potassium phosphate and 3.2 mmol/L $MgCl_2$, pH 6.8. Substrate (200 µL), 102 µL Buffer B (prepared by adding 0.5 mmol/L potassium phosphate and 16 mmol/L $MgCl_2$, pH 6.8.), 120 µL sample solution (0.6 mg/mL in dimethylsulfoxide) at various concentration (25-500 µg), 282 µL water were mixed. Enzyme solution (200 µL) was added after the mixture was pre incubated in water bath at 37 °C for 5 minutes in a 96 well plate, allowed to stay at 37 °C for 30 minutes for enzyme reaction to take place. 1.2 ml of 0.4 mol/L glycine buffer (pH=10.4) was added to terminate the reaction. Absorbance of the released p-nitrophenol was measured at 410 nm using Multiplate Reader. 1-Deoxynojiromycin hydrochloride (DNJ) was used as standard. and negative control was mixture of reagents without test compound. All analyses were carried out in triplicate. Percentage Inhibitory activity and the concentration that induces inhibition halfway between the minimum and maximum response of each compound (relative IC_{50}) was determined [19-20].

Antioxidant activity

Antioxidant activity was measured by using the 2,2-diphenyl-1-picryl-hydrazil (DPPH) radical method. DPPH solution (0.3 mM) was prepared in ethanol. Five microlitres of each sample of different concentration (62.5 µg - 500 µg) was mixed with 95 µl of the solution. The mixture was dispersed in 96 well plate and incubated at 37° C for 30 min. The absorbance at 517 nm was measured by microtitre plate reader (Spectramax plus 384 Molecular Device, USA) and percent antioxidant activity was determined by comparison with the methanol treated control. BHA was used as standard [15].

Insecticidal Activity

Insect/Sample Preparation

Sitophilus zeamais (Maize weevil) was cultured in a dark incubator for 30 days. Maize seeds (10.0 g) were weighed into separate containers. The methanol extract (2.0 mg) of *A. zygia* was dissolved in 2.0 mL of methanol to make the stock solution of 100%. Half concentration (50%) was prepared from this. 2.0 mL of the prepared concentrations of extract was added to the weighed seeds and the solvent was left to evaporate at room temperature. Positive control (Control I) was prepared by adding 2.0 mL of methanol to the seeds while negative control (control II) was 10.0 g of the maize seed without the solvent [21,22].

Procedure for Insecticidal Activity

The method of Jbilou *et al* [21] and Coelho, *et al.* [23] were used but with little modification. The immobilized insects (refrigerated for one hour before use) were added to the prepared seeds. Male and female insects (three each) were added to each of the container, containing the prepared seeds. The plastic containers were covered with a mesh net to prevent suffocation and escape of the insects. The insects were observed at intervals of 3, 6, 12 and 24 hours for three days. Numbers of dead insects were recorded to determine mortality at different time intervals. The test was performed in triplicate. Lethal dose at 50% and 100% concentration was determined [22].

Statistical Analysis: *Data processing & IC_{50} Determination*

Absorbance measurements are expressed as mean absorbance ± SD of triplicate analysis. All

experimental data were analyzed statistically by one-way analysis of variance (ANOVA), processed on SPSS software package (version 15.0; SPSS, Inc., Chicago, Illinois, USA) for more than two means. The following equation was employed; Inhibitory activity (%) = (1 − As/Ac) ×100 where, As is the absorbance in the presence of test substance or sample and Ac is the absorbance of control from which $IC s_0$ was calculated. The IC_{50} value was obtained from curves obtained by linear regression using GraphPad Prism 5 version 5.01 (Graph pad software, Inc., La Jolla, CA, USA.) software.

Results And Discussion

Lupeol (AZ1), colourless crystalline solid. Mpt: 215-216°C, UV [EtOH] nm (log ε): 201.52 (0.3120). IR (KBr) V_{max} cm^{-1}: 3382.6 (O-H), 2986.2, 2920.3, 2850.2 (C-Hstr); ^1H NMR (400 MHz; $CDCl_3$): δ 5.10 (2H, s, CH_2, ethylene), 4.79 (1H, s, O-H), 2.16 (1H, 2xCH, cyclopentane), 1.55 - 1.67 (2H, $2xCH_2$, cyclopentane), 1.49 - 1.76 (2H, $8xCH_2$, cyclohexane), 1.38-1.42 (1H, 4x CH, cyclohexane), 1.82 (3H, s, CH_3, methyl), 0.99- 1.00 (3H, 6x CH_3, methyl), Molecular formulae $C_{30}H_{50}O$.

Betulin (AZ2), colourless needle like crystals. Mpt: 256-257 °C UV [EtOH] nm (log ε): 202.11 (0.0820). IR (KBr) V_{max} cm^{-1}: 3384.3 (O-H) 2986.2, 2923.9, 2851.9 (C-Hstr); ^1H NMR (400 MHz; $CDCl_3$): δ 5.10 (2H, s, CH_2, ethylene), 4.82 (1H, s, O-H), 4.79 (1H, s, O-H), 2.16 (1H, 2xCH, cyclopentane), 1.82 (2H, s, CH_2, methylene), 1.55 - 1.67 (2H, $2xCH_2$, cyclopentane), 1.49 - 1.76 (2H, $8xCH_2$, cyclohexane), 1.38-1.42 (1H, 4x CH, cyclohexane), 0.99- 1.00 (3H, 6x CH_3, methyl), Molecular formulae $C_{30}H_{50}O_2$.

4',7-dihydroxyflavanone (AZ3), yellow powder, Mpt: 310-311°C, UV [EtOH] nm (log ε): 249.89 (1.0020), 285 (0.0098), 349.89 (1.0020). IR (KBr) V_{max} cm^{-1}: 3409.8 (O-H), 2988.3, 2926.3, 2854.4 (C-Hstr), 1735.9 (C=O), 1564.6,1479.1, 1033.7 (C-O), 942.09, 843.38 and 708.07 (aromatic system); ^1H NMR (400 MHz; $CDCl_3$): δ 9.85 (1H, s, O-H, aromatic), 9.40 (1H, s, O-H, aromatic), 7.66 (1H, s, CH, aromatic), 7.17 (1H, d, 2xCH, aromatic), 6.60-6.69 (1H, d, 4xCH, aromatic), 5.53 (1H, t, CH, methine), 3.12, 3.38 (2H, dd, CH_2, methylene), Molecular formulae $C_{15}H_{12}O_4$.

3,4',7-Trihydroxyflavone (AZ4), yellow powder. Mpt: 310-311°C UV [EtOH] nm (log ε): 289.89 (1.1030), 3198(0.0067), 386.75 (1.0020). IR (KBr) V_{max} cm^{-1}: 3418.7 (O-H), 2975.3, 2921.7, 2858.3 (C-Hstr), 1698.1 (C=O), 1542.1,1568.3, 1051.3 (C-O), 918.04, 845.21 and 698.06 (aromatic system); ^1H NMR (400 MHz; $CDCl_3$): δ 10.62 (1H, s, O-H, aromatic), 9.87 (1H, s, O-H, aromatic), 9.45 (1H, s, O-H, aromatic), 6.41-7.98 (1H, d, 7xCH, aromatic), Molecular formulae $C_{15}H_{10}O_5$.

4',5,7-trihydroxy-3'-methoxyflavone (AZ5), yellow powder. Mpt: 315 - 320°C UV [EtOH] nm (log ε): 291.76 (2.0001), 298.32 (0.1016), 376.89 (1.2010). IR (KBr) V_{max} cm^{-1}: 3408.6 (O-H), 2989.2, 2920.6, 2850.9 (C-Hstr), 1739.8 (C=O), 1565.6,1480.1, 1033.7 (C-Ostr methoxyl), 943.08, 842.37 and 707.06 (aromatic system); ^1H NMR (400 MHz; $CDCl_3$): δ 11.89 (1H, s, O-H, aromatic), 10.25 (1H, s, O-H, aromatic), 9.81 (1H, s, O-H, aromatic), 5.93- 7.19 (1H, d, 6xCH, aromatic), 3.85 (3H, s, CH_3, methyl), Molecular formulae $C_{16}H_{12}O_6$.

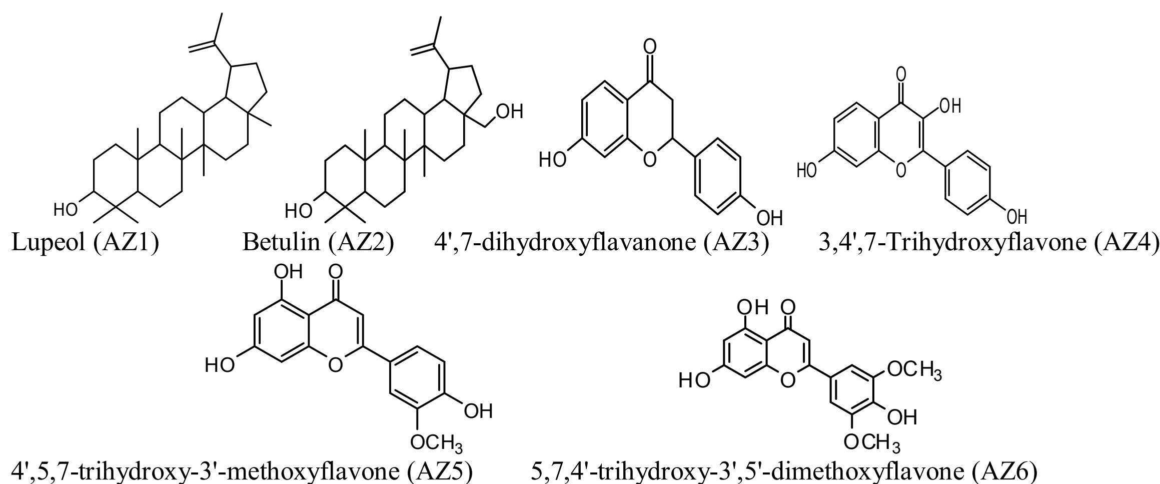

Figure 1: Structures of Lupeol (AZ1), Betulin (AZ2), 4',7-dihydroxyflavanone (AZ3) 3,4',7-Trihydroxyflavone (AZ4), 4',5,7-trihydroxy-3'-methoxyflavone (AZ5) and 5,7,4'-trihydroxy-3',5'-dimethoxyflavone (AZ6)

5,7,4'-trihydroxy-3',5'-dimethoxyflavone (AZ6), yellow powder. Mpt: 330 - 331°C UV [EtOH] nm (log ε): 291.76 (2.0001), 298.32 (0.1016), 376.89 (1.2010). IR (KBr) V_{max} cm^{-1}: 3408.6 (O-H), 2989.2, 2920.3, 2850.4 (C-Hstr), 1739.8 (C=O), 1565.6,1480.1, 1032.6 (C-Ostr, methoxyl), 943.08, 842.37 and 707.06 (aromatic system); ^1H NMR (400 MHz; $CDCl_3$): δ 11.89 (1H, s, O-H, aromatic), 10.25 (1H, s, O-H, aromatic), 8.81 (1H, s, O-H, aromatic), 5.93- 6.74 (1H, d, 5xCH, aromatic), 3.85 (3H, s, 2x CH_3, methyl), Molecular formulae $C_{17}H_{14}O_7$.

The isolated compounds generally showed absorption peaks at 3390 cm^{-1}, 2900 cm^{-1}, 2800 cm^{-1} and 1287.5 cm^{-1} in the infra red spectroscopy indicative of O-H, C-H, C-O functional groups. Lupeol (AZ1) and Betulin (AZ2) are secondary alcohols and pentacyclic triterpenoids that is lupane in which the hydrogen at the 3 beta position is substituted by a hydroxyl group. Betulin has another hydroxyl group in position C28. Absorption in the infra red region at 3382.6 cm^{-1} and 2986.2 cm^{-1} are typical of O-H and C-Hstr signals; while the ^1H NMR in the range of δ 5.10- 5.29 is assigned to the two proton signal of ethylene, 4.79-4.91 for O-H, 1.49 - 1.76 for proton signals in the cyclohexane ring while 0.99- 1.00 for proton signal of methyl. The flavonoids isolated include 4',7-dihydroxyflavanone (AZ3), 3,4',7-Trihydroxyflavone (AZ4), 4',5,7-trihydroxy-3'-methoxyflavone (AZ5), and 5,7,4'-trihydroxy-3',5'-dimethoxyflavone (AZ6). The infra red signals for the flavonoids around 3418.7 cm^{-1}, 2976.5 cm^{-1}, 1698.1 cm^{-1}, 1051.3 cm^{-1} are typical signals representing O-H, C-H, C=O, and C-O functional groups, The aromatic system showed bands in the region 918.04, 845.21 and 698.06 cm^{-1}. The proton NMR spectrum showed aromatic signals indicating the absence of high field proton of C ring on the flavonoid nucleus. The proton NMR are characterized by signals in the range δ 9-10.62 for aromatic O-H and 6-8 for aromatic CH for 4',7-dihydroxyflavanone (AZ3) and 3,4',7-Trihydroxyflavone (AZ4), The methoxyl substituted flavonoids (4',5,7-trihydroxy-3'-methoxyflavone (AZ5) and 5,7,4'-trihydroxy-3',5'-dimethoxyflavone (AZ6) in the infra red showed hydroxyl (O-H) band at 3408.6 cm^{-1}. Peaks around 2976.5 cm^{-1} and 1033.7 cm^{-1} were assigned to C-H and C-O stretch respectively. These compounds showed proton signals in the range δ 9 - 11.8 for the aromatic OH, 5.93- 7.19 for aromatic CH, and 3.85 for methyl functional groups. Compounds obtained from this plant are important because lupeol and betulin have been reported to have beneficial activity against inflammation, tumors, cancer, arthritis, diabetes, heart diseases, renal toxicity and hepatic toxicity. The flavonoids too are antineoplastic agent, used as antioxidant and as metabolite. The isolated compounds were identified by interpretation of the ^1H NMR spectra as well as by comparison with published data. The results obtained from this phytochemical study are in agreement with previous data reported for other *Albizia* species [1-3,5-7,10-11,24-25].

Table 1: Compounds detected in *Albizia zygia* fixed oil

S/N	KI	Name of compound	M. Formula	Rel%	Class of compound
1.	722	Methyl pyruvate	C4H6O3.	0.21	Ester
2.	853	Methyl malonate	C5H8O4	0.24	Ester
3.	1115	Malic acid, dimethyl ester	C6H10O5	0.18	Ester
4.	1181	2.Ethyl linalool	C12H22O	0.16	Alcohol
5.	1212	2-Decenal, (E)-	C10H18O	0.28	Alkene
6.	1239	5-Dodecen-7-yne, (Z)-:	C12H20	0.11	Alkene
7.	1250	Geranyl vinyl ether	C12H20O	5.43	Ester
8.	1357	1-Undecanol	C11H24O	0.44	Alcohol
9.	1389	2-Decanynoic acid	C10H16O2:	0.66	Acid
10.	1417	Methyl 10-methylundecanoate	C13H26O2	0.31	Ester
11.	1449	Dihexyl ketone	C13H26O	0.44	Ketone
12.	1461	Undecylenic Acid	C11H20O2	0.73	Acid
13.	1481	Methyl laurate	C13H26O2	1.46	Ester
14.	1516	1-Pentylheptyl acetate	C14H28O2	1.58	Ester
15.	1570	Dodecanoic acid	C12H24O2	0.79	Carboxylic acid
16.	1575	3-Tetradecanol	C14H30O	1.14	Alcohol
17.	1586	Geranyl isovalerate	C15H26O2	1.09	Ester
18.	1609	9-Tetradecenal, (Z)-	C14H26O	0.88	Aldehnyde
19.	1673	3-Tetradecyn-1-ol	C14H26O	1.29	Alcohol
20.	1680	Methyl myristate	C15H30O2	1.46	Ester

S/N	KI	Name of compound	M. Formula	Rel%	Class of compound
21.	1733	3-Hydroxylauric acid	C12H24O3	1.61	Acid
22.	1754	Hexahydrofarnesyl acetone	C18H36O	20.34	Ketone
23.	1763	(Z)6-Pentadecen-1-ol	C15H30O	0.61	Alcohol
24.	1769	Myristic acid	C14H28O2	0.99	Acid
25.	1774	2-Hexadecanol	C16H34O	0.54	Alcohol
26.	1787	Z-10-Tetradecen-1-ol acetate	C16H30O2	1.08	Ester
27.	1814	Methyl 14-methylpentadecanoate	C17H34O2	1.35	Ester
28.	1816	7,11-Hexadecadienal	C16H28O	0.72	Aldehnyde
29.	1822	10-Methyl-8-tetradecen-1-ol acetate	C17H32O2	0.75	Ester derivative
30.	1886	Methyl (2E)-2-hexadecenoate	C17H32O2	0.35	Ester
31.	1890	2-Methylhexadecan-1-ol:	C17H36O	0.91	Alcohol
32.	1899	Isophytol	C20H40O	1.17	Alcohol
33.	1914	Methyl isoheptadecanoate	C18H36O2	1.35	Ester
34.	1915	Palmitic acid, methyl ester	C17H34O2	6.49	Ester
35.	1968	Palmitic acid	C16H32O2	8.35	Acid
36.	2003	Methyl (10E)-10-heptadecen-8-ynoate	C18H30O2	1.25	Ester
37.	2007	13-Octadecenal	C18H34O	3.17	
38.	2021	Palitamide	C16H33NO	0.47	Amide
39.	2045	trans-Phytol	C20H40O	1.19	Alcohol
40.	2077	Stearic acid, methyl ester	C19H38O2	2.41	Ester
41.	2085	Oleic acid, methyl ester	C19H36O2	8.17	Ester
42.	2104	2-Methyl-Z, Z-3,13-octadecadienol	C19H36O	0.41	Alkenol
43.	2112	Methyl 13,16-octadecadiynoate	C19H30O2	0.58	Ester
44.	2153	n-Nonadecanol-1	C19H40O	0.81	Alcohol
45.	2175	Oleic Acid	C18H34O2	6.13	Acid
46.	2220	Palmitic acid, 2-hydroxyethyl ester	C18H36O3	4.18	Ester
47.	2276	Methyl eicosanoate	C21H42O2	0.91	Ester
48.	2308	Methyl arachidonate:	C21H34O2	1.49	Ester
49.	2390	cis-8,11,14-Eicosatrienoic Acid	C20H34O2	0.65	Acid
50.	2419	Stearic acid, 2-hydroxyethyl ester	C20H40O3	0.41	Ester
51.	2482	α-Monopalmitin	C19H38O4	0.73	Acid derivative
52.	2498	Palmitic acid β-monoglyceride	C19H38O4	0.69	Acid derivative
53.	2499	Methyl (8E,11E,14E)-8,11,14-docosatrienoate	C23H40O2	0.42	Ester
54.	2618	2-Hydroxyethyl icosanoate	C22H44O3	0.44	Ester
				Total%	100%

KI- Kovats retention index, (relative to n-alkane hydrocarbon standards. (Retention Data. NIST Mass Spectrometry Data Center., 2007)

The fixed oil as analysed by GC-MS gave esters of fatty acids as the major component (Table 1). Other classes of compounds detected include alcohol, carboxylic acid, alkenes, and ketones. Hexahydrofarnesyl acetone (20.34%), oleic acid, methyl ester (8.17%), oleic acid (6.13%), geranyl vinyl ether (5.43%), palmitic acid, 2-hydroxyethyl ester (4.18%), 13-octadecenal (3.17%), are however the major compounds In a previous study, the essential oil from *A. zygia* leaves' oil contain linalool, thymol, carvacrol, heneicosane, ocatanal, cymene, and α – pinene as the major component while linalool, thymol, carvacrol, heneicosane, γ – terpinene, cymene, β – caryophyllene, ocatanal, humulene appeared in appreciable quantities in the essential oil obtained from the stem- bark. The results obtained in this study showed that the major constituent of the fixed oil of *A. zygia* oil is different from that obtained for the essential oil [8-9].

Table 2: IC_{50} (µM) values of compounds in the Urease, α-Glucosidase and Antioxidant inhibitory assays.

Compounds and standard	Urease Inhibition	α-Glucosidase Inhibition	Antioxidant assay
AZ1	69.8 ± 0.22	65.8 ± 0.55	55.3 ± 0.12
AZ2	62.8 ± 0.83	53.8 ± 0.32	65.8 ± 0.98
AZ3	86.8 ± 0.87	43.5 ± 0.45	21.5 ±0.32
AZ4	Nil	48.9 ± 0.16	41.2 ± 0.30
AZ5	78.9± 0.33	Nil	40.7 ± 0.21
AZ6	88.9 ± 0.39	Nil	31.2 ± 0.10
AZ7	Nil	Nil	32.9 ± 0.56
BHA	-	-	44.2 ± 0.09
Thiourea	21.6 ± 0.12	-	-
DNJ	-	3.9 ± 0.08	-

* BHA-Butylatedhydoxyanisole, DNJ-1-Deoxynojiromycin hydrochloride

The IC_{50} (µM) values of the isolated compounds in the urease, α-glucosidase and antioxidant inhibition assays is shown in Table 2. The terpenoids and flavonoids showed only moderate activity in both the urease and α-glucosidase inhibition activity. Activity was compared with thiourea and 1-deoxynojiromycin hydrochloride (DNJ). All the compounds however showed significant antioxidant activity. 4',7-dihydroxyflavanone (21.5 ±0.32), 5,7,4'-trihydroxy-3',5'-dimethoxyflavone (31.2 ± 0.10) and the fixed oil (32.9 ± 0.56) were the most active in the antioxidant assay when activity was compared with butylated hydroxylanisole. The renewed interest in search for anti urease agents from nature that can eradicate the invasion and presence of *Helicobacter pylori* strains would be an advantage in traditional medicine. Plants have been reported to be used for the treatment of *H. pylori* [26,27]. due to the presence of secondary metabolites. Therefore, compounds from *A. zygia* would add to the repository of drugs for the treatment of diseases related to *H. pylori* strains. The extracts showed little or no lethal effect on the insects used in the insecticidal screening. *A. zygia* leaves had the highest mortality rate (33%) at 50% concentration. *A. zygia* bark had a mortality rate of 17% at both 50% and 100% concentrations. This showed that the plant had little or no activity as insecticidal plants. Since the insecticidal activity of the crude extract was not significant, the isolates were not investigated for insecticidal activity.

Conclusion

Terpenoids and flavonoids isolated from *Albizia zygia* (Leguminosae) were shown to possess enzyme inhibition (urease and α-glucosidase) and antioxidant activities, when activities were compared with thiourea, 1-deoxynojiromycin hydrochloride (DNJ) and butylated hydroxylanisole used as standards in the three assays. The fixed oil was dominated with hexahydrofarnesyl acetone, palmitic acid, 2-hydroxyethyl ester, oleic acid, and oleic acid, methyl ester. *A. zygia* therefore could be a promising source of metabolites of emerging natural remedies.

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