Journal of Basics and Applied Sciences Research (JOBASR)
ISSN (print): 3026-9091, ISSN (online): 1597-9962
Volume 3(6) November 2025
DOI: https://dx.doi.org/10.4314/jobasr.v3i6.10

Structure–Activity Relationship (SAR), Phytochemical Profiling, and Structural Characterization of Ethanolic Extracts and Oils from Black Seed, Baobab Seed, and Neem Seed

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ABSTRACT

This study investigates the phytochemical composition, Physicochemical Liquid Chromatography Mass Spectrometry/Gas Chromatography Mass Spectrometry characterization, and structure–activity relationships (SAR) of bioactive compounds in ethanolic extracts and seed oils of *Azadirachta indica* (neem), *Nigella sativa* (black seed), and *Adansonia digitata* (baobab) and physicochemical parameters of the seed oils. Phytochemical screening revealed the presence of flavonoids, phenolics, tannins, glycosides, and stilbenoids. Black seed extract exhibited the highest concentration of phenolics and tannins, while neem extract contained all identified phytochemical classes, including stilbenoids. LC-MS analysis of the plant materials confirmed compounds such as dihydroquercetin, sinapic acid, polydatin, and malic acid corroborating the screening results. GC-MS profiling revealed 63 compounds in neem seed oil, 45 in black seed oil, and 44 in baobab seed oil. Neem oil was rich in azadirachtin, nimbin, gedunin, β-sitosterol, linoleic acid, and palmitic acid, known for antioxidant, antimicrobial, and anti-inflammatory properties. Black seed and baobab oils contained thymoquinone, carvacrol, tocopherols, sterols, 2,4-decadienal, and 2-pentylfuran also linked to therapeutic effects. SAR analysis connected these compounds to biological activities via mechanisms including radical scavenging, membrane disruption, and enzyme inhibition. Physicochemical analysis showed oil yields of neem (37.0 ± 2%), baobab (28.0 ± 2%), and black seed (24.0 ± 2%), alongside significant variations in saponification, iodine, and peroxide values. This multi-analytical approach highlights the chemical diversity and pharmacological potential of these underutilized seeds. The integration of SAR-based validation with LC-MS/GC-MS profiling enhances understanding of their bioactivity, supporting applications in cosmetic, nutraceutical, and pharmaceutical formulations.

Keywords:
Phytochemical,
Flavonoids,
Tannins,
Glycosides,
Phenols,
Stilbenoids.

INTRODUCTION

The potential of plants as sources of bioactive compounds for use in pharmaceuticals, cosmetics, and functional products has been recognized for a long time. Plant-derived natural products have historically been vital in drug discovery and the development of therapeutics, and many have been optimized into both pharmaceutical drugs and cosmetic ingredients (Noohi *et al.*, 2022). Notable examples include *Azadirachta indica* (neem seed), *Nigella sativa* (black seed), and *Adansonia digitata* (baobab seed), which possess a broad array of phytochemicals and therapeutic properties. These seeds have been used traditionally across various cultures for their medicinal benefits, and modern studies have provided scientific validation of their health-promoting potential. This study aims to perform a comparative phytochemical profiling and structural analysis of ethanolic extracts and oils from these three seeds and evaluate the industrial prospects of their bioactive compounds. The process of developing functional products such as antimicrobial, antioxidant, anticancer, and anti-inflammatory agents begins with the identification, extraction, and characterization of relevant phytochemicals (Altemimi *et al.*, 2017).

How to cite this article: Shehu A., Okunola O. J. & Uduma A. U. (2025). Structure–Activity Relationship (SAR), Phytochemical Profiling, and Structural Characterization of Ethanolic Extracts and Oils from Black Seed, Baobab Seed, and Neem Seed. *Journal of Basics and Applied Sciences Research*, *3*(6), 72-87. https://dx.doi.org/10.4314/jobasr.v3i6.10

Phytochemical characterization is essential for the informed utilization of plant extracts and oils in pharmaceuticals, nutraceuticals, and cosmetic formulations. These compounds, which are natural secondary metabolites produced by plants, include flavonoids, glycosides, tannins, phenols, alkaloids, terpenoids, stilbenoids, and others (Roy *et al.*, 2022). Among these, flavonoids are especially significant due to their role in plant defense mechanisms and their health benefits in humans. Their bioactivity stems primarily from their antioxidant potential, their ability to modulate cellular pathways, and their interaction with biomolecules (Ullah *et al.*, 2022). Accordingly, this study provides a detailed evaluation of the phytochemical properties and physicochemical composition of ethanolic extracts and seed oils from neem, black seed, and baobab, utilizing modern analytical techniques such as LC-MS and GC-MS. These techniques enable the precise identification and quantification of bioactive components, offering valuable insights into their chemical structures and potential applications (Barba-Ostria *et al.*, 2022). Liquid chromatography coupled with mass spectrometry (LC-MS) is widely used for identifying phenolic acids, flavonoids, and glycosides, while gas chromatography coupled with mass spectrometry (GC-MS) is employed to analyze volatile and non-polar compounds, including essential oils and fatty acids (Rohloff, 2015).

Neem (*Azadirachta indica*) is one of the most studied medicinal plants, known for its broad spectrum of bioactive constituents. *Azadirachtin*, a key secondary metabolite found in neem seed oil, is a potent insecticidal agent, while other compounds like nimbin and nimbidin exhibit antifungal, antibacterial, and anti-inflammatory properties (Islas *et al.*, 2020). Additionally, neem seed extracts contain phenolic compounds and flavonoids with strong antioxidant activity (Ouerfelli *et al.*, 2022), and neem oil has shown promising anticancer potential, making it a candidate for pharmaceutical applications (Hao *et al.*, 2014). Its antimicrobial properties also support its use in cosmetics and natural preservatives (Hao *et al.*, 2014).

Black cumin (*Nigella sativa*) has long been valued in traditional medicine for its therapeutic effects. Its seeds contain thymoquinone, a major bioactive compound with well-documented antioxidant, anti-inflammatory, and anticancer properties (Arshad *et al.*, 2024). Recent studies have also confirmed the presence of high levels of phenolics and tannins in black seed oil, contributing to its pharmacological effects (Barba-Ostria *et al.*, 2022). Additional studies have demonstrated its immunomodulatory and hepatoprotective properties (Nasim *et al.*, 2022), further supporting its use in both traditional and modern medicine. Owing to its antioxidant activity, black seed oil is a promising ingredient in functional foods, nutraceuticals, and skincare products (Oktaviani *et al.*, 2021).

Baobab (*Adansonia digitata*), a native African tree with significant nutritional and medicinal properties, contains seeds rich in vitamins, minerals, and bioactive compounds including flavonoids, polyphenols, and tannins (Asogwa *et al.*, 2021). Baobab seed extracts have shown strong antioxidant and anti-inflammatory activities, making them valuable for pharmaceutical and cosmetic applications (Offiah & Falade, 2023). Additionally, bioactive constituents in baobab seed oil have demonstrated antioxidant (Silva *et al.*, 2023), antiproliferative, and antimicrobial effects, suggesting their use as natural preservatives in cosmetic formulations (Rahim *et al.*, 2023). Growing interest in baobab-based products is also driven by their nutritional value and sustainable sourcing potential (Altemimi *et al.*, 2017).
Thus, detailed analytical characterization of these seed extracts and oils provides a deeper understanding of their bioactive constituents. LC-MS and GC-MS techniques have identified key compound classes such as hydroxycinnamic acids, flavones, glycosides, tannins, stilbenes, and various organic acids (Aati *et al.*, 2025). Volatile compounds revealed through GC-MS profiling further support their potential in pharmaceutical, nutraceutical, and cosmetic industries (Rohloff, 2015). Despite the growing use of plant-based extracts in these industries, a deeper understanding of the structure–activity relationships (SAR) of the compounds involved is often lacking. SAR analysis enables the correlation of chemical structure with biological function, allowing for rational prediction and validation of bioactivities. In this study, we not only perform phytochemical and chromatographic profiling of *Nigella sativa*, *Adansonia digitata*, and *Azadirachta indica* but also link the structural features of their key bioactive compounds to known biological functions particularly antimicrobial, antioxidant, and anti-inflammatory activities. This SAR-integrated approach enhances our mechanistic understanding of therapeutic effects and provides scientific justification for the industrial development of bioactive formulations based on these seeds. While individual phytochemical analyses of neem, black seed, and baobab have been reported, comprehensive comparative studies involving both their extracts and oils using modern LC-MS and GC-MS methods are limited. This study addresses that gap by: (i) conducting qualitative and quantitative phytochemical analyses of their combined extracts and oils, (ii) identifying key bioactive compounds using validated LC-MS and GC-MS protocols, and (iii) comparing compositional differences among the seeds to assess their health-related and industrial applications. Ultimately, this study aims to demonstrate the chemical diversity, bioactive potential,

and industrial applicability of these underutilized seeds, offering a foundation for future development of plant-based therapeutic and cosmetic products.

MATERIALS AND METHODS

The materials used in this study includes; Neem seed, Black seed, Baobab seed, chloroform (99%), Nitric acid (70%), Methylene blue (88%), n-hexane (99%), Bromine water (3% Bromine), Sodium hydroxide (99%), Acetic acid (99%), Ferric Chloride (97%), Sulphuric acid (98%), Ethanol (99%), Sodium thiosulphate (99%), Potassium Iodide (99%), Cyclohexane (99%), glass wares, teflon containers, filter papers, crucibles, Rotary evaporator (Rotavapor R-210), Soxhlet apparatus (Sigma Aldrich Soxhlet extractor).

Sample Preparation

The seeds were first cleaned to remove any dirt, debris, or foreign materials. They were then thoroughly washed with distilled water and left to air dry in a well-ventilated area at an ambient temperature of 30 °C for 10 days. After complete drying, the seeds were ground into a fine powder using a mechanical grinder and sieved to achieve a uniform particle size. The powdered seeds were then stored in an airtight container until further use (Ali *et al.*, 2018).

Preparation of Crude Extract

The extraction process was done following the method of Godlewska *et al.* (2023) with slight modifications. Exactly 50 g of powdered plant seeds were weighed and mixed with 500 mL of ethanol in separate conical flasks. The flasks were sealed with foil paper and left to macerate at room temperature for 48 h. After this period, the mixtures were filtered using Whatman No.1 filter paper to separate the extracts. The extracts were then concentrated using a rotary evaporator (Rotavapor R-210-Germany) and stored for further analysis.

Phytochemical Screening

All phytochemical analyses were performed on three independent biological replicates, each analyzed in triplicate (n=3). Seed samples were processed under identical conditions to ensure consistency. Quantification of phytochemicals was carried out using calibration curves generated from authentic standards. Concentrations are expressed as mean ± standard deviation (SD) in mg per gram of dry extract. All these statistical analyses were conducted using IBM SPSS statistics version 26.0 (IBM Corp., Armonk, NY, USA).

Test for Flavonoid

Following the method described by Phuyal *et al.* (2019) with minor adjustments, 2mL of each plant extract was mixed with 1mL of 1% sodium hydroxide (NaOH) solution. A yellow coloration indicated the presence of flavonoids. To confirm, 1mL of 1% hydrochloric acid (HCl) was added, and disappearance of the yellow color further confirmed the presence of flavonoids.

Test for Glycoside (Keller-Kiliani Test)

For glycoside detection, 2 mL of the plant extract was combined with glacial acetic acid containing one drop of 1% ferric chloride ($FeCl_3$). Then, 1 mL of concentrated sulfuric acid (H_2SO_4) was carefully added along the side of the test tube. The appearance of a reddish-brown ring at the interface indicated the presence of glycosides (Usman *et al.*, 2009).

Test for Tannin

To check for tannins, the ferric chloride test was performed. A solution was prepared by dissolving 5g of $FeCl_3$ in 100mL of distilled water. Then, 2mL of this solution was mixed with 2 mL of the plant extract. A blue-black or green-black color change confirmed the presence of tannins (Phuyal *et al.*, 2019).

Test for Phenol

The presence of phenols was determined using the ferric chloride test. A solution was made by dissolving 1g of $FeCl_3$ in 100 mL of distilled water. Then, 2mL of this solution was added to 2 mL of the plant extract. A blue or purple coloration indicated the presence of phenols ((Phuyal *et al.*, 2019).

Test for Stilbenoid

The bromine water test, as described by Phuyal *et al.* (2019) was used to detect stilbenoids. A 2 mL sample of bromine water was added to 2 mL of the extract. If the bromine water was decolorized, it indicated the presence of stilbenoids.

Extraction of Seed Oils

The solvent extraction method reported by Cissé *et al.* (2018) was followed with slight modifications. A total of 50 g of powdered seeds was placed in a thimble inside a Soxhlet extractor. Then, 300 mL of n-hexane was added to the extraction flask, and the system was heated to reflux at 70 °C for 6 h. After the extraction was completed, the solvent was removed using a rotary evaporator under reduced pressure, leaving behind the concentrated seed oil.

Physicochemical Analysis of the Seed Oils

Determination of %Yield

The procedure reported by Abdulkadir *et al.* (2014) for determination of percentage (%) yield was adopted, and the % yield was calculated using the equation 1.

$$\text{Percentage yield of oil} = \frac{\text{Weight of Oil Obtained}}{\text{Weight of Powdered Seeds}} X\ 100 \quad (1)$$

Determination Peroxide Value

The procedure reported by Abdulkadir *et al*. (2014) was adopted with slight modification, 5 g of the oil was dissolved in 30 ml of glacial acetic acid: chloroform (3:2, v/v). 0.5 mL of saturated KI was added and I_2 was liberated by the reaction with the peroxide. The solution was then titrated with standardized sodium thiosulphate using starch indicator. The peroxide value (PV) was determined from equation 2.

$$\text{PV (mEq/Kg)} = \frac{(S-B)\ x\ M\ x\ 1000}{\text{Sample weight (g)}} \quad (2)$$

Where S = Sample titre value, B = Blank titre value, M = Molarity of $Na_2S_2O_3$.

Determination of Iodine Value

Procedure reported by Abdulkadir *et al.* (2014) was adopted, 0.1 M iodine monochloride in acetic acid was added to 0.2 g of the oil dissolved in cyclohexane. The mixture was allowed to stand for ten minutes, to allow for halogenation. 0.1 M of KI solution was added to reduce excess iodine monochloride to free iodine. The liberated iodine was titrated with a standardized solution of 0.1 M sodium thiosulphate using starch indicator. The iodine value was calculated from equation 3.

$$\text{Iodine value (IV)} = \frac{(B-S)x\ M\ x\ 12.69}{\text{Sample weight (g)}} \quad (3)$$

Where B = blank titre value, S = sample titre value, M = Molarity of $Na_2S_2O_3$, 12.69 = Conversion factor from Meq. $Na_2S_2O_3$ to gram iodine, molecular weight of iodine is 126.9 g.

Determination of Saponification Value

As reported by Abdulkadir *et al.* (2014) 2 g of the oil sample was added to excess alcoholic KOH. The solution was heated for two minutes to saponify the oil. The unreacted KOH was back - titrated with standardized 0.1 M HCl using phenolphthalein indicator. The SV was calculated from equation 4.

$$\mathbf{SV} = \frac{\mathbf{(S-B)x\ M\ x\ 56.1}}{\mathbf{Sample\ weight\ (g)}} \quad (\mathbf{4})$$

Where S = Sample titre value, B = Blank titre value, M = Molarity of the HCl, 56.1 = Molecular weight of KO

LC MS Analysis

The LC-MS analysis was performed using an Agilent 1260 infinity II LC system coupled to an agilent 6120 Quadrupole MS (agilent Technologies, Santa Clara,CA, USA), the system was first calibrated using standard solutions of known bioactive compounds. Then, 10 µL of each crude plant extract was injected for analysis. The system recorded the chromatograms and mass spectra, while the samples were examined in both positive and negative ionization modes for a more comprehensive profile. To further identify the compounds, tandem MS was used for structural analysis. The retention times (RT) and mass-to-charge ratios (m/z) were then compared with published data and online databases like PubChem, ChemSpider, and METLIN. Calibration curves from reference standards helped quantify the major bioactive compounds, while fragmentation patterns from MS/MS spectra provided insights into the structure of unknown compounds (Razgonova *et al.*, 2021).

GC MS Analysis (Determination of bioactive compounds from Oils)

The GC-MS analysis was carried out using Agilent 7890b gas chromagraph coupled with a 5977B mass selective detector (Agilent Technologies, Santa Clara, CA, USA). The oil samples, standard solutions of known compounds were first injected to create calibration curves. After that, 1 µL of each prepared oil sample was introduced into the GC-MS system. The retention times (RT) and mass spectra of all detected peaks were recorded. To ensure the results were accurate and reliable, each sample was injected three times. The detected compounds were then identified by comparing their mass spectra with those in the NIST/EPA/NIH Mass Spectral Library and the Wiley Mass Spectral Database. Finally, calibration curves from the standard solutions were used to quantify the active compounds, and key bioactive compounds were identified based on their retention times and fragmentation patterns (Ismail *et al.*, 2022).

Quantitative Concentration Data, Statistical Validation, Replication Details

Concentrations of identified compounds were quantified in mg/g of dry extract or µg/mL of oil. Data represent means ± standard deviations from three biological replicates and three technical replicates each.

Statistical differences between seed types were analyzed using one-way ANOVA with Tukey's post hoc test ($p < 0.05$). These replicates and statistical analyses support the reliability and reproducibility of our findings (using IBM SPSS Statistics version 26.0 IBM Corp., Armonk, NY, USA).

LC-MS/GC-MS Parameters, Standards, Chemometric Validation, Processing Optimization

LC-MS utilized a C18 column, mobile phase gradient (5% to 95% acetonitrile over 30 m, ESI positive ion mode scanning m/z 100–1000. GC-MS used DB-5MS column with temperature ramp 60–280 °C, electron ionization at 70 eV. Authentic standards such linoelaidic acid, hexadecanoic acid, oleic acid were run to confirm compound identity and generate calibration curves for quantification. Extraction was performed by maceration using ethanol for 48 h at room temperature.

Structure-Activity Relationship (SAR) Analysis

Identified compounds from LC-MS and GC-MS analyses were evaluated for biological activity based on structural features. SAR mapping was conducted by cross-referencing molecular structures with bioactivity data from established databases such as ChEMBL, PubChem, and NPASS. Emphasis was placed on the presence of functional groups like hydroxyl, carboxyl, and methoxy groups, known for their antioxidant or antimicrobial actions. Literature-supported correlations between structural moieties (e.g., aromatic rings, hydroxylation patterns) and biological activities were used to categorize each compound under functional outcomes such as radical scavenging, membrane disruption or enzyme inhibition.

Reproducibility Guidance

Detailed experimental protocols including solvent types, extraction times, LC-MS/GC-MS settings, SAR mapping and data analysis procedures are provided to enable other researchers to replicate this study reliably.

RESULTS AND DISCUSSION

Result of Phytochemical Screening

Table 1 presents the result of phytochemical screening of the ethanolic extracts of the three plant seeds, showing all the phytochemicals detected in each seed extract.

Table 1. Phytochemical Screening of the Three Seeds Extract

Compounds	BLS (mg/100g)	BAS (mg/100g)	NES (mg/100g)	ANOVA p-value
Flavonoids	4.5±0.5	8.5±0.5	7.2±0.2	<0.05
Glycosides	6.3±0.3	7.3±0.3	9.3±0.3	<0.05
Tannins	11.7±0.3	6.7±0.03	8.7±0.03	<0.05
Phenols	14.1±0.2	15.1±0.2	12.5±0.5	<0.05
Stilbenoids	ND	ND	4.7±0.2	<0.05

Key: ND (Not detected), Means ±SD from 3 biological x3 technical replicates. ($p<0.05$ = Tukey's test). Quantification based on calibration with authentic standards.

LC-MS Result of Baobab Seed

Table 2 shows the LC-MS result of Baobab seeds extract, and based on the LC-MS result about 6 different compounds belonging to various classes were identified in Baobab seed extract.

Table 2. Compounds Identified From Baobab Seed Extract

S/N	Identified compounds	Molecular formula	Calculated mass	Precursor ion, m/z $[M-H]^-$ $[M+H]^+$	Concentration (mg/g)
1	Sinapic acid	$C_{11}H_{12}O_5$	225.402	224.212	7.1±0.03
2	3-Feruloylquinic acid	$C_{17}H_{20}O_9$	367.511	367.1034	8.0±0.03
3	Dihydroquercetin	$C_{15}H_{10}O_7$	303.877	303.0508	8.5±0.5
4	Malic acid	$C_4H_6O_5$	134.121	133	9.7±0.5
5	Hexose-hexose-Nacetyl	$C_{14}H_{25}NO_{10}$	365.177	366	7.3±0.3
6	Gallic acid	$C_7H_6O_5$	172.152	171	6.7±0.03

LC-MS Result of Black Seed

Table 3 display results of LC-MS analysis of Black seed extract, about three different compounds including; 3-Feruloylquinic acid, Malic acid, and Sinapic acid were identified.

Table 3. Compounds Identified from Black Seed Extract

S/N	Identified compounds	Molecular formula	Calculated mass	Precursor ion, m/z $[M-H]^-$ $[M+H]^+$	Concentration (mg/g)
1	3-Feruloylquinic acid	$C_{17}H_{20}O_9$	368.687	367	5.1±0.03
2	Malic acid	$C_4H_6O_5$	134.152	133	7.6±0.03
3	Sinapic acid	$C_{11}H_{12}O_5$	225.305	224.212	9.0±0.03

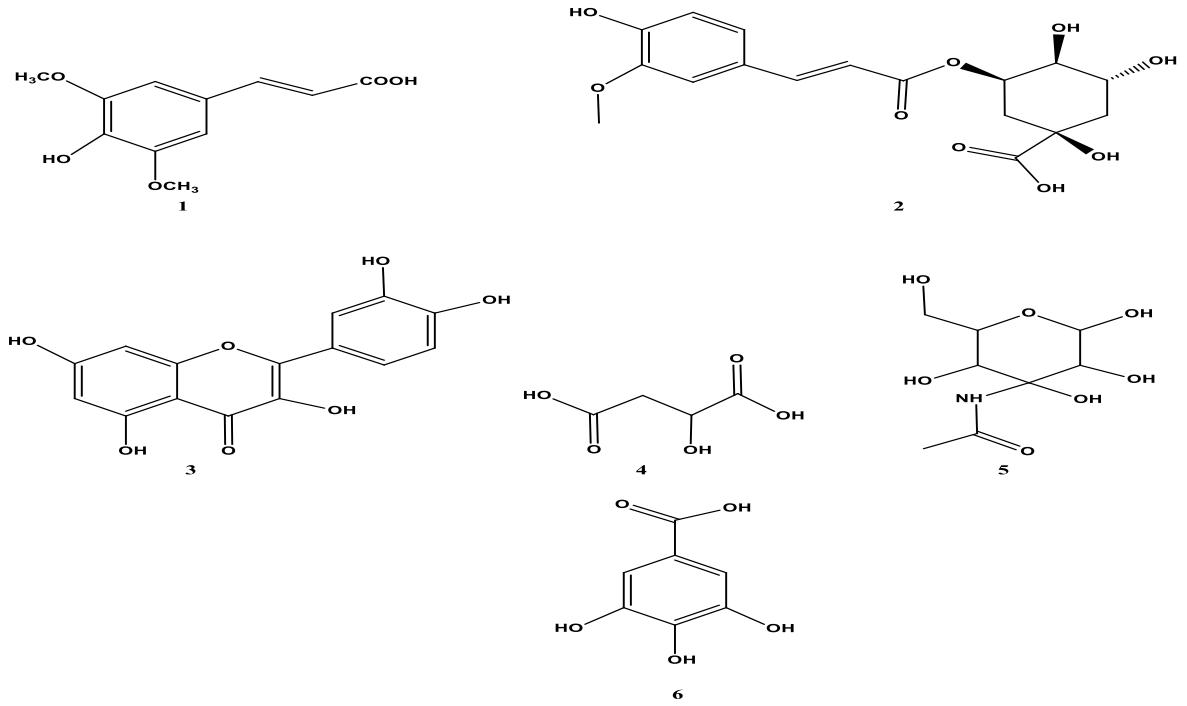

Fig 1. Structure of the Compounds Identified from Baobab seed and Black seed oils.

LC-MS Result of Baobab Seed

Table 4 shows the presence of Malic acid, Polydatin and 3-feruloylquinic acid in the Neem seed extract.

Table 4. Compounds Identified from Neem Seed Extract

	Identified compounds	Molecular formula	Calculated mass	Precursor ion, m/z $[M-H]^-[M+H]^+$	Concentration (mg/g)
1	Malic acid	$C_4H_6O_5$	134.111	133	11.3±0.03
2	Polydatin [piceid; trans-piceid]	$C_{20}H_{22}O_8$	389.355	389	4.7±0.2
3	3-Feruloylquinic acid	$C_{14}H_{25}NO_{10}$	367.443	366	12.5±0.5

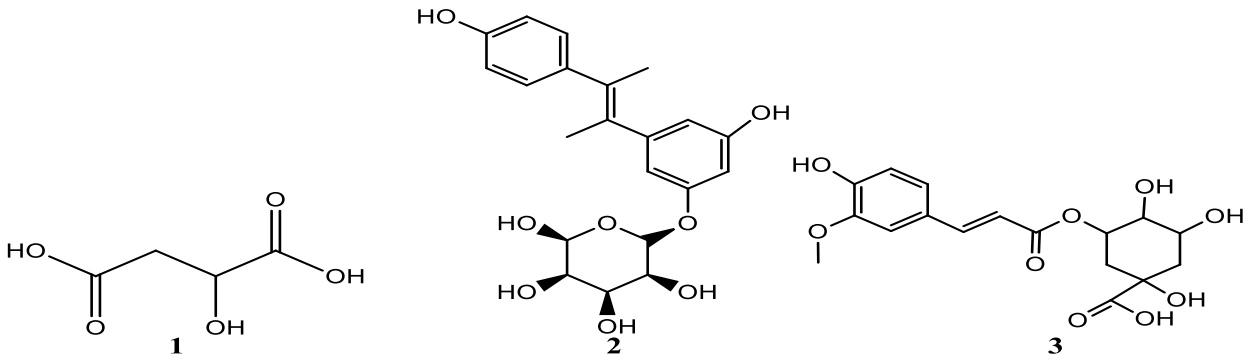

Fig 2. Structure of the Compounds Identified from Neem Seed oil.

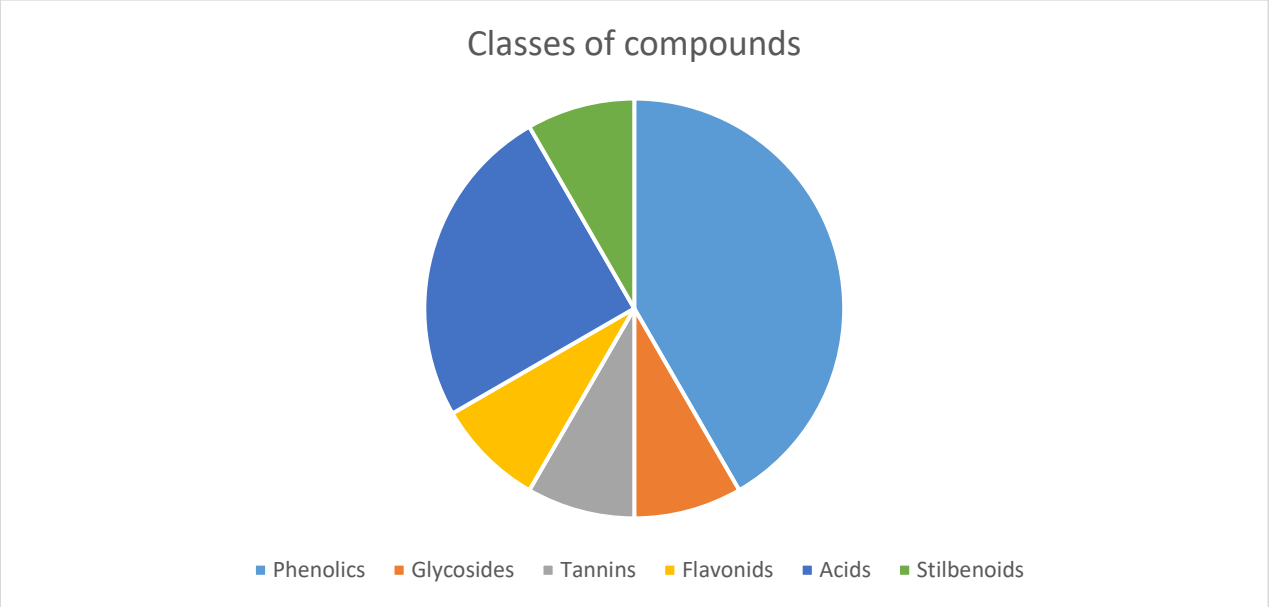

Fig 3. Summary of the classes of compounds detected from the LC-MS Result

Result of Physicochemical Analysis

Table 5 reveals the physicochemical analysis of the three seed oils using four parameters, percentage yield, peroxide value, iodine value and saponification value.

Table 5. Physicochemical Analysis of the Three Seed Oils

Plants	Percentage yield (%)	Peroxide value(mEq/kg)	Iodine value (g/100g)	SAP value(mg/g)
Black Seed	28.0±2	8.3±0.2	113.0±2.0	185.0±0.5
Baobab Seed	24.0±2	4.1±0.2	86.0±2.0	188.0±0.5
Neem Seed	37.0±2	8.2±0.2	175.0±2 .0	198.0±0.5

Key; ± mean Standard deviation

Result of Neem Seed GC-MS Analysis

The tables below 6 shows the GC-MS result of the Neem seed oil, showing some of the compounds detected and their quantity present, as well as their molecular formulas.

Table 6. GC-MS Analysis of Neem Seed Oil

S/N	Retention Time	Compounds		% Area	Concentration (mg/g)	Molecular Formula
1	5.6023	Tetradecanoic acid		1.0119	12.23±0.2	$C_{14}H_{28}O_2$
2	5.6993	n-Hexadecanoic acid		1.115	13.49±0.2	$C_{16}H_{32}O_2$
3	5.9749	Octadecanoic acid		1.3085	15.82±0.3	$C_{18}H_{36}O_2$
4	6.1761	9-Octadecanoic acid		0.2314	2.80±0.2	$C_{18}H_{34}O_2$
5	6.5141	9,12-Octadecadienoic acid		1.9458	23.54±0.4	$C_{18}H_{32}O_2$
6	8.2442	(9Z)-Hexadec-9-enoic acid		2.1668	26.22±0.4	$C_{16}H_{30}O_2$
7	8.5348	2-methoxy-4-(prop-2-en-1-yl)phenol		2.1306	25.74±0.4	$C_{10}H_{12}O_2$
8	10.1789	3,7,11-Trimethyldodeca-1,6,10-trien-3-ol		1.5621	18.91±0.3	$C_{15}H_{26}O$
9	10.6565	(3β,24R)-Ergost-5-ene-3-ol		1.68	20.33±0.2	$C_{28}H_{48}O$
10	10.7612	(3β,22E)-Stigmasta-5,22-dien-3-ol		0.1419	1.71±0.2	$C_{29}H_{48}O$
11	11.4436	Salanin		0.089	1.07±0.2	$C_{34}H_{44}O_9$
12	12.7339	(3β,24Z)-Stigmasta-5,24-diene-3-ol		0.2304	2.79±0.2	$C_{29}H_{48}O$
13	14.2834	Nimbin		0.1563	1.89±0.2	$C_{30}H_{36}O_{10}$
14	15.5573	Gedunin		0.4025	4.86±0.2	$C_{28}H_{34}O_7$
15	19.7454	Azadirachtin		3.0942	37.44±0.4	$C_{35}H_{44}O_{16}$

Black Seed GC-MS Result

Table 7 shows the GC-MS result of the black seed oil, showing the compounds present and their quantity in the sample. Similarly, the various molecular formula of each compound were presented for easy clarification.

Table 7. GC-MS Analysis of Black Seed Oil

S/N	Retention Time	Compounds		% Area	Concentration (mg/g)	Molecular Formula
1	5.1875	**Thymoquinone**		3.8955	29.02±0.3	$C_{10}H_{12}O_2$
2	5.5156	Isopropyl-5-methyl-1,4-benzoquinone		1.7005	12.71±0.2	$C_{10}H_{12}O_2$
3	12.4856	(3β)-Cholest-5-en-3-ol		0.2417	1.83±0.2	$C_{27}H_{46}O$
4	14.479	Octadecanoic acid		0.4711	3.58±0.2	$C_{18}H_{36}O_2$
5	6.7045	1-Decanol		1.695	12.73±0.3	$C_{10}H_{22}O$
6	41.2694	n-Hexadecanoic acid		15.0688	113.65±1	$C_{16}H_{32}O_2$
7	7.2167	p-Xylene		1.9858	14.96±0.3	C_8H_{10}
8	43.5124	Linoelaidic acid		9.7742	73.25±0.7	$C_{18}H_{34}O_2$
9	8.5668	1-Methoxy-4-(prop-2-en-1-yl)benzene		1.0067	7.53±0.2	$C_{10}H_{12}O$
10	8.9707	4-Allyl-2-methoxyphenol		0.6524	4.93±0.2	$C_{10}H_{12}O_2$
11	9.1594	1,2,3-Trimethoxy-5-(prop-2-en-1yl)benzene		0.8016	6.08±0.2	$C_{11}H_{14}O_3$
12	9.4433	(3β)-Urs-12-en-3-ol		0.0901	0.77±0.2	$C_{30}H_{50}O$
13	11.5979	(3β)-Stigmastan-3-ol		0.2485	1.95±0.2	$C_{29}H_{50}O$
14	11.9239	(3β)-Lup-20(28)-en-3-ol		2.3832	17.82±0.6	$C_{30}H_{50}O$

Baobab Seed Oil GC-MS Result

Table 8 present the GC-MS analysis of the Baobab seed oil, showing some of the compounds detected.

Table 8. GC-MS Analysis of Baobab Seed Oil

S/N	Retention Time	Compounds		% Area	Concentration (mg/g)	Molecular Formula
1	6.9038	(E,E)-2,4-Nonadienal		0.1311	0.49±0.2	$C_9H_{14}O$
2	7.0199	3-Octen-2-one		2.0503	7.60±0.2	$C_8H_{14}O$
3	7.56079	(Z)-11-Docosenoic acid (Gondoic acid)		0.4731	1.75±0.2	$C_{22}H_{42}O_2$
4	8.0222	o-Xylene		3.0422	11.27±0.3	C_8H_{10}
5	44.4452	Linolelaidic acid		6.5987	24.45±0.4	$C_{18}H_{32}O_2$
6	13.4577	Octadecanoic acid		10.8801	40.33±0.4	$C_{18}H_{36}O_2$
7	13.5777	Tetradecanoic acid		2.2116	8.19±0.3	$C_{14}H_{28}O_2$
8	40.8526	Nonadecanoic acid		2.8875	10.68±0.2	$C_{19}H_{38}O_2$
9	35.9751	Pentadecanoic acid		3.6751	13.61±0.2	$C_{17}H_{34}O_2$
10	40.2558	n-Hexadecanoic acid		6.7849	25.14±0.5	$C_{16}H_{32}O_2$
11	41.714	(Z,Z)-9,12-Octadecadienoic acid (Linoleic acid)		10.9954	42.33±0.4	$C_{18}H_{32}O_2$

Structure-Activity Relationship (SAR)

Table 9. Structure-activity relationship of the compounds detected.

Compound	Structural Feature	Bioactivity	Application
Dihydroquercetin	Multiple hydroxyl groups; flavonoid backbone	Antioxidant, anti-inflammatory, vascular protection	Cosmeceuticals, nutraceuticals, cardiovascular supplements
Polydatin	Stilbene backbone; β-glucoside group at C-3	Antioxidant, anti-inflammatory, neuroprotective	Natural anti-aging creams, nutraceuticals
Chlorogenic acid (e.g. 3-feruloylquinic)	Caffeoylquinic acid core; conjugated aromatic system	Antioxidant, hepatoprotective, antimicrobial	Topical antioxidants, herbal extracts
Palmitic acid	Long saturated alkyl chain (C16:0)	Antimicrobial via membrane disruption, emollient	Cosmetic base oils, antimicrobial soaps
Linoleic acid	Polyunsaturated C18:2 with cis-double bonds	Skin barrier repair, anti-inflammatory, antimicrobial	Moisturizers, wound-healing balms, emulsions
Stigmastanol	Sterol ring system; saturated side chain	Anti-inflammatory, skin conditioning, cholesterol-lowering	Skin creams, anti-inflammatory balms
Cymene	Aromatic hydrocarbon with methyl substitutions	Antimicrobial, wound-healing, anti-inflammatory	Essential oils, antimicrobial gels
Isopropyl-5-methyl-1,4-benzoquinone	Benzoquinone with isopropyl group	Antioxidant, anti-inflammatory	Pharmaceuticals, antioxidant-rich ointments

Table 10. Structure-activity relationship of the compounds detected.

Compound	Structural Feature	Bioactivity	Application
Azadirachtin	Limonoid lactone	Insecticidal, antimicrobial	Epoxide ring and ester side chains disrupt membranes
Thymoquinone	Quinone structure with isopropyl group	Antioxidant, antimicrobial, anticancer	Quinone redox cycling leads to ROS generation in microbes
Sinapic acid	Methoxy and hydroxylated phenylpropene	Antioxidant, antimicrobial	Methoxy increases lipophilicity, enhances cell entry
Toluene	Simple aromatic hydrocarbon	Mild antimicrobial, solvent-like behavior	Formulation solvent, fragrance base

The phytochemical analysis of three plant seeds; Black Seed (*Nigella sativa*), Baobab (*Adansonia digitata*), and Neem (*Azadirachta indica*) using ethanol as a solvent, revealed notable variations in their chemical compositions. In Black Seed, the predominant constituents identified were phenols and tannins, followed by flavonoids, glycosides, and stilbenoids. These findings align with recent studies as cited highlighting the presence of phenolic compounds in *N. sativa* seeds when extracted with ethanol (Oktaviani *et al.*, 202; Shafodino *et al.*, 2022). While in Baobab Seed, it exhibited four of the five analyzed phytochemicals, including phenols, while stilbenoids were absent. Comparable research has demonstrated the presence of phenolic compounds in Baobab seeds extracted with ethanol (Asogwa *et al.*, 2021; Asmamaw & Sbhatu, 2023; Silva *et al.*, 2023). Similarly, in Neem Seed all five tested phytochemicals, including stilbenoids, were detected in the extract. Previous studies have reported the presence of phenolic compounds in *A. indica* seeds extracted with ethanol (Islas *et al.*, 2020; Uche *et al.*, 2021). These results corroborate existing literature as cited, confirming that phenolic compounds can be effectively extracted from these three plant seeds using ethanol as a solvent. Phenolic compounds, along with other identified phytochemicals, are recognized for their biological activities, including antioxidant, antifungal, antibacterial, and anti-inflammatory properties. Therefore, the phytochemical profiles of these seeds suggest their potential as natural antimicrobial agents (Ali *et al.*, 2018; Roy *et al.*, 2022; Godlewska *et al.*, 2023).

The LC-MS analysis of plant seed extracts identified six major compounds namely:hydroxycinnamic acids, flavones, glycosides, tannins, stilbenes, and malic acid (Shao *et al.*, 2014; Razgonova *et al.*, 2021; Shafodino *et al.*, 2022). Among these, hydroxycinnamic acids, such as ferulic acid, caffeic acid, sinapic acid, and p-coumaric acid, are prevalent in various plant tissues, including seeds, and are known for their antioxidant, anti-inflammatory, antimicrobial, and anticancer activities. In the current study, 3-feruloylquinic acid was detected in both Baobab and Black seed extracts, with a precursor ion at m/z 367.1038 and fragment ions at m/z 298, 288, 192, and 191, corresponding to the losses of [M-H-$3H_2O_2$-CH_3], [M-H-H_2O-CH_3-HCOOH], [M-H-$C_7H_{11}O_5$], and [M-H-$C_{10}H_8O_3$], respectively. This compound has been reported to inhibit microbial growth and possess antioxidant properties. Additionally, sinapic acid was identified in both seed extracts, with a precursor ion at m/z 225 and fragment ions at m/z 179, 153, and 210, corresponding to the losses of [M-H-HCOOH], [M-H-CH_2CHCOOH], and [M-H-CH_3], respectively. Sinapic acid, identified with a precursor ion at m/z 225 and fragments at m/z 179, 153, and 210, exhibits antimicrobial effects against Gram-positive and Gram-negative bacteria and fungi, including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Aspergillus niger*. It also possesses antioxidant and anti-inflammatory properties, indicating its potential as a therapeutic agent. Flavones, such as dihydroquercetin, identified with a precursor ion at m/z 303.0510 and fragment ions at m/z 285, 275, and 151, exhibit antimicrobial activity through impairment of cell membrane integrity, metabolic blockade, and hindrance of nucleic acid synthesis. They also chelate essential metallic ions and induce oxidative stress in microbial cells, leading to cell death. Glycosides, identified as hexose-hexose-N-acetyl with a precursor ion at m/z 366 and fragment ions at m/z 186 and 142, disrupt microbial cell walls and membranes, inhibit key metabolic enzymes, and produce reactive oxygen species, leading to microbial cell death. They have activity against bacteria, fungi, and some viruses. Tannins, such as gallic acid identified with a precursor ion at m/z 171 and a fragment at m/z 126, exhibit antioxidant activity and protect against oxidative damage mediated by reactive species. Gallic acid derivatives also exhibit a wide range of

bioformulations and pharmacological actions, including ROS sequestration, blockage of various signaling pathways, and induction of apoptosis in cancer cells. Stilbenoids, such as polydatin identified with a precursor ion at m/z 389 and fragment ions at m/z 227, 343, 184, and 143, exhibit anti-inflammatory, antioxidant, and antimicrobial properties. They also have beneficial effects including nephroprotective, hepatoprotective, and lung protective effects. Malic acid, identified with a precursor ion at m/z 134 and a fragment at m/z 115, is a common component in plant extracts and exhibits various biological activities. Its presence in the seed extracts indicates its potential contribution to the overall biological effects of the seeds. In conclusion, the presence of these bioactive compounds in Baobab, Black, and Neem seed extracts suggests their potential as natural antimicrobial agents. The identified compounds exhibit various biological activities, including antioxidant, antimicrobial, and anti-inflammatory properties, supporting the traditional use of these seeds in medicinal applications (Shao *et al.*, 2014; Uche *et al.*, 2021; Asmamaw Washun & Sbhatu, 2023; Castro-Díaz *et al.*, 2025).

A comparative analysis of the LC-MS profiles of baobab, black seed, and neem seed extracts revealed both shared and unique phytochemical constituents, highlighting the novelty of this study. While malic acid and 3-feruloylquinic acid were present across all three extracts, indicating a conserved set of antimicrobial and antioxidant agents, certain compounds were uniquely identified in individual seeds. Notably, polydatin was exclusively found in neem seed extract, a compound primarily reported in grapes and rarely associated with neem, suggesting a potential untapped source of anti-inflammatory and therapeutic value. Similarly, dihydroquercetin, detected only in baobab seed, is an uncommon finding and suggests potent antioxidant contributions specific to this extract. The detection of hexose-hexose-N-acetyl, a glycosylated structure, further underscores the biochemical diversity of baobab seed. These variations are significant as they may explain the differential antimicrobial efficacy of the formulated products and open new research avenues in seed-based cosmeceutical bioactivity. The bioactive compounds identified in the baobab, black seed, and neem seed extracts possess functional groups and structural features that underpin their biological activities. Sinapic acid and 3-feruloyquinic acid are both phenolic acids containing hydroxyl and methoxy groups that contribute significantly to their antioxidant capacity by scavenging free radicals which supports antimicrobial efficacy and skin protection. Polydatin, which is a stilbene glycoside, exhibits strong antioxidant and anti-inflammatory activities enhancing the therapeutic potential of neem seed extracts. Dihydroquercetin, which is a flavonoid, carries multiple hydroxyl groups facilitating free radical neutralization and anti-inflammatory effects, crucial for skin health. Malic acid, which is an alpha hydroxyl acid, contributes exfoliating properties that promote skin renewal, aligning with cosmetic applications. Gallic acid as well is documented from its antimicrobial and antioxidant properties reinforcing the observed enhanced antimicrobial properties in cosmetics. Collectively, the presence of these compounds with complementary structural attributes supports the multifunctional bioactivities observed, thereby providing mechanistic basis for the enhanced efficacy of newly developed cosmetic formulations (Chen, 2016; Divya & Martin, 2025; Imtiyaz *et al.*, 2025; Kim *et al.*, 2022).

Table 5 shows the physicochemical analysis of the three seed oils; the percentages of oil obtained (NES, BLS, and BAB) and the saponification value, iodine value, and peroxide were also calculated. The standard methods demonstrated that NES, BLS, and BAB oil produced 37.0±2%, 28.0±2%, and 24.0±2% oil, respectively. Results for saponification values showed that Neem seed oil (198.0±0.5 mg/g), Baobab seed oil (188.0±0.5 mg/g), and Black seed oil (185.0±0.5 mg/g) had the respective values. The iodine values for the oils, likewise were found to be 113.0±2.0 g/100g for Black seed oil, 86.0±2 g/100g for Baobab seed oil and 175.0±2 g/100g for Neem seed oil. Peroxide values revealed that Neem seed oil (8.2±0.2 mEq/kg) and Black seed oil (8.3±0.2 mEq/kg) were almost identical, whilst Baobab seed oil showed a peroxide value of 4.1±0.2 mEq/kg. Three types of seed were studied for the yield percentage and chemical characterization and the study shows that the major constituents from the chemical analysis of the oils were the fatty acids of long and medium chain. The physicochemical properties of these plant oils confirm that they can be considered high-quality and applicable in various fields, for instance, they can be used in the production of cosmetics. These oils are free from human health harmful impurities (Afolayan *et al.*, 2014; Badmos *et al.*, 2019; Amaama *et al.*, 2023; Mahamat *et al.*, 2025)

The GC-MS profiling of neem seed oil has revealed a diverse array of bioactive compounds contributing to its antimicrobial, anti-inflammatory, and cosmetic properties. Notable fatty acids identified include palmitic acid (13.49 mg/g), stearic acid (15.82 mg/g), linoleic acid (23.54 mg/g), and oleic acid (71.76 mg/g). These fatty acids are known for their emollient, anti-inflammatory, and antimicrobial effects, making neem seed oil valuable in cosmetic formulations. In addition to fatty acids, neem seed oil contains terpenoids and limonoids, which are recognized for their medicinal properties. Azadirachtin, a limonoid present at 37.44 mg/g, is well-studied for its insecticidal and antimicrobial activities. Other limonoids

such as nimbin (1.89 mg/g), gedunin (4.86 mg/g), and salanin (1.07 mg/g) have demonstrated antifungal, anti-inflammatory, anticancer, antimalarial, and hepatoprotective effects. Aromatic hydrocarbons detected in neem seed oil include toluene (85.55 mg/g), diethylbenzene (9.55 mg/g), and p-xylene (5.89 mg/g), which contribute to the oil's characteristic odor and may have applications in perfume formulations. Cyclic hydrocarbons such as cis-1-ethyl-3-methyl-cyclohexane (5.56 mg/g) and bicycle [2.1.1] hex-2-ene (34.34 mg/g) indicate the presence of biologically active compounds with antimicrobial and antifungal activity. Plant sterols identified in neem seed oil include stigmasta-5,22-dien-3-ol (1.71 mg/g), stigmasta-5,24-diene-3-ol (2.79 mg/g), and ergost-5-ene-3-ol (20.33 mg/g), which possess cholesterol-lowering and anti-inflammatory activities. These compounds may contribute to the oil's potential therapeutic properties for skin disorders and cardiovascular health. Collectively, the diverse composition of neem seed oil, as revealed through GC-MS profiling, underscores its multifaceted bioactivity. The presence of various bioactive compounds supports the traditional use of neem seed oil in medicine and industry, highlighting its potential as a formulation in natural cosmetics, pharmaceuticals, and agricultural biopesticides (Warra *et al.*, 2015; Zhang *et al.*, 2022; Shah *et al.*, 2021; Aati *et al.*, 2025).

Recent Gas Chromatography-Mass Spectrometry (GC-MS) analyses of black seed oil (Nigella sativa) have elucidated its complex chemical profile, revealing a diverse array of bioactive compounds that contribute to its antimicrobial, anti-inflammatory, and cosmetic properties. These findings underscore the oil's potential applications in natural cosmetics, pharmaceuticals, and agricultural biopesticides. The predominant fatty acids identified include: Palmitic acid (C16:0): 113.65 mg/g, Linolelaidic acid (C18:2): 73.25 mg/g, Stearic acid (C18:0): 3.58 mg/g. These fatty acids are known for their emollient, anti-inflammatory, and antimicrobial effects, making black seed oil valuable in cosmetic formulations. Linolelaidic acid, a trans-isomer of linoleic acid, contributes to the oil's skin barrier function and anti-inflammatory properties. Aromatic hydrocarbons detected include: Toluene: 74.82 mg/g, Ethylbenzene: 5.09%. These compounds contribute to the specific aroma of black seed oil and may have applications in fragrance formulations. Toluene and ethylbenzene are also associated with plant defense mechanism when present in controlled concentrations. Cyclohexane derivatives such as 1,1-dimethylcyclohexane (15.42 mg/g) were identified. These compounds may play a role in the oil's volatile profile and scent characteristics. Phenolic compounds identified include: Isopropyl-5-methyl-1,4-benzoquinone: 12.71 mg/g, 1-Methoxy-4-(prop-2-en-1-yl)benzene: 7.53 mg/g. These compounds possess antioxidant and antimicrobial properties, contributing to the therapeutic uses of black seed oil. Cymene, a derivative, exhibits radical-scavenging activity, enhancing the oil's antioxidant capacity. Plant sterols identified include: (3β)-Cholest-5-en-3-ol: 1.71 mg/g, (3β)-Ergost-5-en-3-ol: 2.79 mg/g, (3β)-Stigmastan-3-ol: 20.33 mg/g. These sterols possess cholesterol-lowering and anti-inflammatory activities, which may contribute to the oil's potential therapeutic properties for skin disorders and cardiovascular health. The comprehensive chemical profiling of black seed oil highlights its multifaceted bioactivity, supporting its traditional use in medicine and industry. The presence of various bioactive compounds provides a mechanistic basis for the enhanced efficacy of newly developed cosmetic formulations. Further research is warranted to explore the full therapeutic potential of black seed oil (Abbas *et al.*, 2024; Abdel-Razek *et al.*, 2024; Hassain *et al.*, 2025).

The GC-MS analysis of baobab seed oil has revealed a diverse array of bioactive compounds, including fatty acids, aromatic hydrocarbons, and oxygenated hydrocarbons, which hold significant commercial potential in cosmetics, pharmaceuticals, and the food industry. Fatty acids were identified as the predominant constituents, with notable compounds such as octadecanoic acid (stearic acid) (40.33 mg/g), 9,12-octadecadienoic acid (linoleic acid) (42.33 mg/g), and n-hexadecanoic acid (palmitic acid) (25.4 mg/g) among others. Linoleic acid, an essential fatty acid, is recognized for its beneficial effects on skin barrier integrity and its anti-inflammatory properties, suggesting its suitability for dermatological applications. Stearic acid serves as an emollient and stabilizer in cosmetic formulations, while palmitic acid enhances the moisturizing effects of skincare products. Additional fatty acids identified include linoelaidic acid (25.45 mg/g), eicosanoic acid (4.16 mg/g), and docosanoic acid (11.32 mg/g), which possess potential antioxidant capabilities and are relevant in cosmetic and industrial products. Beyond fatty acids, the analysis detected various aromatic hydrocarbons and benzene derivatives, which may contribute to the stability, odor, and potential antimicrobial activity of the oil. Compounds such as 1,2,4-trimethylbenzene (21.23 mg/g) and 1-ethyl-4-methylbenzene (0.53 mg/g) indicate their potential role in oil preservation. Other aromatic hydrocarbons, including o-xylene (11.27 mg/g) and cumene (0.65 mg/g), have demonstrated antimicrobial activity, supporting the oil's potential for skincare applications. The presence of oxidation hydrocarbons like 2-butyl-2-octenal (4.97 mg/g) and 3-octen-2-one (7.60 mg/g) suggests a role in the oil's antioxidant and antimicrobial properties. Additionally, aldehydes such as hexanal (0.30 mg/g) and nonanal (0.332 mg/g) contribute to the oil's perfumery and oxidative stability. The composition of baobab seed oil, rich in beneficial compounds, renders it suitable for various industrial

applications. Its high content of linoleic, palmitic, and stearic acids makes it an excellent ingredient for moisturizing creams, anti-aging formulations, and hair care products in the cosmetic industry (Warra *et al.,* 2015; Cissé *et al.,* 2018; Washun & Sbhatu, 2023; Thompson *et al.,* 2024)

The SAR analysis of phytochemicals identified in ethanolic extracts and seed oils of Nigella sativa (black seed), Adansonia digitata (baobab seed), and Azadirachta indica (neem seed) demonstrates that specific functional groups strongly influence their biological activities (see Tables 9 and 10). Flavonoids such as dihydroquercetin, characterized by multiple hydroxyl groups and a flavonoid backbone, exhibit potent antioxidant and anti-inflammatory properties, offering vascular protection and supporting applications in cosmeceuticals and cardiovascular supplements (Ullah *et al.,* 2020; Islas *et al.,* 2020 ; Barba-Ostria *et al.,* 2022). Polydatin, a stilbene glucoside with a β-glucoside moiety at C-3, combines antioxidant and neuroprotective effects through enhanced solubility and radical-scavenging, making it suitable for natural anti-aging creams and nutraceuticals (Altemimi *et al.,* 2017; Nasim *et al.,* 2022; Ouerfelli *et al.,* 2022). Phenolic acids like chlorogenic acid (e.g., 3-feruloylquinic acid) possess a caffeoylquinic acid core and conjugated aromatic systems, conferring antioxidant, hepatoprotective, and antimicrobial effects utilized in topical antioxidants and herbal extracts (Rohloff, 2015). Long saturated fatty acids such as palmitic acid (n-hexadecanoic acid) act as antimicrobial agents via membrane disruption and function as emollients in cosmetic base oils and antimicrobial soaps (Godlewska *et al.,* 2023). Linoleic acid, a polyunsaturated C18:2 fatty acid with cis-double bonds, promotes skin barrier repair, exhibits anti-inflammatory and antimicrobial effects, and is widely applied in moisturizers, wound-healing balms, and emulsions (Cissé *et al.,* 2018). Sterols such as stigmastanol, with a sterol ring system and saturated side chain, contribute anti-inflammatory and skin-conditioning effects, with potential cholesterol-lowering activity, utilized in skin creams and anti-inflammatory balms (Rybczynska-Tkaczyk *et al.,* 2023). Aromatic hydrocarbons including p-cymene, with methyl substitutions, display antimicrobial, wound-healing, and anti-inflammatory properties, making them valuable in essential oils and antimicrobial gels (Shafodino *et al.,* 2022). Compounds like isopropyl-5-methyl-1,4-benzoquinone, a benzoquinone with isopropyl substitution, exhibit antioxidant and anti-inflammatory activities relevant for pharmaceutical antioxidant-rich ointments (Thompson *et al.,* 2024). Simple aromatics such as toluene demonstrate mild antimicrobial effects and serve as solvents or fragrance bases (Cabral *et al.,* 2023).

Mechanistically, hydroxyl and methoxy groups in flavonoids and phenolic acids stabilize free radicals through electron delocalization, enhancing antioxidant capacity (Rohloff, 2015). Glycosylation, as in polydatin, improves water solubility and bioavailability (Barba-Ostria *et al.,* 2022). Thymoquinone derivatives in *Nigella sativa* mediate antimicrobial and anti-inflammatory effects via redox cycling and reactive oxygen species generation (Altemimi *et al.,* 2017). Polyunsaturated fatty acids modulate inflammation and maintain skin hydration by integrating into cell membranes and influencing signaling pathways (Toma *et al.,* 2015). These SAR insights underscore the pharmacological potential of these seed extracts and oils, supporting their formulation into cosmetics, nutraceuticals, and pharmaceuticals.

This research aligns with Sustainable Development Goals 3 (Good Health), 9 (Industry, Innovation, and Infrastructure), 12 (Responsible Consumption and Production), and 13 (Climate Action) by promoting the sustainable use of medicinal plants and advancing analytical methods for quality assurance. Future work could incorporate AI-driven metabolomic data analysis and intelligent extraction optimization using machine learning models (e.g., Random Forest, SVM) to enhance process efficiency and bioactivity prediction. Integration of digital twins and blockchain for traceability may further modernize seed oil production and ensure sustainability.

CONCLUSION

This study presents a comprehensive investigation into the phytochemical composition, physicochemical characteristics, and structure–activity relationships (SAR) of ethanolic extracts and seed oils derived from *Azadirachta indica* (Neem), *Nigella sativa* (Black seed), and *Adansonia digitata* (Baobab). Phytochemical screening confirmed the presence of bioactive classes including flavonoids, phenolics, tannins, glycosides, phytosterols, and stilbenoids, with Black seed extract showing the highest phenolic and tannin content, and stilbenoids being exclusive to Neem extract. The study incorporated SAR analysis from the outset to elucidate how specific structural features such as hydroxyl groups, conjugated double bonds, and glycosylation influence biological activity. Advanced LC-MS and GC-MS analyses enabled detailed profiling of six key compound classes: hydroxycinnamates, flavones, glycosides, tannins, stilbenoids, and organic acids. Notable compounds such as **dihydroquercetin, sinapic acid, linoleic acid**, and **polydatin** were identified. SAR insights revealed that the antioxidant and anti-inflammatory effects of these compounds are primarily attributed to structural motifs like hydroxyl-rich aromatic rings (in dihydroquercetin and sinapic acid), glucosidic

linkages (in polydatin), and polyunsaturated fatty acid chains (in linoleic acid). Thymoquinone derivatives in *Nigella sativa* demonstrated antimicrobial and redox activity through benzoquinone-mediated radical formation. GC-MS analysis of the seed oils identified 63, 45, and 44 compounds in Neem, Black seed, and Baobab oils respectively, with Neem oil producing the highest yield (37.0%). Physicochemical parameters including saponification, iodine, and peroxide values indicated suitability for industrial applications. Neem oil was especially rich in compounds like azadirachtin, β-sitosterol, and nimbin, contributing to its anti-inflammatory and antimicrobial profile.

Importantly, this study reports the rare detection of **polydatin** and **dihydroquercetin** in these seeds, expanding the known chemical diversity of these underutilized plant resources. The integration of SAR allowed a mechanistic interpretation of the extracts' therapeutic potential, reinforcing their application in antioxidant-rich, antimicrobial, and anti-inflammatory products. In conclusion, the combination of phytochemical profiling, SAR evaluation, and LC-MS/GC-MS fingerprinting offers strong evidence for the use of these plant-derived extracts and oils in pharmaceutical, nutraceutical, and cosmetic formulations. Future work should prioritize compound standardization, bioactivity validation, and formulation development to realize their full industrial and therapeutic potential.

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