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DETECTION AND CHARACTERIZATION OF SAPONINS IN SOME INDIGENOUS PLANTS USING UV, FTIR AND XRD SPECTROSCOPY

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ABSTRACT: *Detection, evaluation and characterization of steroidal saponins extracted from wild yam, yellow yam and ginger were carried out using the UV, FTIR and XRD spectroscopy. Air dried samples from the local plants were ground and sieved to smoothness with 250µm sieve to achieve constant particle size. Methanol and ethanol were used differently as the extraction solvent. The FTIR spectra were collected using Potassium bromide powder which was mixed with the extracted powdered samples and compressed to a thin potassium bromide (KBr) pellet. The resulting signal at the detector presented unique spectral, typically from 4000 to 650cm^{-1}, representing the diagnostic (functional group) region and the fingerprint region of the samples. The outcome of the spectra demonstrated characteristic steroidal absorption peaks of the functional groups OH, C=H, C=O, C=C. The glycoside linkages to the aglycone or sapogenin were indicated by the absorptions of C-O. The UV absorbance of saponin extract from yellow yam had two peaks, the main peak absorbance at 369nm and shoulder peak at 450nm. The UV absorbance of saponin extracts from wild yam and ginger had main peaks at 367nm and 369nm and shoulder peaks were at 455nm and 466nm respectively. Results of the UV analysis of the various samples suggested the presence of steroidal saponins. The XRD analysis performed showed that the samples were a group of amorphous colloidal glycosides. The different analyses indicated that detection and characterization of steroidal saponins are feasible with* UV analysis, infrared and XRD spectroscopy.

KEYWORDS: Steroidal, Molecular fingerprints, Aglycone, Colloidal glycosides

INTRODUCTION

Saponins are glycosides which exist in three different forms as steroids, triterpeneoids and alkaloids depending on the hydrophobic, lipid soluble aglycone unit which bonds covalently with the polar sugar molecules (Augustin *et al.,* 2011; El-Aziz *et al.,* 2019; Nowrouzi *et al.,* 2020). Saponins are classified as nonionic biosurfactants, as they do not have any charges on their polar head group but are comprised of water hating tail and water loving head.

The type of aglycones, carbohydrates and different attachment positions result in the several kinds of saponins. In the course of extraction, processing and storage, chemical structures of saponins

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may undergo biotransformation as a result of hydrolysis, microbial and chemical reactions. The amorphous region of the saccharide units as well as chemical components of saponins may change due to fermentation, thereby modifying their biochemical properties (Yuliana *et al.*, 2017; Deng *et al.*, 2013). Hossain *et al*., 2016 reported that appropriate extraction; processing and storage method are key components of any efficient technology. Saponins can be classified according the number of carbon atoms present in the aglycone unit. The steroidal glycosides have three methyl groups detached, whereas, in the triterpenoid glycosides, all 30 C-atoms are retained (Kregiel *et al.*, 2017). This means that the carbon skeleton of triterpenoid glycosides has 30-C atoms while the steroidal glycosides have 27-C atoms.

Alkaloid glycosides have similar structures with steroidal glycosides, the difference is that alkaloid glycosides have six-membered ring containing N-atom (piperidine ring) instead of pyranose ring (six-membered ring containing O-atom) as in steroid glycosides (El-Aziz *et al.*, 2019). Both triterpenoid and steroidal saponins usually have a glycosidic linkage at the third carbon atom which has the OH functional group. Glycosides have been used in many industrial applications ranging from the preparation of steroid hormones and modern drugs in the pharmaceutical industry, where they function as building blocks to utilization as food additives, exploring their nonionic and amphiphilic properties (Kregiel *et al.*, 2017; Sheng and Sun, 2011).

Saponins are used to enhance immune system, due to their anti-oxidant properties. They exhibit anti-inflammatory, hypocholesterolemic, anti-cancer and other biological properties (Liu *et al.*, 2016; Cheeke *et al.*, 2006; Marrelli *et al.*, 2016). This may be due to the various elemental compositions of the crude saponins. Steroidal glycosides are predominant found in wild plants, mainly the monocotyledonous angiosperms and have been used as alternative medicine in combating different ailments, thus aiding health maintenance.

MATERIALS AND METHODS

Dewaxing was carried out to remove the oil/wax from the plant samples. This was performed in a 1000ml capacity distillation flask using n-hexane as dewaxing solvent. A known mass of dewaxed sample was weighed in a round bottom flask containing a constant volume of ethanol and the mixture was carefully stirred for 60 minutes. The central composite method was employed for the experimental design. Reflux extraction technique was adopted in the extraction of saponin from the different plant materials (Zhang *et al.*, 2018: Sharma *et al.*, 2014). Ethanol and Methanol were used differently as extraction solvents. It involved the condensation of vapours and the return of the condensate to the system from where it originated.

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To test for presence of saponins, about 5mls of distilled water was added to the plant extract from the various biomaterials in test tubes and were shaken fervently. The formation of stable honey-comb foam indicated the presence of saponins. The lather when mixed with few drops of oil (olive) and shaken vigorously, led to the formation of emulsion as established by Gul *et al.,* 2017. The UV spectrophotometry which is based on adsorption of visible or ultra-violet light by molecular compounds was used to obtain the maximum wavelength of the extracted samples. This leads to production of spectra due to the interaction between light and matter which resulted in excitement of the electrons from the ground state to a higher energy state. Potassium bromide powder was mixed with the extracted powdered samples of yellow yam, wild yam and ginger, compressed to a thin potassium bromide (KBr) pellet, using a mortar and pestle. The resulting signal at the detector presented unique spectral, typically from 4000 to 650cm^{-1}, representing molecular functional group region and fingerprint region of the samples as established by Titus *et al.,* 2019. The functional group of each molecule or chemical structure of the saponins produced a unique spectral or the diagnostic region. The XRD analysis was performed to determine the crystalline nature of the extracted saponins. The saponin samples were irradiated with incident X-rays and the intensities and scattering angles of the X-rays leaving the materials were measured. The samples were analysed using the reflection-transmission spinner stage of Theta-Theta settings. Two-Theta starting position was 4 degrees and ends at 75 degrees with a two-theta step of 0.026261 at 8.67 seconds per step. The various samples/nomenclature were; GG-AD-ME: ginger, air dried methanol extract; GG-AD-ET: ginger, air dried ethanol extract; YY-AD-ME: yellow yam, air dried methanol extract; YY-AD-ET: yellow yam, air dried ethanol extract; WY-AD-ET: wild yam, air dried ethanol extract and WY- AD-ME: wild yam, air dried methanol extract.

RESULTS AND DISCUSSIONS

Results of Quantitative Analysis of Saponin

Quantitative determination of saponin was done using the method by Ejikeme *et al.,* 2014. The estimated amount of saponin contained in the yellow yam was 7.8%, wild yam gave 6.6%, while ginger was 4.9%.

Results of Qualitative Analysis of Saponin

The qualitative determination of Saponins from yellow yam, wild yam and ginger was carried out using the double beam spectrophotometer (UV-6300PC). The UV absorbance of saponins extracts from yellow yam had two peaks. The main peak absorbance at 369nm and shoulder peak at 450nm (figure 3.1).

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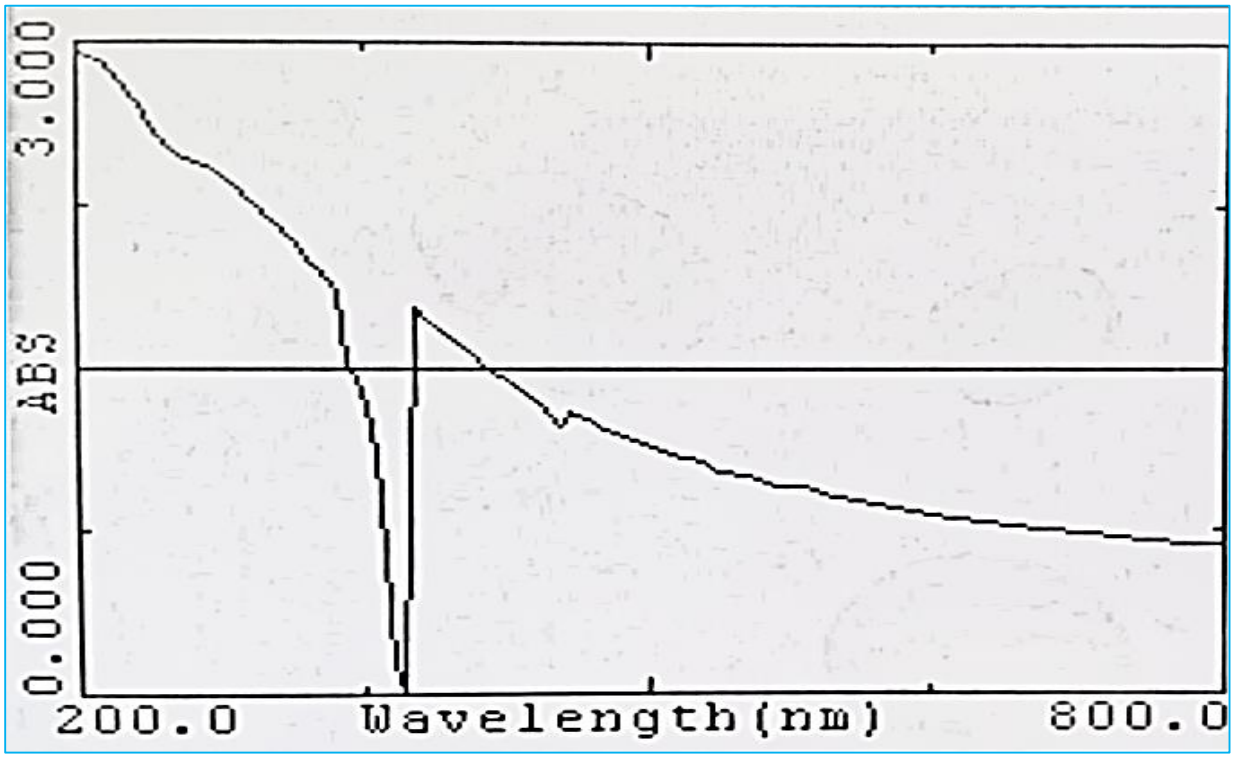

Figure 3.1: UV absorption spectrum of saponin from yellow yam

Similarly, the UV absorbance of crude saponin from wild yam and ginger had main peaks at 367nm and 369nm respectively; as shown in figures. 3.2 and 3.3 below, while the shoulder peaks were at 455nm and 466nm. The results of the UV analysis of the various samples from yellow yam, wild yam and ginger (yellow) suggested that steroidal saponins were present in the three samples. The steroidal saponins produced may have undergone microbial transformation during processing, storage and converted into diosgenin. This is similar to the work by Liu *et al*., 2021; they developed a new bioprocess of clean Diosgenin production through hydrolysis and microbial biotransformation from Dioscorea *zingiberensis* (yellow ginger). Research by Adiukwu *et al.*, 2017; showed the UV-vis detection of *Veronia amygdalina* saponin at 365nm. This was a representative of the pure steroidal saponin component and was determined using the double beam detector. Another research by Chapagain and Wiesman, 2005 determined the UV absorption spectrum of standard sapogenin (diosgenin) purchased from Sigma Incorporated. The diosgenin level was determined by measuring the absorbance at 430nm.

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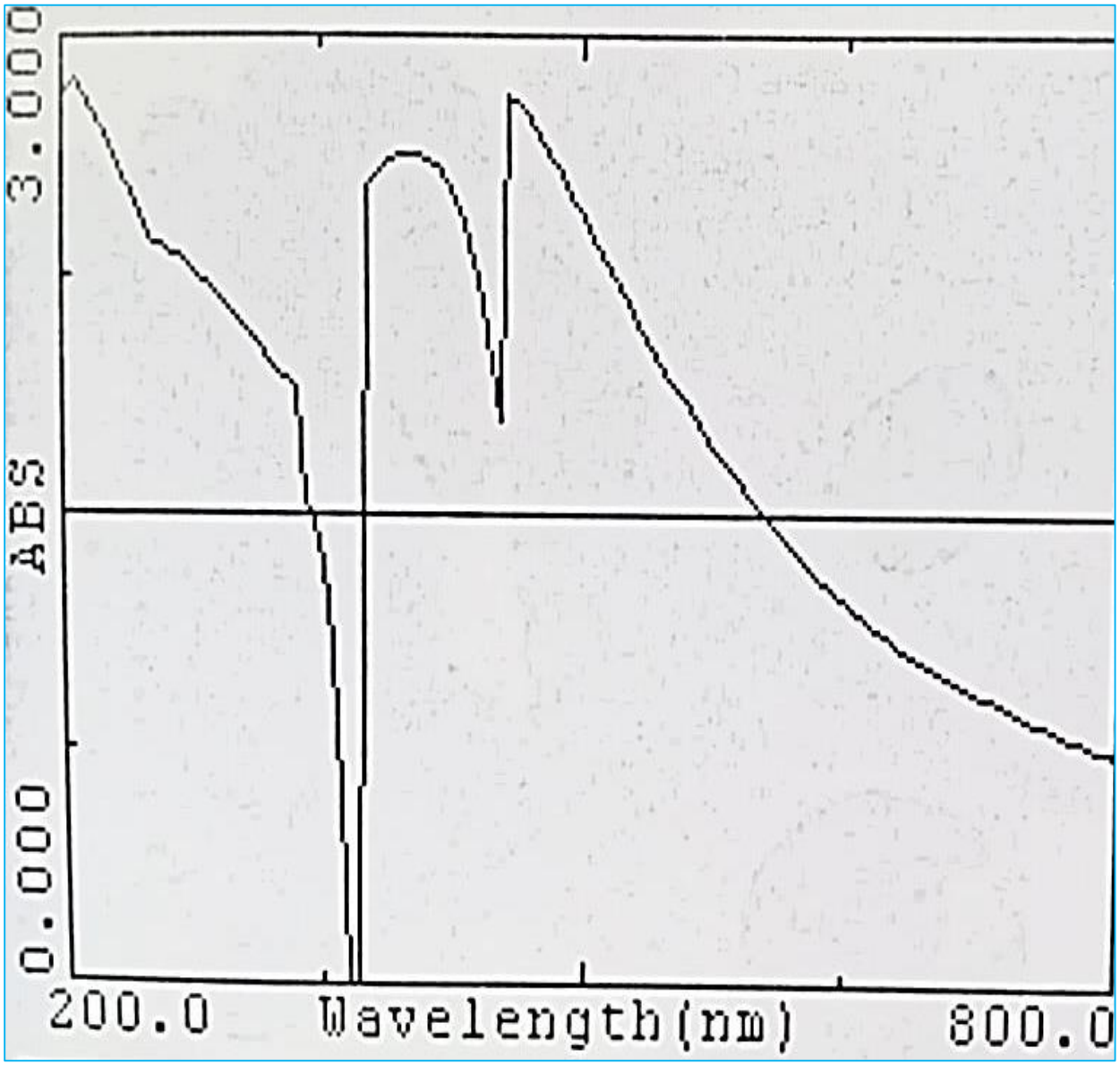

Figure 3.2: UV absorption spectrum of saponin from wild yam

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Figure 3.3: UV absorption spectrum of saponin from yellow ginger.

Fourier Transform Infrared (FTIR) Spectroscopy

The spectra for wild yam, yellow yam and ginger were collected in region of 4000 – 650 cm^{-1}, using the Agilent Cary 360 spectroscopy. The outcome of the FTIR spectra of saponins demonstrated characteristic steroidal absorptions of the functional groups OH, C=H, C=O, C=C and the finger print groups. The glycoside linkages to the aglycone or sapogenin were indicated by the absorptions of C-O. The aglycone or sapogenin may consist of either a sterol or the common triterpene unit.

The spectra for crude saponin extract for wild yam, ginger and yellow yam (Figures 3.4 – 3.9) showed a broad infrared absorbance of the hydroxyl group (OH) at 3283.8 and 3246.50 cm^{-1} which is similar to the previous studies by Wisetkomolmat *et al.*, 2020. They gave the characteristic absorbance of the hydroxyl (OH) group crude of methanolic and aqueous extracts of some detergent plants gave the peaks at wavenumbers 3325.52cm^{-1} and 3261.94cm^{-1}. Jahanbin et al., 2017 reported that it was a characteristic of polysaccharide. Almutairi and Ali, 2015 established in their study that saponins from soapnut showed a characteristic infrared absorbance of the OH group at 3407cm-1 in aqueous extract, 3419cm^{-1} in 95% ethanolic extract and that the absorbance ranged from 3525cm^{-1} to 3281cm^{-1} in the standard Quillaja saponin. Similar research by Bajad *et*

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al., 2019 showed the various characteristic bands of standard saponin. Absorbance of the hydroxyl group (OH) was evident at 3459 and 3323 cm^{-1}.

Carbon – hydrogen (C-H) absorption range 2922.2 – 2929.7 cm^{-1} was observed for all the extracted samples. The existing range correspond to the vibration of C-H stretching, especially methyl (CH_3) group (Liu *et al.*, 2017, Rashid *et al.*, 2018). This is similar to the IR spectra of saponins, as reported by Bajad *et al*., 2019 which showed the peak of 2924cm-1 for methyl (CH_3) group. C-H absorption at 2923.23 of the methanolic extract of L. *glutinosa* had been reported (Wisetkomolmat *et al*., 2020). The absorption of some phyto-based saponins from medicinal plants with carbon-hydrogen (C-H) absorbance at 2922cm^{-1} to 2929cm^{-1} had been mentioned (Kareru *et al*., 2008). Carbon-hydrogen (C-H) absorption was distinct at 2931cm^{-1}. An Indication of the aliphatic stretching vibrations (El Barky and Mohamed, 2020).

There C=C absorbance for all the samples (wild yam, yellow yam and ginger) ranged between 1632.6cm^{-1} to 1636.3cm^{-1}. This is similar to the work of Bajad *et* al., 2019 who recorded the C=C stretching at 1636cm^{-1} for IR spectra of saponins. In the study by Wisetkomolmat *et al*., 2020 they reported that the detergent plant (*A. concinna*) showed absorbance at 1620.33cm^{-1} while *L.* glutinosa had absorbance at 1606cm^{-1}

Oligosaccharide linkage absorption to sapogenins, that is C-O-C (typical of the finger print region) were found in all the extracted saponin samples, the absorbance was at 1073 – 1077cm^{-1}. This is similar to the work carried out on some detergent plants by Wisetkomolmat et al., 2020, where the absorbance varied in the methanolic extract from 1035.12cm^{-1} (*S. rarak*) to 1026.26cm^{-1} (*A. concinna*) and aqueous extracts of *S. rarak* with absorbance at 1034.78cm^{-1} and 1033.22cm^{-1} in *L. glutinosa.* Bajad *et al*., 2019 reported the absorbance of the C-O-C stretching of the glycoside linkage of Oligosaccharide to sapogenin to be at 1076cm^{-1}. Oligosaccharide linkage absorptions to sapogenins, that is C-O-C, were apparent at 1096 cm^{-1} region (Bajad *et* al., 2019; El Barky *et al.*, 2016).

EL Barky and Mohamed, 2020 established that bands at 1465 and 1388cm^{-1} were on behalf of the vibration of the C-H deformation, the signals at 1245 and 851cm^{-1} were referred to as fingerprint area of carbohydrates, among which the bands at 1018, 1082cm^{-1} were the characteristic absorptions of the pyranose ring.

Infrared spectroscopy is quite useful to characterize the specific kind of bonds, finger print region and functional group (diagnostic) region in plant extracts. The interpretation of these results is that saponins are present and detectable in the locally available plants (wild yam, yellow yam and

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ginger), extracted with ethanol and methanol using the FTIR spectroscopy as shown in the various finger prints below.

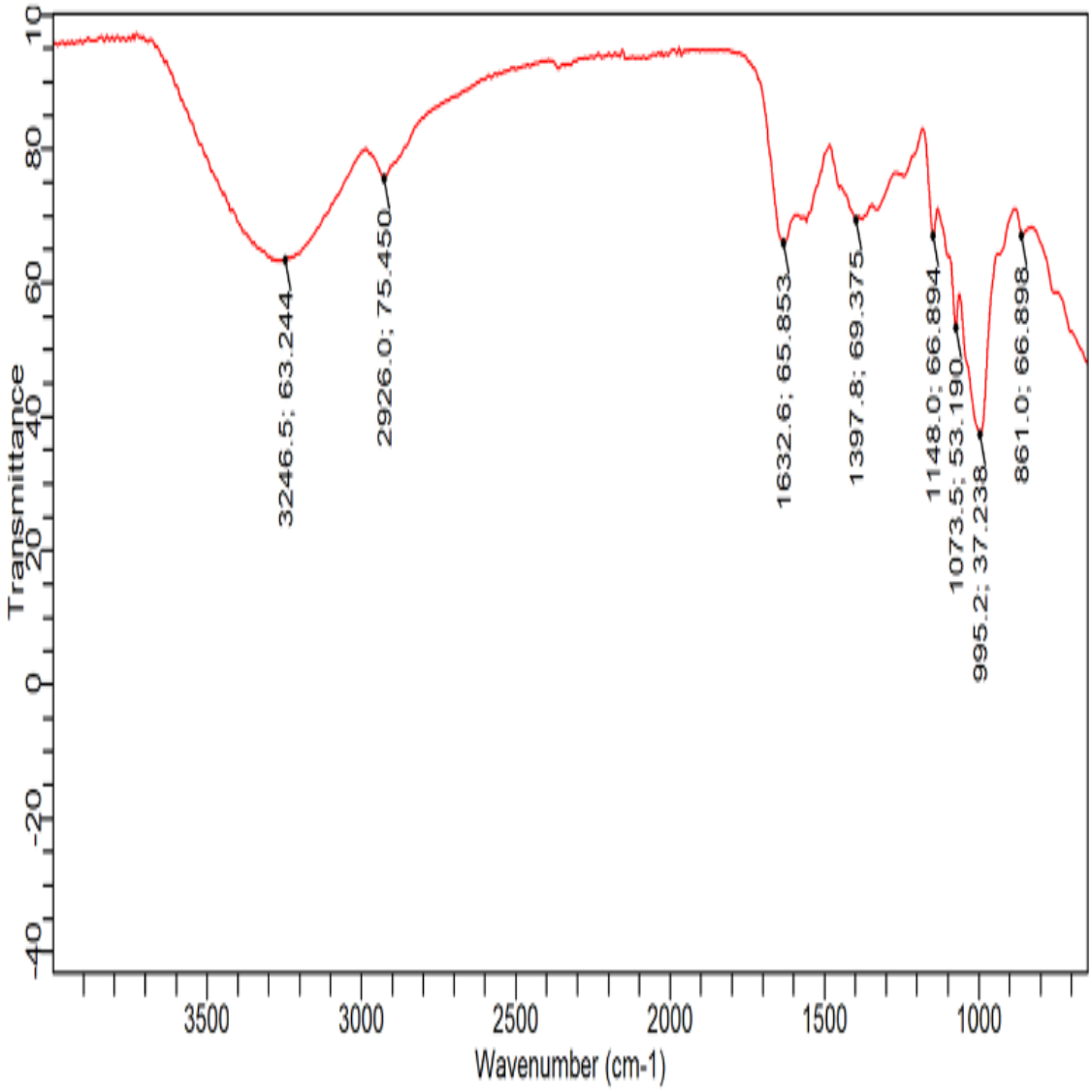

Figure 3.4: IR Absorbance of GG-AD-ME

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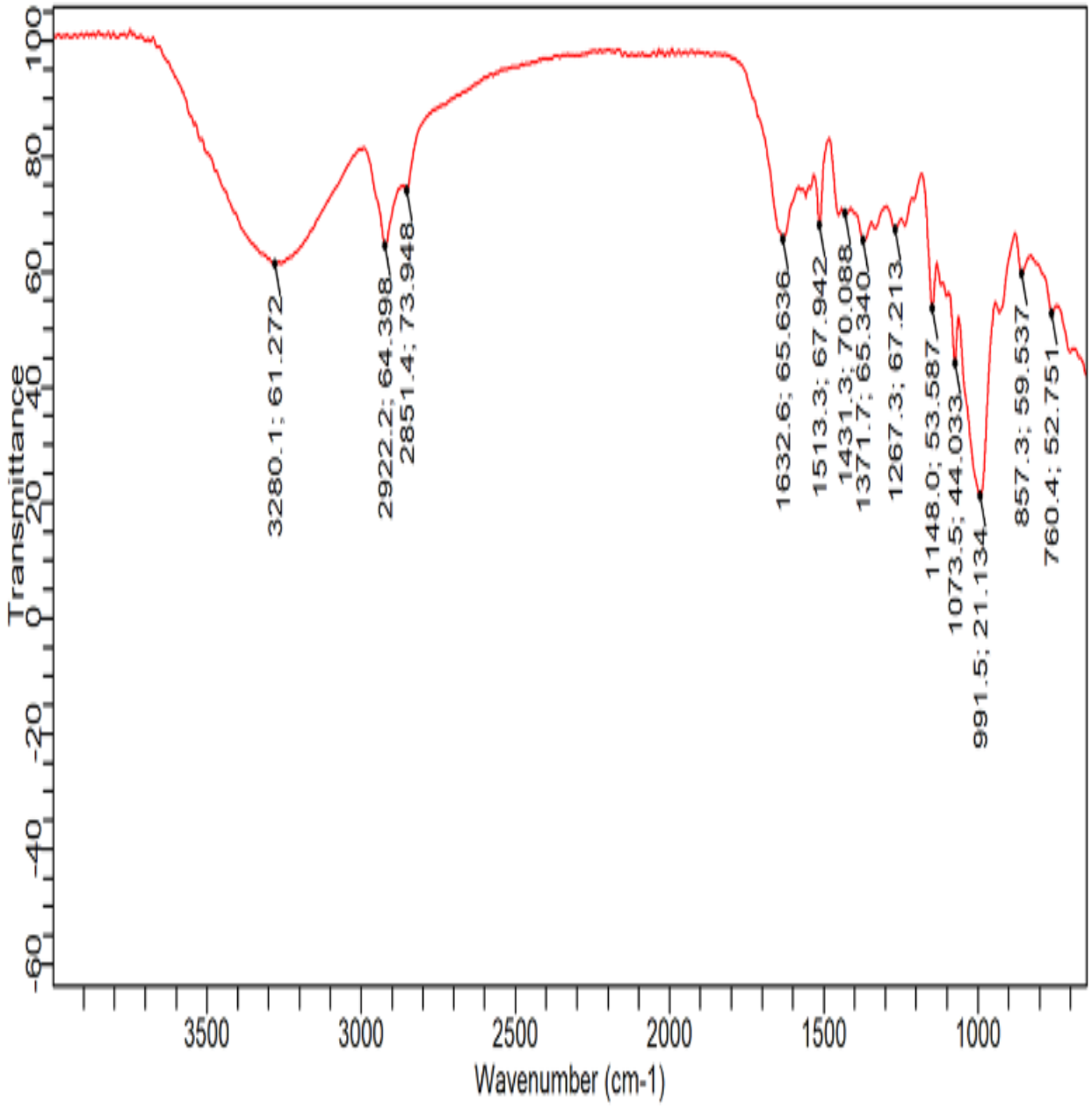

Figure 3.5: IR Absorbance of GG-AD-ET

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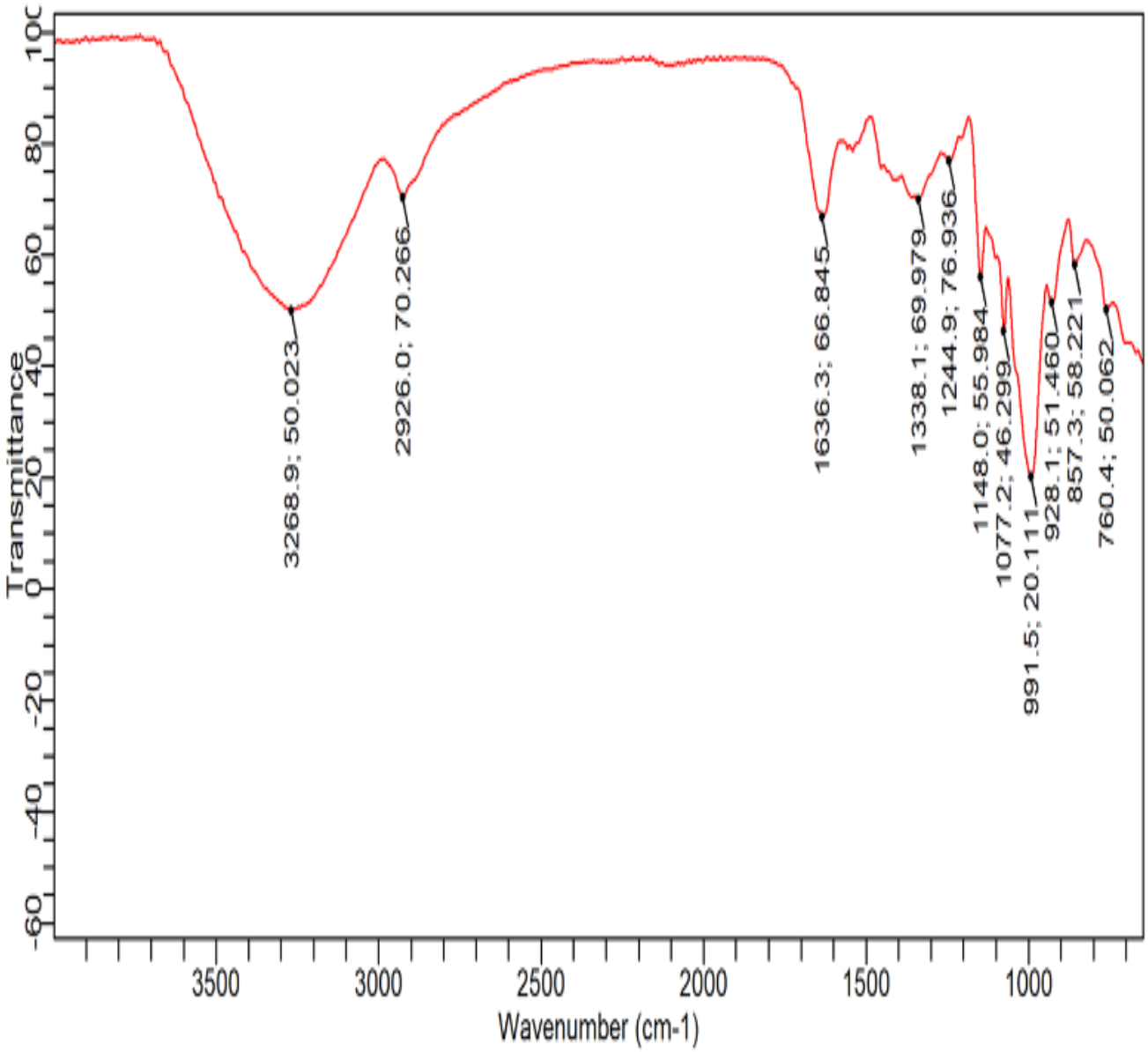

Figure 3.7: IR Absorbance of YY-AD-ME

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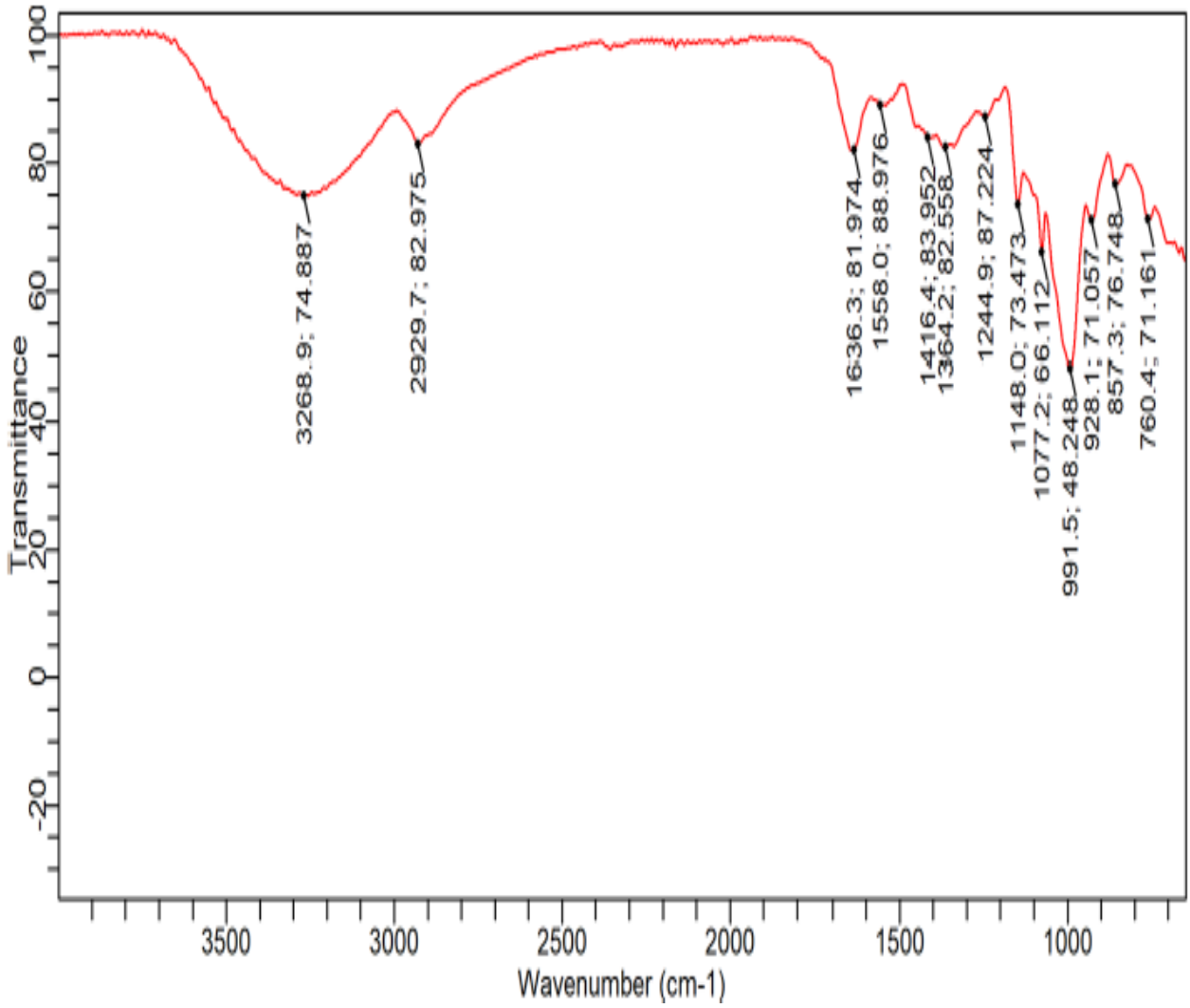

Figure 3.8: IR Absorbance of WY-AD-ET

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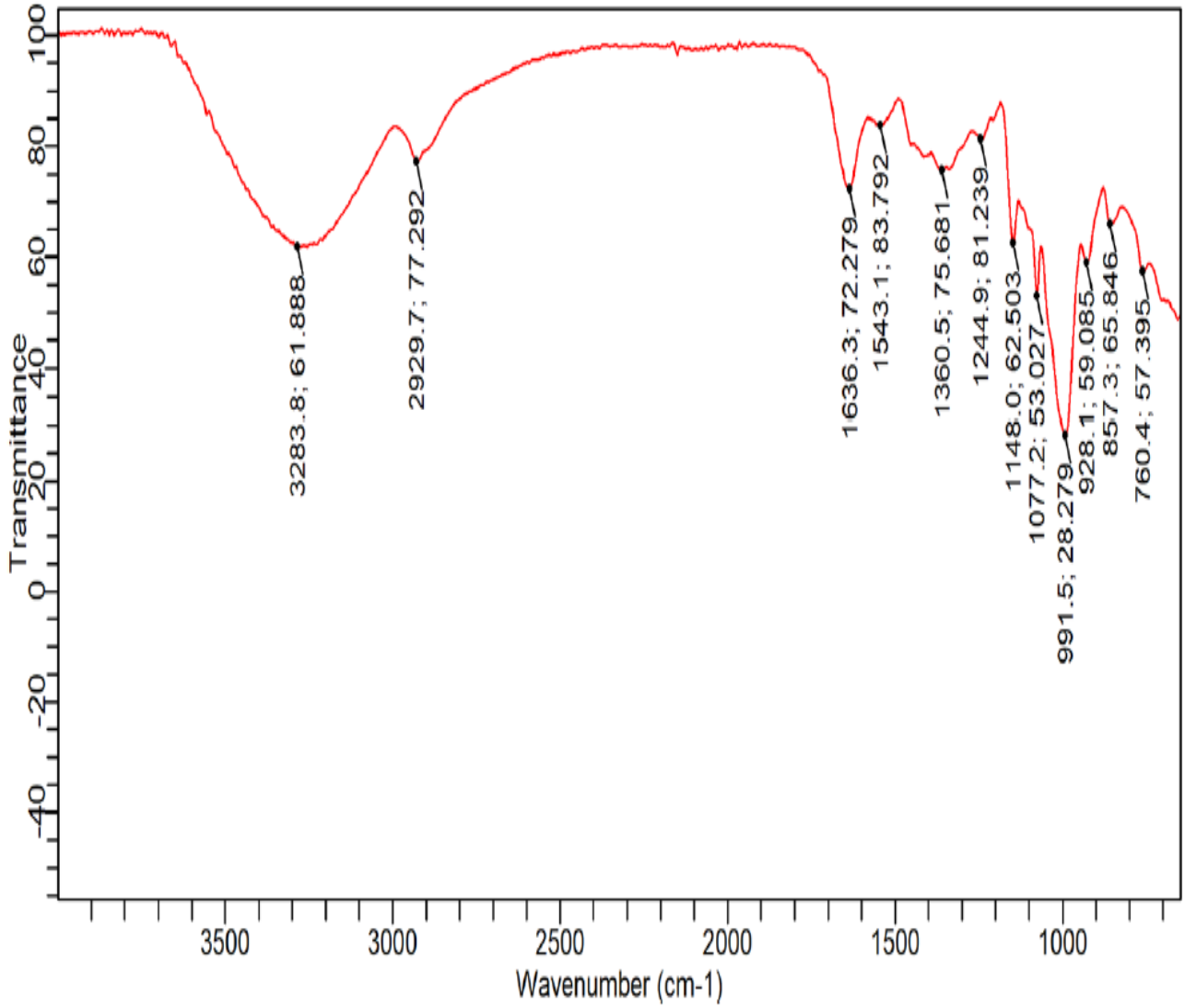

Figure 3.9: IR Absorbance of WY-AD-ME

The XRD analysis was performed to determine the crystalline nature of the extracted compounds and to provide the qualitative information of different elements in these compounds. Amorphous

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materials are have a broad hump without sharp and definite diffraction peaks. The presence of sharp diffraction peaks is an indication of crystallinity.

Table 3.0: Saponins Samples and Diffraction Peaks

Saponin Sample	Diffraction Peaks at 2θ values
WY-AD-ME	5.44, 14.72, 17.12, 19.50, 22.03, 23.96, 26.27, 34.20^0
WY-AD-ET	5.33, 15.07, 16.94, 21.85, 23.78, 26.16, 34.21^0
YY-AD-ET	5.39, 14.85, 17.01, 21.97, 24.13, 26.00, 34.19^0
YY-AD-ME	5.62, 14.91, 16.90, 22.06, 24.14, 26.16, 34.23^0
GG-AD-ET	15.06, 17.04, 17.96, 26.31^0
GG-AD-ME	15.05, 16.80, 17.95, 26.31^0

Results of the various saponin samples of wild yam, yellow yam and ginger depicted as WY-AD-ME, WY-AD-ET; YY-AD-ET, YY-AD-ME and GG-AD-ET, GG-AD-ME showed their diffraction peak at 2θ values as shown in table 3.0. It revealed that saponins are a group of amorphous colloidal glycosides and are well distributed in these plants (figures 3.12- 3.15). Their spectra did not possess distinctive and sharp diffraction peaks

However, GG-AD-ET and GG-AD-ME samples displayed diffraction peaks at 2θ values (figures 3.10- 3.11). This revealed that they are not entirely amorphous solids. Samples (wild yam, yellow yam and ginger) did not form crystals large enough, hence were analyzed by the X-ray powder diffraction (XRPD) technique. Since amorphous materials have higher energy, they have an increased tendency to undergo physical and chemical transformation (Nunes *et al*., 2005). During processing and storage, saponins show increased solubility and dissolution rates. This characterization is similar to the study carried out by El Barky *et* al., 2020. Their results were shown as 2θ (peak positions) and X-ray counts (intensity) in the form of x-y plot of the XRD spectra of a reference standard (commercial) saponin. Their diffraction peak values at 2θ were 12.70, 16.56, 19.67, 20.13, 21.41, 23.89, 27.73, 31.86, 37.63, 38.73 and 40.90°. El Barky *et* al., 2020; El Aziz *et al*., 2019 reported that the isolated solid saponins have a high molecular weight and they contain between 27 to 30 carbon atoms in a non-saccharide (aglycone) portion and were amorphous

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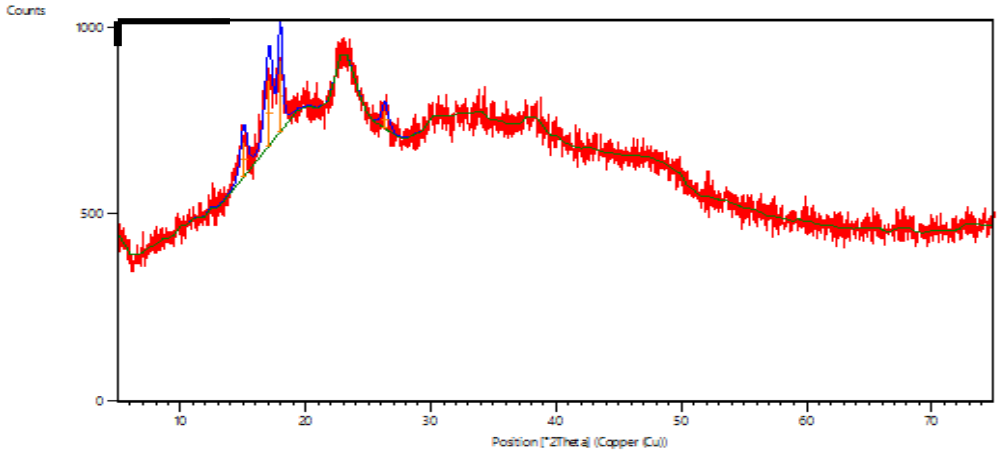

Figure 3.10: XRD Spectra of GG-AD-ET

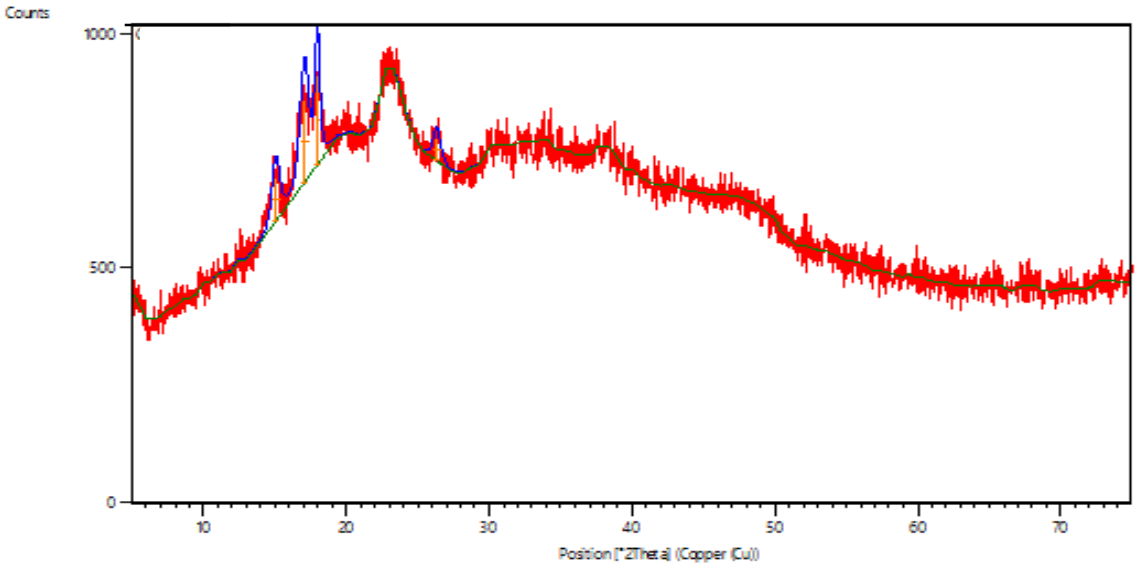

Figure 3.11: XRD Spectra of GG-AD-ME

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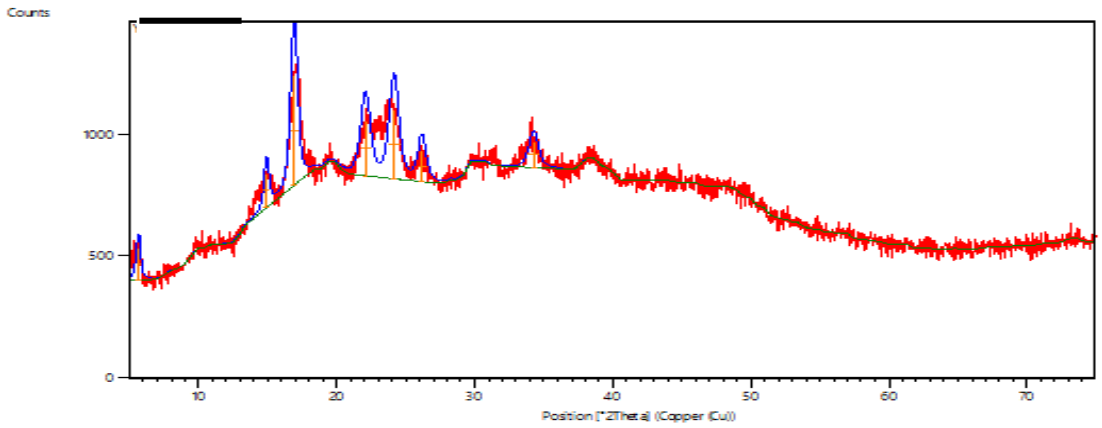

Figure 3.12: XRD Spectra of YY-AD-ME

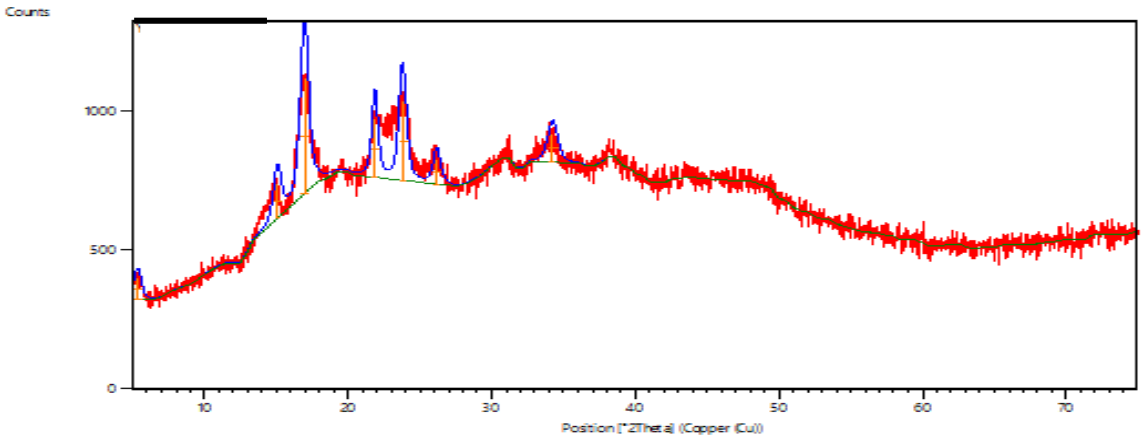

Figure 3.13: XRD Spectra of YY-AD-ET

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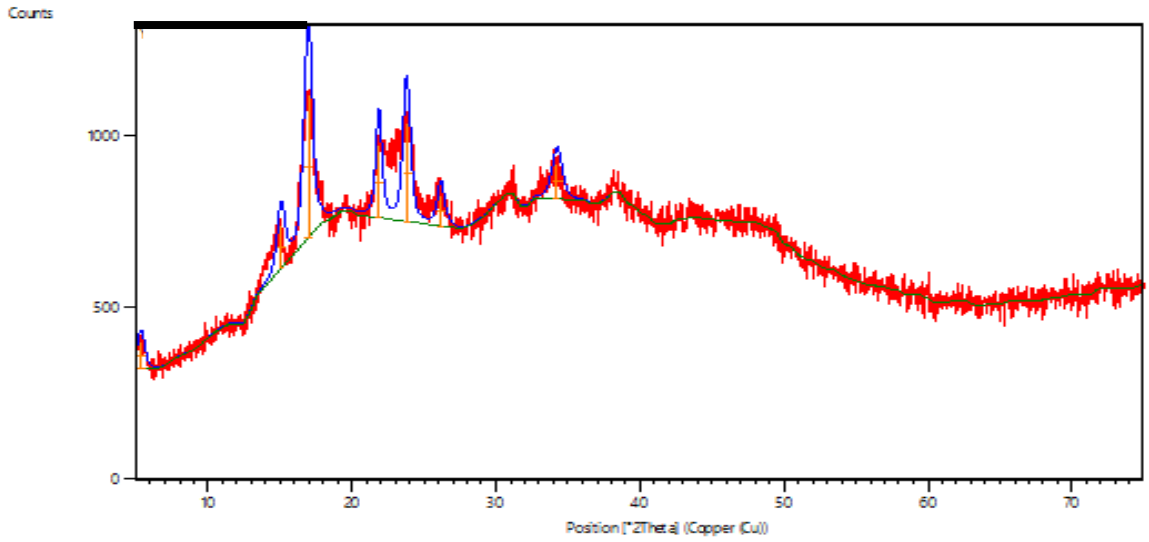

Figure 3.14: XRD Spectra of WY-AD-ET

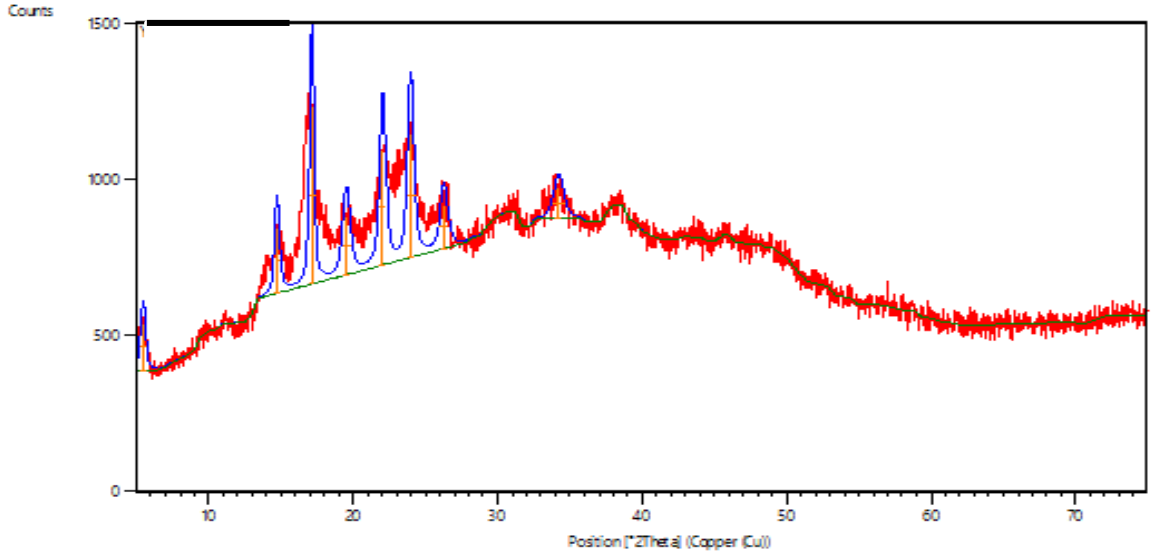

Figure 3.15: XRD Spectra of WY-AD-ME

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CONCLUSIONS

The interpretation of results of the various phytochemical screening established that steroidal saponins were present and detectable in the locally available plants. The detection and characterization of extracted saponins can be performed directly using spectroscopic methods of analysis such as FTIR, XRD and UV visible spectrophotometry. The methods were simple, fast and economical and results were comparable with standards and reference materials found in open literature.

REFERENCES

1. Adiukwu, P. C., Bonsu, M., Okon-Ben, I., Peprah, P., Mensah-Kane P., Jato, J., Nambatya, G. (2017). Ultraviolet spectroscopic evaluation of bioactive saponin fraction from the aqueous extract of *Veronia amygdalina* (esteraeceae) leaf. *International Journal of Biological and Chemical Sciences,*11(4): 1893-1902

2. Almutairi, M. S., Ali, M. (2015). Direct detection of saponins in crude extracts of soapnuts by FTIR. *Natural Products Research,* 29(13): 1-5

3. Augustin, J. M., Kuzina, V., Anderson, S. B., Bak, S. (2011). Molecular Activities, biosynthesis and evolution of triterpenoid Saponins. *Phytochemistry,* 72, 435- 457

4. Bajad, P. N., Pardeshi, A. B., Pagore, v. p. (2019). Extraction, isolation and quantification of Saponin from *Dodonaea viscosa* JACQ. *The Pharma Innovative Journal*, 8(5): 41-44

5. Chapagain, B., Wiesman, Z. (2005). Variation in diosgenin level inseed kernels among different provenances of *Balanites aegyptiaca* Del (Zygophyllaceae) and its correlation with oil content. *African Journal of Biotechnology,* 4(11): 1209-1213.

6. Cheeke, P. R., Piacente, S., Oleszek, W. (2006). Anti-inflammatory and anti-arthritic effects of Yucca Schidigera: A review. *Journal of Inflammation,* 3 (1) 6-13

7. Deng, F. M., Mu, T. H., Zang M., Abegunde, O.K. (2013). Composition Structure and Physico-Chemical Properties of Sweet Potato Starches Isolated by Sour Liquid Processing and Centrifugation. *Starch-Starke*, 65 (1-2): 162 – 171.

8. Ejikeme, C. M., Ezeonu, C. S., Eboatu, A. N. (2014). Determination of Physical and phytochemical Constituents of Some Tropical Timbers Indigenous to Niger Delta Area of Nigeria. *European Scientific Journal,* 10(18): 247-270

9. El Aziz, M., Ashour, A., Melad, A., A review on Saponins from Medicinal plants; chemistry, isolation and determination. *Journal of Nanomedicine Research,* 8(1): 282-288, 2019.

10. El Barky, A. R., Mohamed, T. M. (2020). Isolation, Characterization and Biological Activity of Some Natural Components of Marine Sea Cucumber and Orange Peel. *Biomedical Journal of Scientific and Technical Research,* 27(2): 20554-20565

11. El Barky, A. R., Hussein, S. A., Alm Eldeen, A. A., Hafez, Y. A., Tarek, M. (2016). Anti-diabetic activity of Holothuria thomasi Saponin. *Biomedicine and Pharmacotherapy,* 84, 1472-

1487
12. Gul, R., Jan, S. U., Faridullah, S., Sherani, S., Jahan, N. (2017).Preliminary Phytochemical Screening, Quantitative Analysis of Alkaloids, and Antioxidant Activity of Crude Plant Extracts from Ephedra intermedia Indigenous to Balochistan. *The Scientific World Journal* 2017, 1-7
13. Hossain, M. B., Brunton, N. P., Rai, D. K. (2016). Effect of drying methods on the steroidal alkaloid content of potato peels, shoots and berries. *Molecules*, 21 (4) 403-413
14. Jahanbin, K., Abbasian, A., Ahang, M. (2017). Isolation, purification and structural characterization of a new water-soluble polysaccharide from Eremurus stenophyllus (bioss. &buhse) baker roots. *Carbohydrate Polymers*, 178, 386-393
15. Kareru, P., Keriko, J., Gachanja, A., Kenji, G. (2008). Direct detection of triterpenoid saponins in medicinal plants. *African Journal of Traditional Complementary and Alternative Medicines*, 5(1): 56-60
16. Kregiel, D., Witonska, I. A., Antolak, H., Proestos, C., Berlowska, J., Babic, M., Zhang, B., Babic, L. (2017). Saponin Based Biological-Active Surfactants from plants. In book: Application and Characterization of Surfactants. InTech, (6): 183-205
17. Liu, D., Sun, Q., Xu, J., Li, N., Lin, J., Chen, S., Li, F. (2017). Purification, characterization and bioactivities of polysaccharide from mycelial fermentation of *Bjerkandera fumosa*. *Carbohydrate Polymers*, 167, 115-122.
18. Liu, W., Xiang, H., Zhang, T., Pang, X., Su, J., Liu, H., Ma, B., Yu, L. (2021). Development of a New Bioprocess for Clean Diosgenin Production through Submerged Fermentation of an Endophytic Fungus. *American Chemical Society Omega* 6, 9537-9548
19. Liu, J., Li, Y., Shi, H., Wang, T., Wu, X., Sun, X., Yu, L. (2016). Components characterization of total tetraploid jiaogulan (*Gynostemma pentaphyllum*) saponin and its cholesterol lowering properties. *Journal of Functional Foods*, 23, 542-555. .
20. Marrelli, M., Conforti, F., Araniti, F., Statti, G.A. (2016). Effects of saponins on lipid metabolism: A review of potential health benefits in the treatment of obesity. *Molecules*, 21, (10): 1404-1424.
21. Nowrouzi, I., Mohammadi, A. H., Mashad, A. K. (2020). Water-oil interfacial tension (IFT) reduction and wettability alteration in surfactant flooding process, using extracted Saponin from Anabasis setifera plant. *Journal of Petroleum Science and Engineering*, 189, 1-13
22. Numes, C, J., Suryanarayanan, R., Mahendrasingam, A. (2005). Quantification of Crystallinity in Amorphous Materials by Synchrotron X-ray Powder Diffractometry. *Pharmaceutical Research*, 22(11): 1942-1953
23. Rashid, F. Hussain, S., Ahmed, Z. (2018). Extraction, purification and characterization of galactomannan from fenugreek for industrial utilization. *Carbohydrate Polymers*, 180, 88-95
24. Sharma, R., Saxena, N., Thakur, G. S., Sanodiya, B. S., Jaiswal, P. (2014). Conventional Method for Saponin Extraction from Chlorophytum Borivilianum. Sant. et Fernard. *Global Journal of Research on Medicinal Plants & indigenous Medicine*, 3(2): 33-39.

https://doi.org/10.37745/ijeats.13

25. Sheng, H., Sun, H. (2011). Synthesis, biology and clinical significance of pentacyclic triterpenes:a multi-target approach to prevention and treatment of metabolic and vascular diseases. *Natural Product Reports,* 28(3): 543-593.
26. Titus, D., Samuel, J. J., Roopan, S. M. (2019). Nanoparticle characterization techniques. In book: Green Synthesis, Characterizationand Applications of Nanoparticles. *Micro and Nano Technologies*, pp. 303-319
27. Wisetkomolmat, J., Suksathan, R., Puangpradab, R., Kunasakdakul, K., Jantanasakulwong, K., Rachtanapun, P., Sommano, S. R. (2020). Natural surfactant saponin from tissue of Litsea Glutinosa and Its Alternative Sustainable Production. *Plants*, 9(11): 1521-1536.
28. Yuliana, N., Nurdjanah, S., Setyani, S., Novianti, D. (2017). Improving Properties of Sweet Potato Composite Flour: Influence of Lactic Acid Fermentation. *AIP Conference Proceedings*. 1854 (1): 1-5.
29. Zhang, Q., Lin, L., Ye, W. (2018). Techniques for extraction and isolation of natural products: A comprehensive review. *Chinese Medicine,* 13(1): 20-46