Applied Chemical Engineering (2025) Volume 8 Issue 3 ISSN: 2578-2010 (O)
doi: 10.59429/ace.v8i3.5719

ORIGINAL RESEARCH ARTICLE

Chemical composition and evaluation of antioxidant activity of extract and essential oil of Aloysia citrodora in Morocco

Zineb Maaroufi[1,*], Driss Azdem[1], Youssef El Mourabit[2], Ibrahim Alsayer[3], Jamal Mabrouki[1], Souad El Hajjaji[1]

[1] *Laboratory of spectroscopy, molecular modeling, materials, nanomaterials, water and environment, Faculty of Sciences, University Mohammed V .4 avenue ibn Batouta BP1014 RP, in Rabat - Morocco*

[2] *Science and Technical Research Lab, Higher School of Education and Training, University of Ibn Zohr, Agadir, 80000, Morocco*

[3] *Department of Chemical and Materials Engineering, College of Engineering, Northern Border University, Arar, 73551, Saudi Arabia*

***Corresponding author:** Zineb Maaroufi, zineb2maaroufi@gmail.com

ABSTRACT

Driven by the growing interest in natural antioxidants for applications in the pharmaceutical and food industries, medicinal plants have become prominent candidates for the discovery of bioactive molecules. This study investigates the antioxidant properties and phytochemical composition of Aloysia citrodora (lemon verbena) through various extraction approaches. Leaves of the plant were subjected to Soxhlet extraction using two solvents of distinct polarity: methanol and hexane, yielding the Methanolic Extract (ME) and Hexane Extract (HE), respectively. The total phenolic and flavonoid contents were quantified using established colorimetric assays, confirming the richness of both extracts in antioxidant secondary metabolites. Antioxidant potential was assessed via the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. The methanolic extract exhibited the highest free radical neutralization capacity (IC_{50} = 0.024 µg/mL), outperforming the hexane extract (IC_{50} = 0.812 µg/mL) and the essential oil (IC_{50} = 1.771 µg/mL). Furthermore, GC-MS analysis of the hexane extract identified 15 volatile constituents, with major components including cis-Sesquisabinene hydrate (29.96%), Spathulenol (25.19%), α- Curcumene (13.06%), and Geranyl-p-cymene (4.95%). These findings highlight the significant antioxidant potential of Aloysia citrodora, particularly when extracted with methanol, and support its traditional use as a medicinal herb. The integration of phytochemical quantification, chromatographic profiling, and bioactivity evaluation provides a robust framework for future development in pharmaceutical and nutraceutical applications.

Keywords: Aloysia citrodora; phenolic compounds; radical scavenging activity; Soxhlet extraction; GC-MS analysis

ARTICLE INFO

Received: 4 August 2025
Accepted: 15 September 2025
Available online: 30 September 2025

1. Introduction

Medicinal plants are important for pharmacological research and drug development, not only when plant components are used directly as therapeutic agents, but also as raw materials for drug synthesis or as models for pharmacologically active compounds[1,2]. These medicinal plants contain many active ingredients where some are the result of secondary metabolism. Plants already produce 70% of our drugs[3,4]. Approximately 170,000 bioactive molecules have been identified from plants[5]. To this end, secondary metabolites**.** are the subject of much research, which leads to the identification of the main active elements of the plant[6,7]. Many plants, foods or medicines contain Antioxidant ingredients. Regular intake of phytonutrients with antioxidant power Significantly associated with diseases linked to oxidative stress (cancer, cardiovascular diseases and atherosclerosis). This is why we are interested in evaluating the antioxidant activity of verbena[8,9]. Aloysia citrodora known by the vernacular name "verbena" is an aromatic and medicinal plant of the Verbenaceae family, for food purposes[10,11]. It has had a long history in traditional medicine such as the treatment of asthma, colds, fever and flu, it is used to fight flatulence, colic, diarrhea, indigestion, insomnia and anxiety. Lemon verbena is also used against nervous conditions, palpitations, migraines, ringing in the ears and vertigo, it is also used to lower blood sugar levels[12,13]. The essential oils of this plant are used in the treatment of cancers. Analyzes carried out in vitro using various tests have shown the antioxidant, antispasmodic and anti-inflammatory properties of the infused[14,15]. Numerous investigations have addressed the chemical profile and pharmacological attributes of Aloysia citrodora. For instance, [15,16], underscored the abundance of phenolic and flavonoid compounds in methanolic extracts, alongside their marked antioxidant capacity. Moreover, essential oils derived from this species have been associated with anti-inflammatory effects and promising anticancer activity[17,18]. Despite these findings, limited attention has been devoted to a comprehensive comparison of how different extraction solvents influence yield, chemical composition, and antioxidant efficacy particularly through the combined application of gas chromatography–mass spectrometry (GC-MS) and biological assays such as DPPH radical scavenging[19,20]. The present work seeks to fill this gap by systematically evaluating methanolic and hexane extracts, as well as the essential oil of A. citrodora[21,22]. Through the integration of quantitative phytochemical assessments, GC-MS profiling, and antioxidant testing via the DPPH assay, this study provides novel insights into the relationship between chemical constituents and biological activity[23]. Such an integrative methodology enhances our understanding of the therapeutic potential of A. citrodora and supports its valorization in both pharmaceutical and functional food sectors[24,25].

2. Materials and methods

2.1. Plant material

This study investigates the biological properties of *Aloysia citrodora* (lemon verbena) collected in January 2018 from the Missour region (Boulemane Province, Morocco; coordinates: 33.05°N, 3.99°W). After collection, the plant material was sorted and air- dried under shade at ambient temperature to preserve its biochemical integrity. Once dried, the leaves were manually separated from other plant parts and finely pulverized using a laboratory grinder[26,27]. The resulting plant powder was stored in airtight glass containers to prevent moisture exposure, in preparation for solvent-based extraction using methanol and hexane[28,29].

2.2. Experimental procedures

2.2.1. Extraction of essential oil

The essential oil was obtained via hydrodistillation using a Clevenger-type apparatus, following the protocol of[30,31]. Approximately 100 g of dried verbena leaves were immersed in a round-bottom flask filled to two-thirds with distilled water and subjected to boiling for six hours. The steam carrying volatile components was condensed, yielding a distillate in which the oil phase separated naturally from the aqueous phase[28,32]. The essential oil was then recovered using a micropipette and stored in ambervials at 4°C. The extraction yield was calculated as a percentage of oil mass relative to the dry weight of the plant material[33,34].

2.2.2. Solvent extraction of bioactive compounds

Soxhlet extraction was conducted using 40 g of dried *A. citrodora* leaf powder[35]. The material was placed in an extraction cartridge and processed separately with two solvents of distinct polarity: methanol and hexane (both 99.9% purity; Merck, Germany)[36]. The apparatus operated under reflux for six hours until plant material decolorization indicated exhaustion. Post-extraction, the solvents were removed under reduced pressure using a rotary evaporator (BUCHI R-100, B-100 bath, V-700 vacuum pump). The concentrated residues (crude extracts) were stored at 5°C in sealed containers for further analysis. Extraction efficiency (yield) was calculated using the formula (1) [37]:

$$Yield(\%) = \left(\frac{m_{extract}}{m_{initial}} \right) \times 100 \quad (1)$$

Where: mextract is the mass of dried extract m initial is the mass of starting plant material.

2.3. Analytical and biological assays

2.3.1. GC-MS characterization of essential oil

Gas chromatography–mass spectrometry (GC-MS) was used to identify volatile constituents of the essential oil[38]. The analysis was performed on a Perkin Elmer Clarus 680 GC coupled to a Q-8 MS detector, equipped with an Rxi-5Sil MS capillary column and an autosampler. The system employed helium as a carrier gas (1 mL/min), with electron ionization at 70 eV. Oven temperature was initially set to 40°C for 2 minutes, then increased at 10°C/min up to 290°C[39]. The injector temperature was maintained at 260°C, and a 0.5 µL sample was injected. Compound identification was based on mass spectral matching and retention indices.

2.3.2. Quantification of total polyphenols

Total phenolic content was estimated using the Folin–Ciocalteu method as described by Zugazua-Ganado et al[40]. A reaction mixture containing 200 µL of extract, 800 µL of 7.5% Na_2CO_3, and 1 mL of diluted Folin reagent (1:10) was incubated at room temperature for 2 hours. Absorbance was measured at 765 nm using a UV-Vis spectrophotometer (LLG-Uni SPEC2). Gallic acid was used to construct a standard calibration curve (40–140 µg/mL), and results were expressed as µg gallic acid equivalents (GAE) per mg of extract.

2.3.3. Flavonoid content determination

Flavonoid levels were measured by the aluminum chloride colorimetric assay[41]. Equal volumes (1 mL each) of extract and 2% methanolic $AlCl_3$ were mixed and incubated for 15 minutes at room temperature. Absorbance was read at 430 nm. Quercetin served as the standard (0–300 µg/mL), and flavonoid concentration was expressed in µg quercetin equivalents (QE) per mg of extract.

2.3.4. Tannin content estimation

Tannins (hydrolyzable type) were quantified using the vanillin-HCl method[42]. A mixture of 500 µL extract, 3 mL of 4% vanillin-methanol solution, and 1.5 mL HCl was incubated for 15 minutes. Absorbance was measured at 500 nm, and catechin was used as the reference standard. Tannin content was expressed in µg catechin equivalents (CE) per mg of extract.

2.3.5. Antioxidant activity assessment

- **DPPH Radical Scavenging Assay**

Free radical scavenging capacity was evaluated using the DPPH method[43]. A 0.3 mM methanolic DPPH solution was mixed with various concentrations (40–350 µg/mL) of the extracts or essential oil. After a 30-minute dark incubation at room temperature, absorbance was recorded at 517 nm. Ascorbic acid served as a positive control. The inhibition percentage (I%) was calculated as follows:

$$\%Inhibition = \left(\frac{Acontrol - \text{Asimpl}}{Acontrol} \right) \times 100 \quad (2)$$

IC_{50} values, defined as the concentration required to reduce DPPH activity by 50%, were determined to compare antioxidant strength[44].

- **FRAP (Ferric Reducing Antioxidant Power) Assay**

The reducing power of the extracts was assessed using the FRAP method[45]. A mixture of extract, phosphate buffer (pH 6.6), and 1% potassium ferricyanide was incubated at 50°C for 20 min. After adding 10% trichloroacetic acid, the solution was centrifuged, and the supernatant was mixed with $FeCl_3$. Absorbance was measured at 700 nm. The CE_{50} value, corresponding to the concentration yielding 0.5 absorbance, was used to express reducing potential, with comparisons made to ascorbic acid[46].

3. Results

3.1. Extraction yields

The extraction yields of *Aloysia citrodora* leaves collected in the Missour region are illustrated in **Figure 1**. Among the tested extraction methods, the methanolic extract exhibited the highest yield (24.08%), significantly surpassing both the hexane extract (2.72%) and the essential oil obtained by hydrodistillation (0.1945%). These findings highlight the superior efficiency of methanol, a polar solvent, in extracting a widerange of polar secondary metabolites.

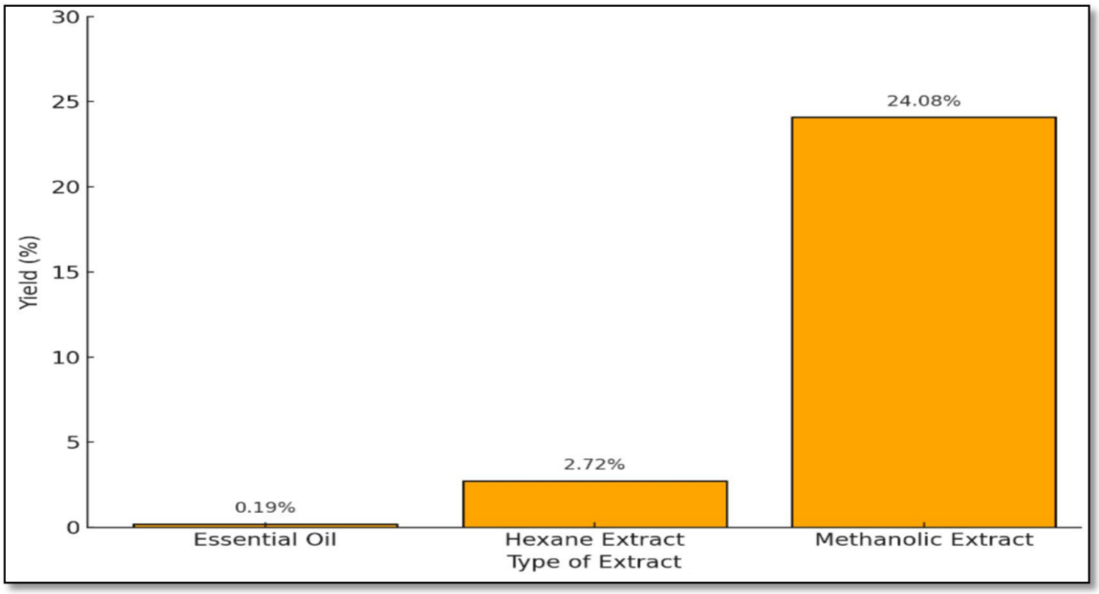

Figure 1. Comparison of extraction yields (%) of essential oil, hexane extract, and methanolic extract from Aloysia citrodora leaves collected in the Missour region (Morocco).

Conversely, the lower yield obtained with hexane, a non-polar solvent, suggests selective extraction of non-polar compounds[47]. Although the essential oil yield remains low, it nevertheless confirms the presence of volatile constituent's characteristic of *A. citrodora*'s aromatic profile. Overall, the observed variation in yields emphasizes the critical role of solvent polarity in phytochemical recovery, and supports the use of complementary extraction techniques to fully characterize the plant's chemical potential.

Table 1 below summarizes the results of the study of the yield of verbena extracts collected in the Missour region.

Table 1. Yields of different extract.

	Essential oil	Hexane extract	Methanolic extract
Yields %	0.1945	2.72	24.08

3.2. Chemical characterization of essential oil by GC-MS

The volatile constituents of *Aloysia citrodora* essential oil, extracted from leaves collected in the Missour region, were identified using gas chromatography–mass spectrometry (GC-MS). The resulting total ion chromatogram (TIC) is displayed in **Figure 2**, while the detailed chemical composition and retention times of the major compounds are listed in **Table 2**.

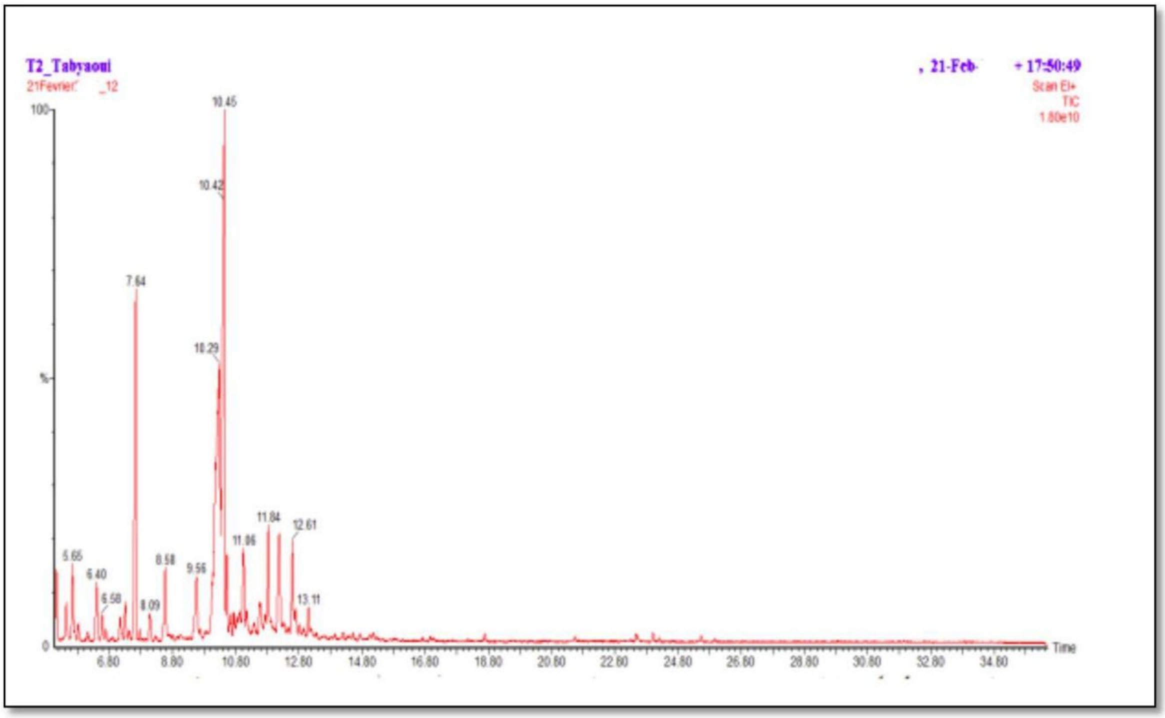

Figure 2. GC-MS chromatographic profile of *A. citrodora* essential oil.

A total of 97.38% of the essential oil composition was successfully identified, highlighting the high analytical resolution of the method employed. The essential oil was predominantly composed of sesquiterpene derivatives, with cis-Sesquisabinene hydrate (29.96%) and Spathulenol (25.19%) representing the most abundant constituents. Other notable components included alpha-Curcumene (13.06%), Geranyl-p-cymene (4.95%), and γ-Cadinene (3.34%). These results suggest that the essential oil is rich in oxygenated sesquiterpenes and sesquiterpene hydrocarbons, compounds widely recognized for their pharmacological and aromatic properties[48].

Table 2. Chemical composition of *Aloysia citrodora* essential oil from the Missour region.

Compounds	Percentage %	Retention time
Copaene	1.287	5.446
(-) -bêta-bourbonene	2.603	5.643
Caryophyllene	2.633	6.407
Alloaromadendrene	1.165	7.314
Alpha-Curcumene	13.058	7.641
Germacrene D	1.066	8.091
γ-Cadinene;	3.342	8.578
Nerolidol	3.070	9.562
Spathulenol	25.185	10.286
Cis-Sesquisabinene hydrate	29.961	10.443

Compounds	Percentage %	Retention time
(-) -cuparene	1.983	10.529
Alpha-Terpinene	1.490	11.587
(-) -bêta-Cadinene	2.937	11.837
Geranyl-p-cymene	4.947	12.184
Isosteviol	2.654	12.604
Total	97.381	
Reste	Traces 2.619	

Table 2. (*Continued*)

The dominance of oxygenated sesquiterpenes such as Spathulenol and cis- Sesquisabinene hydrate is consistent with previous reports associating these molecules with anti-inflammatory, antimicrobial, and antioxidant activities. The presence of monoterpenes and other minor constituents further contributes to the oil's complexity and potential bioactivity.

Taken together, the GC-MS analysis provides a detailed chemical fingerprint of *A. citrodora* essential oil, supporting its traditional medicinal use and laying the foundation for further pharmacological investigations.

3.3. Determination of total polyphenol content

The quantification of phenolic compounds in the different extracts of *Aloysia citrodora* was performed using the Folin–Ciocalteu method, and the results were extrapolated from a standard calibration curve constructed using gallic acid. As shown in **Figure 3**, the curve exhibits a strong linear correlation between absorbance and concentration, with a determination coefficient of **R^2 = 0.9966**, indicating high reliability of the analytical method. The linear regression equation obtained was **$y = 0.0116x + 0.0347$**.

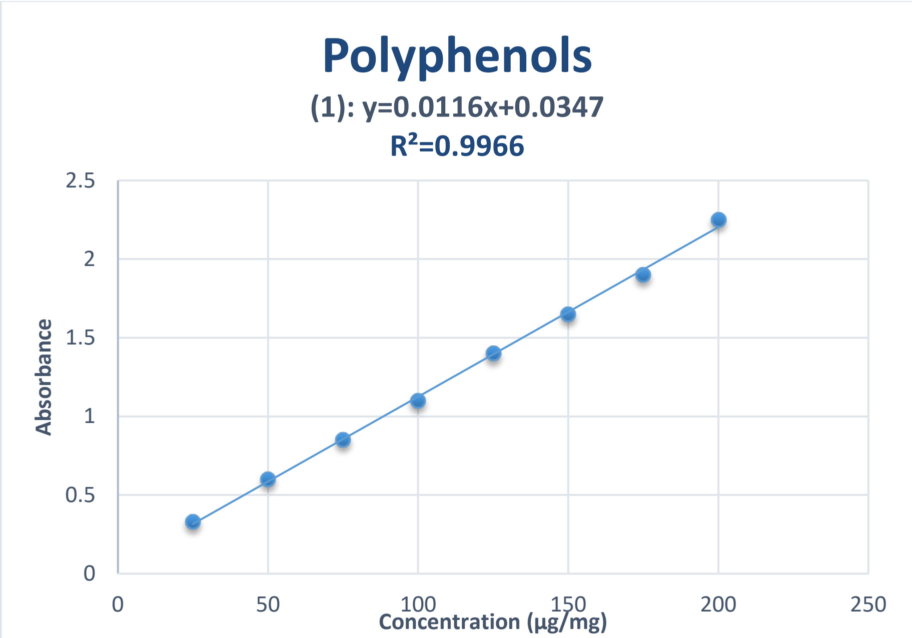

Figure 3. Calibration curve of gallic acid used for the determination of total phenolic content in *A. citrodora* extracts.

Based on this calibration, the methanolic extract demonstrated the highest concentration of total polyphenols, reaching 215 µg GAE/mg of extract, followed by the hexane extract with a lower value of 165 µg GAE/mg (**Table 3**).

Table 3. Total polyphenol content (µg GAE/mg of extract) in methanolic and hexane extracts of Aloysia citrodora.

Methanolic Extract	215
Hexane Extract	165

These findings confirm the greater efficiency of methanol as a polar solvent in extracting phenolic compounds, which are known to be key contributors to antioxidant activity[49].

The difference in polyphenol content between the two extracts can be attributed to the solvent's polarity and its ability to dissolve hydrophilic phytochemicals. Methanol, being highly polar, facilitates the extraction of polyphenols, whereas hexane, a non- polar solvent, extracts fewer polar constituents. The high phenolic content in the methanolic extract is consistent with its stronger radical scavenging capacity observed in the DPPH assay. These results reinforce the significance of solvent selection in optimizing the recovery of phenolic antioxidants and support the potential use of methanolic extracts of A. citrodora in nutraceutical and pharmaceutical applications.

3.4. Determination of total flavonoid content

The total flavonoid content in *Aloysia citrodora* extracts was assessed using the aluminum chloride colorimetric method, with quercetin serving as the reference standard. The calibration curve, constructed over a range of known concentrations, exhibited excellent linearity with a determination coefficient of $R^2 = 0.998$, and the regression equation was established as $y = 0.015x – 0.0296$ (**Figure 4**).

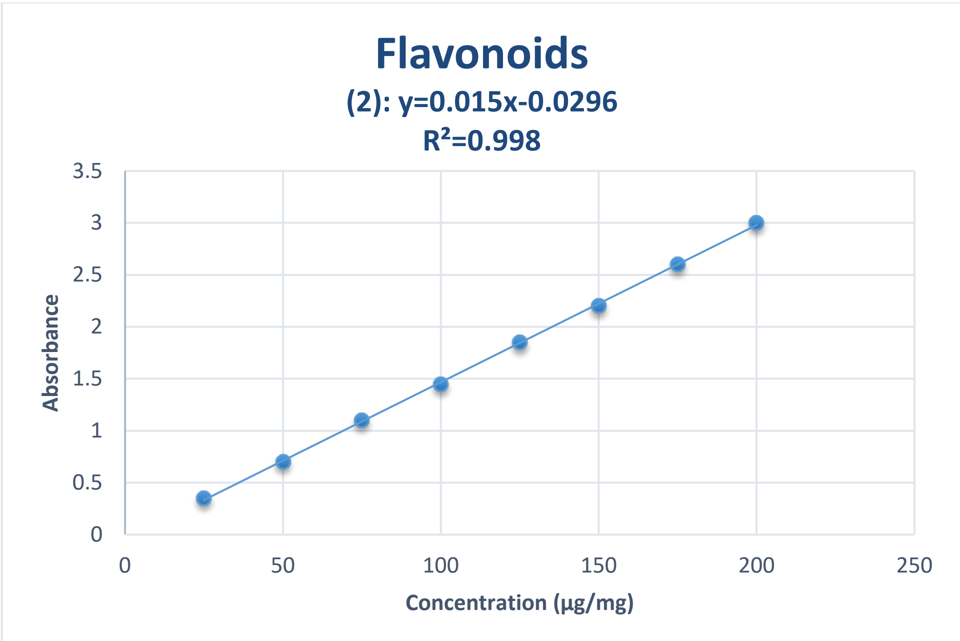

Figure 4. Calibration curve of quercetin for the quantification of total flavonoids in *A. citrodora* extracts.

Based on this calibration, flavonoid concentrations were expressed in micrograms of quercetin equivalents per milligram of extract (µg QE/mg). As shown in **Table 4**, the methanolic extract contained the highest level of total flavonoids, with a value of 125 µg QE/mg, whereas the hexane extract presented a significantly lower concentration of 66 µg QE/mg[50].

Table 4. Total flavonoid content (µg QE/mg of extract) in methanolic and hexane extracts of Aloysia citrodora.

Methanolic Extract	125
Hexane Extract	66

3.5. Determination of tannin content

The quantification of tannins in *Aloysia citrodora* extracts was carried out using the vanillin–HCl colorimetric assay, with catechin employed as the reference compound.

The calibration curve generated from a series of catechin standards demonstrated excellent linearity, with a correlation coefficient of $R^2 = 0.9937$ and a regression equation of $y = 0.011x + 0.0366$ (**Figure 5**).

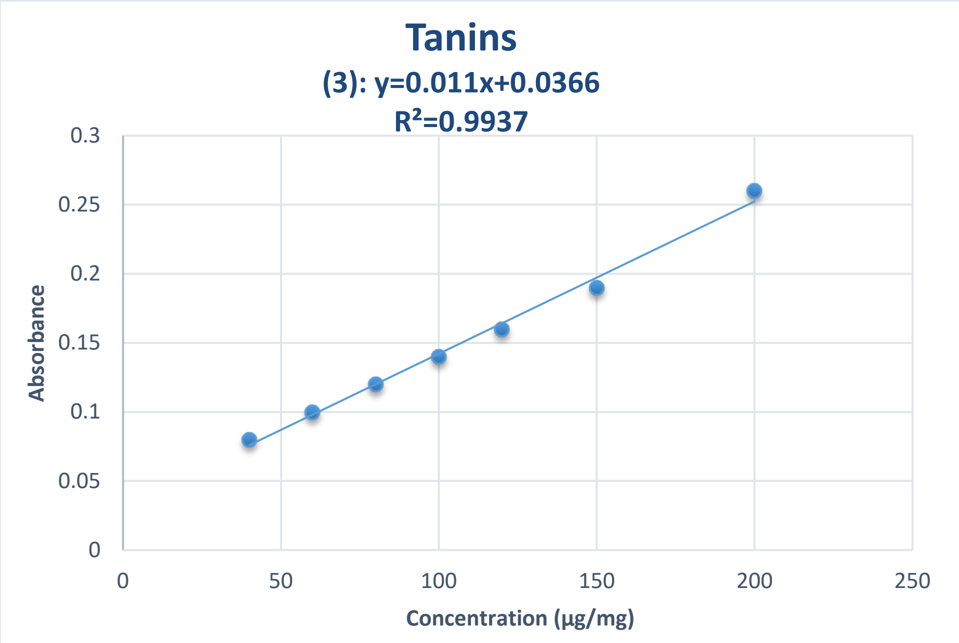

Figure 5. Calibration curve of catechin for the determination of tannin content in *Aloysia citrodora* extracts.

Tannin concentrations in the extracts were expressed as micrograms of catechin equivalents per milligram of extract (µg CE/mg). As shown in **Table 5**, the methanolic extract exhibited a notably higher tannin content (65 µg CE/mg) compared to the hexane extract, which recorded only 28 µg CE/mg**.** These results reaffirm the superior efficacy of methanol in extracting phenolic-type compounds, including hydrolyzable tannins[51].

This enhanced extraction capacity is primarily attributed to the high polarity of methanol, which facilitates the solubilization of polyphenolic structures, in contrast to hexane, whose non-polar character limits the recovery of such hydrophilic constituents.

Table 5. Comparison of tannin content (µg catechin equivalent per mg of extract) between methanolic and hexane extracts.

Methanolic Extract	65
Hexane Extract	28

Taken collectively, the tannin content data along with previously discussed phenolic and flavonoid levels support the conclusion that methanol is the most suitable solvent for maximizing the extraction of antioxidant phenolics from *A. citrodora*. These findings underscore the plant's potential as a natural source of bioactive compounds for pharmaceutical or nutraceutical formulations.

3.6. Evaluation of antioxidant activity

- **DPPH Radical Scavenging Assay**

The antioxidant potential of *Aloysia citrodora* extracts and essential oil was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay, a well- established and widely applied method due to its simplicity and reproducibility[52]. This assay is based on the ability of antioxidant compounds to donate hydrogen atoms or electrons to neutralize the DPPH radical, resulting in the formation of a stable, non-radical molecule (DPPH-H). The reduction in DPPH absorbance correlates directly with the scavenging ability of the tested sample.

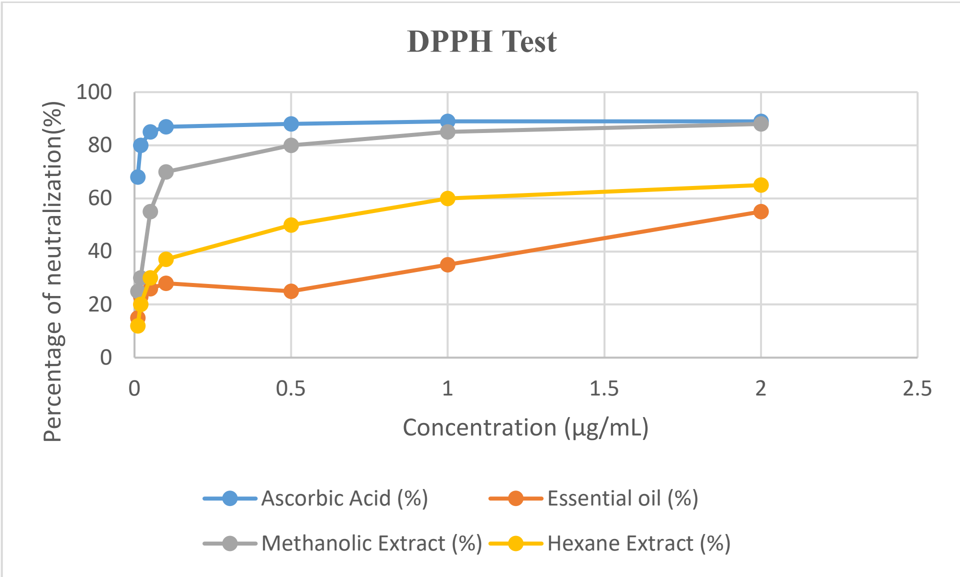

Figure 6. DPPH radical scavenging activity (%) of *A. citrodora* extracts, essential oil, and ascorbic acid at varying concentrations.

As shown in **Figure 6**, an increase in the concentration of all tested samples led to a corresponding enhancement in radical scavenging activity. Ascorbic acid, used as a reference antioxidant, exhibited the highest neutralization capacity, reaching over 90% inhibition at low concentrations. Among the natural samples, the methanolic extract showed superior activity compared to both the hexane extract and the essential oil, with a pronounced dose-dependent response.

The antioxidant effectiveness of each sample was quantitatively expressed using the IC_{50} value, defined as the concentration required to inhibit 50% of DPPH radicals.

These values, determined by regression analysis of the dose-response curves, are presented in **Table 6**. Lower IC_{50} values indicate stronger antioxidant activity. The methanolic extract demonstrated the most potent scavenging capacity with an IC_{50} of 0.024 µg/mL, followed by the hexane extract (0.812 µg/mL) and the essential oil (1.771 µg/mL). Ascorbic acid, as expected, exhibited the strongest activity overall (IC_{50} = 0.007 µg/mL).

Table 6. IC_{50} values (µg/mL) of the tested samples in the DPPH assay.

Methanolic Extract	IC50 = 0.024
Hexane Extract	IC50 = 0.812
Essential oil	IC50 = 1.771
Ascorbic Acid	IC50 = 0.007

These results confirm the richness of the methanolic extract in bioactive antioxidant compounds, likely phenolics and flavonoids, aligning with previous phytochemical findings. The relatively weaker activity of the hexane extract and essential oil reflects the lower polarity and thus reduced extraction of hydrophilic antioxidant molecules.

- **FRAP Assay**

The reducing capacity of *Aloysia citrodora* extracts and essential oil was evaluated using the Ferric Reducing Antioxidant Power (FRAP) assay, which measures the electron-donating ability of antioxidant compounds to reduce Fe^{3+} to Fe^{2+} in the presence of potassium ferricyanide. The assay is based on spectrophotometric detection at 700 nm, where increased absorbance reflects greater reducing power[53].

As shown in **Figure 7**, the absorbance values increased proportionally with sample concentration for all extracts tested, as well as for the reference standard, ascorbic acid. The methanolic extract demonstrated the strongest reducing ability among the tested samples, exhibiting a steep concentration-dependent increase in absorbance. In contrast, the hexane extract and essential oil showed much lower responses, indicative of weaker electron-donating activity.

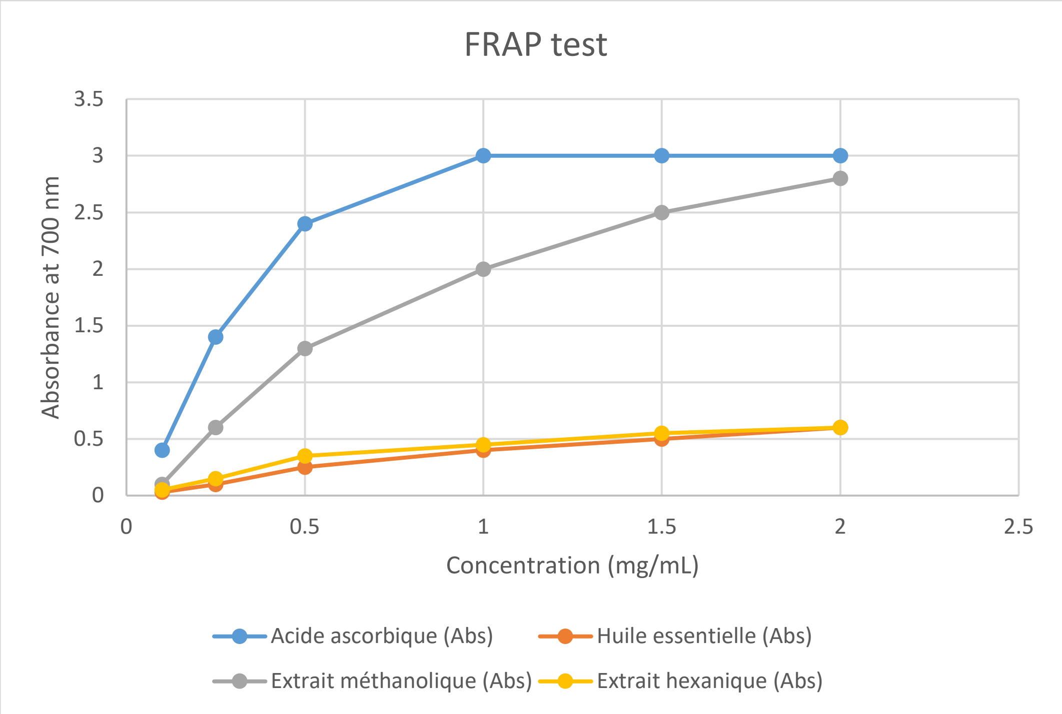

Figure 7. Reducing power of *A. citrodora* extracts and essential oil assessed by the FRAP method.

To quantitatively compare the antioxidant power of the samples, the EC_{50} value (the effective concentration required to reach an absorbance of 0.5) was calculated. As presented in **Table 7**, the methanolic extract exhibited an EC_{50} of 0.158 mg/mL, outperforming both the hexane extract (1.225 mg/mL) and the essential oil (1.578 mg/mL). As expected, ascorbic acid showed the strongest reducing activity with an EC_{50} of 0.049 mg/mL.

Table 7. EC_{50} values (mg/mL) of tested samples in the FRAP assay.

Methanolic Extract	EC50 = 0.158
Hexane Extract	EC50 = 1.225
Essential oil	EC50 = 1.578
Ascorbic Acid	EC50 = 0.049

These findings are consistent with the previous DPPH assay results and further validate the superior antioxidant potential of the methanolic extract. The enhanced reducing capacity observed can be attributed to the presence of phenolic and flavonoid compounds, which are known to act as effective electron donors.

4. Discussion

Our study aimed to evaluate certain biological characteristics of extracts and essential oil of Aloysia citrodora harvested with different extraction methods (contents of polyphenols, flavonoids, and tannins). The results obtained highlighted the respective importance of these different factors for obtaining extracts of biological or even therapeutic interest from verbena. The results obtained showed a significant variability in the contents of phenolic compounds in the extracts of the aerial parts (leaves) of verbena (Aloyisia citrodora). The extracts obtained by Soxhlet in a methanolic medium are significantly richer in polyphenol flavonoids and tannins than those obtained in a hexanic medium. Previously, **Michielin et al.**, had already reported that the contents of polyphenol flavonoids and tannins obtained by Soxhlet are higher when the extraction is carried out with ethanol. It is well established that extraction with a Soxhlet type device makes it possible to isolate the active ingredients of plants without degrading them. Based on these results, methanol constitutes the ideal solvent for the extraction of phenolic compounds from the verbena plant[54].

This result is inconsistent with the observations of **Fernández-Agulló et al.**, who reported that the polyphenol contents of extracts of walnut leaves (Juglans regia), another species of phytotherapeutic interest, are higher when the extraction is carried out with ethanol rather than water[55]. These differences in solubility of phenolic compounds depending on the polarity of the extraction solvent essentially depend on the nature, structure, degree of polymerization and interaction of these compounds with each **other Naczk & Shahidi, 2004; Maisuthisakul et al., 2006**[56,57].

Regarding antioxidant activity, the results obtained (DPPH test) showed that the methanolic extract exhibited higher antioxidant activity than the hexanic extract and the essential oil across the entire range of concentrations studied. Similarly, the results obtained (FRAP test) demonstrated that the methanolic extract had greater reducing activity than both the hexanic extract and the essential oil. Thus, the lowest IC50 and EC50 values for free radical scavenging and iron-reducing activities were recorded with the methanolic extracts, indicating better antioxidant activity when extraction is performed with methanol rather than hexane.

Furthermore, **Sheyla et al. (2011)** reported that ethanolic extracts of Verbena officinalis have a stronger antioxidant power (IC50 = 21 mg/mL) than aqueous extracts (IC50 = 33 mg/mL)[62].

In the more specific context of antioxidant activity, our work also highlighted the importance of the geographical origin of verbena on the antioxidant potential of the plant's extracts, with those from the Boulemane region exhibiting significantly stronger activity (lower IC50).

This variation among plants of the same species but from different localities/origins in Morocco has already been documented in the literature, particularly for another medicinal plant, the nodiflorous ice plant **[58]**. Environmental factors (sunlight exposure, rainfall), especially edaphic conditions (water deficit, excessive salinity), explain these differences through their influence on the plants' ability to synthesize and accumulate total polyphenols, and consequently, their antioxidant potential[59]. Concerning the Analysis of the oil by GC-MS the result showed that the main components of the essential oil were alpha-Curcumene (13.058%), Spathulenol (25.185%), cis-Sesquisabinene hydrate (29.961%), and Geranyl-pcymene (4.947%). On the other hand, **Bellakhdar** analyzed the essential oil from the flowering tops of the fragrant verbena Aloyisia triphylla grown in the Agadir region (Morocco) which was found to contain 9.9% geranial, 6.9% neral, 7, 4% 6-methyl-5-hepten-2-one and 12.4% 1,8-cineole[60].

In Türkiye, **Özek** noted that verbena EO from the leaves contains 14.8% limonene and 17.9% citrals while that from leafy branches contains 18.6% limonene and 27.9% citrals. (Ibrahim et al., 2015) found in the essential oil of fragrant verbena cultivated in Egypt: d-limonene (6.3 -16.2%), 1.8 cineole (4.7% - 7.3%) and citral (19.9% - 28.8%). These specifications are of great practical interest and could be used for the standardization of fragrant verbena, by characterizing, among other things, varieties rich in toxic substances, in this case β-thujone[61].

5. Conclusion

The findings of this study reinforce the relevance of Aloysia citrodora as a valuable botanica source of bioactive molecules, particularly antioxidants. Through comprehensive phytochemical screening and antioxidant evaluation, the methanolic extract consistently demonstrated superior performance in terms of phenolic, flavonoid, and tannin content, as well as in its radical scavenging (DPPH) and reducing (FRAP) activities. This efficiency is strongly attributed to methanol's high polarity, enablingthe effective solubilization of diverse polar secondary metabolites. Gas chromatography mass spectrometry (GC-MS) analysis of the essential oil revealed a chemical profil dominated by oxygenated sesquiterpenes, including cis-Sesquisabinene hydrate and Spathulenol, which are compounds frequently associated with antioxidant, anti-inflammatory, and antimicrobial properties. The distinct chemotype observed suggests that geographical factors, such as soil composition and climate in the Boulemane region, may influence both the qualitative and quantitative composition of secondary metabolites. Antioxidant testing clearly established the methanolic extract as the most potent among the tested samples, with the lowest IC_{50} and EC_{50} values in both DPPH and FRAP assays, confirming its capacity to neutralize free radicals and reduce ferric ions effectively. In contrast, the hexane extract and essential oil exhibited weaker antioxidant responses, correlating with their lower content in polar phenolic compounds.

Collectively, these results support the use of methanol as the solvent of choice for the extraction of antioxidant constituents from *A. citrodora*. The study also highlights the importance of extraction methodology and plant origin in shaping the phytochemical profile and biological efficacy of herbal preparations. These insights lay the groundwork for further pharmacological investigations and valorization of Aloysia citrodora in the formulation of antioxidant-rich nutraceuticals and herbal therapeutics.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgement

The authors extend their appreciation to the Deanship of Scientific Research at Northern Border University, Arar, KSA for funding this research work through the project number "NBU-FFR-2025-2148-10".

References

1. Falleh, H., Ksouri, R., Boulâaba, M., Guyot, S., Abdelly, C., Magné, C. (2012). Phenolic nature, occurrence and polymerisation degree as marker of environmental adaptation in the edible halophyte Mesembryanthemum edule. S Afr J Bot, 79, 117-124
2. Chaachouay, N., & Zidane, L. (2024). Plant-derived natural products: A source for drug discovery and development. Drugs and Drug Candidates, 3(1), 184-207.
3. Azrour, M., Mabrouki, J., & Ahmad, S. (2025). IoT and Advanced Intelligence Computation for Smart Agriculture. CRC Press.
4. Jangpangi, D., Patni, B., Chandola, V., & Chandra, S. (2025). Medicinal plants in a changing climate: Understanding the links between environmental stress and secondary metabolite synthesis. Frontiers in Plant Science, 16, 1587337.
5. Sarwar, R., Maisam, M., Khan, M. W., Xue, Y., & Hassan, S. (2025). Bioactive Compounds in Herbal Remedies. In Herbal Pharmacopeia (p. 89-121). CRC Press.
6. Agoud, S., Azdem, D., Khabouche, H., Elmoustaqim, K., Mabrouki, J., & Slaoui, M. (2025). Opportunities and Challenges of Integrating Methanization and Biogas Technologies into the Green Hydrogen Economy. Obstacles Facing Hydrogen Green Systems and Green Energy, 301-312.

7. Ennejjai, I., Ariss, A., Mabrouki, J., & Ziti, S. (2025). Enhancing misinformation detection using long short-term memory (LSTM) and bidirectional LSTM (Bi-LSTM) with word embedding techniques. Discrete Mathematics, Algorithms and Applications, 17(04),2450052.
8. Azdem, D., Mabrouki, J., & El Hajjaji, S. (2025). MarocFarm: A Digital Revolution for Agriculture in Morocco. In Circular Economy Applications in Energy Policy (p.137-152). IGI Global Scientific Publishing.
9. El Mouedden, M., Azdem, D., Mabrouki, J., & El Hajjaji, S. (2025). MarocFarm: A digital revolution for agriculture in Morocco. In Circular Economy Applications in Energy Policy (p. 141-155). Scopus. https://doi.org/10.4018/979-8-3693-6680-6.ch010
10. Agoud, S., Khabouche, H., Mabrouki, J., & Slaoui, M. (2025). Smart Anaerobic Reactors by Utilizing Artificial Neural Networks and Nonlinear Regression to Predict Biogas Output. In Circular Economy Applications in Energy Policy (p. 209-218). IGI Global Scientific Publishing.
11. Ajjoun, M., Oualdi, I., Diass, K., Touzani, R., Bussmann, R. W., & Elachouri, M. (2024). Aloysia citrodora Paláu Araceae. In Ethnobotany of Northern Africa and Levant (p.225-232). Springer.
12. Afrasiabian, F., Mirabzadeh Ardakani, M., Rahmani, K., Azadi, N. A., Alemohammad, Z. B., Bidaki, R., Karimi, M., Emtiazy, M., & Hashempur, M. H. (2019). Aloysia citriodora Palau (lemon verbena) for insomnia patients: A randomized, double-blind, placebo-controlled clinical trial of efficacy and safety. Phytotherapy Research, 33(2),350-359.
13. Elshaikh, A., Abdo, G., Salih, A., Ladisa, G., Bogliotti, C., Sokrab, I., Barsi, M., & Mabrouki, J. (2025). Sustainable Pathways: Best Practices for Enhancing Production While Managing the Water-Energy-Food Nexus. In Circular Economy Applications in Energy Policy (p. 243-256). IGI Global Scientific Publishing.
14. Elouardi, M., Mabrouki, J., & El Belghiti, M. A. (2023). Evaluation of algal absorbed CO2 sequestration techniques: A review. Euro-Mediterranean Journal for Environmental Integration, 8(3), 481-491.
15. El Alouani, M., Alehyen, S., Aouan, B., Mabrouki, J., & Saufi, H. (2023). Application of mesoporous fly ash as a low-cost adsorbent for effective removal and immobilization of hazardous pollutants in aqueous environments. Modeling Earth Systems and Environment, 9(3), 3539-3552.
16. Alrub, M. A. (2023). Unveiling the chemical profiling, antioxidant, anticancer and antibacterial activities of essential oils derived from fennel (Foeniculum vulgare), M. fruticose and Alyosia citriodora. Fruticose and Alyosia Citriodora. [Google Scholar].
17. El Mouedden, M., Mabrouki, J., Azdem, D., & El Hajjaji, S. (2025). The development of intelligent agriculture, the contribution of the Internet of Things (IoT) to modernization and its implications for precision farming: A review. Euro-Mediterranean Journal for Environmental Integration. https://doi.org/10.1007/s41207-025-00834-x
18. El Alouani, M., Saufi, H., Aouan, B., Bassam, R., Alehyen, S., Rachdi, Y., ... & Barka, N. (2024). A comprehensive review of synthesis, characterization, and applications of aluminosilicate materials-based geopolymers. Environmental Advances, 16, 100524.
19. Elshaikh, A., Elsheikh, E., & Mabrouki, J. (2024). Applications of artificial intelligence in precision irrigation. J. Environ. Earth Sci, 6.
20. Fattah, G., Ghrissi, F., Mabrouki, J., & Kabriti, M. (2021). Control of physicochemical parameters of spring waters near quarries exploiting limestone rock. In E3S web of conferences (Vol. 234, p. 00018). EDP Sciences.
21. Mabrouki, J., Azrour, M., Boubekraoui, A., & El Hajjaji, S. (2022). Simulation and optimization of solar domestic hot water systems. International Journal of Social Ecology and Sustainable Development (IJSESD), 13(1), 1-11.
22. Elshaikh, A., & Mabrouki, J. (2024). Impact of Agricultural Soil Degradation on Water and Food Security. In Artificial Intelligence Systems in Environmental Engineering (p. 13-24). CRC Press.
23. Azdem, D., Mabrouki, J., Moufti, A., & Fatni, A. (2024). Assessment of heavy metal contamination in seawater in Agadir coastline, Morocco. Desalination and Water Treatment, 317, 100129.
24. Benchrifa, M., Mabrouki, J., Ariss, A., Ennejjai, I., Hmouni, D., & El-Moustaqim, K. (2024). Study of the physicochemical properties of lavender essential oil (Lavandula Stoechas). In Advanced Systems for Environmental Monitoring, IoT and the application of Artificial Intelligence (p. 329-341). Springer.
25. Fattah, G., Elouardi, M., Benchrifa, M., Ghrissi, F., & Mabrouki, J. (2023). Modeling of the coagulation system for treatment of real water rejects. In Advanced technology for smart environment and energy (pp. 161-171). Cham: Springer International Publishing.
26. Al-Jadabi, N., Laaouan, M., El Hajjaji, S., Mabrouki, J., Benbouzid, M., & Dhiba, D. (2023). The dual performance of Moringa oleifera seeds as eco-friendly natural coagulant and as an antimicrobial for wastewater treatment: A review. Sustainability, 15(5), 4280.
27. Drhimer, F., Rahmani, M., Regraguy, B., El Hajjaji, S., Mabrouki, J., Amrane, A., Fourcade, F., & Assadi, A. A. (2023). Treatment of a food industry dye, brilliant blue, at low concentration using a new photocatalytic configuration. Sustainability, 15(7), 5788.
28. Elouardi, M., Fattah, G., Benchrifa, M., Mabrouki, J., Zair, T., & Belghiti, M. A. E. (2023). The evaluation of the valorization of cannabis residues for the production of energy by combustion. In Advanced technology for smart environment and energy (p. 247-256).
29. Özek N. T. Kirimer K. H.C. Baser & Tümen G. (1996). Composition of the Essential Oil of Aloysia triphylla (L'Herit.) Britton Grown in Turkey. Journal of Essential Oil Research, 8:581-583

30. Fattah, G., Ghrissi, F., Mabrouki, J., & Cherifi, H. (2022). Impact of limestone deposit operation on the yield of surrounding agricultural land: Case of the western rif, Morocco. E3S web of conferences, 337, 02005.
31. Zayani, R., BenSalem, E., Khouja, M., Bouhjar, A., Boussaid, M., & Messaoud, C. (2025). Optimization of Tunisian Myrtus communis L. Essential Oil Extraction by Complet Factorial Experimental Design. Metabolites, 15(6), 369.
32. Elouardi, M., Zair, T., Mabrouki, J., Fattah, G., Benchrifa, M., Qisse, N., & El Belghiti, M. A. (2022). A review of botanical, biogeographical phytochemical and toxicological aspects of the toxic plants in Morocco. Toxicologie Analytique et Clinique, 34(4), 215-228.
33. Batista, B. F., Bianchini, N. H., Pavlack, A. S., Andrade, G. E., Muniz, M. F. B., & Heinzmann, B. M. (2025). INFLUENCE OF DIFFERENT DRYING METHODS ON YIELD, DENSITY, COLOR, AND CHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF Ocotea lancifolia (SCHOTT) Mez LEAVES. Revista Árvore, 49, e4912.
34. Lkima, K., Salcedo, F. P., Mabrouki, J., & Aziz, F. (2022). Precision agriculture: Assessing water status in plants using unmanned aerial vehicle. In IoT and Smart Devices for Sustainable Environment (p. 139-153). Springer.
35. Adenan, N. S., Zahir, N. M., Daud, N. M., Sulaiman, W. R. W., Hasham, R., Ya'akob, H., & Hamid, M. A. (2024). Antioxidants activities of Backhousia citriodora leaf extract by ultrasonic assisted extraction. Materials Today: Proceedings, 110, 191-196.
36. Yu, Z.-W., Wang, J., Lin, F.-H., He, F.-M., Li, H.-S., Wang, S.-S., Fang, M.-J., Li, B.-C., & Qiu, Y.-K. (2024). A comprehensive study on solvent effect and establishment of n- hexane solvent system based normal-phase liquid chromatography× reversed-phase liquid chromatography for isolation of natural products. Journal of Chromatography A, 1733, 465278.
37. Amanullah, M. F., Rasamani, P., & Sukumar, M. (2024). Process kinetics optimization for extraction of essential oil from the peel of Citrus sinensis for the development of food packaging film. Journal of Food Process Engineering, 47(4), e14613.
38. Xu, M., Cai, J., Wang, L., Zhu, S., Chen, Y., Chen, Y., Zhong, J., Li, J., Hu, P., & Ye, Q. (2025). Identification of Chemical Components in Three Types of Rose Essential Oils Based on Gas Chromatography-Mass Spectrometry (GC-MS) and Chemometric Methods. Molecules, 30(9), 1974.
39. Verma, A., Vyas, M., & Sahu, S. K. (2025). Unveiling Anticancer Phytoconstituents: A Comprehensive Review of GC–MS Applications in Natural Products. ChemistrySelect, 10(9), e202405724. https://doi.org/10.1002/slct.202405724
40. Zugazua-Ganado, M., Bordagaray, A., Ezenarro, J., Garcia-Arrona, R., Ostra, M., & Vidal, M. (2024). Adaptation of the Folin-Ciocalteu and Fast Blue BB spectrophotometric methods to digital image analysis for the determination of total phenolic content: Reduction of reaction time, interferences and sample analysis. LWT, 193, 115756.
41. Sultana, S., Hossain, M. L., Sostaric, T., Lim, L. Y., Foster, K. J., & Locher, C. (2024). Investigating Flavonoids by HPTLC Analysis Using Aluminium Chloride as Derivatization Reagent. Molecules, 29(21), 5161.
42. Palacios, C. E., Nagai, A., Torres, P., Rodrigues, J. A., & Salatino, A. (2021). Contents of tannins of cultivars of sorghum cultivated in Brazil, as determined by four quantification methods. Food Chemistry, 337, 127970.
43. Huong, D. Q., Tai, P. D. T., Trung, N. Q., Thong, N. M., Tam, N. M., Phong, N. H., & Nam, P.C. (2024). Investigation of the free radical scavenging ability of l-tryptophan and its derivatives using experimental methods and quantum chemical calculations. RSCadvances, 14(51), 38059-38069.
44. Brand-Williams, W, Cuvelier, M.E., Berset, C. "Use of a free radical method to evaluate antioxidant activity". Food. Sci. Technol, 28, p 25–30; (1995).
45. Oyaizu M. Studies on products of browning reaction: antioxidative activity of products of browning reaction prepared from glucosamine. The Japanese Journal of Nutrition and Dietetic ,1986, vol.44, n°6, p.307–315
46. Ozyurt, D., Demirata, B., & Apak, R. (2007). Determination of total antioxidant capacity by a new spectrophotometric method based on Ce (IV) reducing capacity measurement. Talanta, 71(3), 1155-1165.
47. Regraguy, B., Ellouzi, I., Mabrouki, J., Rahmani, M., Drhimer, F., Mahmou, C., ... & El Hajjaji, S. (2022). Zinc doping of different nanoparticles of TiO2 Sachtopore for improved elimination of the methyl orange by photocatalysis. Emergent Materials, 5(6), 1945-1958.
48. De Sousa, D. P., Damasceno, R. O. S., Amorati, R., Elshabrawy, H. A., de Castro, R. D., Bezerra, D. P., Nunes, V. R. V., Gomes, R. C., & Lima, T. C. (2023). Essential oils : Chemistry and pharmacological activities. Biomolecules, 13(7), 1144.
49. Zainal-Abidin, M. H., Eng, J. J., Kristianto, S., Mahmood, W. M. A. W., Al-Fakih, A. M., Matmin, J., Wahab, R. A., Abdullah, F., Mohamad, M. F., & Hashim, S. E. (2024). Effectiveness of ammonium-based deep eutectic solvents in extracting polyphenol from Chlorella vulgaris. Algal Research, 79, 103436.
50. Shah, M., Linyan, Z., Qasim, M., Zewdie, Y., Qiu, L., & Ayaz, M. (2025). HPLC/MALDI-TOF-MS characterization and nutraceutical potential of Seriphedium quettense extracts: A polarity directed solvent extraction and antioxidant kinetics. Journal of Molecular Liquids, 426, 127349.
51. Lizárraga-Velázquez, C. E., Leyva-López, N., Hernández, C., Gutiérrez-Grijalva, E. P., Salazar-Leyva, J. A., Osuna-Ruíz, I., Martínez-Montaño, E., Arrizon, J., Guerrero, A., & Benitez-Hernández, A. (2020). Antioxidant molecules from plant waste: Extraction techniques and biological properties. Processes, 8(12), 1566.

52. Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. Nature, 181(4617), 1199–1200. – for DPPH assay methodology.
53. Abraham, A. (2025). Phytochemical Analysis and Antioxidant Potential of Myxopyrum serratulum AW Hill. EC Pharmacology and Toxicology, 13, 01-13.
54. Michielin, E.M.Z., Wiesse, L.P.L., Ferreira, E.A., Pedrosa, R.C., Ferreira, S.R.S. (2011). Radical-scavenging activity of extracts from Cordia verbenacea DC obtained by different methods. J Supercrit Fluids, 56, 89-96.
55. Fernández-Agulló, A., Pereira, E., Freire, M.S., Valentão, P., Andrade, P.B., González-Álvarez, J., Pereira, J.A. (2013). Influence of solvent on the antioxidant and antimicrobial properties of walnut (Juglans regia L.) green husk extracts. Ind Crops Prod, 42, 126-132
56. Naczk, M., Shahidi, F. (2004). Extraction and analysis of phenolics in food. J Chromato, 1054, 95-111.
57. Maisuthisakul, P., Suttajit, M., Pongsawatmanit, R. (2006). Assessment of phenolic content and free radical-scavenging capacity of some Thai indigenous plants. Food Chem, 32, 59-72
58. D'Archivio, M., Filesi, C., Di Benedetto, R. (2007). Polyphenols, dietary sources and bioavailability. Ann Ist Super Sanita, 43, 348-361
59. Benchrifa, M., Benchrifa, K., Alsayer, I., Azdem, D., Mabrouki, J., & Hmouni, D. (2025). Valorization of argan fruit residues to produce thermal energy by combustion. Sustainable Chemistry One World, 100066.
60. Bellakhdar J., Il Idrissi A., Canigueral S., Iglesias J. & Vila R., (1994). Composition of Lemon Verbena (Aloysia triphylla (L'Herit.) Britton) oil of Moroccan origin. Journal of Essential Oil Research, 6: 523-526.
61. Ghizlane, F., Mabrouki, J., Ghrissi, F., & Azrour, M. (2022). Proposal for a high-resolution particulate matter (PM10 and PM2. 5) capture system, comparable with hybrid system- based internet of things : Case of quarries in the western rif, Morocco. Pollution, 8(1),169-180
62. Sheyla, R., Olman, H., De Cirano, M.G.I., Navarro, I., Astiasarán, I., Ansorena, D., Yolanda, C.R., Calvo, M.I. (2011). Chemical composition, mineral content and antioxidant activity of Verbena officinalis L. LWT-Food Sci Technol, 44, 875-88