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Bioassay Guided Fractionation and α-Amylase Inhibitory Activity of Lupeol from the stem bark of *Faidherbia albida Del.* Mimosaceae

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Abstract: *The present study was designed to isolate active compound(s) through activity guided isolation using α-amylase inhibitory activity of crude extract and fractions of Faidherbia albida . The crude and fractions were subjected to inhibitory effect on α-amylase. The ethylacetate fraction showed 91.63% inhibition of the enzyme, column chromatography of the ethylacetate fraction afforded the Compound EFAE 5 which inhibited the enzyme by 82.88% and showed significant α-amylase inhibition. Spectral analysis of the isolated compound confirmed it to be Lupeol by comparison of the spectral data with literature.*
Keywords: *lupeol, -α amylase inhibitors, antidiabetic activity, Faidherbia albida*

I. Introduction

Herbal medicine is increasingly becoming a mainstream healthcare program owing to the report that about 80% of the population in developing countries depend on it for their well-being due to affordability and availability (Prabhakar et al., 2013). Plant extract and their derivatives are being used to manage Diabetes mellitus (DM). It is a metabolic disease of perpetual hyperglycaemia or expanded blood glucose levels with disturbing influences in fat, starch and protein metabolism due to decreased or total absence of insulin secretion (Alberti and Zimmet, 1998). One of the control methods for the observed hyperglycaemia is to retard the assimilation of ingested carbohydrate through the inhibition of hydrolyzing enzyme- α-amylase. Inhibition of α-amylase reduces post prandial rise in blood glucose. Enzyme inhibitors can be a potential focus in the numerous ranges of ailment control and treatment as enzymes catalyse the most vital biochemical pathways (Kim and Nho , 2004). α-Amylase inhibitors can be utilized to treat disease such as diabetes as well as test for activity of new plants and their metabolites.

Faidherbia albida Delile (Mimosaceae) is a deciduous legume tree native to Africa and the Middle East, formerly included in the genus Acacia. It is the only member of the genus Faidherbia. Common names include Apple ring acacia, Winter thorn and in Hausa ethnomedicine of northern Nigeria, it is called Gawo. Traditionally, the plant and its various parts is used to treat diseases like leprosy, pneumonia, diarrhea, difficulty in breathing (Orwa et al, 2009) Stem bark extract is used to treat chest pain and trypanosomiasis in Northern Nigeria. The root bark of the plant and the seed have been shown to possess mild hypoglycemic activity (Gaber et al., 2013, Salisu et al 2009).Some phytoconstituents such as α-pinene (Ogunbinu et al 2010), fatty acids and fatty acid esters (Fadipe et al 2015) have been isolated from F. albida. In our continuing search for alternative drugs for the management of diabetes mellitus especially from Nigeria ethnomedicinal plants, we report herein, the bioassay guided fractionation and isolation of Lupenol and its α-amylase inhibitory activity for the first time for this plant.

II. Materials and Methods

Plant collection: The stem bark of *F. albida* was collected from its trees in Bauchi, North East Nigeria in February 2015. It was identified and authenticated by Mr JJ Azila of Federal College of Forestry, Jos where a voucher specimen was deposited for future reference.

Preparation of extracts: The stem bark was shade dried for 21 days, then pulverized. 1000g of the plant stem bark was macerated with 2.5L of 50% ethanol for 72 hours and filtered with cotton wool, the marc further extracted twice for 48 hours each with 50% ethanol and filtered. The combined filterate was concentrated on a rotary evaporator and subsequently dried on a water bath at 50°C. The dried extract weighed 76g (7.6% w/w). 50g was successively partitioned in a separating funnel with n-hexane (4x 200ml), dichloromethane (3X 200ml) ethylacetate (3x200ml)and n-butanol (3x200ml). The fractions were dried and kept in a dessicator for use The fractions were tested for their potential to inhibit the enzyme α-amylase. The ethylacetate and n-butanol fractions were the promising fractions.

Alpha amylase inhibitory activity

The alpha amylase inhibitory activity was carried out by the method devised by Miller (1959). Briefly, the total assay mixture containing 200 µl of 0.02M sodium phosphate buffer, 20 µl of enzyme (α-amylase) and the plant extracts at a concentration of 100 µg/ml were incubated for 10 min at room temperature followed by addition of 200 µl of 1% starch in all the test tubes. The reaction was terminated with addition of 400 µl of di-nitro salicylic acid color reagent, placed in boiling water bath for 5 min, cooled to room temperature and diluted with 15 ml of distilled water and the absorbance measured at 540 nm (Systronic- UV-VIS spectrophotometer). The control samples were also prepared accordingly without any plant extracts and were compared with the test samples containing the plant extracts prepared with different solvents. The results were expressed as % inhibition calculated using the formula:

Inhibition activity % = Abs control - Abs extract ×100/ Abs control

Where Abs= Absorbance

Ethylacetate fraction was selected for isolation and subjected to flash column chromatography over silica gel. 5g of the ethylacetate fraction was packed in a column (2cm x 50cm) and gradiently eluted with n-hexane (100%), n-hexane: ethylacetate mixtures; 95:5, 90:10, 80:20, 70:30,60:40, 50:50, 40:60,30:70,20:80, ethylacetate 100% and finally 5% methanol in ethylacetate. The elution was monitored using TLC with solvent systems consisting of n-hexane: ethylacetate 9:1 (System 1), 4:1 (System 2). About 160 fractions of 10mls were collected. Fractions 45- 75 revealed similar spots containing 4 spots were pooled to give 0.21g of yellow powdery compound which was then separated using a 1cm x 50cm column.The column was eluted starting with n-hexane 100% (100ml) followed gradiently by eluting with n-hexane- Ethylacetate 99:1 (100ml), 98:2 (100ml), 95:5 (200ml) and 90:10 (200ml). The fractions were monitored using TLC . Fractions with similar spot(s) were pooled and the most intense single spot were combined and the solvent allowed to evaporate.. The resulting compound was then re-crystalised using acetone- methanol mixture (40:60) to give a white amorphous powder (15mg) with R_f value 0.45 (System 1).

Statistical analysis

Data are expressed as mean ± S.E.M. The data was analyzed to ascertain the level of significance using GraphPad InStat version 3.05 for Windows. Values of $p<0.05$ were considered statistically significant.

III. Results

Alpha amylase inhibitory activity

Table 1: α-Amylase inhibition of *F.albida* fractions and isolated constituents

FA fractions	Concentration µg/ml	α-amylase (% inhibition)
Compound 1	100	82.88
Hexane	100	21.67
Dichloromethane	100	87.45
Ethylacetate	100	91.63
n-Butanol	100	88.97
Acarbose	100	85.17
Control	-	-

The results of α-amylase inhibitory activity (Table 1) showed that acarbose 100mcg/ml exhibited 85.17% while ethylacetate fraction exhibited 91.63% α-amylase inhibitory activities. Compound EFAE 5, inhibited α-amylase by 82.88%

Identification of the chemical structure of the isolated compound

The compound was characterized by comparing the spectroscopic data with those reported in literature (Reynolds et al.,1986, Chaturvedula and Prakash, 2012). Thus enabling to conclude that compound EFAE 5 is Lupeol.

Compound EFAE 5, a white amorphous solid (15.17 mg), ^{1}H- NMR (CDCl3): δ = 4.57, 1H (H-29b), 4.69, 1H (H-29a), 3.20 (m), 1H (H-3); 1.70, 3H (s), (H-30); 1.05, 3H (s), (H-27); 0.99, 3H (s) (H-26); 0.97 3H (s) (H-2); 0.85, 3H(s), (H-28); 0.81, 3H (s), (H-25); and 0.78, 3H (s), (H-24)

^{13}C- NMR: (62.5MHz) CDCl3: 38.7(C-1); 27.4 (C-2); 79.0(C-3); 38.8 (C-4); 53.3 (C-5); 18.3 (C-6); 29.3 (C-7); 40.8 (C-8); 50.4 (C-9); 37.2 (C-10); 20.9 (C-11); 25.2 (C-12); 38.7 (C-13); 42.3 (C-14); 27.5 (C-15); 35.6 (C-16); 43.0 (C-17); 48.3 (C-18); 48.0 (C-19); 150.9 (C-20); 29.9 (C-21); 40.0 (C-22); 28.0 (C-23); 15.4 (C-24); 16.1 (C-25); 15.9 (C-26); 14.6 (C-27); 18.0 (C-28); 109.3 (C-29); 19.3 (C-30).

IV. Discussion and Conclusion

Alpha amylase inhibitory activity demonstrated by the fractions of *Faidherbia albida* led to the isolation of lupeol from the ethylacetate fraction. Lupeol through its α-glucosidase inhibitory activities was recently isolated from the flower of banana and found to be responsible for its antihyperglycemic activities(Ramith et al 2014) Banana peel was also reported to posess antihyperglycemic activities due to a related compound- Lupenone (Hongmei et al 2016). The % inhibiton values for Lupeol and acarbose were 82.88% and 85.17% respectively. The value obtained for lupeol is lower that the inhibition value for ethylacetate fraction (91.63%). This may imply that in addition to the activity of Lupeol, there may be other compounds present in the fraction in trace quantity that could be potent or potentiate the activity of lupeol. The isolation of lupeol justify the use of *F.albida* in the traditional management of diabetes mellitus.

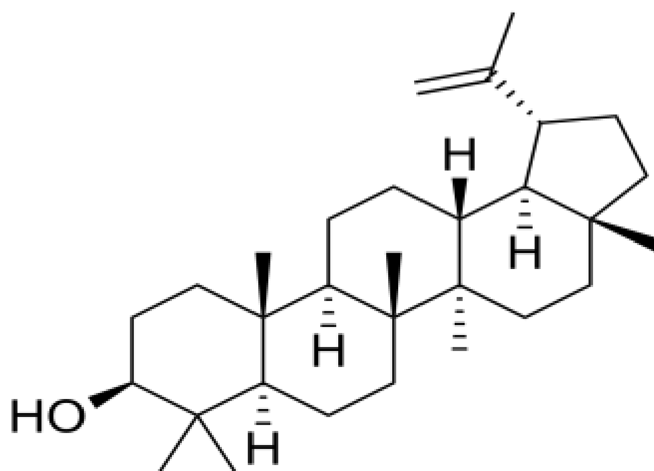

Figure 1: Lupeol

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