Comparative Study of Saponin Extraction from Tropical Plants and Their Prospective Application in Crude Oil Dispersion and Bioremediation – 1

By

Fatima Mohammed Maude[a], Abdulrahman Abdu Audu[a], Saudatu Chinade Ja'afaru[b], Haruna Tsoho Bala[c], Asma'u Mohammed Maude[d] and Amina Aminu Garba[e]

Abstract

Crude oil contamination poses significant environmental challenges, requiring eco-friendly remediation strategies. This study comparatively evaluated the extraction, purification, and functional performance of saponin-rich extracts from five tropical plants: Ziziphus mauritiana *(ZM),* Balanites aegyptiaca *(BA),* Glycine max *(GM),* Erythrina senegalensis *(ES), and* Euphorbia hirta *(EH). Ultrasound-assisted extraction with 70% ethanol was employed, followed by fermentation-based purification. Extracts were assessed for yield, total saponin content, purity, surface tension, critical micelle concentration (CMC), droplet size distribution, and emulsion stability. Results revealed substantial differences among species. BA showed the highest extraction yield (99.38%), whereas ES and EH contained the greatest total saponin content (653.95 and 525.80 mg/g, respectively). Purification efficiency was highest for ES (60.20%) and ZM (56.30%). Surface activity analysis demonstrated that BA and ZM achieved the lowest surface tension (30.53 and 31.31 mN/m) and CMC values (56.42 and 60.21 mg/L), indicating strong micellization. Dynamic Light Scattering showed ZM produced the smallest droplets (24.63 nm) and lowest PDI (0.208), yielding stable nanoemulsions with superior emulsification stability (81.39%). Comparative ranking indicated ZM > GM > ES > BA > EH, with ZM and GM emerging as the most promising biosurfactant sources. Overall, this work highlights the potential of tropical plant-derived saponins, particularly from ZM and GM, as sustainable alternatives to synthetic surfactants for crude oil dispersion and bioremediation.*

Keywords: Saponins; Biosurfactants**;** Tropical plants; Crude oil dispersion**;** Bioremediation

Introduction

Petroleum exploration and associated industrial activities have contributed significantly to global energy supply but at the expense of serious environmental challenges, particularly crude oil contamination of soils and aquatic ecosystems[1]. Hydrocarbon pollution leads to the deterioration of soil fertility, disruption of microbial communities, and long-term ecological damage due to the persistence of petroleum hydrocarbons in the environment[2]. Conventional treatment methods, including mechanical recovery, synthetic dispersants, and soil washing,

[a] Department of Pure and Industrial Chemistry, Bayero University Kano, Kano State, Nigeria
[b] Department of Chemistry, Aliko Dangote University of Science and Technology, Wudil, Kano State, Nigeria
[c] Department of Biochemistry, Bayero University Kano, Kano State, Nigeria
[d] Department of Microbiology, Federal University of Technology, Minna, Niger State, Nigeria
[e] Department of Physical Sciences, Kano State Polytechnic, Kano State, Nigeria.
*Corresponding Author; Fatima Mohammed Maude, Department of Pure and Industrial Chemistry, Bayero University Kano, Kano State, Nigeria, +2348035917809, fm.maude@yahoo.com

[1] Adeola AO, Akingboye AS, Ore OT, Oluwajana OA, Adewole AH, Olawade DB, et al. Crude oil exploration in Africa: socio-economic implications, environmental impacts, and mitigation strategies. Environment Systems and Decisions. 2022;42(1):26-50.

[2] Mohanta S, Pradhan B, Behera ID. Impact and remediation of petroleum hydrocarbon pollutants on agricultural land: a review. Geomicrobiology Journal. 2024;41(4):345-59.

though widely employed, often present drawbacks including high cost, limited efficiency, and secondary pollution [3]. As a result, environmentally friendly and sustainable alternatives are being considered to minimize the environmental impact of crude oil pollution.

Among nature derived compounds, saponins (a class of amphiphilic glycosides which are found extensively in plants) have been shown the promise as environmentally friendly surfactants. Recently, studies by Kaur, Rasanpreet and co-workers among other researchers have reported saponins as potential eco-friendly surfactants [4 5 6]. Saponins have hydrophilic sugar moieties and hydrophobic aglycone structures that can lower surface and interfacial tension, emulsify hydrophobic substances, and promote the solubilization of lipophilic non-polar contaminants [5]. Saponins are characterised by having hydrophilic sugar molecules and at least one hydrophobic aglycone residue, thus is able to act and reduce surface tension, emulsifying the hydrophobic matter and solubilizing contaminants [5]. Their biodegradable and low-toxic nature, as well as their abundance render them convenient for use in soil and water purification processes [7]. Apart from their commercial applications in pharmaceutical, cosmetic and food industries, saponins are known to improve crude oil dispersion and increase the bioavailability of crude oil for enhanced microbial degradation of hydrocarbons [8].

Tropical plants can be a less studied source of saponins but may contain high levels of saponins. Plants like Ziziphus mauritiana (ZM), Balanite aegyptiaca L (BA), Glycine Max (GM) and Erythrina senegalensis DC. (ES) and Euphorbia hirta L.(EH) are widely distributed throughout Sub-Saharan Africa and they have for longtime been made use of in traditional medicine and some local industries [9 10]

Previous studies have highlighted these tropical plants as saponin containing species with a wide range of structural variations and functional properties [11]. Despite this, little comparative

[3] Gertsen MM, Arlyapov VA, Perelomov LV, Kharkova AS, Golysheva AN, Atroshchenko YM, et al. Environmental Implications of Energy Sources: A Review on Technologies for Cleaning Oil-Contaminated Ecosystems. Energies (19961073). 2024;17(14).

[4] Kaur R, Mishra V, Gupta S, Sharma S, Vaishnav A, Singh SV. Industrial and environmental applications of plant-derived saponins: an overview and future prospective. Journal of Plant Growth Regulation. 2024;43(9):3012-26.

5 Diaz-Cruces E, Tom T, Gomez-Lopez VM, Negrete-Bolagay D, Hermoso-Gil J, Miro-Colmenarez PJ, et al. Saponins as Natural Emulsifiers: Challenges, Regulatory Landscape, and Future in Biomedical and Cosmetic Fields. Industrial & Engineering Chemistry Research. 2025;64(12):6217-39.

6 El-Saadony MT, Saad AM, Mohammed DM, Korma SA, Alshahrani MY, Ahmed AE, et al. Medicinal plants: bioactive compounds, biological activities, combating multidrug-resistant microorganisms, and human health benefits-a comprehensive review. Frontiers in immunology. 2025;16:1491777.

7 Garcia-Garcia A, Muñana-González S, Lanceros-Mendez S, Ruiz-Rubio L, Alvarez LP, Vilas-Vilela JL. Biodegradable natural hydrogels for tissue engineering, controlled release, and soil remediation. Polymers. 2024;16(18):2599.

8 Pandit NK, Meena SS. Exploring Sustainable Biosurfactant Production Through Waste Valorization: Emerging Research Trends and Industrial Applications. Waste and Biomass Valorization. 2025:1-35

9 Chisoro P, Mazizi B, Jaja IF, Assan N, Nkukwana T. Sustainable utilization of wild fruits and respective tree byproducts as partial feed ingredients or supplements in livestock rations. Frontiers in Animal Science. 2025;6:1501412.

10 Jan A, Adil S, Ali T, Ahmed B, Ahmed Z, Hussain Z. Desert and Medicinal Plants as Novel Sources of Antimicrobial Agents for Crop Protection. Planta Animalia. 2025;4(3):197-218.

[11] Puspitasari YE, De Bruyne T, Foubert K, Aulanni'am Aa, Pieters L, Hermans N, et al. Holothuria triterpene glycosides: a comprehensive guide for their structure elucidation and critical appraisal of reported compounds. Phytochemistry Reviews. 2022;21(4):1315-58.

research was conducted on how best to extract, purify, and apply their saponin-rich extracts in environmental remediation.

Purifying crude saponin extracts is crucial for improving their effectiveness and consistency in such applications. Conventional solvent-based purification methods often leave behind impurities, which can limit performance and make large-scale use difficult. In contrast, fermentation-based purification has recently gained attention as a greener alternative, capable of selectively increasing saponin concentration while minimizing unwanted secondary metabolites[12].

In this study, we explore the extraction and purification of saponins from selected tropical plants and assess the potential of the purified extracts to improve crude oil dispersion in contaminated soils. By combining phytochemical characterization with environmental performance testing, our work contributes new insights into how plant-derived surfactants can be harnessed sustainably for the bioremediation of hydrocarbon-polluted environments.

2.0 Methodology

2.1 Sampling and Identification of Plant Materials

The plant samples were freshly plucked from their respective trees in various locations in Kano State, Nigeria and were taxonomically identified and certified by a botanist in Plant Science Department, Bayero University Kano. Table 1 and Figure 1 are respectively a list and pictures of the plants used in this study.

Table 1: Saponin-Rich Plants Utilized in the Study

Scientific Names	English Common Names	Hausa Names	Parts Used
Balanite aegyptiaca L	Desert date	Aduwa	Fruits
Euphorbia hirta L.	Asthma weed/plant	Nonon-Kurciya	Whole plant
Glycine max	Soya bean	Waken suya	Seeds
Ziziphus mauritiana	Jujube, plum tree	Magarya	Leaves
Erythrina senegalensis DC.	Coral tree/flower	Minjirya	Stem-bark

[12] Xu X, Wang G, Xie F, Song X, Zhang H, Xiong Z, et al. Fermentation of Plant-Based Yogurt and Its Effects on Phytochemicals: A Review. Food Reviews International. 2025:1-22.

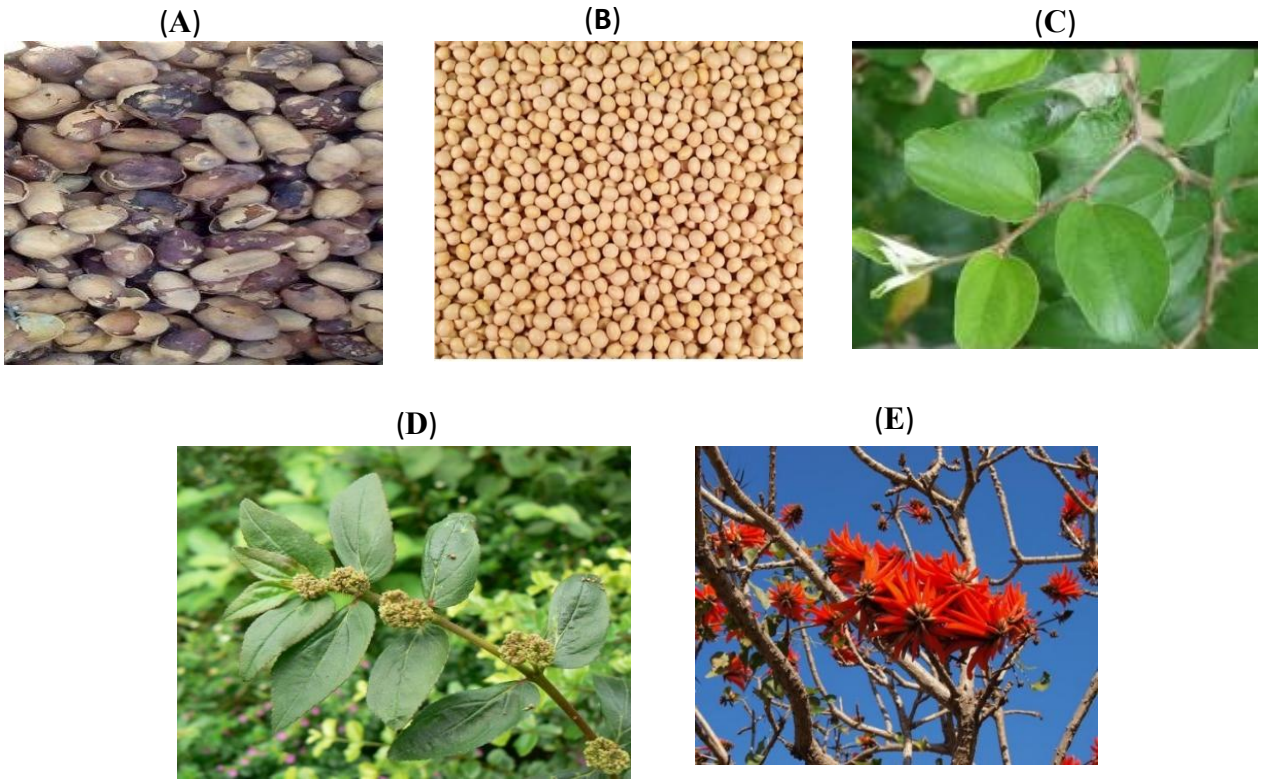

Figure 1: Pictorial illustrations of the plant extracts employed (**A**) - Desert date fruits, (**B**) - Soya Bean Seeds, (**C**) - Jujube/Plum Tree, (**D**) - Asthma weed/plant and (**D**) - Coral tree/flower

2.2 Sample Preparation

The leaves, fruit, seed or stem bark (as the case may be) of the saponin rich plants were separated from their stalks, rinsed with clean water, and air dried. Prior to analyses, each of the dried sample was pulverized into a fine powder using a mechanical mill and passed through a sieve of approximately 0.5mm and stored in an air tight jar [13]. Prior to extraction process, the pulverized samples were oven-dried at 40°C for 1 hour to eliminate any residual moisture [14].

2.3 Extraction of Saponins

Extraction of saponin from the various plant parts was carried out using ultrasound-assisted extraction (UAE) method. The procedure was conducted using the optimized ultrasonic-assisted conditions [15]. Aqueous ethanol (70%) at a ratio of sample to solvent of 1:15 (w/v) was used to extract the powdered plant parts (leaves, seeds or stem-bark) for 20 minutes and repeated in triplicate. The ultrasonic conditions were set to a frequency of 800 Hz at 20°C. The mixture was then centrifuged at 3400 × g for 10 minutes and the supernatants were then dried

[13] Imuetinyan H, Agi A, Gbadamosi A, Junin R. Extraction, characterization and evaluation of saponin-based natural surfactant for enhanced oil recovery. Arabian Journal of Geosciences. 2022;15(3):226
[14] Rai S, Kafle A, Devkota HP, Bhattarai A. Characterization of saponins from the leaves and stem bark of Jatropha curcas L. for surface-active properties. Heliyon. 2023;9(5).
[15] Zhang L, Li J, Huo Y, Yang W, Chen J, Gao Z, et al. Ultrasonic extraction and antioxidant evaluation of oat saponins. Ultrasonics sonochemistry. 2024;109:106989.

under vacuum using a rotary evaporator at 50°C[16]. The Extraction Yield (EY) was estimated and expressed as shown in equation 1:

$$\text{Extraction Yield (\%)} = \frac{\text{Weight of Extract}}{\text{Weight of Plant Material}} \times 100 \quad (1)$$

2.4 Purification of Saponins by Fermentation

2.4.1 Yeast Fermentation of Crude Saponin Extract

Yeast Activation

Distilled water (15 mL) and crude saponin extract (15 mL) were used to rehydrate *Saccharomyces cerevisiae* dry yeast (3 g). The yeast solutions were then activated by placing them in a water bath at 35°C for 30 min and were used for further fermentation [17].

Fermentation Method

The crude saponin extract (150 mL) was used to inoculate the activated yeast and air tight containers were incubated at 25-35 °C and kept away from direct sunlight for 4 days (96 hours) [17]. Upon sampling the fermented extract, the yeast cells were eliminated by centrifugation at 8000 rpm for 10 mins [18] and the supernatant obtained was used to determine :

(i) Total saponins according to the Vanillin-Sulfuric acid assay [19].
(ii) Content of soluble solids
(iii) Purity %= $\frac{Wi}{WS} \times 100$

Where, purity% represents the purity of total saponins, *Wi* (g) is the weight of total saponins in the extract, and *Ws* (g) is the soluble solid content [20].

2.5 Screening for Saponin (Frothing Test)

The extract (2.5 mL) was combined with 1.25 mL of distilled water which was then shaken vigorously resulting in the formation of a significant amount of lather, indicating the presence of saponins[21].

---

[16] Del Hierro JN, Herrera T, García-Risco MR, Fornari T, Reglero G, Martin D. Ultrasound-assisted extraction and bio accessibility of saponins from edible seeds: Quinoa, lentil, fenugreek, soybean and lupin. Food research international. 2018;109:440-7.
[17] Le X-T, Tran-Thi T-A, Phuong K-T, Nguyen-Kim M-T, Dao TP. Improvement in extraction and sensory properties of soapnut extract by fermentation. Polish Journal of Chemical Technology. 2023;25(2).
[17] Le X-T, Tran-Thi T-A, Phuong K-T, Nguyen-Kim M-T, Dao TP. Improvement in extraction and sensory properties of soapnut extract by fermentation. Polish Journal of Chemical Technology. 2023;25(2).
[18] Heng W, Ling Z, Na W, Youzhi G, Zhen W, Zhiyong S, et al. Extraction and Fermentation-Based Purification of Saponins from Sapindus mukorossi Gaertn. Journal of Surfactants and Detergents. 2015;18(3):429-38.
[19] Madhu M, Sailaja V, Satyadev T, Satyanarayana M. Quantitative phytochemical analysis of selected medicinal plant species by using various organic solvents. Journal of Pharmacognosy and Phytochemistry. 2016;5(2):25-9
[20] Chen C, Li R, Li D, Shen F, Xiao G, Zhou J. Extraction and purification of saponins from Sapindus mukorossi. New Journal of Chemistry. 2021;45(2):952-60.
[21] Ajuru MG, Williams LF, Ajuru G. Qualitative and quantitative phytochemical screening of some plants used in ethnomedicine in the Niger Delta region of Nigeria. Journal of food and Nutrition Sciences. 2017;5(5):198-205.

2.6. Total Saponin Content

The vanillin–sulphuric acid colorimetric assay was used to determine the total saponin content (TSC) of the plant extracts following the procedure of Du MengHao and colleagues, with slight modifications[22]. Briefly, 1.0 mL of fermented plant extract was transferred into a 10 mL stoppered test tube and mixed with 1.0 mL of 8% (w/v) vanillin in ethanol. 8 mL of 72% (v/v) sulphuric acid was then slowly added to the tubes placed in an ice water bath, and the mixture was incubated for 30 mins in a water bath at 60 °C. The tubes were allowed to cool to room temperature in an ice-water bath. The absorbance reading of the resulting solutions were measured at 560 nm with UV-VIS spectrophotometer.

Using various concentrations of a commercial pure saponin solution as a standard, a calibration curve was generated. The saponin concentration in the extracts were calculated from a linearized equation obtained from the calibration plot. Based on the total saponin amount and the initial mass of the powdered extract, the total saponin content (mg/g) was calculated according to equation 2 [23]

$$\text{Total Saponin Content (mg/g)} = \frac{Total\ saponin\ amount\ (mg)}{Initial\ mass\ of\ powdered\ extract\ (g)} \quad (2)$$

2.7 Preliminary/ Performance Analysis of the Saponin-Rich Extracts

2.7.1 Solubility Test

Dried saponin-rich extracts, placed in a desiccator over calcium oxide (CaO), were tested for their solubility in different solvents (water, methanol, ethanol, chloroform, petroleum ether, dilute NaOH, and dilute HCl). For each test, approximately 3 mg of each extract was added to 3 mL of solvent until saturation was obtained, and solubility was determined [14].

2.7.2 Measurement of Surface Activity and Critical Micelle Concentration (CMC)

As adopted by Huck-Iriart and team workers, capillary rise method was used to measure the surface tension of the different saponin-rich extracts [24]. Prior to the experiment, various concentrations of the plant extracts (20, 50, 100, 200, and 400 mg/L) were prepared. Capillary tubes which were uniquely labelled were selected and cleaned thoroughly upon usage. Each tube was immersed in a beaker of the extract solution to ensure that both their interior and exterior surfaces were fully wet, a step necessary to prevent errors related to trapped air or incomplete wetting. An optical pin was positioned so that its tip just contacted the liquid surface; this served as the reference level. The pin was then left in place while the microscope was aligned so its horizontal cross-wire was tangential to the pin's tip, and the corresponding microscope reading was recorded as the baseline.

[22] Du MengHao DM, Guo ShaoHai GS, Zhang JinPing ZJ, Hu LiSong HL, Li MingZe LM. Quantitative analysis method of the tea saponin. 2018.

[23] Aryanti N, Khoiriyah Ll, Nafiunisa A, Widiasa IN, Zakki A, Adina AR. Microwave-assisted extraction of eco-friendly surfactant from Jatropha curcas for sustainable solubilization of reactive dyes. Communications in Science and Technology. 2025;10(1):52-8.

[24] Huck-Iriart Cn, De-Candia A, Rodriguez J, Rinaldi C. Determination of surface tension of surfactant solutions through capillary rise measurements: An image-processing undergraduate laboratory experiment. Journal of Chemical Education. 2016;93(9):1647-51.

Subsequently, the beaker of the extract solution was returned to its original position, and one of the wetted capillary tubes was carefully inserted into the solution, taking care that no droplets remained on its surface. Through the microscope, the meniscus of the extract solution rising within the tube was brought into focus such that it lay tangential to the horizontal cross-wire, and that reading was recorded. The height of the capillary rise, h, was determined by subtracting the baseline (pin) reading from the meniscus reading.

This procedure was repeated for each of the remaining capillary tubes under the same conditions for the given concentration and thereafter repeated sequentially for each extract concentration (20, 50, 100, 200, 400 mg/L). To determine the internal radius r of each capillary tube, measurements of internal diameter were taken using the travelling or vernier microscope by focusing on opposite inner walls ideally in two perpendicular orientations to negate any deviation from circularity and averaging these measurements before halving them.
Using the measured values of capillary rise h, internal radius r, the density of the liquid *p,* (at the recorded temperature), and standard acceleration due to gravity g, the surface tension T of the liquid was calculated using the capillary rise equation 3:

$$T = \frac{Pghr}{2} \quad (3)$$

After completing measurements across all concentrations, surface tension values were plotted against concentration and the critical micelle concentration (CMC) was estimated by the intersection between the straight lines formed in the surface tension plot as a function of surfactant concentration [25].

2.7.3 Emulsification Stability Test

The stability of an emulsion serves as a key indicator of surfactant activity. The procedure of da Silva and colleagues and that of Hajimohammadi and team-members was adopted to measure the emulsification index of the various saponin-rich extracts [26] [27] Equal volume of crude oil and each extract in a test tube was vortexed for 1 min and the emulsification stability was calculated after 24 hours (E_{24}) according to equation 4:

$$E_{24} = E/H \times 100 \quad (4)$$

Where *E* is the measured height of the emulsion layer and *H* the total height of the mixture, both expressed in cm. Tests were performed in triplicate.

[25] Kariyawasam T, Prenzler PD, Howitt JA, Doran GS. Eucalyptus saponin-and sophorolipid-mediated desorption of polycyclic aromatic hydrocarbons from contaminated soil and sediment. Environmental Science and Pollution Research. 2023;30(8):21638-53.
[26] da Silva IGS, de Almeida FCG, da Rocha e Silva NMP, de Oliveira JTR, Converti A, Sarubbo LA. Application of green surfactants in the remediation of soils contaminated by hydrocarbons. Processes. 2021;9(9):1666.
[27] Hajimohammadi R, Hosseini M, Amani H, Najafpour GD. Production of saponin biosurfactant from Glycyrrhiza glabra as an agent for upgrading heavy crude oil. Journal of Surfactants and Detergents. 2016;19(6):1251-61.

2.7.4 Droplet size Distribution Analysis

The mean droplet size of the prepared water-in-oil (W/O) emulsions was assessed using Dynamic Light Scattering (DLS) equipment. Measurements were repeated at least 6 times per sample to calculate the average diameter and polydispersity index (PDI). The analysis was carried out at a temperature set at 25 °C with an equilibration time of 3 mins, and a laser wavelength of 658 nm. The procedure involved automatic laser beam regulation, with optics adjusted for each measurement and the autocorrelation functions were registered for backscattered light at a specific detection angle of 175° [28].

3.0 Results and Discussion

Extraction Yield of Saponin-Rich Extracts

The extraction yields of the selected plants using ultrasound-assisted extraction with 70% ethanol varied markedly (Figure 2). *BA* recorded the highest yield (99.38%), followed by moderate yields in *ZM* (30.71%), *EH* (20.7%), and *GM* (17.91%), while *ES* produced the lowest (10.89%). The exceptional yield from *BA* is attributed to the complete dissolution of its pericarp, enabling almost full solubilization of saponins, as similarly observed in other saponin-rich fruits [16]. Conversely, the limited recovery in *ES* likely reflects restricted release due to a denser tissue matrix.

The use of ultrasound-assisted extraction (UAE) significantly improved recovery across all samples by facilitating cell wall disruption and enhancing solvent penetration [29 30]. UAE has been widely reported to outperform conventional techniques by increasing yield, reducing extraction time, and preserving bioactive compounds [31 32]. In addition, the choice of 70% ethanol proved effective in solubilizing both hydrophilic and moderately lipophilic saponins, consistent with reports identifying aqueous ethanol (50–80%) as optimal for total saponin extraction [33 34]. The difference in extraction yields among the studied plants may also be linked to differences in saponin biosynthesis and tissue distribution. For instance, legumes such as *GM* are known to contain moderate levels of saponins distributed within complex cellular matrices, which may hinder their efficient recovery compared to fruits such as *BA* with readily extractable pericarps [16].

---

[28] Jarzębski M, Siejak P, Smułek W, Fathordoobady F, Guo Y, Pawlicz J, et al. Plant extracts containing saponins affects the stability and biological activity of hempseed oil emulsion system. Molecules. 2020;25(11):2696.

[29] Quaratesi I, Calinescu I, Lavric V, Ferrara V, Badea E, Chipurici P, et al. Loop-ultrasound-assisted extraction: An efficient approach for the recovery of bioactive compounds from oak bark. Agronomy. 2024;14(7):1452.

[30] Gavrila AI, Tatia R, Seciu-Grama A-M, Tarcomnicu I, Negrea C, Calinescu I, et al. Ultrasound assisted extraction of saponins from Hedera helix L. and an in vitro biocompatibility evaluation of the extracts. Pharmaceuticals. 2022;15(10):1197.

[31] Wen L, Zhang Z, Sun D-W, Sivagnanam SP, Tiwari BK. Combination of emerging technologies for the extraction of bioactive compounds. Critical reviews in food science and nutrition. 2020;60(11):1826-41.

[32] Usman I, Hussain M, Imran A, Afzaal M, Saeed F, Javed M, et al. Traditional and innovative approaches for the extraction of bioactive compounds. International Journal of Food Properties. 2022;25(1):1215-33.

[33] Hadidi M, Ibarz A, Pagan J. Optimisation and kinetic study of the ultrasonic-assisted extraction of total saponins from alfalfa (Medicago sativa) and its bioaccessibility using the response surface methodology. Food chemistry. 2020;309:125786.

[34] Espinoza CR, Ruiz CAJ, Ramos OPF, Solano MAQ, Quiñonez GH, Mallma NES. Optimization of the ultrasoud-assisted extraction of saponins from quinoa (Chenopodium quinoa wild) using response surface methodology. Acta Scientiarum Polonorum Technologia Alimentaria. 2021;20(1):17-23.

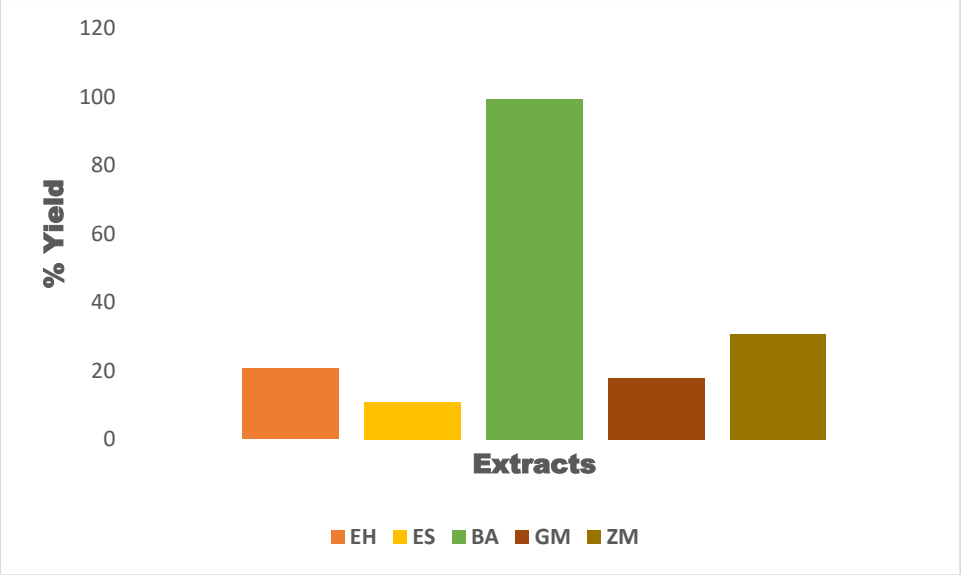

Figure 2: Percentage Extraction Yield of Extracts.

Qualitative Test for Saponins

The froth test confirmed the presence of saponins in all selected plants (Table 2). *ZM* exhibited strong froth formation (++++), followed by strong levels (+++) in *GM* and *BA.* Moderate froth was observed in *EH* (++), while *ES* showed weak froth formation (+).

A high concentration of saponins were observed in ZM as indicated by the strong froth formation. Notably, this result is in line with previous reports that saponin-rich plants (*Sapindus mukorossi* and *Glycyrrhiza glabra*) produce potent biosurfactants that enhance the solubility and dispersion of petroleum hydrocarbons [27] [35]. The strong froth levels in GM and BA further support their potential for application as effective, eco-friendly surfactants in crude oil remediation.

Interestingly, even plant extracts that showed moderate to weak froth formation, such as EH and ES may still contribute meaningfully to crude oil bioremediation. Research have shown that even at low saponin concentrations, hydrocarbon bioavailability can be enhanced in contaminated soil (Hoang). Additionally, some hydrocarbon-degrading bacteria can thrive with saponins, facilitating effective synergy between plant- microbe remediation [36].

[35] Arumugam A, Fang C, Selvin J, Kuppusamy S, Devi OR, Zhang F, et al. Plant biomass extracted eco-friendly natural surfactant enhanced bio-electrokinetic remediation of crude oil contaminated soil. Environmental Research. 2024;245:117913.

[36] Octaviany E, Suharjono S, Mustafa I. Isolation and identification of hydrocarbon-degrading bacteria that tolerant to saponin of Sapindus rarak plant. Jurnal Biodjati. 2019;4(1):79-88.

Table 2: Qualitative Test for Saponins

Samples	Froth Formation	Froth Level
ZM	Y	+ +++
GM	Y	+ + +
BA	Y	+ + +
EH	Y	+ +
ES	Y	+

Key: froth formation is represented as follows: : **'Y'** (presence of froth) **'+ + + +'** (very strong froth) **'+ + +'** (strong froth) . **'+ +'** (moderate froth) **'+'** (weak froth).

Total Saponin Content of the Extracts

Significant variation in the total saponin content was observed among the plant extracts ($p<0.05$). The results presented as mean±SEM (Table 3).

The highest concentration was recorded in *ES* (653.95 ± 0.41 mg/g), followed by *EH* (525.80 ± 8.05 mg/g) and *BA* (483.10 ± 0.37 mg/g). Lower levels were observed in *ZM* (222.43 ± 0.99 mg/g) and *GM* (223.55 ± 0.99 mg/g).

The differences in the total saponin content among plants reflects their distinct biosynthetic capabilities and tissue compositions. The vanillin-sulfuric acid assay utilized in this study, remains a widely accepted saponin quantification method due to its reproducibility, sensitivity, and simplicity[37].

The high concentrations of saponins in ES and EH makes them promising candidates for saponin-based biosurfactant applications. Similarly high levels of triterpenoid saponins have been reported in *Camellia sinensis and Sapindus mukorosi* known for their potent surface-active properties [38]. The substantial amount of saponin exhibited by BA combined with its high extraction yield make it ideal for large-scale bioremediation applications.

Alternatively, the relatively lower saponin concentrations in ZM and GM may be as a result of species-specific metabolic differences, the diversity of saponin's structure or limited recovery due to the accumulation of these compounds within complex cellular matrices [39]. While the vanillin-sulfuric acid assay is reliable, it is worth noting that the process can be affected by the type of solvent, extraction parameters and interfering substances and also to improve accuracy, modifications such as solvent evaporation steps have been proposed [37].

[37] V. Le A, E. Parks S, H. Nguyen M, D. Roach P. Improving the vanillin-sulphuric acid method for quantifying total saponins. Technologies. 2018;6(3):84.
[38] Wu X, Jia L, Wu J, Liu Y, Kang H, Liu X, et al. Simultaneous determination and quantification of triterpene saponins from Camellia sinensis seeds using UPLC-PDA-QTOF-MS/MS. Molecules. 2019;24(20):3794.
[39] de Aguiar NS, Hansel FA, Reis CAF, Lazzarotto M, Wendling I. Optimizing the Vanillin-Acid Sulfuric Method to Total Saponin Content in Leaves of Yerba Mate Clones. Chemistry & Biodiversity. 2024;21(4):e202301883.

Table 3: Quantitative Saponin Content of Extracts (mg/g)

Sample	Total Saponin Content ± SEM
ES	653.95± 0.41
EH	525.80 ± 8.05
BA	483.10 ± 0.37
GM	223.55 ± 0.99
ZM	222.43 ± 0.99

Key: Values are expressed as mean± standard error of mean (SEM)

Purity of the Fermented Purified Extracts

The purity of fermented extracts from each plant species was assessed, with average values shown in Figure 3. The highest saponin purity was displayed in ES (60%), followed by ZM (56.30%). Moderate purity was shown by GM (46.48%), while EH and BA had lower purity levels. The variations in their purity pattern reflects differences in initial saponin content, matrix complexity and the efficiency of the purification method. The relatively high purification efficiency shown in ES and ZM suggest the presence of abundant saponins and minimal interference by other secondary metabolites during fermentation. This finding is in consistent with previous studies noting that triterpenoid-rich saponins are efficiently separated during fermentation owing to their structural stability [40] [41].

The moderate purity observed in GM can be attributed to the amphiphilic and heterogeneous components of soy saponins. This corresponds with previous reports that purification of soybean-derived saponin often yield intermediate values due to co-extracted compounds that complicate separation [42]. Conversely, the lower purity levels in EH (26.86%) and BA (11.58%) is likely due to high levels of non-saponin compounds in their crude extracts interfering with purification efficiency [43].The variation in saponin purity across these species reflects differences in phytochemical constituents and fermentation-based purification effectiveness. These findings aligns with previous studies emphasizing the importance of extraction matrix complexity and microbial selectivity in determining saponin purity [44] [45].

[40] He Y, Hu Z, Li A, Zhu Z, Yang N, Ying Z, et al. Recent advances in biotransformation of saponins. Molecules. 2019;24(13):2365.
[41] Yao L, Wang J, He J, Huang L, Gao W. Endophytes, biotransforming microorganisms, and engineering microbial factories for triterpenoid saponins production. Critical Reviews in Biotechnology. 2021;41(2):249-72.

[42] Zhang X, Shan T, Jia H, Guo C, Wang Z, Yue T, et al. Comparative evaluation of the effects of natural and artificial inoculation on soybean paste fermentation. Lwt. 2022;155:112936.
[43] Belemlilga MB, Ouedraogo S, Boly GAL, Dao DH, Coulibaly JT, Ouedraogo JCRP, et al. Optimization and Standardization of the Extraction Method of Balanites aegyptiaca Del. Seeds (Zygophyllaceae) Used in the Formulation of an Antiparasitic Phytomedicine. Pharmaceuticals. 2024;17(12):1698.
[44] Wang Y, Ma Y, Tao L, Zhang X, Hao F, Zhao S, et al. Recent advances in separation and analysis of saponins in natural products. Separations. 2022;9(7):163.
[45] Ligor M, Ratiu IA, Kiełbasa A, Al-Suod H, Buszewski B. Extraction approaches used for the determination of biologically active compounds (cyclitols, polyphenols and saponins) isolated from plant material. Electrophoresis. 2018;39(15):1860-74.

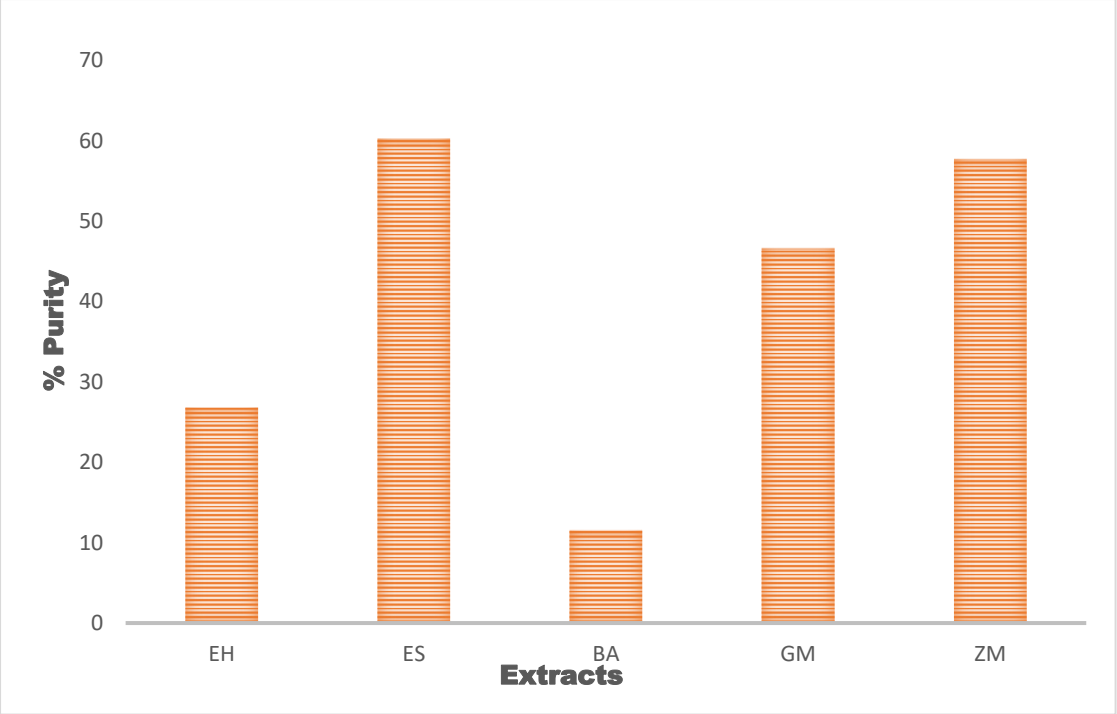

Figure 3: Purity of the Fermented Purified Extracts Extract

Emulsification Stability of Saponin-Rich Extracts

The emulsification stability of saponin-rich extracts obtained from different plant species was investigated and the results are reported in Table 4. Notable differences were identified in the surface-active behaviour of the tested extracts. Among them, ZM and ES revealed the highest stability values (>70%), implying that their saponins are remarkably effective in decreasing interfacial tension and retaining oil-in-water emulsions. This potent emulsifying properties aligns with earlier findings on saponins from *Quillaja saponaria* and tea, which are recognized to generate stable interfacial films that restrict oil droplet aggregation and creaming [46 47].

The stabilization of emulsions by saponins is primarily due to the formation of viscoelastic interfacial layers in addition to repulsive forces from electrostatic and steric interaction around dispersed oil droplets (Schreiner et al., 2021). The exceptional performance of ZM and ES may therefore be attributed to their higher saponin levels and optimal structural configuration such as sugar chain length and polarity that boost interfacial performance. Similar structure-function relationships have been observed for triterpenoid saponins, where glycosylation and acylation patterns strongly impact emulsification efficiency [48].

[46] Zhu Z, Wen Y, Yi J, Cao Y, Liu F, McClements DJ. Comparison of natural and synthetic surfactants at forming and stabilizing nanoemulsions: Tea saponin, Quillaja saponin, and Tween 80. Journal of colloid and interface science. 2019;536:80-7.
[47] Yuan Y, Chen C, Guo X, Li B, He N, Wang S. Noncovalent interactions between biomolecules facilitated their application in food emulsions' construction: A review. Comprehensive reviews in food science and food safety. 2024;23(1):e13285.
[48] Pradhan A, Bhuyan S, Chhetri K, Mandal S, Bhattacharyya A. Saponins from Albizia procera extract: Surfactant activity and preliminary analysis. Colloids and Surfaces a: Physicochemical and Engineering Aspects. 2022;643:128778.

Notably, GM and EH showed moderate emulsification stability which suggest that while they contain appreciable saponins, their emulsifying performance may be restricted by lower purity, structural limitations or presence of co-extracted compounds, aligning with previous observations made by Lv and colleagues highlighting the impact of extract composition and saponin heterogeneity on interfacial activity [49].

The lowest emulsification stability recorded for BA, contradicts to its high extraction yield (Figure 2), likely due to the presence of co-extracted non-saponin compounds (e.g., protein, sugar, tannins) interfering with the formation of a stable film at the interface and destabilizing emulsion .

Table 4: Emulsification Stability (%) of Saponin- Rich Extracts from Various Plant Sources

Samples	% Emulsification Stability ± SEM
EH	46.17 ± 4.22
ES	75.73 ± 1.07
BA	41.36 ± 3.37
ZM	81.39 ± 6.46
GM	62.10 ± 2.09

Key: values are mean and ± SEM

Surface Tension of Saponin-Rich Extracts

As the concentration of each saponin-rich extract increased from 20 to 400 mg/L, all samples demonstrated a marked decrease in surface tension, confirming their surfactant properties (Table 5). BA and ZM consistently achieved the lowest surface tension values across the tested range, reaching 30.53 mN/m and 31.31 mN/m, respectively at the highest concentration. ES and GM displayed intermediate surface tension reductions, while EH retained relatively higher surface tension values at comparable concentrations, suggesting weaker interfacial activity.

The reduction in surface tension observed for all studied extracts highlighted the amphiphilic character of saponins, which gives them the ability to adsorb at the air-water interface and interfere with hydrogen bonding between water molecules. The superior performance of BA and ZM, which lowered surface tension to ~30-31 mN/m, is in close agreement with values reported for highly active triterpenoid saponins from *Quillaja saponaria* and *Sapindus mukoross* [50 51]. These values also fall within the typical range for commercial biosurfactants, indicating strong potential for practical applications.

The high interfacial activity of BA and ZM may be attributed to favourable structural features of their saponins, such as monodesmosidic glycosidic linkages and balanced hydrophobic-hydrophilic ratios, which enhance their potential to reduce surface tension efficiently [50].

[49] Lv S, Zhang Y, Tan H, Zhang R, McClements DJ. Vitamin E encapsulation within oil-in-water emulsions: Impact of emulsifier type on physicochemical stability and bio accessibility. Journal of agricultural and food chemistry. 2019;67(5):1521-9.

[50] Böttcher S, Drusch S. Saponins—Self-assembly and behavior at aqueous interfaces. Advances in Colloid and interface Science. 2017;243:105-13.

[51] Timilsena YP, Phosanam A, Stockmann R. Perspectives on saponins: food functionality and applications. International Journal of Molecular Sciences. 2023;24(17):13538.

On the contrary, ES and GM displayed moderate reductions implying that their surface -active saponins are being affected by structural differences or impurities that decrease efficiency. This is consistent with the research of Schreiner and team' s observation highlighting that crude saponin extracts can approach synthetic surfactant activity but are influenced by purity and saponin composition [52].

Table 5: Surface Tension (mN/m) of Saponin-Rich Extracts

Samples	Concentrations mg/L				
	20	**50**	**100**	**200**	**400**
BA	$43.62\pm0.81^{b\,D}$	$38.85\pm0.64^{b\,C}$	$33.71\pm0.70^{a\,B}$	$32.02\pm0.76^{a\,A}$	$30.53\pm0.49^{a\,A}$
ZM	$40.40\pm0.57^{a\,D}$	$35.41\pm0.59^{a\,C}$	$34.78\pm0.74^{a\,BC}$	$33.48\pm0.57^{ab\,B}$	$31.31\pm0.52^{a\,A}$
EH	$71.51\pm0.8^{e\,E}$	$62.60\pm0.75^{f\,D}$	$54.35\pm0.69^{e\,C}$	$47.80\pm0.63^{d\,B}$	$40.69\pm0.58^{c\,A}$
ES	$45.42\pm0.72^{b\,E}$	$41.07\pm0.66^{c\,D}$	$37.28\pm0.61^{b\,C}$	$34.03\pm0.55^{ab\,B}$	$31.87\pm0.49^{a\,A}$
GM	$59.42\pm0.88^{d\,D}$	$41.77\pm0.74^{c\,C}$	$39.73\pm0.69^{c\,C}$	$36.83\pm0.63^{c\,B}$	$33.77\pm0.57^{b\,A}$

Key: Values are mean±SD (n = 3). Different lowercase superscript letters in the same column indicate significant differences among extracts at the same concentration ($p < 0.05$). Different uppercase superscript letters in the same row indicate significant differences across concentration for same extract ($p < 0.05$).

The relatively higher surface tension values retained by EH across all concentrations indicate less efficient interfacial activity. This could be due to the presence of more steroidal-type saponins or interfering phytochemicals, which are generally less effective in reducing surface tension compared to triterpenoid forms[53]. Such structural limitations may also reflect a higher critical micelle concentration (CMC), as suggested by the slower reductions at lower concentrations. Below the CMC, saponins remain largely as monomers, covering the surface less effectively, a behaviour consistent with other natural surfactants such as quinoa-derived saponins.

[52] Rai S, Acharya-Siwakoti E, Kafle A, Devkota H, Bhattarai A. Plant-Derived Saponins: A Review of Their Surfactant Properties and Applications. Sci 2021, 3, 44. s Note: MDPI stays neutral with regard to jurisdictional claims in published …; 2021.

[53] Esmaeelian M, Jahani M, Feizy J, Einafshar S. Foam and emulsion properties of crude saponin extract from saffron (Crocus sativus L.) corm. Journal of Food Engineering. 2024;370:111956.

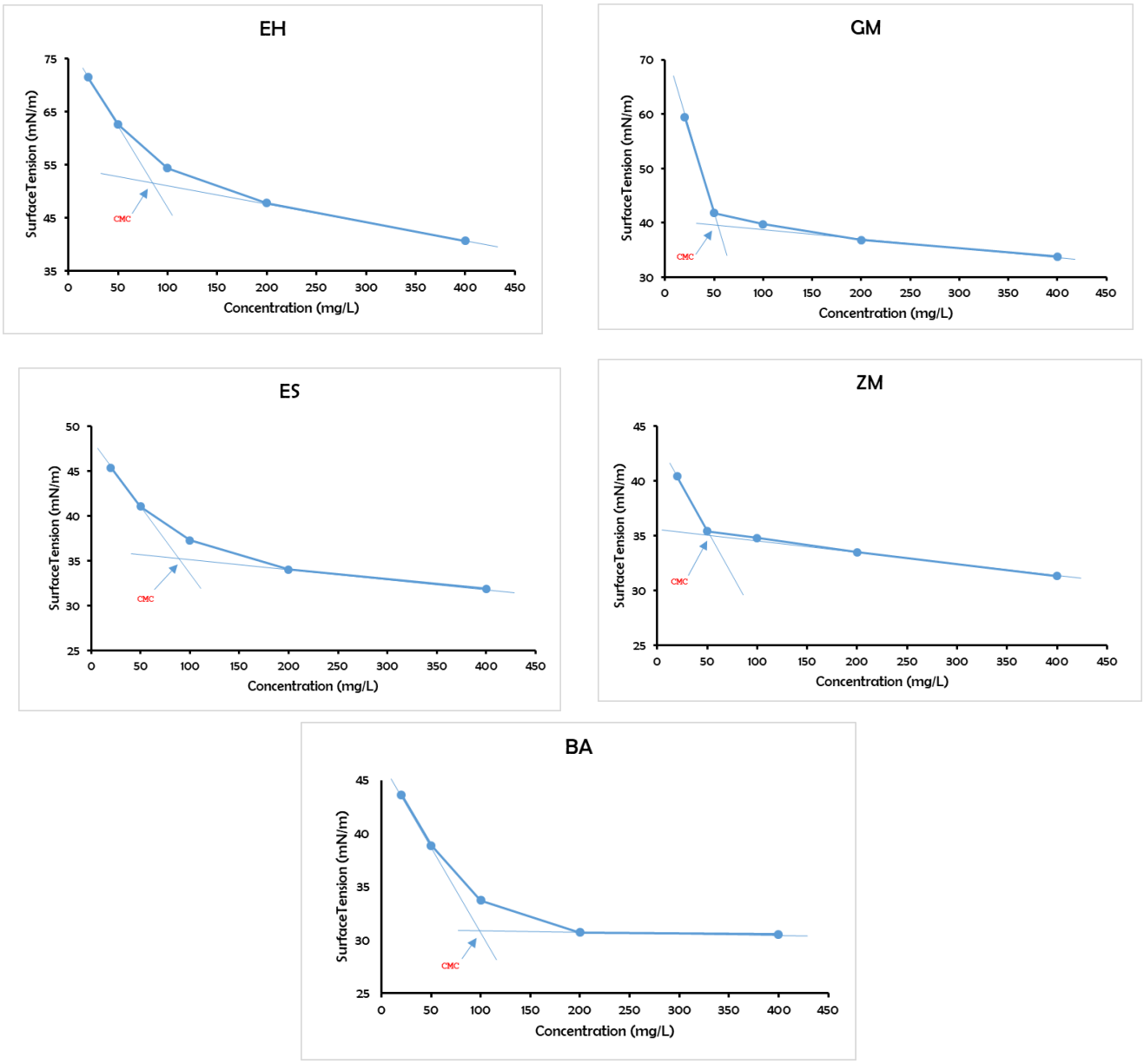

Figure 4: Plots of Surface Tension vs Concentration Showing the CMC of The Extracts (BA; GM; ES; EH; ZM)

Critical Micelle Concentration (CMC) of Saponin-Rich Extracts

The critical micelle concentration (CMC) of the selected saponin-rich extracts was evaluated by plotting surface tension against concentration (Figure 4). The CMC corresponds to the point at which a plateau in surface tension occurs, signifying micelle formation. The CMC values for the extracts are presented in Figure 5. Among the extracts, **BA** and **ZM** showed the lowest CMC values at **56.42 mg/L** and **60.21 mg/L**, respectively, highlighting their superior efficiency in micellization.**ES** exhibited an intermediate CMC, while **EH** and **GM** recorded higher CMCs, reflecting comparatively lower micellization efficiency.

The comparatively low CMC values observed for **BA** and **ZM** indicate that these extracts can form micelles at relatively low concentrations, a property that is particularly advantageous for

applications where minimal surfactant input is desired to achieve effective interfacial modification, such as in emulsification, detergency, and crude oil dispersion. The strong micellization ability of these extracts is likely related to the prevalence of **triterpenoid saponins**, which possess a favourable balance of hydrophobic aglycone and hydrophilic sugar moieties. This structural balance enhances interfacial packing and facilitates hydrophobic interactions within micelles, resulting in reduced CMC values [54].

By contrast, **EH** and **GM** required higher concentrations to achieve micellization, suggesting either a predominance of less surface-active saponins (e.g., steroidal types) or the influence of co-extracted impurities such as polyphenols, proteins, or polysaccharides, which may compete for interfacial adsorption and hinder micelle formation [51]. The intermediate CMC value observed for **ES** suggests that while it contains effective surfactant saponins, the extract composition may include additional constituents that reduce its overall efficiency compared to BA and ZM.

The CMC range reported here (56–90 mg/L) is consistent with literature values for crude plant saponin extracts, which generally fall between 50–150 mg/L depending on extraction method, purity, and plant source. For example, quinoa saponins exhibit CMCs in the range of 60–80 mg/L[55], comparable to our findings for ZM and ES. Notably, the particularly low CMC of **BA** (56.42 mg/L) supports earlier reports emphasizing the strong interfacial activity of *BA* saponins [55].

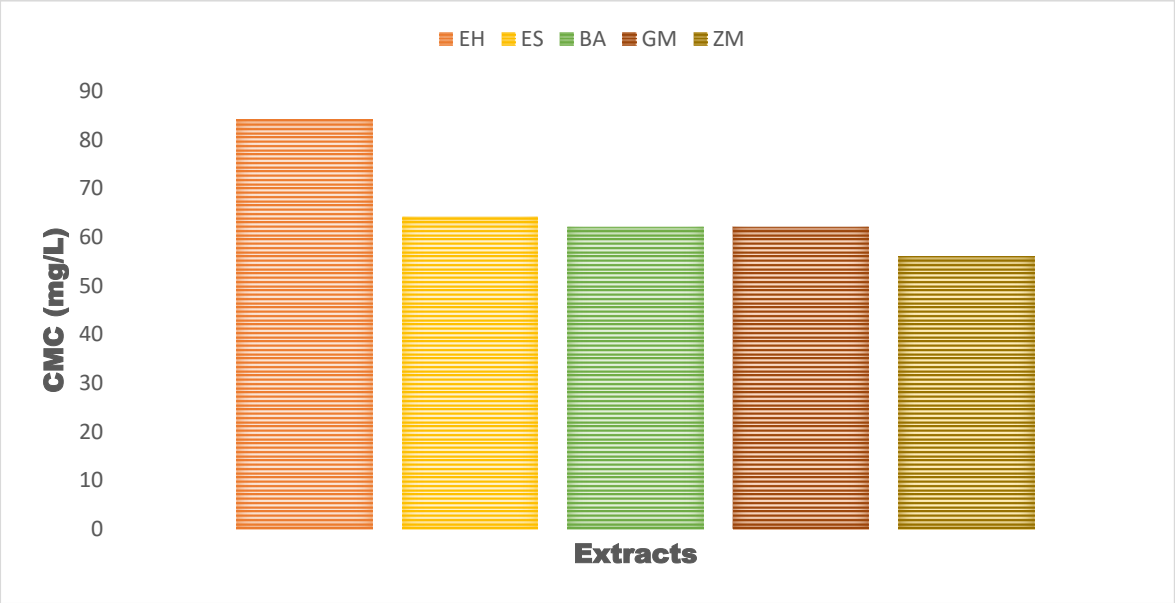

Figure 5: Critical Micelle Concentration (CMC) of Saponin-Rich Extracts

[54] Schreiner TB, Dias MM, Barreiro MF, Pinho SoP. Saponins as natural emulsifiers for nanoemulsions. Journal of Agricultural and Food Chemistry. 2022;70(22):6573-90.
[55] Vincken J-P, Heng L, de Groot A, Gruppen H. Saponins, classification and occurrence in the plant kingdom. Phytochemistry. 2007;68(3):275-97.

Droplet size Distribution and Polydispersity Index (PDI) of the Saponin-Rich Extracts

Dynamic Light Scattering (DLS) analysis revealed clear differences in the emulsification performance of the saponin-rich extracts (Figure 6). ZM (24.63 nm, PDI 0.208) produced the most uniform and stable nanoemulsion, with the smallest droplet size and a sharp unimodal peak, confirming its superior interfacial activity and dispersion quality. Such small, uniform droplets are consistent with findings of Nejatian and colleagues as well as several other researchers who reported that emulsions below 50 nm with PDI < 0.3 exhibit superior interfacial stability and environmental robustness [56] [57] [58]. BA (64.73 nm, PDI 0.333) and GM (64.92 nm, PDI 0.241) also generated nanoscale droplets with moderately narrow, unimodal distributions, suggesting efficient emulsification with limited polydispersity.

In contrast, ES (97.71 nm, PDI 0.574) and EH (112.4 nm, PDI 0.475*)* exhibited broad, multimodal or bimodal distributions with higher droplet sizes, indicating heterogeneous emulsions prone to droplet coalescence and reduced stability over time. These findings demonstrate that ZM is the most effective emulsifier, while BA and GM show promising but less pronounced performance. EH and ES, with larger droplet sizes and higher polydispersity, appear less suited for stable nanoemulsion formation without further optimization.

BA and GM also demonstrated effective emulsification, forming nano-sized droplets (~64 nm) with PDIs of 0.241 - 0.333. These values fall within the accepted stability threshold (PDI < 0.4), suggesting moderate-to-good uniformity and resistance to coalescence [28] [59]. The amphiphilic structure of soy saponins likely accounts for GM's efficient interfacial adsorption, as noted by Gao and others while the relatively narrow peaks observed for BA resemble reports of optimized saponin emulsions near their CMC [60]. Together, both extracts represent promising candidates for biosurfactant applications in soil remediation.

In contrast, ES and EH formed fewer uniform systems, with droplet sizes of 97.71 nm and 112.4 nm, respectively, and high PDIs (0.475–0.574). These values exceed the standard monodispersity cut-off (PDI < 0.3), suggesting multimodal or broad size distributions prone to flocculation and coalescence. Such instability is typical of crude plant extracts containing co-solutes that disrupt interfacial stabilization [46, 61]. Moreover, as Schmitt and colleagues highlighted, polydisperse emulsions complicate DLS interpretation due to scattering biases, showing that unoptimized biosurfactant concentrations promote broad size distributions

[56] Rai S, Acharya-Siwakoti E, Kafle A, Devkota HP, Bhattarai A. Plant-derived saponins: a review of their surfactant properties and applications. Sci. 2021;3(4):44.
[57] Sabri N, Moulai-Mostefa N. Formulation and characterization of oil-in-water emulsions stabilized by saponins extracted from Hedera Helix Algeriensis using response surface method. Bio interface Res Appl Chem. 2020;10:6282-92.
[58] Nejatian M, Abbasi S. Formation of concentrated triglyceride nanoemulsions and nanogels: natural emulsifiers and high power ultrasound. RSC advances. 2019;9(49):28330-44.
[59] Banerjee A, Binder J, Salama R, Trant JF. Synthesis, characterization and stress-testing of a robust quillaja saponin stabilized oil-in-water phytocannabinoid nanoemulsion. Journal of cannabis research. 2021;3(1):43.
[60] Dahlawi SM, Nazir W, Iqbal R, Asghar W, Khalid N. Formulation and characterization of oil-in-water nanoemulsions stabilized by crude saponins isolated from onion skin waste. RSC-advances. 2020;10(65):39700-
[61] Zhao S, Wang Z, Wang X, Kong B, Liu Q, Xia X, et al. Characterization of nanoemulsions stabilized with different emulsifiers and their encapsulation efficiency for oregano essential oil: Tween 80, soybean protein isolate, tea saponin, and soy lecithin. Foods. 2023;12(17):3183.

(Schmitt *et al.*, 2014; [62]. The need for co-emulsifiers or advanced homogenization to reduce polydispersity in natural saponin systems was further emphasized [63]. As a result, despite the ability of ES and EH to form nano-range droplets, they seem to be less suitable as independent emulsifier for the remediation of crude oil unless further enhancement or formulation optimization is carried out.

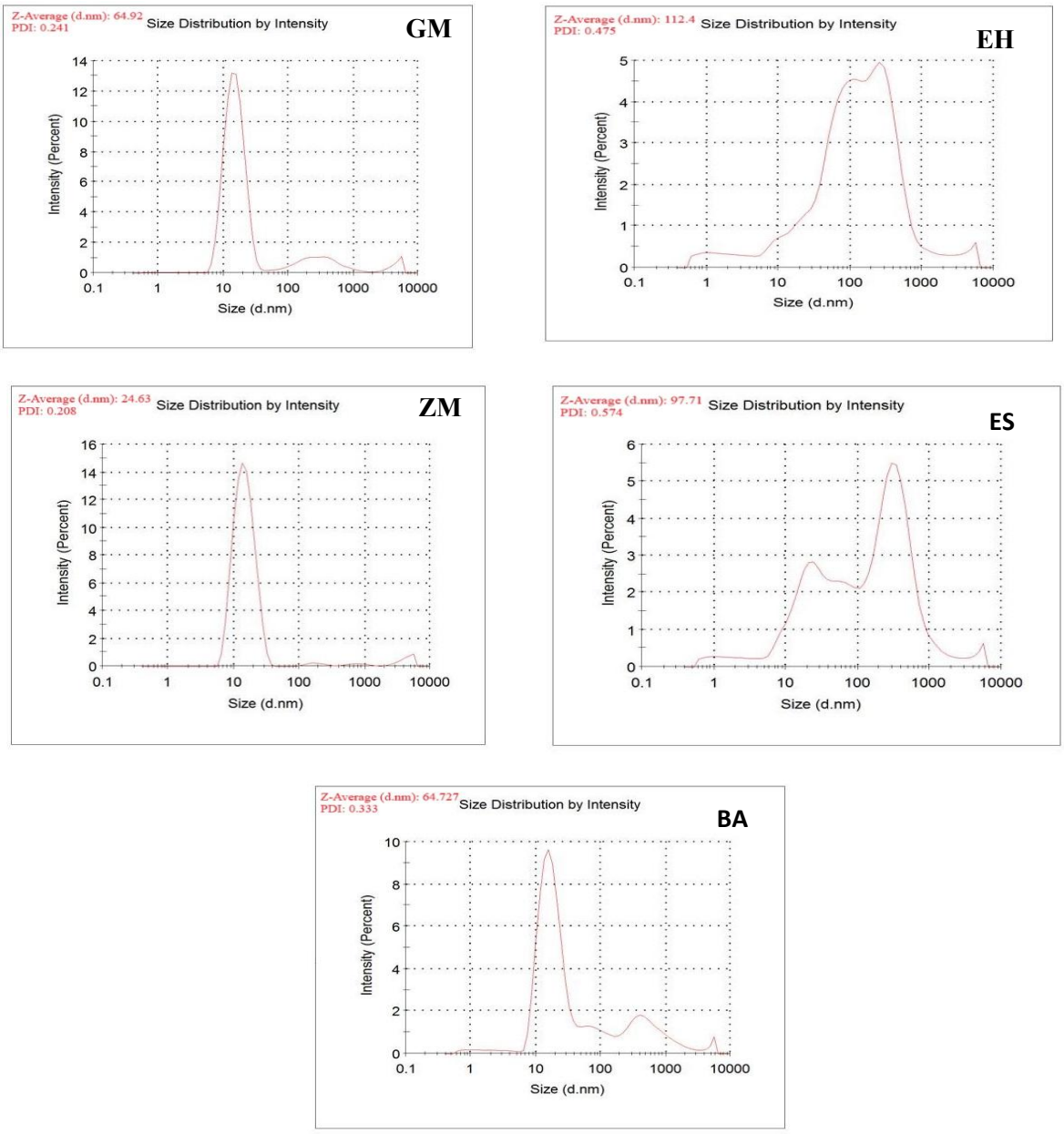

Figure 6: Intensity Distribution Curve of The Extracts GM; EH; ES; ZM*;* and BA

[62] Schreiner TB, Santamaria-Echart A, Ribeiro A, Peres AM, Dias MM, Pinho SP, et al. Formulation and optimization of nanoemulsions using the natural surfactant saponin from quillaja bark. Molecules. 2020;25(7):1538.

[63] Sotomayor-Gerding D, Morales E, Rubilar M. Comparison between quinoa and quillaja saponins in the formation, stability and digestibility of astaxanthin-canola oil emulsions. Colloids and Interfaces. 2022;6(3):43.

Conclusion

This findings revealed significant differences in extraction yield, purification efficiency and functional performance among saponins from tropical plants. Notably, BA recorded the highest yield, ZM proved to be the most effective extract, characterized by high purity, potent surfactant activity, low CMC, uniform nanoscale droplets formation, and excellent emulsion stability. GM also demonstrated strong potential balancing moderate purity with effective dispersion and stability. Whereas ES displayed partial potential but with limitations in droplet size and uniformity. Conversely, despite some beneficia properties, BA and EH were generally less effective. Ultimately, ZM and GM emerged as the most suitable candidates for biosurfactant-based crude oil dispersion and bioremediation, emphasizing their potential as eco-friendly alternatives to synthetic surfactants.

Funding Acknowledgement
The author acknowledges the financial support provided by the Petroleum Technology Development Fund (PTDF) under Reference Number PTDF/ED/ISS/PHD/FMM/2055/21.