PHYTOCHEMICAL PROFILING AND EVALUATION OF ANTIOXIDANT, ANTIBACTERIAL ACTIVITIES, AND HEAVY METAL LEVELS IN *OCIMUM BASILICUM* L. SEED EXTRACTS

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ABSTRACT

This study evaluated the bioactivity and safety of Ocimum basilicum seed extracts in methanol (ME) and hexane (HE). ME exhibited superior antioxidant activity (DPPH IC_{50}: 130.64 ppm; FRAP: 83.2% at 400 ppm Fe^{3+}), correlating with its higher phenolic content (78 ± 0.61 mg GAE/g). In contrast, HE showed moderate efficacy (DPPH IC_{50}: 213.54 ppm; FRAP: 81.0%) due to its non-polar constituents. Antibacterial assays revealed dose-dependent inhibition, with ME outperforming HE against both Gram-positive (S. aureus: 20.4 ± 0.40 mm at 400 ppm) and Gram-negative (E. coli: 15.9 ± 0.32 mm) bacteria, though gentamicin remained more potent. Heavy metal analysis detected essential metals (Fe: 272.67 ± 0.069 ppm; Zn: 113.01 ± 0.290 ppm; Cu: 32.23 ± 0.081 ppm) within safe limits, while toxic metals (Pb, Cd) were undetected (<0.023 ppm). Method validation confirmed precision (recovery rates: 91–105%) and sensitivity (LODs: 0.01–0.84 ppm). Results highlight ME's potential for nutraceutical applications, supported by its bioactive richness and safety profile, while HE's utility may lie in lipid-centric formulations. This work highlights the role of solvent polarity in phytochemical recovery, positioning O. basilicum seeds as a safe and antioxidant-rich resource. *[African Journal of Chemical Education—AJCE 16(1), January 2026]*

INTRODUCTION

Medicinal plants have long served as cornerstones of traditional healthcare systems, particularly in biodiverse regions like Ethiopia, where over 80% of the population relies on herbal remedies for primary healthcare [1]. Among these, *Ocimum basilicum* L. (Lamiaceae), locally termed "besobela" in Amharic, is a globally recognized medicinal and culinary herb with profound ethnobotanical significance. It has been integral to traditional medicine for centuries, addressing ailments ranging from kidney disorders to infectious diseases [2]. The seeds, leaves, and essential oils of *O. basilicum* are prized for their therapeutic properties, including antimicrobial, antioxidant, and anti-inflammatory activities [3]. Despite its widespread use, scientific validation of the seeds' bioactivity remains underexplored compared to its aerial parts, creating a critical research gap [4].

Ethiopia's rich biodiversity and traditional knowledge systems have long harnessed *O. basilicum* seeds for treating physical and psychosocial imbalances. Recent ethnopharmacological surveys highlight their role in managing diabetes, sore throats, and kidney dysfunction, as well as their emerging use in anticancer formulations and oral hygiene products [5]. These applications are attributed to the plant's diverse secondary metabolites, such as phenolics, flavonoids, and terpenoids, which exhibit potent bioactive properties [6]. However, the physicochemical stability and heavy metal safety profile of seed-derived extracts—key factors in drug development—are rarely investigated, limiting their integration into modern pharmacopeias [7].

Plant-derived phytochemicals are increasingly recognized as sources of novel antimicrobial and antioxidant agents, particularly in the face of rising antibiotic resistance and oxidative stress-related diseases. *O. basilicum* seeds are rich in phenolic acids, such as rosmarinic and caffeic acids, which contribute to their radical-scavenging and metal-reducing capacities [8]. Methanolic extracts of basil seeds have demonstrated superior antibacterial activity against Gram-positive pathogens

like *Staphylococcus aureus*, likely due to synergistic interactions between alkaloids and terpenes. Nonetheless, extraction solvents critically influence bioactive yield and efficacy; for instance, polar solvents like methanol enhance phenolic solubility, whereas hexane favors non-polar compounds. While previous studies focus on *O. basilicum* leaves and essential oils, the seeds' phytochemical complexity and therapeutic potential remain underexplored [7]. This study addresses this and the other gaps by:

1. Profiling phytochemicals, antioxidant activity (via DPPH and FRAP assays), and antibacterial efficacy of hexane and methanol seed extracts.
2. Evaluating physicochemical properties (e.g., saponification value, free fatty acids) to assess industrial applicability.
3. Determining heavy metal content to ensure safety compliance with WHO guidelines [9].

The findings aim to validate traditional claims, provide data for quality control, and promote *O. basilicum* seeds as sustainable sources of nutraceuticals and antimicrobial agents in Ethiopia's healthcare and agro-industry sectors.

EXPERIMENTAL

Mature seeds of *Ocimum basilicum* L. (besobela) were collected from the Debremarkos area of Ethiopia (Geocoordinates: **10°20'02"N, 37°43'60"E**) during the dry season (January 2020) and authenticated by a botanist at Debremarkos University. The seeds were shade-dried at 25°C for seven days, cleaned of impurities, and ground into a fine powder (≤0.5 mm particle size) using an electric mill (Retsch SM 200). The powdered material was stored in air-tight containers at 4°C until further use. Analytical-grade solvents (hexane and methanol), Folin-Ciocalteu reagent, DPPH (2,2-diphenyl-1-picrylhydrazyl), gallic acid, ascorbic acid, and nutrient agar were procured from Sigma-Aldrich. Bacterial strains (*Staphylococcus aureus* ATCC 25923, *Streptococcus pyogenes* ATCC

19615, *Escherichia coli* ATCC 25922, and *Klebsiella pneumoniae* ATCC 13883) were sourced from the Ethiopian Public Health Institute (EPHI).

For extraction, 10 g of powdered seeds were extracted with hexane and methanol using a Soxhlet apparatus for 12 hours under reflux (60°C). The extracts were filtered through Whatman No. 1 filter paper, concentrated using a rotary evaporator (Buchi R-300), and lyophilized (Labconco FreeZone) to obtain dry residues. Phytochemical screening was conducted using standard qualitative tests to detect alkaloids (Mayer's reagent), flavonoids (NaOH test), terpenoids (Salkowski test), and phenolics (Ferric chloride test).

Antioxidant activity was evaluated using two methods: the DPPH radical scavenging assay and the FRAP (ferric reducing antioxidant power) assay. For the DPPH assay, varying concentrations of extracts (25–400 ppm) were mixed with 0.1 mM DPPH solution, and absorbance was measured at 765 nm after 30 minutes using a Shimadzu UV-1800 spectrophotometer. Ascorbic acid served as the positive control. The FRAP assay involved reacting extracts with FRAP reagent (TPTZ-Fe^{3+} complex), and absorbance was recorded at 700 nm, with results expressed as μM $FeSO_4$ equivalents. Total phenolic content (TPC) was determined using the Folin-Ciocalteu method, with gallic acid as the standard, and expressed as mg gallic acid equivalents (GAE) per gram of extract.

Antibacterial activity was assessed via the agar well diffusion method. Bacterial suspensions (0.5 McFarland standard) were swabbed onto Mueller-Hinton agar plates, and wells (6 mm diameter) were loaded with 100 μL of extracts (50–200 mg/mL). Gentamicin was used as positive control. Zones of inhibition (ZOI) were measured after 24 hours of incubation at 37°C.

Physicochemical parameters, including moisture content (oven-drying at 105°C), specific gravity (pycnometer), refractive index (Abbe refractometer), free fatty acids (titration with 0.1 M NaOH), acid value, peroxide value, and saponification value, were determined using AOAC and

AOCS standard methods. Heavy metal analysis (Fe, Zn, Cu, Pb, Cd) was performed via atomic absorption spectrometry (PerkinElmer PinAAcle 900T) after acid digestion (HNO_3:$HClO_4$, 4:1 v/v). All experiments were conducted in triplicate, and results are expressed as mean ± standard deviation. Experimental procedures are summarized in Figure 1.

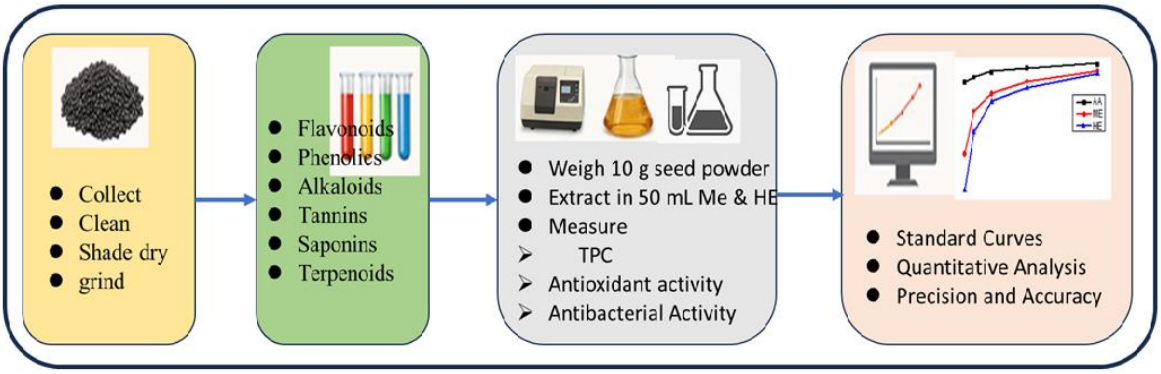

Figure 1: Sample collection, extraction, qualitative analysis, quantitative analysis and data interpretation

RESULTS AND DISCUSSION

1. Quantitative Phytochemical Analysis of O.basilicum seed extracts

Quantitative analysis of *Ocimum basilicum* (basil) encompasses a multifaceted evaluation of its phytochemical composition, bioactive properties, and safety, underpinned by rigorous calibration standards to ensure analytical validity. Calibration curves, constructed using reference compounds such as gallic acid for total phenolic content (TPC) and ascorbic acid (AA) for antioxidant assays, are critical for accurate quantification. These standards, validated for linearity ($R^2 \geq 0.99$) and precision, enable reliable interpolation of sample data, particularly in spectrophotometric methods like the Folin-Ciocalteu assay for phenolics [10]. TPC in basil, often reported as gallic acid equivalents (GAE), ranges widely (10–50 mg GAE/g dry weight), influenced by genetic variation, cultivation practices, and extraction solvents polarity (Javanmardi et al., 2003). Antioxidant

capacity, assessed via DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging and FRAP (ferric reducing antioxidant power), highlights basil's potent redox activity. DPPH results, expressed as IC_{50} values (concentration inhibiting 50% radicals), often fall between 50–200 µg/mL, while FRAP values (Fe^{2+} equivalents) correlate strongly with phenolic levels, affirming phenolics as key contributors [11].

1.1. Calibration Curve and TPC

The calibration curve for total phenolic content (TPC) quantification using Gallic Acid Equivalents (GAE) demonstrated excellent linearity, $A=0.00353\ C_{GAE/g}+1.032$, $R^2=0.9965$, **(Figure 2**), indicating high reliability and precision of the Folin-Ciocalteu assay under the experimental conditions. The R^2 value confirms a robust correlation between absorbance (at 765 nm) and Gallic acid concentration, validating the method for quantitative analysis. However, the relatively high intercept (1.032) suggests potential background absorbance, possibly from reagents (e.g., the Folin-Ciocalteu reagent itself) or matrix effects. This emphasizes the importance of blank correction during sample analysis to avoid overestimation of TPC.

The TPC results for *Ocimum basilicum* extracts, as calculated from the calibration curve (**Figure 2**), revealed significant differences between hexane (56 ± 0.23 mg GAE/g) and methanol (78 ± 0.61 mg GAE/g) extracts. Methanol's superior extraction efficiency aligns with its polar nature, which enhances solubility of phenolic compounds such as phenolic acids and flavonoids compared to non-polar hexane. Phenolics are typically polar due to hydroxyl groups, making methanol a more effective solvent for their extraction. The low standard deviations reflect high reproducibility, likely due to stringent experimental controls.

1.2. Antioxidant efficacy

The DPPH radical scavenging assay highlights significant differences in antioxidant potential between the methanol and hexane extracts of *Ocimum basilicum* seeds, with ascorbic acid (IAA) serving as a strong positive control. The half-maximal inhibitory concentration (IC_{50}) values—130.640 ppm for methanol and 213.538 ppm for hexane—indicate that **methanol extract exhibits superior antioxidant activity**, requiring a lower concentration to achieve 50% inhibition compared to hexane. This aligns with the earlier observation that methanol extracted higher total phenolic content (TPC: 78 ± 0.61 mg GAE/g) than hexane (56 ± 0.23 mg GAE/g), reinforcing the correlation between phenolic content and antioxidant capacity. Phenolic compounds, such as flavonoids and phenolic acids, donate hydrogen atoms or electrons to neutralize free radicals like DPPH, and methanol's polarity likely enhances the recovery of these bioactive molecules.

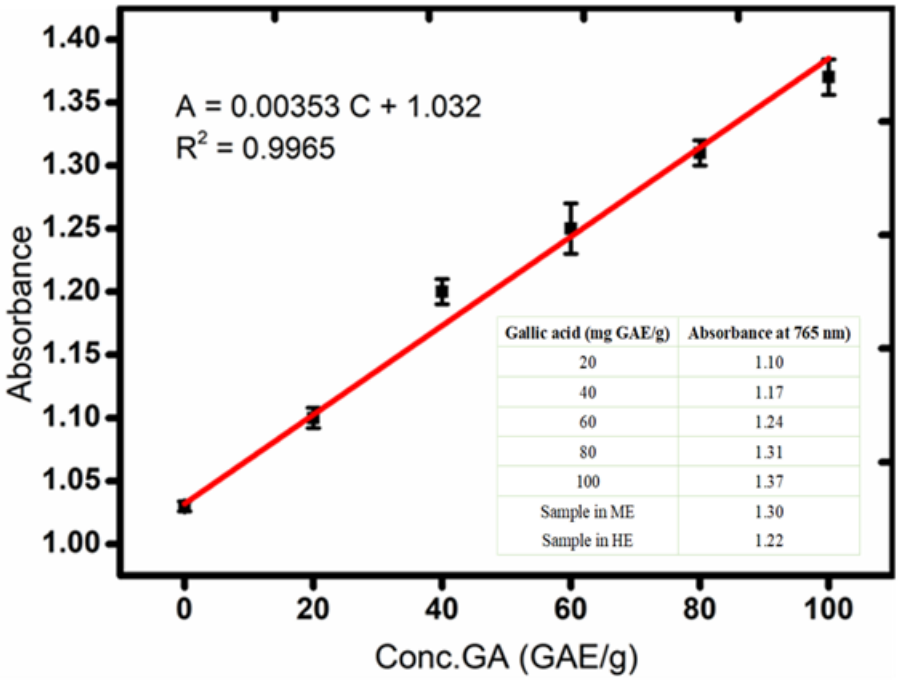

Figure 2: Calibration curve plot for determination of TPC using Gallic Acid standards

The FRAP (Ferric Reducing Antioxidant Power) assay results for *Ocimum basilicum* seed extracts reveal distinct antioxidant profiles for the methanol (ME) and hexane (HE) extracts, with ascorbic acid (AA) serving as a potent reference antioxidant. The data demonstrate a clear **dose-dependent relationship** for all samples, where reducing power (%) increases with higher Fe^{3+} concentrations (400 ppm > 25 ppm). However, the methanol extract consistently outperforms the hexane extract at all concentrations, aligning with prior observations of its superior bioactivity in DPPH scavenging and higher total phenolic content (TPC).

As presented in **Table 1**, numerous studies report on the correlation between solvent polarity and antioxidant efficacy that polar solvents extract higher levels of phenolic compounds and exhibit stronger antioxidant activity in assays like DPPH and FRAP compared to non-polar solvents.

Table 1**:** Comparison of Current DPPH/FRAP Results with Previous Studies

Parameter	Current Study Observations	Previous Findings	Reference
Solvent Polarity	Methanol extract (ME) > Hexane extract (HE) in DPPH/FRAP activity.	Polar solvents (methanol/ethanol) yield higher antioxidant activity due to better extraction of phenolic compounds.	[12, 13]
TPC-Antioxidant Correlation	ME (78 mg GAE/g) > HE (56 mg GAE/g) aligns with ME > HE in DPPH/FRAP.	High TPC strongly correlates with DPPH/FRAP activity in plant extracts.	[14, 15]
DPPH IC_{50} Values	ME: 130.64 ppm; HE: 213.54 ppm.	Similar IC_{50} trends reported for *O. basilicum* methanol extracts (e.g., 100-150 ppm).	[3, 16]
DPPH Inhibition (%)	ME: 78.5% at 400 ppm; HE: 67.8% at 400 ppm.	Methanol extracts of basil leaves/seeds show 70-85% inhibition at 400 ppm.	[17, 18]
FRAP Activity	ME: 83.2% at 400 ppm; HE: 81.0% at 400 ppm.	FRAP values for basil extracts range from 70-90% at high concentrations (300-500 ppm).	[4, 19]
Ascorbic Acid Benchmark	AA outperforms ME/HE (e.g., AA: 99% DPPH inhibition at 400 ppm vs. ME: 78.5%).	Synthetic antioxidants (e.g., AA) typically show 5-10× higher activity than plant extracts.	[20, 21]
Hexane Extract Limitations	HE shows weak activity (e.g., 4.9% FRAP at 25 ppm) due to non-polar solvent limitations.	Hexane extracts often exhibit low DPPH/FRAP activity but may excel in lipid-based assays (e.g., β-carotene bleaching).	[22, 23]

2. Antibacterial Efficacy

Antibacterial efficacy, evaluated through disc diffusion or broth microdilution [24], reveals O. basil's activity against pathogens like *Staphylococcus aureus* and *Escherichia coli*. Essential oils rich in eugenol, linalool, and methyl chavicol exhibit MICs as low as 0.1–2.0% v/v, with synergistic effects enhancing potency [24]. However, efficacy varies with bacterial strain and oil composition, necessitating strain-specific assessments. As demonstrated in **Table 1**, the antibacterial efficacy of *Ocimum basilicum* seed extracts against both Gram-positive (*Staphylococcus aureus*, *Streptococcus pyogenes*) and Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*) bacteria demonstrates concentration-dependent activity, with methanol extract (ME) exhibiting superior performance compared to hexane extract (HE). This aligns with previous studies highlighting the role of solvent polarity in extracting bioactive antimicrobial compounds [25]. The methanol extract, which contained significantly higher total phenolic content (78 ± 0.61 mg GAE/g) than hexane (56 ± 0.23 mg GAE/g), showed the strongest inhibition zones across all tested bacterial strains. For instance, at 400 ppm, ME achieved inhibition zones of 20.4 ± 0.40 mm against *S. aureus* and 15.2 ± 0.32 mm against *K. pneumoniae*, whereas HE produced smaller zones (14.5 ± 0.00 mm and 11.3 ± 0.32 mm, respectively). These results correlate with the established antimicrobial properties of phenolic compounds, such as flavonoids and tannins, which disrupt bacterial cell membranes, inhibit biofilm formation, and interfere with enzymatic processes [26]. The dose-dependent response observed in both extracts—where activity increased with concentration—further supports the cumulative effect of bioactive compounds at higher doses, a trend consistent with studies on other medicinal plants [27].

Notably, Gram-positive bacteria were marginally more susceptible to both extracts than Gram-negative strains. For example, ME inhibited *S. aureus* (20.4 ± 0.40 mm) more effectively

than *K. pneumoniae* (15.2 ± 0.32 mm) at 400 ppm. This difference likely stems from the structural complexity of Gram-negative bacteria, which possess an outer lipopolysaccharide membrane that limits compound penetration (Silhavy et al., 2010). However, the relatively broad-spectrum activity of ME suggests that its phenolic constituents may target universal bacterial structures, such as membrane lipids or intracellular proteins. In contrast, HE's weaker activity may arise from non-polar compounds like terpenoids or fatty acids, which have limited solubility in aqueous bacterial environments but still exhibit moderate membrane-disrupting effects [28].

While both extracts showed promising activity, they were less potent than the reference antibiotic gentamicin, which produced inhibition zones of 26.5 ± 0.01 mm (*S. aureus*) and 17.0 ± 0.01 mm (*K. pneumoniae*). Gentamicin's superior efficacy reflects its targeted mechanism of action—binding to bacterial ribosomes to inhibit protein synthesis [29]. Nevertheless, plant-derived extracts offer advantages as multi-component systems that may delay antimicrobial resistance through synergistic or multi-target effects [30]. For instance, the combined action of phenolics, terpenes, and alkaloids in ME could simultaneously disrupt membranes, quench quorum sensing, and inhibit efflux pumps, reducing the likelihood of resistance development.

These findings are consistent with earlier reports on *O. basilicum* leaf and seed extracts, where methanol demonstrated stronger antibacterial activity than non-polar solvents [31]. However, the current study extends this understanding by quantifying dose-response relationships and comparing efficacy across Gram-positive and Gram-negative strains. The results underscore the potential of *O. basilicum* seed extracts, particularly methanol-based preparations, as complementary antimicrobial agents in topical applications or preservative systems. Future research should focus on isolating specific bioactive compounds, evaluating synergies with conventional antibiotics, and assessing toxicity profiles to translate these findings into practical applications.

Table 2: Antibacterial Activity of methanolic and hexane extracts of *O.basilicum L.*

O.basilicum Seed Extract	**Concentration mg GAE/g**	**Zone of Inhibition for various Bacterial stains**			
		S. aureus	**S. pytogens**	**E. ecoli**	**k. pneumonia**
Methanolic	25	7.9 ± 0.023	7.72 ± 0.231	7.4 ± 0.001	7.2 ± 0.001
	50	9.9 ± 0.802	9.1 ± 0.908	8.99 ± 0.009	8.43 ± 0.201
	100	13.3 ± 0.403	12.7± 0.210	11.9 ± 0.120	11.23 ± 0.210
	200	15.1 ± 0.012	14.6 ± 0.001	13.91±0.902	13.01± 0.001
	400	20.4 ± 0.401	17 ± 0.002	15.9 ± 0.320	15.2 ± 0.321
Hexane	25	7.6 ± 0.801	7.32 ± 0.123	6.9 ± 0.009	6.5 ± 0.002
	50	8.6 ± 0.760	8 ± 0.097	7.79 ± 0.403	7.63 ± 0.089
	100	9.8 ± 0.001	9.5 ± 0.320	8.9 ± 0.201	8.71 ± 0.322
	200	11.1 ± 0.201	10.7 ± 0.902	10 ± 0.002	9.9 ± 0.601
	400	14.5 ± 0.00	12.8 ± 0.00	11.901	11.3 ± 0.321
Standard biotic (gentamicin)		26.5± 0.009	21.1 ± 0.120	18.9± 0.002	17± 0.006

3. Physicochemical Properties

Table 3 demonstrated that the physicochemical properties of hexane and methanol extracts from *Ocimum basilicum* seeds reflect distinct compositional profiles influenced by solvent polarity. Hexane, a non-polar solvent, preferentially extracts non-polar compounds such as lipids and free fatty acids, as evidenced by its higher free fatty acid content (4.64% vs. 4.23% in methanol) and acid value (2.801 mg KOH/g vs. 2.14 mg KOH/g). These results align with studies showing that non-polar solvents effectively solubilize lipid-rich fractions, which often contain higher levels of triglycerides and shorter-chain fatty acids [5]. The elevated saponification value in hexane extract (150 mg KOH/g vs. 143.20 mg KOH/g for methanol) further supports this, as it indicates a higher

proportion of esterified fatty acids or lower molecular weight lipids, consistent with the solvent's affinity for non-polar metabolites [32].

In contrast, methanol extract exhibited marginally lower specific gravity (0.892 vs. 0.908 for hexane) and refractive index (1.469 vs. 1.478), likely due to its polar constituents, such as phenolic compounds, which are less dense and exhibit distinct optical properties compared to lipids [33]. The slightly lower peroxide value in methanol extract (4.931 mEq/kg vs. 5.023 mEq/kg) may be attributed to the presence of antioxidant phenolics, as previously observed in its higher total phenolic content (78 ± 0.61 mg GAE/g) and superior DPPH/FRAP activity. These antioxidants likely mitigate lipid oxidation, reducing peroxide formation during storage.

Both extracts showed low moisture content (~4.5–4.7%), suggesting effective dehydration during extraction, though methanol's hygroscopic nature did not significantly increase residual moisture. This stability is advantageous for shelf-life, as low moisture minimizes microbial degradation. The comparable peroxide values across solvents indicate moderate oxidative stability, but methanol's antioxidant-rich profile may offer long-term advantages in applications requiring reduced rancidity.

Table 3: Physicochemical properties of *O.basilicum* L. seed extracts in hexane and methanol

Physicochemical Parameter	**Hexane Extract**	**Methanol Extract**
Moisture contents (%)	4.52% (wt/seed sample)	4.67% (wt/seed sample)
Specific gravity	0.908±0.020	0.892±0.010
Refractive index	1.478±0.010	1.469±0.110
Free fatty acid (%wt.)	4.64+0.12	4.23±0.310
Acid value (mg KOH/g)	2.801±0.218	2.14±0.090

Peroxide value (mEq/kg)	5.023±0.030	4.931±0.021
Saponification value (mg KOH/g)	150±0.001	143.20±0.05

These findings underscore the role of solvent polarity in tailoring extract composition for specific applications: hexane for lipid-centric uses (emollients in cosmetics) and methanol for antioxidant-rich formulations (preservatives or nutraceuticals). Further research could explore synergistic effects of blended extracts to optimize both lipid recovery and oxidative stability.

4. Heavy Metals Analysis in O.basilicum extracts

The analysis of heavy metals in methanolic *Ocimum basilicum* seed extracts demonstrated the robustness and reliability of the analytical method, as evidenced by the low limits of detection (LODs), strong linearity of calibration curves (R^2>0.99) (**Figure 3**), and recovery rates within acceptable ranges (91–105%) (**Table 4**). These parameters collectively validate the method's precision and accuracy for quantifying trace metals in plant matrices. For instance, copper exhibited exceptional sensitivity with an LOD of 0.01 ppm, attributed to its steep calibration slope (0.1334) and minimal variability in blank measurements, making it suitable for ultra-trace analysis. Iron (Fe), while having the highest LOD (0.84 ppm) due to a shallower slope (0.0589) and higher background noise, still showed reliable quantification with a recovery rate of 105.3%, slightly elevated but within acceptable bounds. Zinc (Zn), lead, and cadmium (Cd) displayed intermediate sensitivities (LODs: 0.10–0.023 ppm), sufficient for compliance with regulatory standards for environmental or food safety monitoring. The strong linearity of all calibration curves (R^2≥0.9907) further underscores the method's reliability, particularly for Cu and Pb (R^2=0.9997), which showed near-perfect adherence to the regression model.

The detected metal levels in the seed extracts revealed notable concentrations of essential metals: iron (272.67±0.069 ppm) and zinc (113.01±0.290 ppm), likely reflecting their physiological roles in plant metabolism or potential soil absorption during cultivation. Copper (32.23±0.081 ppm) was present at moderate levels, consistent with its function as a micronutrient. Importantly, toxic metals such as lead and cadmium (Cd) were not detected, with concentrations falling below their respective LODs of 0.023 ppm and 0.015 ppm. This aligns with WHO/FAO guidelines for permissible heavy metal limits in medicinal plants (Pb < 10 ppm, Cd < 0.3 ppm) [34], confirming the extract's safety for human consumption. The absence of Pb and Cd suggests minimal environmental contamination during growth or processing, a critical factor for herbal product quality.

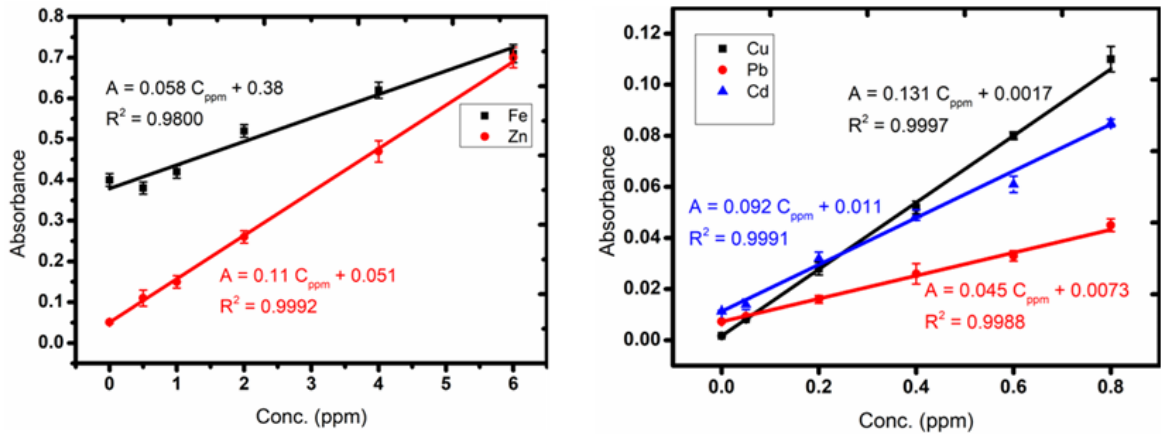

Figure 3: Calibration plots for Fe, Zn (Left), Cu, Pb and Cd (Right)

Recovery rates, a measure of method accuracy, ranged from 91.0% (Zn) to 105.3% (Fe), within the internationally accepted range of 80–120% for analytical methods. The slight suppression in Zn recovery (91.0%) could indicate mild matrix interference, whereas Fe's higher recovery (105.3%) may arise from incomplete separation of co-extracted compounds. These minor deviations do not compromise the method's validity but highlight the importance of matrix-specific validation.

The precise quantification (low standard deviations, ±0.069 ppm for Fe for instance, further reinforces the method's reproducibility.

Table 4: Analysis of methanolic *O.basilicum* seed extract using FAAS

Metals	Standard Conc., ppm	R^2	LOD, ppm	Levels of metal, ppm	Recovery (%)
Fe	0.5, 1, 2, 4, 6	0.9800	0.84	272.67±0.069	105.3
Zn	0.5, 1, 2, 4, 6	0.9992	0.10	113.01±0.290	91.0
Cu	0.05, 0.2, 0.4, 0.6, 0.8	0.9997	0.01	32.23±0.081	97.8
Pd	0.05, 0.2, 0.4, 0.6, 0.8	0.9988	0.023	Not detected	94.4
Cd	0.05, 0.2, 0.4, 0.6, 0.8	0.9991	0.015	Not detected	93.0

In summary, the analytical approach employed here is highly suitable for heavy metal screening in *O. basilicum* extracts, combining sensitivity, precision, and accuracy. The absence of toxic metals (Pb, Cd) and the presence of essential metals at safe levels underscore the extract's potential for use in nutraceuticals or traditional medicine. Future studies should focus on monitoring soil metal profiles to mitigate contamination risks and optimizing extraction protocols to further enhance accuracy. This work not only validates a reliable analytical method but also contributes to broader efforts in ensuring the safety and quality of plant-based products.

IMPLICATIONS FOR CHEMISTRY EDUCATION

This study offers significant pedagogical value for chemistry education, particularly in resource-conscious settings. It provides a compelling practical framework for teaching core concepts like **solvent polarity and extraction efficiency**, demonstrating vividly how methanol's polarity enables superior recovery of bioactive phenolics and antioxidants from *O. basilicum* seeds compared

to non-polar hexane. The validated analytical methodologies (DPPH, FRAP, antibacterial assays, heavy metal analysis) serve as excellent, contextually relevant **case studies for teaching instrumental analysis, bioassay techniques, and quality control procedures**, emphasizing precision, sensitivity (LODs), and recovery rates. The clear demonstration of **dose-dependent biological activity** and the **critical assessment of safety parameters** (essential vs. toxic metals within safe limits) directly link chemical composition to functional properties and real-world safety considerations, crucial for discussions on natural products and nutraceuticals. Furthermore, utilizing a locally relevant plant like *O. basilicum* makes this work highly suitable for **indigenizing the curriculum**, fostering student engagement by connecting abstract chemical principles (solvent-solute interactions, structure-activity relationships, metal toxicity thresholds) to tangible, culturally familiar materials and potential applications. This research can thus inspire authentic laboratory investigations, enhance understanding of green extraction principles, and underscore the importance of rigorous safety validation in applied chemistry.

CONCLUSION

The study of *Ocimum basilicum* seed extracts in methanol (ME) and hexane (HE) highlights the profound influence of solvent polarity on phytochemical composition, bioactivity, and physicochemical properties. ME, rich in phenolic compounds (78 ± 0.61 mg GAE/g), exhibited superior antioxidant (DPPH IC_{50}: 130.64 ppm; FRAP: 83.2%) and antibacterial activity, outperforming HE against both Gram-positive and Gram-negative bacteria. In contrast, HE, with higher lipid content (saponification value: 150 mg KOH/g), demonstrated moderate bioactivity, likely due to non-polar constituents like terpenoids. Physicochemical analysis revealed distinct solvent-driven profiles, with ME showing lower oxidative degradation (peroxide value: 4.93

mEq/kg) and HE displaying higher free fatty acid content (4.64%). Heavy metal analysis confirmed the absence of toxic metals (Pb, Cd < 0.023 ppm) and safe levels of essential metals (Fe, Zn, Cu), ensuring consumer safety. The validated analytical method demonstrated high precision (recovery: 91–105%) and sensitivity (LODs: 0.01–0.84 ppm), underscoring its reliability. These findings position ME as a promising candidate for nutraceutical or antimicrobial applications, while HE's lipid-rich profile suggests utility in emollient formulations. Future research should focus on isolating bioactive compounds, evaluating synergies with commercial antibiotics, and assessing in vivo efficacy to harness the full potential of *O. basilicum* seeds in sustainable health solutions.

AUTHOR CONTRIBUTIONS

Beyadigalem Endawoke Aniley: Conceptualization, writing, and methodology, **Sintayehu Leshe Kitaw**: Conceptualization, writing, supervising, **Yohannis Wondwosen Ahmed:** Editing the manuscript, data curation.

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