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Analysis of Crude Saponin from the Leaves of Vernonia Amygdalina

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ABSTRACT
Saponin, which is naturally occurring glycosides, was extracted and studied. Vernonia Amygdalina is a shrub plant commonly found in Asia and Africa that contain various bioactive compounds, which have been used for treating illness and diseases. The saponin was extracted from the leaves of Vernonia amygdalina by ultrasonic. The crude extract was further studies by Fourier Transform Infrared (FTIR) and High Performance Liquid Chromatography (HPLC). The FTIR spectral gives peaks at 3281 for the hydroxyl group (OH), 2926 for the Carbon-hydrogen (C-H), 1557 for the Carbon double bond (C=C), 2116 for the Carbon-Oxygen (C=O) and 1069 for the Carbon-Oxygen-Carbon (C-O-C). This shows the presence of functional groups in the crude saponin. The HPLC studies of saponin from solid crude extract of Vernonia amygdalina shows the peak at 280 nm and when matched with the standard saponin it confirms the presence of saponin in the extracts. The yield of saponin from Vernonia amygdalina is 18%, which is confirmed by the phytochemical analysis done by Udochukwu et al.

Keywords: Saponin, Vernonia Amygdalina, Ultrasonic, FTIR and HPLC

I. INTRODUCTION

Saponins are a group of naturally occurring plant glycosides, characterized in aqueous solution by their strong foam-forming properties (Sahu et. al, 2011). They are found in a vast number of plant species in parts including roots, shoots, flowers and seeds. Saponins have mainly found an industrial interest as surface-active or foaming agents. A natural surfactant named Quillaja Saponaria Molina, which was extracted from a Soar bark tree in Chile was one of the recent saponin extract (Rigano and Lionetti, 2009).

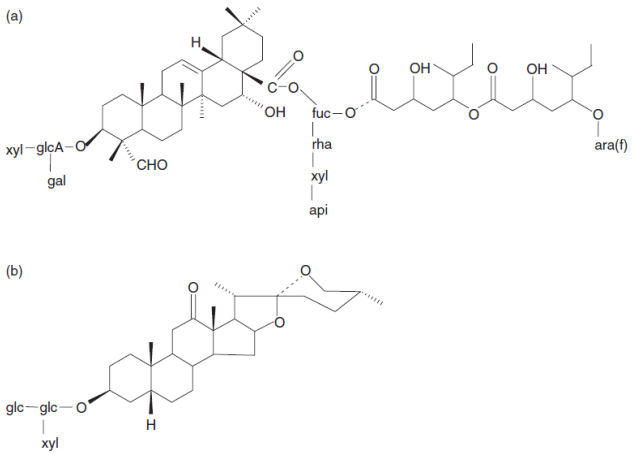

Figure 1: Standard saponin structure; (a) triterpene saponin from Quillaya saponaria (b) steroidal saponin from Yucca schidigera (Wieslaw and Arafa, 2010)

II. METHODS AND MATERIAL

Extraction of Saponin from Vernonia Amygdalina

Extraction was carried according to Wu et al. (2001) with small modification. Plant leaves were collected from Johor-Skudai areas, air dried for 5 days and grounded into powder form. The powered plant material was sieved through 0.5mm. Then 2 g of the powdered sample was dispersed in 100 ml of distilled water in a beaker. This was shaken for 2 minutes on an orbital shaker (Protech Model 720) after which the beaker was then placed in an ultrasonic water bath (Crest Ultrasonics, Trenton, NJ, USA). The extraction was done for 1-2 hrs during which the bath temperature was between 27-35°C. After extraction, the liquid samples were centrifuged at 2000 rpm for 20 minutes and then filtered using an ash-less filter paper. These were placed in a freeze dryer to evaporate to dryness under vacuum at 45°C.

Qualitative Test

Before extraction, 10 ml of distilled water was added to 100 mg of grounded leaves in test tubes and shaken vigorously for 2 minutes. After extraction, 0.5g of extracted saponin was dissolved in 10 ml of distilled water and stirred for 10 minutes. Both gives a stable froth indicating the presence of saponin.

III. RESULTS AND DISCUSSION

The extracted saponin gives a fine light brown powder with a density of 0.1g/cc. The qualitative test of the crude extract indicate presence of saponin by the formation of a persistence foam. FTIR was recorded to observe the functional group present. Infrared spectra were recorded at a wavelength between 4000 and 500 cm^{-1}.

Saponins showed characteristic infrared absorbance of the hydroxyl group (OH) is 3281 cm-1 in the solid extract and from 3525 to 3281 cm-1 in the standard saponin. Carbon-hydrogen (C-H) absorption is 2926 cm-1 in the solid extract and 2924 cm-1 in standard saponin. The C=C absorbance was observed at 1557 cm-1 in the solid extract and at 1588 cm-1 for standard saponin. For C=O absorbance, solid extract gives a value of 2116 cm-1 while the standard saponin gives a value of 1727 cm-1. Oligosaccharide linkage absorptions to sapogenins, that is C-O-C, was evident at 1069 cm-1 for the solid extract and 1074 cm-1 in standard saponin.

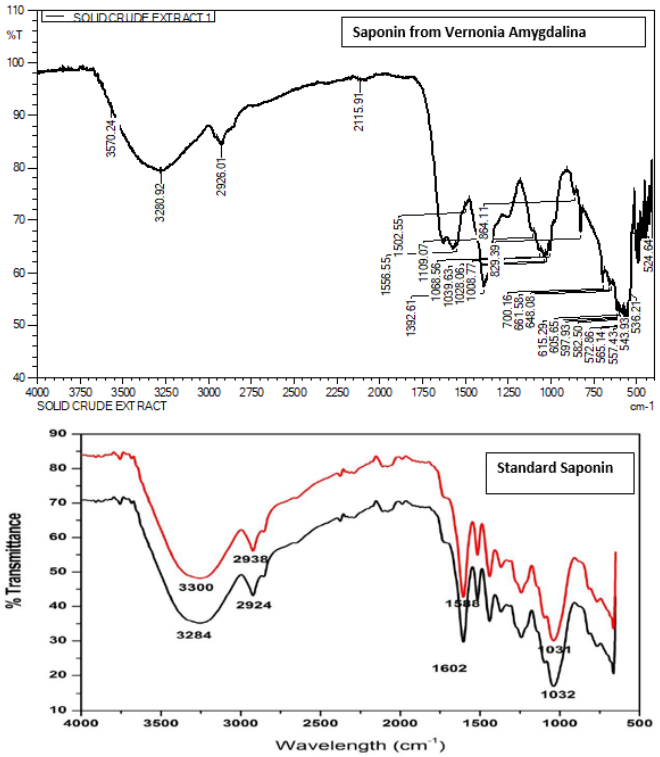

Figure 2: FTIR spectra for saponin extract from Vernonia amygdalina and standard saponin

The HPLC condition were modelled after Kim and Wimpler (2009), where the saponin extract from Vernonia amygdalina was diluted 3-fold using deionized water and filter through 0.45 µm syringe filter prior to injection. The chromatograph was acquired from a HPLC system using UV-Vis detector run at 0.8 mL/min. The mobile phase is a buffer solution of o-phosphoric acid with a pH of 2.4 that was run for 30 min at 0.8 mL/min. It shows peak at 280 nm.

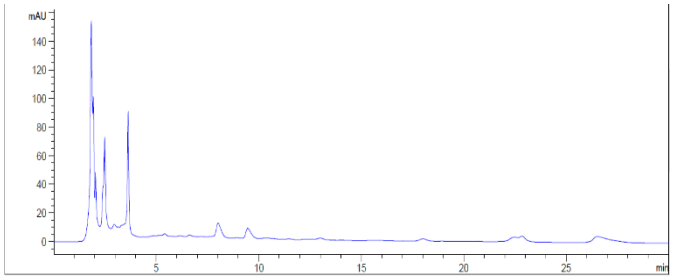

Figure 3: HPLC Analysis of saponin from Vernonia Amygdalina

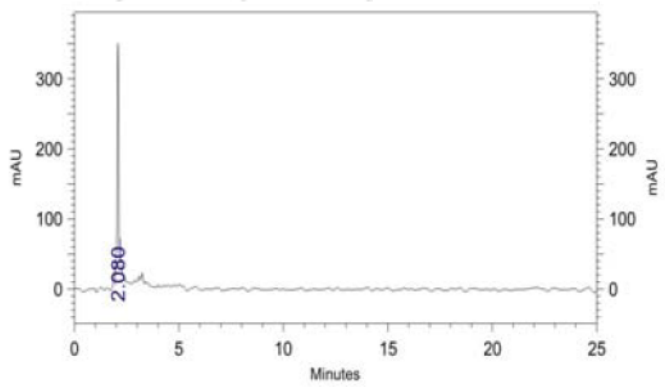

Figure 4: HPLC Analysis of standard saponin

On comparing the retention times on both chromatograms, standard saponin shows a retention time of 2.1 minutes while saponin extracts shows multiple retention time of 1.8, 1.9, 2.5, 3.6 minutes, and this correspond to common saponin constituents. The amounts of saponin present in Vernonia amygdalina were calculated after extraction to be approximately 18%.

IV. CONCLUSION

Saponin, which is characterized by its foam forming abilities, are present in Vernonia Amygdalina and was easily extracted by ultra-sonication. This is a more efficient means of extraction than the use of soxhlet apparatus because of its short extraction time. The solid extract was obtained by freeze-drying under vacuum; this gives saponin without damaging the structural makeup by application of heat. FTIR and HPLC are powerful method to determine the presence and amount of saponin. This work add Vernonia amygdalina to the list of plant that contain extractable saponin.

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