**SHORT COMMUNICATION**

# Title: Direct detection of saponins in crude extracts of soapnuts by FTIR

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**Authors: MeshariSaadAlmutairi[a] (Corresponding author) & Muhammad Ali[b]**

[a&b]*School of Civil Engineering & Surveying,*

*University Portsmouth,*

*Portland Building, Portland Street,*

*Portsmouth, United Kingdom PO1 3AH*

***Tel: +447848688849***

***Email:meshari.almutairi@myport.ac.uk***

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# Direct detection of saponins in crude extracts of soapnuts by FTIR

**Abstract:** Direct detection of saponins in soapnuts (*Sapindus mukorossi)* using Fourier Transform Infrared (FTIR) spectroscopy is investigated in this project. Potassium bromide (KBr) powder was mixed with extracted powder of soapnuts and compressed to a thin pellet for examination process. The outcome of the FTIR spectra of saponin demonstrated characteristic triterpenoid saponin absorptions of OH, C=O, C-H, and C=C, while the glycoside linkages to the sapogenins were indicated by the absorptions of C-O. The significance of this study is that saponin absorption peaks are directly detectable in crude aqueous and 95% ethanol extracts of soapnuts powder using FTIR spectroscopy thereby eliminating the need of further expensive and exhaustive purification steps. The extracts of soapnuts were screened for saponins along with controls by phytochemical tests andadvanced spectroscopic techniques like Ultra Fast Liquid Chromatography and UPLC QTOF MS/MSwere also implemented to validate the saponins.

**Keywords:**saponin; soapnuts; FTIR; extraction; phytochemical.

## 1.0 Introduction

Saponins are a large assembly of glycosides classified as non-ionic bio-surfactants. They are extensively distributed in the territory of green plants Roy et al. (1997), especially vegetables, soapnut pericarp, soybeans, peas, beans and herbs whereas saponins are commercially produced from *Yucca schidigera* and *Quillaja saporina*. The name 'saponin' is originated from the word 'sapo' which in Latin means soap, owing to the foam generating characteristic in aqueous solution by stirring action. The solutions are amphiphilic glycosides in which the polar components include sugars (pentoses, hexoses or uronic acids) which are connected covalently to a non-polar group which includes an aglycone known as sapogenin that can be in the form of either streroid, alkaloidal or triterpene depending on the nature of the aglycone. Saponin's structure is in the form of lipid soluble polycyclic aglycones connected to a single or multiple sugar chains and water-soluble sugar residues (Hong et al. 2002).

In general, the glycone elements of saponins are oligosaccharides. It is possible for oligosaccharides to be connected to the sapogenin by means of either ether or ester bond at one or two glycosylation sites resulting in the monodesmosidic or bidesmosidicsaponins. The amphipathic nature of the compounds which is the main reason for their detergent-like characteristics is attributed to the uneven distribution of their hydrophobic (aglycone) and hydrophilic (sugar) moieties. The saponin's unique foaming property is resulted from the mixture of components within their non-polar (oil soluble) sapogenin and the polar (water soluble) sugar chain molecular structure (Zhou et al. 2013).

Soapnuts are well known to be rich in saponins and this study is aimed at direct determination of saponins in crude extracts of soapnuts. The initial screening for saponins on the extracts was conducted by phytochemical tests like foam and hemolysis test. Ultra-Fast Liquid Chromatography and Ultra Performance Liquid Chromatography Quadrupole-Time of Flight-Mass Spectrometry were also performed for qualitative analysis of samples to verify that the detection of triterpenoid saponins by FTIR will suffice fast, reliable and cost effective system for direct detection of saponins directly from the crude extracts. Despite the fact that UFLC and UFLC QTOF MS/MS are highly sensitive and reliable methods, they are expensive and time consuming and hence FTIR has an advantage over these methods in terms of quick, easy and direct detection of saponins from crude extracts.

## 2.0 Results and Discussion

### *2.1 Phytochemical tests*

The aqueous and alcoholic extracts were shown to be positive for saponins when subjected to phytochemical screening by froth test and hemolysis test as shown in supplementary Figures S1, S2 and Table S1(online only).

### *2.2 Fourier Transform Infra-Red Spectroscopy (FTIR)*

The presence of saponins in the aqueous and 95% ethanol extract were confirmed by infrared absorption spectrum and compared with that of the standard. Saponins showed characteristic infrared absorbance of the hydroxyl group (OH) ranging from 3407 $cm^{-1}$ in the aqueous extract,3419 $cm^{-1}$ in the 95% ethanol extract and from 3525 $cm^{-1}$ to 3281$cm^{-1}$ in the standard Quillaja saponin. Carbon-Hydrogen, C-H absorption was ranging from 2931 $cm^{-1}$ in the aqueous extract, 2931 $cm^{-1}$ 95% ethanol extract, while in standard Quillaja saponin it ranged from 2973 $cm^{-1}$ to 2932 $cm^{-1}$. The C=C absorbance were observed at 1613 $cm^{-1}$, 1636 $cm^{-1}$ and 1609 $cm^{-1}$ in the aqueous extract, 2931 $cm^{-1}$in95% ethanol extract and standard Quillaja saponin respectively. Whereas, C=O absorbance was found to be at 1727 $cm^{-1}$ in the aqueous extract and 1726 $cm^{-1}$ in the 95% ethanol extract, while standard saponin showed a value of 1724 $cm^{-1}$. Oligosaccharide linkage absorptions to sapogenins, that is C-O-C, were evident between 1074 $cm^{-1}$ in the standard, 1045 $cm^{-1}$ in 95% ethanol extract and 1046 $cm^{-1}$in aqueous extract.

The abovementioned infrared functional group absorptions characteristic of saponins has been referred by researchers such as Toshiyuki et al. (2001) and Da Silva et al.(2002). Owing to the oleanolic acid/ester, these oleanane-type triterpenoid saponins are characterized by the C=O infrared absorbance. According to Toshiyuki et al. (2001), Kirmizigul et al. (2002), and Natori et al. (1981), these triterpenoid saponins tend to be bidesmosides as they have two attachments of glycones to the sapogenin namely glycosidic and ester groups. In this study, saponins detected in *soapnuts,* was likely to be bidesmosidic, oleanane-type triterpenoids. The FTIR spectra of standard Quillaja saponin (Fisher) demonstrated triterpenoid saponin absorptions characteristics.

It can be seen from Figure 1, that saponins are clearly detectable in the crude aqueous and alcoholic soapnut extracts even before the purification of saponins. The similarity in the FTIR spectrum of the crude soapnut extracts and the standard Quillaja saponin shows the importance

of detection of saponins right after the extraction and freeze drying step thereby eliminating the need of further extensive purification steps. The significance of this observation is that saponins are detectable in crude aqueous and alcoholic extracts directly using FTIR spectroscopy, therefore shortening the time and the necessity for purification steps before performing analysis.

### *2.3 Ultra Performance Liquid Chromatography Quadrupole-Time of Flight-Mass Spectrometry (UPLC QTOF MS/MS)*

The mass spectroscopic data of the aqueous extract, 95% ethanolic extract was carried (Figure S3, online only) out to substantiate the presence of triterpenoid saponins by screening for characteristic molecular masses, based on similar cases of reports of other researchers. However, a study at the level of detailed molecular identification was not intended at this stage. The uniformity in appearance of 381 m/z, 723 m/z, 1065 m/z and 1407 m/z demonstrates the presence of disaccharide moiety in the glycone element of saponins. This is in agreement with the results of Wong (2014) while another study by Heng et al. (2014), refers to compounds molecular weights of 1206, 1074, 882, 750, 924 and 966 were identified as triterpenoid saponin, primarily the oleanolic acid type. Based on this report the LC-MS/MS data of the aqueous extract, 95% ethanol extract and standard saponin was examined for the compounds with the above masses which are characteristic for triterpenoid saponin. The findings are depicted in Table S2 (online only).

### *2.4 Ultra Fast Liquid Chromatography*

The UFLC chromatograms of aqueous and 95% ethanolic extract of soapnuts and standard Quillaja saponin is shown in Figure S4 (online only). The comparison of retention times of the separated compounds are tabulated in Table S3 (online only).UFLC separation of compounds in all samples was achieved within 6 minutes. It can be interpreted from the chromatograms that an earlier onset of separation of Quillaja saponin than the soapnuts extracts under the same chromatographic conditions is consistent with the study by Shiau et al. (2009). However, the shorter retention times in this study is due to the difference in technique as described in experimental section (see supplementary material). Chromatograms of both the aqueous extract and 95% ethanol extract of soapnuts exhibited close similarity. By comparing the retention times of peaks on the chromatograms of the soapnut extracts and Quillaja saponin it can be established

that peaks corresponding to retention times 2.8, 3.5, 4.1, 4.8 and 5 min correspond to common saponin constituents.

## 3.0 Conclusions

This study has shown that the FTIR absorptions of oleanane-type triterpenoid saponin are characterised by the C=O, which tend to be bidesmosides as they have two attachments of glycones to the sapogenin namely glycosidic and ester groups. It was found that thesaponins detected in soapnuts, was likely to be bidesmosidic, oleanane-type triterpenoids. This claim was verified by the characteristic mass spectrum of the soapnut extracts. Moreover, the FTIR spectra of Quillaja saponin (standard) also demonstrated triterpenoid saponin absorptions characteristics. The saponin powder extracted by distilled water and ethanol (95%) displayed 97% similarity in absorbance to the standard Quillaja saponin. Moreover, to validate the presence of saponin in the soapnuts extract, advanced analytical methods like UFLC and UPLC QTOF MS/MS were carried out for qualitative analysis. The results from this preliminary qualitative analysis of saponin showed that the FTIR absorption spectra of the crude would be sufficient, fast and easy.

## Supplementary material

Experimental details relating to this paper are available online, alongside Table S1-S3 and Figures S1-S4.

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