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Formulation and Characterization of Microemulsion Using Artemisia Extract

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Declaration

I certify that this thesis submitted for the degree of master, is the result of my research, except where otherwise acknowledged, and this thesis has not been submitted for the higher degree to any other university or institution.

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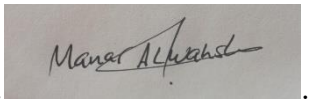
Signed: …  ……

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Manar Al-Wahsh

Abstract

This study presents a novel and validated approach to formulate and characterize microemulsions incorporating Artemisia herba-alba extracts for potential pharmaceutical and cosmetic applications. The microemulsions were developed using biocompatible surfactants (Tween 80 and Span 20) and characterized through pseudo-ternary phase diagrams to determine the optimal emulsification behavior. Methanolic and ethyl acetate extracts of Artemisia leaves and seeds were analyzed for phytochemical content, antioxidant capacity, and antibacterial efficacy. Total phenolic and flavonoid contents were quantified using spectrophotometric methods, with seed extracts yielding higher phenolic content (881 mg GAE/g) compared to leaves (302 mg GAE/g). In comparison, leave extracts exhibited superior flavonoid levels (371.59 mg/g). Antioxidant activity was confirmed via DPPH assay (>74% inhibition), and key phytochemicals such as chlorogenic acid and rutin were identified through HPLC-PDA. Nanoparticle morphology and size were analyzed by Atomic Force Microscopy, revealing particles as small as 4.5 nm. Optical properties were confirmed using UV-Vis and fluorescence spectroscopy. Antibacterial tests against S. aureus, E. coli, and K. pneumoniae showed moderate efficacy. Stable and transparent microemulsions were successfully developed, and a topical cream formulation was prepared and evaluated for physical stability. This is the first report detailing the emulsification behavior and microemulsion-based delivery system of Palestinian Artemisia herba-alba, establishing a foundation for its application in natural therapeutics.

Key words: Artemisia herba-alba, microemulsion, phytochemical analysis, antioxidant activity, antibacterial activity, pseudo-ternary phase diagram, flavonoids, phenolic compounds, HPLC-PDA, Atomic Force Microscopy (AFM), UV-Vis spectroscopy, fluorescence spectroscopy, topical cream formulation, natural therapeutics.

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List of Abbreviations, Symbols, and Terminology:

Abbreviation	Full word
EOs	Essential oils
GC-Ms	Gas chromatography–mass spectrometry
HPLC	High-performance liquid chromatography analysis
DMSO	Dimethyl sulfoxide
TPC	Total Phenolic Content.
TFC	Total Flavonoid Content.
DPPH	Reducing Antioxidant reagent.
GAE	Gallic Acid Equivalent
MHA	Muller Hinton agar
AFM	Atomic Force Microscopy
EDTA	Ethylenediaminetetraacetic acid
NP	Nanoparticle
UV	Ultraviolet
PS	particle size
DSE	droplet size estimate

Chapter One:

Introduction

1.1 Artemisia:

As the global burden of chronic and infectious diseases continues to rise, the search for effective and natural therapeutic alternatives remains a major scientific priority. Medicinal plants have served as one of the most important sources of bioactive compounds throughout human history. Among these, Artemisia herba-alba, commonly known as white wormwood, holds particular significance due to its wide use in traditional medicine across the Middle East and North Africa, including Palestine.

Artemisia herba-alba is a perennial aromatic herb belonging to the Asteraceae family. It typically grows in arid and semi-arid climates, where it thrives in rocky and sandy soils. The plant is characterized by its erect, woody stems, silvery-gray pubescent leaves, and small yellowish flower heads that bloom from September to December. Botanically, it is classified under the genus Artemisia, which includes over 500 species known for their aromatic and medicinal properties (Mohamed et al, 2010).

For centuries, Artemisia herba-alba has played a prominent role in traditional herbal medicine. It has been used to treat a variety of ailments such as digestive problems, respiratory infections, diabetes, parasitic infestations, and inflammatory disorders. In Palestinian folk medicine, decoctions and infusions prepared from its leaves and flowering tops are commonly administered for gastrointestinal discomfort and as antiseptics. Its essential oils and plant extracts have also been used in traditional cosmetics and household remedies (Mohamed et al, 2010, and Abad et al,2012).

Phytochemical investigations have identified a rich profile of bioactive constituents in Artemisia herba-alba, including flavonoids, phenolic acids, monoterpenes, and sesquiterpenes. These compounds are largely responsible for the plant's medicinal potential, displaying antioxidant, antimicrobial, anti-inflammatory, and antispasmodic activities. The essential oils, in particular, are dominated by camphor, α- and β-thujone, and borneol compounds that contribute both to its distinctive scent and its pharmacological actions (Mohamed et al, 2010, and Abad et al,2012).

Although various studies have confirmed the pharmacological potential of Artemisia herba-alba, including antioxidant, antibacterial, and anti-inflammatory activities, a persistent challenge in its therapeutic use lies in the poor solubility, instability, and limited bioavailability of its active compounds. These issues hinder its effectiveness in conventional dosage forms and require innovative drug delivery systems to enhance its pharmacokinetic and pharmacodynamic profiles. The delivery of plant-based compounds through traditional oral or topical formulations often leads to degradation or poor absorption, thereby diminishing therapeutic outcomes (Zhang et al,2020 and Mohammed et al, 2021).

Recent advancements in nanotechnology and formulation science have introduced microemulsions as promising vehicles for the efficient delivery of poorly soluble bioactive compounds. Microemulsions are thermodynamically stable, isotropic mixtures composed of oil, water, surfactants, and co-surfactants. These systems offer several advantages, such as enhanced solubilization, improved permeability, targeted delivery, and sustained release. Despite their established utility in the delivery of synthetic and some natural products, microemulsions have not been adequately explored for the formulation of Artemisia herba-alba extracts (Yadav et al,2023) .

Additionally, no published research has yet characterized the phase behavior of Artemisia herba-alba extracts from Palestinian sources or systematically evaluated their incorporation into optimized microemulsion systems for pharmaceutical or dermatological use. Therefore, there is a significant knowledge gap in the literature regarding the microemulsion-based delivery of Artemisia herba-alba, especially for its seed and leaf extracts. Addressing this gap may lead to the development of more effective and stable natural therapeutic products, enabling broader clinical and commercial utilization (Abubakar et al, 2020).

Recent advancements in natural product research have revived scientific interest in Artemisia herba-alba, especially in the context of developing herbal-based formulations for pharmaceutical use. Ongoing studies are focusing on optimizing the extraction methods and delivery systems of its bioactive compounds to maximize therapeutic efficacy while minimizing toxicity. Given its wide distribution, long-standing traditional use, and diverse pharmacological profile, A. herba-alba represents a valuable yet underutilized medicinal plant deserving of further exploration (Yadav et al, 2023).

This study aims to formulate and characterize a microemulsion system incorporating Artemisia herba-alba extract as the active ingredient. The use of biocompatible surfactants such as Tween 80 and Span 20 will be investigated to optimize the stability and performance of the microemulsion. Characterizing the formulated microemulsions will include physicochemical properties such as droplet size, viscosity, and phase behavior. Additionally, the antioxidant and antimicrobial activities of the Artemisia extract-loaded microemulsions will be assessed to evaluate their therapeutic potential (Yadav et al, 2023).

1.1.2 Phytochemical constituents of Artemisia:

Phytochemicals are biologically active, non-nutrient compounds that plants produce through various secondary metabolic pathways. These substances are responsible for the characteristic color, flavor, and aroma of many plants and have attracted considerable attention due to their broad pharmacological properties, including antioxidant, antimicrobial, anti-inflammatory, and anticancer effects (Chebbac et al., 2021; Cianciosi et al., 2018). More than 200 different phytochemicals have been identified to date, including flavonoids, phenolic acids, terpenoids, coumarins, alkaloids, and chlorophyll derivatives. Their composition and concentration can vary significantly depending on plant species, environmental conditions, and the specific plant part analyzed.

Artemisia herba-alba, commonly known as white wormwood, is a perennial medicinal plant native to arid and semi-arid regions of the Mediterranean basin, particularly in North Africa and the Middle East. It has been widely used in traditional medicine for the treatment of gastrointestinal disturbances, diabetes, infections, and inflammatory conditions. The therapeutic properties of A. herba-alba are closely linked to its rich and complex phytochemical profile, which is influenced by environmental factors, geographical location, and extraction methods (Akrout et al., 2010; El Hawary et al., 2022).

The major classes of phytochemicals identified in A. herba-alba include:

- Essential oils rich in monoterpenes and sesquiterpenes such as camphor, β-thujone, 1,8-cineole, and borneol
- Phenolic acids like chlorogenic acid and caffeic acid
- Flavonoids including rutin, quercetin, and luteolin
- Tannins, coumarins, and alkaloids

Essential oils, in particular, contribute significantly to the plant's medicinal value due to their strong antimicrobial and insecticidal activities. These oils are highly variable in composition, depending on factors such as soil, climate, and harvest season. Camphor and β-thujone are considered chemical markers of Artemisia herba-alba, while flavonoids and phenolic acids are primarily responsible for its antioxidant potential (Boudjelal et al., 2013; Msaada et al., 2015).

The diversity and abundance of these phytochemicals underline the pharmacological importance of Artemisia herba-alba and support its continued exploration as a natural source of therapeutic agents. Recent studies have employed advanced analytical techniques such as high-performance liquid chromatography (HPLC) and spectrophotometry to further characterize its bioactive constituents and confirm their functional role.

1.1.2.1 Phenolic Acids

Phenolic acids are organic compounds characterized by a phenol ring with one or more carboxylic acid groups. They are widely distributed in medicinal plants and contribute significantly to their biological activities (Shahidi & Ambigaipalan, 2015). *In* Artemisia herba-alba, phenolic acids such as chlorogenic acid, caffeic acid, and ferulic acid have been identified as major components (Ben Houda et al., 2020). These acids possess strong antioxidant and anti-inflammatory properties, which help in neutralizing free radicals and protecting cells from oxidative damage. Additionally, phenolic acids in Artemisia herba-alba play a role in antimicrobial activity, supporting the traditional use of the plant in treating infections (Msaada et al., 2017). They also contribute to the characteristic bitter taste of the plant extracts, influencing the sensory profile of herbal formulations.

1.1.2.2 Flavonoid

Flavonoids are a diverse group of polyphenolic compounds involved in plant defense and human health. In Artemisia herba-alba, flavonoids such as quercetin, rutin, and luteolin have been reported as abundant phytochemicals (Zarrouk et al., 2018). These compounds exhibit potent antioxidant, antimicrobial, and anti-inflammatory effects, which enhance the therapeutic potential of the plant (Elshafie & Camele, 2017). Studies indicate that flavonoids from Artemisia herba-alba contribute to the inhibition of microbial growth and reduction of oxidative stress, justifying their use in traditional medicine (Rahmani et al., 2019). Their presence also improves the pharmacological profile when incorporated into modern drug delivery systems like microemulsions, enhancing bioavailability and stability.

1.1.3 Extract Techniques of Artemisia:

To utilize the bioactive compounds in Artemisia herba-alba effectively, an efficient and reproducible extraction method is crucial. In this study, Soxhlet extraction was employed, a widely recognized and traditional technique used for the exhaustive extraction of soluble phytochemicals from solid plant matrices. It is particularly valued in phytochemical and environmental research for its high recovery efficiency and reproducibility.

Soxhlet extraction operates by continuously recirculating solvent over the plant material. The plant sample is placed in a thimble, which is repeatedly washed with freshly condensed solvent. Once the extraction chamber fills, it siphons back into the distillation flask, carrying the solubilized compounds. This cycle continues until the solvent becomes saturated with extractable constituents.

The efficiency of this technique depends on several factors:

- The choice of solvent (in this case, methanol and ethyl acetate),
- The solvent-to-solid ratio,
- Temperature and extraction time,
- And the particle size of the plant material.

The advantages of Soxhlet extraction include complete dissolution of target compounds and the ability to process samples without constant supervision. In this study, extracts were obtained from both seeds and leaves of Artemisia herba-alba, followed by evaporation and lyophilization for use in further characterization assays (De Castro et al, 2000).

1.2- Medical effects of Artemisia

Artemisia species are known for their medicinal properties, including antioxidant, antibacterial, and antimalarial activities. Despite these benefits, limited research has been conducted on developing microemulsion formulations containing Artemisia extracts. Recent studies have explored various approaches to enhance the delivery and efficacy of Artemisia compounds. (Septembre-Malaterre, et al, 2020)

For instance, a study developed a solid formulation containing a microemulsion of a novel Artemisia extract with nematocidal activity for oral administration. This formulation aimed to improve the delivery and effectiveness of the extract against parasitic nematodes (Perez-Roman et al,2020).

Another research focused on enhancing the transdermal delivery of artemisinin, a compound derived from Artemisia species, using a microemulsion vehicle based on ionic liquids and lidocaine ibuprofen. The study demonstrated that this formulation significantly increased the permeation flux of artemisinin through the skin, suggesting its potential for effective transdermal therapeutic applications (Zhang et al, 2020).

These studies highlight the potential of microemulsion systems in improving the delivery and efficacy of Artemisia extracts. Further research in this area could lead to the development of effective therapeutic formulations utilizing the beneficial properties of Artemisia species (Septembre-Malaterre et al, 2020).

1.2.1 Antioxidant effect:

showed excellent antioxidant properties. These antioxidant effects were highly associated with the number of phenolic hydroxyl groups on the phenolics they contained. The results of this study suggested that the wild aromatic herb Artemisia herba-alba Asso. (Wormwood) can be a source of phenolic compounds with natural antioxidant properties, which can be used for potential pharmaceutical applications. This plant is well-known for its healing and medicinal effects and has been used in both conventional and modern medicine (Mohammed et al,2021).

The study of naturally occurring bioactive chemicals is the main focus of current research. Thymus algeriensis and the medicinal plant Artemisia herba-alba are widely used by Moroccans to treat a variety of inflammatory conditions. This study aimed to evaluate the individual and combined antioxidant and analgesic properties of the essential oils that were isolated from these therapeutic plants. Essential oils were extracted by hydro-distillation using an apparatus akin to that of Clevenger. Iron reduction and the scavenging of the free radical DPPH were the two methods

utilized to evaluate the antioxidant activity. The results of the two antioxidant tests indicated that our extract mixture exhibits good iron reduction capacity and very interesting DPPH free radical scavenging power, with an IC50 of around 4.38 ± 0.98 µg/mL, higher than that of the benchmark antioxidant, BHT. These intriguing findings demonstrate the plant EOs' significant antioxidant activity and potent synergistic action, which motivates more thorough research into their pharmacological characteristics (El Ouahdani et al,2021).

In folk medicine, Artemisia herba alba Asso. (Compositae) It is commonly used. This plant has phenolic compounds that might be candidates for some of its biological roles and, as a result, for its potential use in medicine. The polyphenolic components in this study were identified, measured, and extracted using extracts from the leaves of A. herba alba. Flavonoids (luteolin and apigenin) were detected in higher concentrations in the

phenolic acids, including the ethyl acetate phase and protocatechuic acid, caffeic acid, gallic acid, and ferulic acid derivatives. While the aqueous phase has lower amounts of phenolic acids, the chloroform phase contains phenolic acids and aglycon flavonoids. By evaluating how well the extracts and specific phenolic compounds scavenge and block the free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH), the antioxidant properties of these. (Seddik et al,2010).

The antioxidant and cytotoxic properties of the phytochemicals found in Artemisia herba-alba's ethanol extract were investigated. The Folin–Ciocalteau technique was used to quantify the total phenolics after an accelerated solvent extraction and evaluated by GC-MS. Three techniques were used to assess the extract's antioxidant activity, and flow cytometry and the MTT test (which evaluates cellular metabolism) were used to assess its cytotoxicity in vitro on the NCI-N417 tumor cell line. The A. herba-alba extract contained 21 compounds, had a high DPPH-radical-scavenging activity (IC50 = 69.01 ± 0.03 µg mL−1), inhibited β-carotene oxidation (95.75%± 0.013), and reduced power (DO700nm = 0.751 ± 0.001). It was also rich in phenolic compounds (248.56 ± 11.05 µg GAE mg−1 extract). A herba-alba ethanol extract showed antiproliferative and cytotoxic properties. (Amkiss et a,2022)

1.2.2 Anti-inflammatory effect:

One area of current research is the study of naturally occurring bioactive compounds. The Moroccan people employ Artemisia herba-alba, a medicinal plant, extensively for treating several inflammatory diseases. This study aimed to assess the anti-inflammatory properties of the essential oils that were isolated from these therapeutic herbs, both alone and in combination. With a maximum inhibition of edema percentage of 89.99 ± 4.08, the anti-inflammatory test showed that the oral mixture at a 150 mg/kg dose had superior activity compared to 1% Diclofenac. Compared to the mice treated with Tramadol (42.00 ± 2.70), the mice treated with the mixture at a dose of 150 mg/kg experienced considerably fewer cramps (29.80 ± 1.92). These are fascinating (El Ouahdani et al,2021).

One medicinal plant that is well-known for its ability to fend off chronic illnesses is Artemisia herba-alba. The assessment of the anti-inflammatory impact induction was conducted by the glutathione metabolism (glutathione reductase, transferase, and peroxidase), and protein-free

thiols were measured in Jurkat cells incubated with low to high concentrations of Artemisia herba-alba extracts, ranging from 10 to 100 g/mL. It is interesting to note that extracts from the microwave showed a stronger induction, 5 to 10-fold changes at early time points, and maintained over 72 hours. In contrast, Artemisia herba-alba extracts showed a moderate anti-proliferative effect in the leukemic Jurkat line. We concluded that Artemisia herba-alba extracts directly enhance anti-inflammatory cell response, initially through ROS induction, but later vigorously maintained, independently of ROS, indicating the involvement of cellular metabolism in enhancing these effects (Bouchara et al,2021).

This study evaluates the antibacterial and anti-inflammatory properties of Artemisia herba-alba's methanolic leaf extract. The extract, at 40 mg/kg, Comparing the extract to the control, and the anti-hematogenic effects demonstrated a substantial reduction in both dextran-induced inflammation and carrageenan-induced paw edema at 1 and 3 hours. Furthermore, there was a substantial decrease in xylene-induced ear edema inhibition when compared to the control group ($p<0.05$). showed significant reductions in dextran-induced inflammation and carrageenan-induced paw edema, as well as xylene-induced ear edema inhibition. The extract also showed a decrease in xylene-induced ear edema inhibition. The bacterial activity of five strains was assessed using the in vitro agar diffusion method. The results suggest that the extract could be a cost-effective and accessible source of enhanced traditional medicine (Hafidh et al, 2022).

The biological activities of methanolic extracts from plants collected in the center of Tunisia—Artemisia herba-alba, Ruta chalpensis L., and Peganum harmala L.—were examined in this work. Artemisia herba-alba has a significant phenolic composition, according to the results (123.95±4.3g GAE/kg of dry mass). The plant extract demonstrated the highest antioxidant (IC50 (DPPH assay) 20.64±0.84mg/L) and anti-inflammatory (72% inhibition at 150mg/L) activities when tested using various antioxidant assays (DPPH, ABTS, and AAPH/linoleic acid methods) and an IFN-γ/LPS induced RAW 264.7 murine macrophages' assay. IC50 values for A. herba-laba were 81.59±4.4, 59.05±3.66, and 90.96 mg/L, respectively, while the two other extracts, except Peganum harmala L. extract, shown strong anticancer activity against multiple cell lines (human bladder carcinoma RT112, human laryngeal carcinoma Hep2, and human myelogenous leukemia K562) (Khlifi et al,2013).

Millions of people throughout the world suffer with arthritis, a crippling illness marked by pain and inflammation that lowers quality of life. It is essential to comprehend the causes underlying arthritis and create efficient remedies. This study examined the preventive efficacy of Artemisia herba-alba hydroethanolic extract against oxidative stress, lipid peroxidation, and arthritic markers in vitro. Its anti-arthritic efficacy in vivo was also evaluated. Numerous components were found in the extract by the phytochemical examination, including high levels of flavonoids and polyphenols. Because of these substances' many health advantages, A. herba-alba may be a good source of useful phytochemicals. A. herba-alba showed a significant impact on the degree of arthritic pain, paw edema, and body weight loss. Histopathological analysis demonstrated improvements in bone and muscle structure, highlighting its potential as a treatment for chronic (Wahnou et al, 2024).

1.2.3 Antibacterial effect:

The direct TLC bioautography assay was used to evaluate the combined A. herba-alba extracts' antibacterial efficacy. Using the disc-diffusion assay and direct TLC bioautography assay, the antibacterial activity of each fraction was assessed against two Gram-positive (Bacillus cereus and Staphylococcus aureus) and two Gram-negative (Escherichia coli and Proteus vulgaris) pathogens. While Fractions IV and V did not show substantial antimicrobial activity, Fraction I inhibited both P. vulgaris and B. cereus, Fraction II inhibited both B. cereus and E. coli, and Fraction III inhibited everything but P. vulgaris. These extracts showed antibacterial properties, indicating that the various phenolic chemicals contained within them had distinct inhibitory effects on bacterial growth. Phenolic chemicals with inherent antibacterial qualities can be found in Herba-alba (Mohammed et al,2021).

The study investigates the chemical makeup of Artemisia herba-alba essential oil, a medicinal plant used in Algerian herbal medicine. It reveals that the oil contains 19 components, with camphor being the most potent. The oil showed strong antibacterial activity against Klebsiella oxytoca and Acinetobacter baumannnii, with minimal bactericidal and inhibitory concentrations ranging from 10-20 mg mL−1 and 5-10 mg mL−1, respectively. After 24 hours of exposure, all bacteria reached the bactericidal endpoint, with a median lethal dose of 615 mg kg−1. The study suggests that Artemisia herba-alba essential oil may contain natural antibacterial compounds with potential therapeutic uses (Bertella et al,2018).

In this investigation, seven clinical isolates of bacteria were employed. In microtitre plates, biofilm development was initially measured. Using a two-fold dilution series, the biofilm inhibitory concentration (BIC) and minimum inhibitory concentration (MIC) tests were carried out in microtitre plates. The impact of Artemisia herba-alba essential oil on initial adhesion to the polystyrene surface was then tested using the isolate that was the most tolerant.
All isolates were inhibited by Artemisia herba-alba essential oil, although susceptibility varied greatly. It was discovered that the MIC values fell between 0.5 and 4% v/v. Furthermore, the most tolerant isolate (Pseudomonas aeruginosa) showed initial adherence inhibition at sub-inhibitory concentrations when exposed to Artemisia herba-alba essential oil.
All isolates were significantly inhibited by the essential oil of Artemisia herba-alba.
At sub-inhibitory concentrations, it was able to prevent the most tolerant isolate from adhering initially (Al-Shuneigat et al,2014).

Traditional medicines frequently employ Artemisia herba-alba to treat a variety of illnesses. Two new compounds, 1,3,8-trihydroxyeudesm-4-en-7α,11βH-12,6α-olide (1) and 5-β-d-glucopyranosyloxy-7-methoxy-6H-benzopyran-two (2), were isolated and identified from the organic extract of A. herba-alba's aerial parts, along with five known metabolites: 3α,8β-dihydroxygermacr-4(15),9(10)-dien-7β,11αH,12,6α-olide (3), 1β,8α-dihydroxy-11α,13-dihydrobalchanin (4), 11-epiartapshin (5), tomenin (6), and benzoic acid, p-(β-D-glucopyranosyloxy)-methyl ester (7). The chemical structures were demonstrated via spectroscopic analysis, such as ESI-MS and 1 D/2D NMR. Compound 1 inhibited Staphylococcus aureus and Bacillus subtilis, two Gram-positive bacteria. Gram-positive and gram-negative bacteria were both susceptible to the antibacterial action of compounds 2 and 3 (Mohamed et al,2021).

The essential oil, extracted from A. herba-alba cultivated in Jordan, contained 22 identified compounds, with monoterpenoids comprising 71.90% of the oil. The major component was alpha-pinene (17.20%). The oil exhibited antibacterial activity against Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus, methicillin-resistant Staphylococcus aureus (MRSA), and methicillin-resistant Staphylococcus epidermidis (MRSE). The highest inhibitory effect was observed against MRSE (Dmour et al,2024).

1.3 Microemulsion

Microemulsions are thermodynamically stable, transparent or translucent colloidal dispersions composed of oil, water, surfactant, and co-surfactant. These systems form spontaneously and are characterized by small droplet sizes ranging from 10 to 100 nanometers. Their nanometric size and large surface area enhance the solubility, bioavailability, and stability of incorporated active compounds. In recent years, microemulsions have gained substantial attention for the delivery of herbal extracts due to their ability to improve the therapeutic efficacy of poorly water-soluble plant-derived compounds (Tanwar et al., 2020).

The advantages of microemulsion-based delivery systems over traditional formulations include:

- Enhanced solubility and absorption of hydrophilic and lipophilic phytochemicals.
- Improved stability of sensitive bioactive compounds such as polyphenols and flavonoids.
- Controlled release and prolonged therapeutic effects.
- Reduced required dosage, which minimizes side effects and formulation costs (Tanwar et al., 2020; Muhammad et al., 2019).

In the case of Artemisia herba-alba, the microemulsion system facilitates the delivery of key active constituents such as chlorogenic acid, rutin, and flavonoids by protecting them from oxidative degradation and enhancing their transdermal and systemic penetration. The small droplet size contributes to better diffusion across biological membranes, making these formulations ideal for pharmaceutical and cosmetic applications (Rosero, 2019; Khan et al., 2019).

Surfactants and co-surfactants used in this study, such as Tween 80 and Span 20, were selected based on their biocompatibility, emulsification efficiency, and ability to form stable microemulsion systems. Co-surfactants like ethanol help reduce interfacial tension and expand the microemulsion region (Kimura, 2014). The formulation process involved homogenizing oil and surfactant in fixed ratios, followed by the gradual titration of the aqueous phase under constant stirring using a vortex mixer and water bath.

1.4 Pseudo-Ternary Phase Diagram

To optimize microemulsion formulations, pseudo-ternary phase diagrams were constructed to define the appropriate concentration ranges for oil, surfactant/co-surfactant, and aqueous phases. These diagrams are triangular graphs where each vertex represents 100% of one component (oil, water, surfactant), and points within the triangle indicate different compositions.

In this research, pseudo-ternary phase diagrams were constructed by titrating fixed oil-surfactant mixtures with water and observing phase transitions such as clarity, turbidity, or separation. The transparent and monophasic regions were identified as the microemulsion zone. This approach guided the selection of stable and visually uniform formulations that were further analyzed for nanoparticle size, morphology, and bioactivity (Huang et al., 2016).

1.5 Problem Statement

While synthetic drugs continue to dominate pharmaceutical formulations, their use is often associated with undesirable side effects, low bioavailability, and high systemic toxicity. These limitations have prompted a global shift toward natural and plant-based therapies as safer and more sustainable alternatives.

Natural plant extracts like Artemisia herba-alba have strong therapeutic properties but face challenges in delivery:

- Poor solubility
- Low stability
- Inefficient absorption

These limitations hinder the optimal bioavailability and efficacy of Artemisia's active phytochemicals.

To date, no published studies have focused on formulating Palestinian Artemisia herba-alba extract into a microemulsion system, a promising nanocarrier known for improving solubility, enhancing bioavailability, and stabilizing plant based bioactives.

This research addresses a significant knowledge gap by formulating and characterizing microemulsions.

1.6 Aim of the Thesis

- To select the most suitable solvent for extraction and formulation, evaluate the solubility of Artemisia herba-alba extract in different solvents such as water, ethanol, and DMSO.
- To quantify the total phenolic and flavonoid contents of Artemisia herba-alba extract using validated spectrophotometric methods.
- To identify and characterize the major phytochemical compounds in the extract using high-performance liquid chromatography with photodiode array detection (HPLC-PDA).
- To analyze the optical characteristics of the extract and its formulation using UV-Visible spectroscopy and fluorescence spectroscopy.
- To formulate a stable microemulsion system incorporating Artemisia herba-alba extract.
- To investigate the phase behavior of the microemulsion system using pseudo-ternary phase diagrams to determine the optimal component ratios.
- To determine the particle size and surface morphology of the microemulsion using Atomic Force Microscopy (AFM).
- To evaluate the antioxidant potential of the extract using the DPPH radical scavenging assay.

- To assess the antibacterial activity of the extract and its microemulsion formulation against selected bacterial strains using the disc diffusion method.

1.7 **Justification of the Thesis**

- Solubility studies of Artemisia herba-alba extract in solvents such as water, ethanol, and DMSO are essential for identifying the most appropriate extraction and formulation media, directly affecting the efficiency and stability of the final product.
- Quantification of phenolic and flavonoid contents is critical, as these bioactive compounds are primarily responsible for the plant's antioxidant and antimicrobial properties.
- Phytochemical profiling using HPLC-PDA enables the precise identification of active constituents, supporting standardization, quality control, and future therapeutic development.
- Spectroscopic techniques like UV-Vis and fluorescence spectroscopy offer valuable insights into the optical and molecular characteristics of the extract, which are essential for understanding its behavior in different formulations.
- Formulating a stable microemulsion enhances the solubility, bioavailability, and shelf-life of plant-based bioactives, making them more effective in therapeutic applications.
- Studying the phase behavior through pseudo-ternary phase diagrams allows the optimization of microemulsion composition and stability.
- Particle size and surface morphology analysis via AFM is important to evaluate the physical characteristics of the microemulsion, which influences its delivery and performance.
- Biological evaluation of the extract and its formulation, including antioxidant and antibacterial activity, is crucial to validate its potential as a natural therapeutic alternative in line with the global trend toward replacing synthetic agents.

Chapter Two

Literature Review

This chapter provides an overview of previous studies related to the formulation and characterization of microemulsions, with a particular focus on plant-based systems. It highlights the use of Artemisia herba-alba in pharmaceutical and cosmetic applications due to its rich phytochemical composition and biological activities, including antioxidant, antibacterial, and anti-inflammatory.

Relevant literature on extraction techniques, surfactant selection, and the construction of pseudo-ternary phase diagrams for microemulsion systems is reviewed. Additionally, previous works on the physicochemical characterization and biological evaluation of microemulsions containing herbal extracts are discussed to establish a foundation for the present study.

2.1 Phytochemical constituents of Artemisia

The bioactive substances and medicinal uses of Artemisia herba-alba from various geographical locations have been investigated in several studies. Twenty accessions of Artemisia herba-alba obtained from North Africa were studied by Boulanouar et al. (2013), who concentrated on both qualitative and quantitative differences in phytochemical contents. Significant differences in phenolic compounds were found by high-performance liquid chromatography (HPLC) examination; the most prevalent phenolics found were rosmarinic acid and chlorogenic acid.

The essential oil composition of Artemisia herba-alba from Palestinian territories was examined in a study conducted by Al-Rimawi et al. (2017). Using gas chromatography-mass spectrometry (GC-MS), the study found that the main constituents were chrysanthenone (8.9%), camphor (38.5%), and 1,8-cineole (19.6%). These bioactive substances have been connected to antibacterial and anti-inflammatory properties, indicating Artemisia herba-alba's potential as a therapeutic agent.

The impact of ultraviolet (UV)-B irradiation on the phytochemical profile and antioxidant characteristics of Artemisia herba-alba at varying intensities was investigated by El-Rahman et al. (2020). The study used spectrophotometric techniques and ultra-high-performance liquid

chromatography (UHPLC) to measure the total phenolic content (TPC) and total flavonoid content (TFC). The greatest values were recorded at an intensity of 3.6 W/m² following 8 hours of treatment, indicating that UV-B exposure raised TFC and TPC. Furthermore, as demonstrated by improved DPPH radical scavenging activity, UV-B therapy increased antioxidant capacity.

Al-Tamimi et al. (2022) also used MTT tests to examine the antiproliferative effect of Artemisia herba-alba extracts against breast cancer cell lines (MCF-7). According to the study, extracts from UV-B-treated plants showed more cytotoxic effects than untreated samples, suggesting that post-harvest irradiation could be used as a method to improve Artemisia herba-alba's therapeutic qualities.

2.2 Phase Behavior of Artemisia Extract (Emulsification)

Microemulsions are among the many novel chemicals that have been produced as a result of recent developments in combinatorial drug development. Ghosh et al. (2014) explored that these adaptable systems provide benefits like improved drug solubility, thermodynamic stability, and ease of production and permeation. They are made up of a transparent, thermodynamically stable mixture of two immiscible liquids by a surfactant such as Tween 80 and Span 20 in stabilizing microemulsions, emphasizing their impact on droplet size and drug release kinetics. so regulate or enhance the medicinal drugs' bioavailability levels.

The researchers used various hydrophile-lipophile balance (HLB) surfactant systems to create drug-loaded water-in-oil (W/O) microemulsions. Yuan et al. (2006) demonstrated that oil-in-water microemulsions enhance the oral bioavailability of hydrophilic drugs through improved solubilization and absorption.

Microemulsions consist of isotropic mixes of water, oil, surfactant, and co-surfactant that are thermodynamically stable. Their distinct structure allows for bioactive substances' regulated release and effective encapsulation. Microemulsions have been used extensively in pharmaceutical formulations to enhance drug solubility and penetration across biological membranes (Kreilgaard, 2002). Regarding herbal extracts, microemulsions support the medicinal potential of phytochemicals while preserving their bioactivity.

2.3 Medical Effect of Artemisia

The possible therapeutic benefits of Artemisia herba-alba's medicinal qualities have been the subject of much research. To improve the pharmaceutical quality of medicinal plants, Ghasemzadeh et al. (2016) looked at how UV-B irradiation affected the production of flavonoids and phenolic acids. Spectrophotometric techniques, ultra-high-performance liquid chromatography analysis, DPPH assay, and MTT assays were used to measure the total flavonoid content (TFC), total phenolic content (TPC), antiproliferative activity, and antioxidant activity of Artemisia extracts, respectively.

Similarly, bioactive compounds including eicosyl ester, luteolin, naringin, oleic acid, α-tocopherol, apigenin, ascorbic acid, and phenolic compounds were found in Artemisia extracts when Baskaran et al. (2015) investigated their phytochemical composition. The findings showed that Artemisia herba-alba has strong antioxidant and antihypercholesterolemic effects, with the highest inhibition effect of roughly 74% among 25 tested medicinal plant extracts.

2.3.1 Antioxidant effect:

We are interested in examining the physicochemical and phytochemical makeup as well as the antioxidant qualities of various extracts (acetonic, ethanolic, and methanolic extracts) made from the leaves of Artemisia herba-alba and Olea europaea L. as part of the appraisal of regional medicinal plants. According to the physicochemical study of the plant powder by (Fatmi, et al 2022), Olea europaea L. had 8.23% water, 3.51% mineral salts, and 2.81% fat, while Artemisia herba-alba had roughly 7.86% water, 5.99% mineral salts, and 4.94% fat.

According to the results, the methanolic extract of Artemisia herba-alba had the highest concentration of phytochemicals (24.8%), while the ethanolic extract of Olea europaea L. had the highest concentration of bioactive compounds (37.65%). Acetonic extracts of the two plants under study yielded the highest levels of polyphenol, flavonoid, tannin, and chlorophyll pigments.

The acetonic extract for Artemisia herba-alba (IC50 = 0.611 mg/mL, EC50 = 5.03 mg/mL, TAC = 97.91µg AAE/mg dw) and the methanolic extract for Olea europaea L. (IC50 = 0.56 mg/mL, EC50 = 0.83 mg/mL, TAC = 150.49 µg AAE/mg dw) demonstrated the strongest anti-free radical activity with DPPH, reducing power, and total antioxidant capacity (TAC).

Numerous illnesses, such as cancer, neurological conditions, and cardiovascular ailments, are linked to oxidative stress. Because of their high phenolic and flavonoid content, Artemisia herba-alba both show excellent antioxidant properties.

The effects of several extraction techniques on the antioxidant capacity of A. herba-alba were examined in this work by Boutennoun et al. (2017), who confirmed that ethanol extracts had the maximum radical scavenging activity.

Additionally, Mohammedi and Atik (2011) examined the methanolic extracts of A. herba-alba's DPPH radical scavenging activity and found a considerable suppression of free radicals, supporting the plant's usage in traditional medicine to fight oxidative stress.

Similarly, large levels of flavonoids and phenolic acids, which support A. herba-alba's strong antioxidant activity, were discovered when Akrout et al. (2010) investigated the essential oil composition of the plant.

Artemisia herba-alba's antioxidant qualities have been extensively researched for their ability to guard against illnesses linked to oxidative stress. The total phenolic content and free radical scavenging activity of A. herba-alba extracts were assessed by Bekkara et al. (1998), who found that they have strong antioxidant capability on par with synthetic antioxidants.

2.3.2 Anti-inflammatory effect

This study by (Akrout et al. 2021) focuses on the analgesic, anti-inflammatory, and antioxidant qualities of essential oils from Artemisia herba-alba and Thymus algeriensis, which are utilized in Moroccan traditional medicine to treat diseases linked to inflammation. Hydro-distillation was used to extract the extracts, and their characteristics were evaluated. According to the antioxidant tests, the extract mixture exhibited superior iron reduction capacity, DPPH free radical scavenging ability, and a larger IC50 than BHT. With a peak inhibition of edema of 89.99 ± 4.08 and less cramping in animals than Tramadol, the oral mixture demonstrated greater efficacy in the anti-inflammatory test. Further investigation into the pharmacological properties of these plants is supported by the toxicology tests, which revealed no signs of danger.

A Yun et al. (2016) study looked into how Artemisia argyi leaf extract affected mice's contact dermatitis. The extract dramatically decreased the production of pro-inflammatory cytokines like interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and interferon-gamma (IFN-γ) as well as ear swelling. Furthermore, it suppressed the production of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) in macrophage cells, pointing to possible pathways for its anti-inflammatory effects.

2.3.3 Antibacterial effect

One of the most significant classes of plant metabolites, essential oils (EOs) are in charge of the biological activities of plants. The purpose of this study by Amor, Ghita, et al (2019) was to determine the chemical makeup and antibacterial properties of essential oils from Artemisia herba-alba and Origanum majorana against a strain of fungus isolated from spoilt butter as well as some Gram-positive and Gram-negative bacteria. The EOs were extracted by hydrodistillation and subjected to GC-MS analysis after the plants were gathered in the Azzemour region of South West Morocco. The agar paper disc method was used to measure the antibacterial activity. Cis-thujone, trans-thujone, and vanillyl alcohol were the primary constituents of A. herba-alba EO, whereas terpinen-4-ol, isopulegol, and β-phellandrene were the most prevalent in O. majorana EO. Both essential oils demonstrated concentration-dependent growth-inhibiting properties against several microorganism species. According to our findings, the essential oils of O. majorana and A. herba-alba may be useful natural antibacterials in food.

The major way that aromatic plants might disrupt the Mediterranean ecology is by introducing volatile compounds into the surrounding environment. We thus investigated the essential oil that was isolated from the leaves of the Tunisian Artemisia herba-alba Asso, as well as its chemical makeup and potential phytotoxic and antibacterial properties. GC and GC-MS were used to examine the essential oil's chemical makeup after it was extracted using hydrodistillation. A total of twenty-four compounds were found. The primary constituents were cis-thujone (7.8%), chrysanthenone (15.0%), and camphor (39.1%). Raphanus sativus L., Lepidium sativum L.,

Sinapis arvensis L., Triticum durum L., and Phalaris canariensis L. seeds were tested for their germination and first radical growth to assess the essential oil's phytotoxic potential in vitro. The oil had varying effects on the five seeds' radicle elongation, but it did not affect germination. Eight specific bacterial strains were evaluated, and the oil's antibacterial efficacy was shown to be minimal. The chemosystematics of this intricate genus can benefit from knowledge of the chemical makeup of A. herba-alba oil. The biological activity that has been seen, however, does not appear to be important from an ecological or medical standpoint.

Chapter Three:

Materials and Methods

3.1 Materials and Reagents

Artemisia leaves and seeds (Figure 3.1.1) were collected in the winter of 2023 from Wadi Al-Qaf in Tarqumiya in Hebron, Palestine, and the Za'tara desert in Bethlehem, Palestine. methanol 95%, absolute ethanol, distilled water, DPPH, Sodium bicarbonate, Sodium Nitrite, Aluminum chloride, Sodium hydroxide were purchased from Sigma Aldrich.

Catchin standard and Gallic Acid standard (with CAS No. 149-91-7) G7384,7) A9511, Folin–Ciocalteu reagent F9252, Span® 20; CAS Number: 1338-39-2; EC Number: 215-663-3; Synonyms: Sorbitan, Tween 80 were purchased from Sigma-Aldrich.

Figure 3.1.1 Artemisia herba alba leaves and seeds.

3.2 Instruments

PERKIN-ElMER Lambda 5 UV/VIS Spectrophotometer, Analytical balance SHIMADZU ATx324 320g in Balances (S-841), Magnetic Hotplate Stirrer (MS-H-S-Pro), Ultrasonic Bath (Sonicator) IKON INDUTRIES (170VAC – 270VAC)m HPLC, The size and morphology of artemisia microemulsion were assessed using Scanning Prob microscopy (SPM-9700HT, Shimadzu, Japan), Figure(3.2.1)**,** UV-visible (Shimadzu UV-2600i), Figure(3.2.2), SHIMADZU RF6000 Spectro fluorophotometer (Figure 3.2.3). Mechanical grinder, Benchtop B4- centrifuge from Jouan, Refrigerator, Soxhlet apparatus (model FA-46) (Figure 3.2.4), Rotary evaporator from IKA WEREK RV06-ML, Freeze Drying machine (Lyophilizer) from Labconco, Desiccators, Stabili therm incubator from Thermo, Hot plate, Water bath from Jouan, Wire brush, Graduated cylinder, Evaporating dish, Evaporimeter, Test tubes and Test tubes rack, Micro pipets, Spatula, Thermometer, Funnel, Beaker. The Muller-Hinton agar assay typically involves the use of petri dishes. The standard diameter of a Petri dish used for this assay is 90-100 millimeters (mm). As for the thickness of the media in the petri dish, it is generally recommended to pour approximately 4-5 millimeters (mm) of the Muller Hinton agar into the dish.

Figure 3.2.1 Scanning Probe (SPM-9700HT) microscopy (Equip at Palestine Technical University Kadoorie, Nanotechnology research lab).

Figure3.2.2UV-visible (Shimadzu UV-2600i) spectrophotometer instrument (Equipt at Palestine Technical University Kadoorie, Nanotechnology research lab).

Figure 3.2.3 Photoluminescence (SHIMADZU RF6000) spectroscopy instrument. (Equip at Palestine Technical University Kadoorie, Nanotechnology research lab).

Figure 3.2.4 Soxhlet apparatus (model FA-46)

Figure 3.2.5 Evaporimeter (left), creams in 40°C (middle), creams in 40°C+75% RH (right).

3.3-Methods:

3.3.1 Plant Sample:

After removing the artemisia leaves from their stems, the artemisia was thoroughly dried and cleaned with running tap water. The cleaned artemisia leaves were ground into a coarse powder using a mechanical grinder, and allowed to air dry at room temperature in the shade for approximately two days (make sure the leaves are not too many on top of one another and completely dry to prevent mold growth), and then stored in the refrigerator until needed. In contrast, the Artemisia seeds were equally ground into a fine powder using a mechanical grinder after being manually cleaned to remove husks and other unwanted objects (Tarayrah et al, 2021).

3.3.2 Artemisia extraction

3.3.2.1 Preparation of artemisia leaves and seeds methanolic extract

Using 9gm of the dry leave powder in 95% methanol and dried seed powder 6 g in 95%methanol, for two hours to draw out the chemical components found in artemisia, a Soxhlet apparatus (type FA-46) was used to create a methanolic extract of the plant. The plant's powder-to-solvent ratio was 1:16 (wt./vol) using the Soxhlet apparatus method of extraction in 95% methanol. Following extraction, MN615.Ø110 mm filter paper was used to filter the extract.

Following filtration, a rotary evaporator operating at 25°C and then 40°C was used to concentrate Artemisia's crude methanolic and ethyl acetate extract. Lyophilization (freeze-drying) was then carried out at -40°C and 0.095 mbar of pressure until a constant weight was reached. Using a straightforward physics principle known as sublimation, lyophilization is the process of separating a solid material from an aqueous solution by freezing the solution and evaporating the ice under vacuum.

The dried leaves powder obtained was 1.17 g (13%) on a dry weight basis, which was calculated by using the following equation:

$$\text{Percentage yield} = \frac{\text{Weight of dried extracts} \times 100}{\text{Weight of powder taken}}$$

The dried seed powder obtained was 0.6975 g (11.63%) on a dry weight basis, which was calculated by using the following equation:

$$\text{Percentage yield} = \frac{\text{Weight of dried extracts} \times 100}{\text{Weight of powder taken}}$$

The dry extract was kept in a refrigerator in an opaque bottle until it was analyzed by HPLC, AFM images, and optical properties of nanoparticles. For additional pharmacological research and future testing, the same procedure was used to prepare the extracts of leaves and seeds using a variety of solvents (methanol 50%, ethyl acetate, petroleum ether) for various assays (Tarayrah et al, 2021).

3.3.2.2 Solubility study of crude extract

To test the artemisia extract's solubility, distilled water was utilized. By tripling the quantities of artemisia extract, the extract's solubility was ascertained. increasing the concentration gradually till the extract lost its solubility. By gently heating a water bath and shaking it, the extract became as soluble in water as possible.

Table 3.3.2.2.1: Artemisia leaves methanolic extract solubility test in various solvents

Extract: Solvent (w/v)	Solubility	
	Distilled water	Ethanol
1 mg/ml	soluble	Freely soluble
3 mg/ml	soluble	Freely soluble
6 mg/ml	soluble	Freely soluble
9 mg/ml	insoluble	Freely soluble

Table 3.3.2.2.2: Solubility test for methanolic extract of artemisia seeds in DMSO solvents.

Extract: Solvent (w/v)	Solubility DMSO
1 mg/ml	Freely soluble
3 mg/ml	Freely soluble
6 mg/ml	Freely soluble
9 mg/ml	Freely soluble
12 mg/ml	Freely soluble
15 mg/ml	Freely soluble
18 mg/ml	Freely soluble
21 mg/ml	Freely soluble

* DMSO: Dimethyl sulfoxide.

3.3.3 Phytochemical screening

3.3.3.1 Total Phenolic content (Folin-Ciocalteau assay)

Total phenolic content was analyzed using the spectrophotometric method according to the Folin-Ciocalteu assay. Crude artemisia (leaves/seeds) extracts. The extracts were prepared by dissolving 0.1g in 100 mL of 95% ethanol. For the assay, 0.5 mL of the extract was mixed with 2.5 mL of Folin reagent (10% v/v) and 2.5 mL of sodium carbonate solution (7.5% w/v), and the mixture was incubated in the dark for 30 minutes at 38 room temperature. The absorbance was then measured at 760nm in **Figure 3.3.4.1.1**. A calibration curve was prepared using different concentrations of the gallic acid standard (100-500 ppm). As indicated in **Table 3.3.4.1.1**. The results were expressed as mg of gallic acid equivalents (GAE) per gram of sample.

Table 3.3.3.1.1: Absorbance of different concentrations of Gallic Acid(ppm)

Concentration of Gallic acid (ppm)	Absorbance at 760nm.
100	0.677
200	0.918
300	1.102
400	1.353

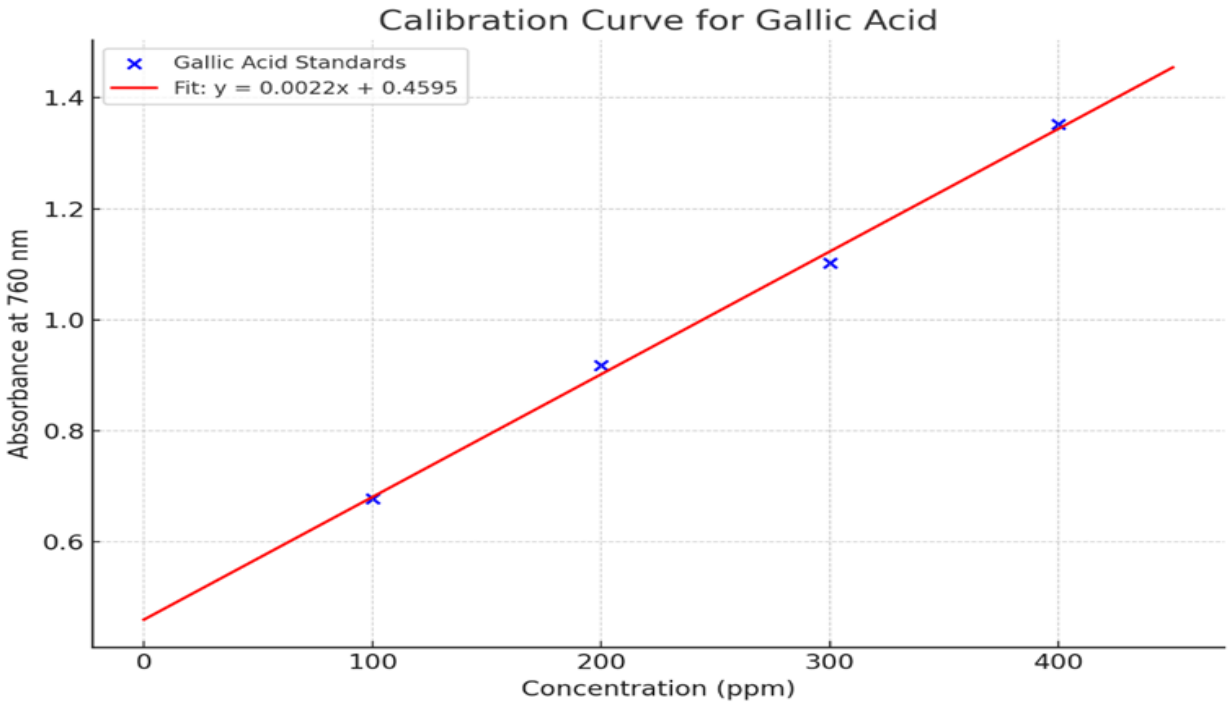


Figure 3.3.3.1.1 The Folin-Ciocalteu technique is used to determine the total phenolic content of Artemisia seed and leave extracts using a standard calibration curve of gallic acid (100–500 ppm). At 760 nm, absorbance was measured.

3.3.3.2 Total Flavonoid Content (TFC)

Total Flavonoid content was analyzed using the spectrophotometric method. Crude artemisia (leaves/seeds) extracts. Firstly, 1 ml of Artemisia extract was mixed with 4 ml of distilled water. Then, 0.3 ml of 5% sodium nitrite solution was added, followed by 0.3 ml of 10% aluminum chloride solution. The resulting mixture was incubated at room temperature (25℃) for 5 minutes. Next, 2 ml of 1 M sodium hydroxide was added to the mixture, and the volume of the reaction mixture was immediately adjusted to 10 ml with distilled water. The mixture was thoroughly mixed, and the absorbance of the pink color developed was measured at 510 nm in **Figure 3.3.3.2.1.** A calibration curve was prepared using aqueous solutions with known concentrations ranging from 50 to 100 ppm as indicated in **Table 3.3.3.2.1**.

Table 3.3.3.2.1. Absorbance for different concentrations of Catechin (ppm)

Concentration of Catechin (ppm)	Absorbance at 510 nm
50	0.255
60	0.282
75	0.353
85	0.396
100	0.496

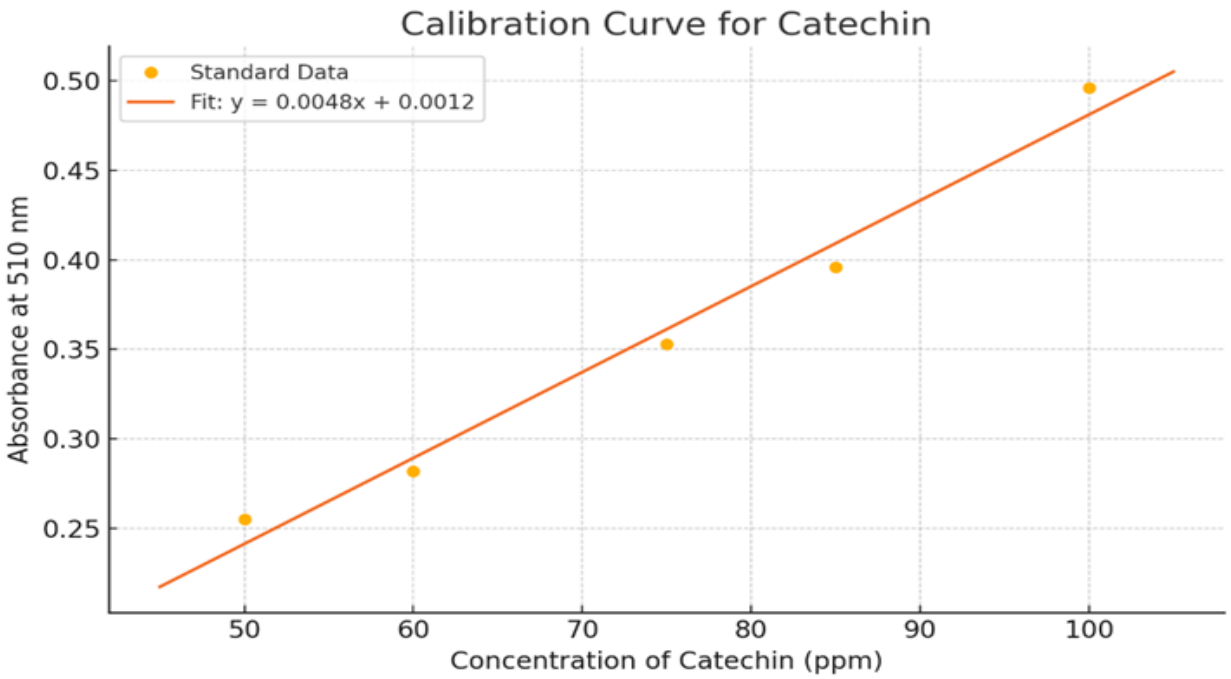


Figure 3.3.3.2.1 Extracts from Artemisia seeds and leaves were tested for total flavonoid concentration. At 510 nm, the absorbance was measured. Solutions of catechins with concentrations ranging from 50 to 100 ppm were used to create a standard calibration curve.

3.4 Antioxidant assay:

Free radical scavenging activity (DPPH Reagent)

The procedure for measuring the scavenging activity of the DPPH radical was carried out by the methodology to initiate the reaction, a sample was combined with a stable DPPH radical in a

solution of ethanol. The reaction mixture consisted of 0.5 mL of the sample, 3 mL of absolute ethanol, and 0.3 mL of DPPH radical solution (0.5 mM in ethanol). The reduction of the DPPH radical occurs when it reacts with an antioxidant compound that can donate hydrogen. The resulting change in color, from deep violet to light yellow, was read as Absorbance (Abs) at 517 nm after 100 minutes of reaction time using a UV/VIS spectrophotometer. A blank solution of ethanol (3.3 mL) and sample (0.5 mL) was used, while the control solution was prepared by mixing ethanol (3.5 mL) and DPPH radical solution (0.3 mL). The scavenging activity percentage (AA%) was determined by the following equation:

DPPH Inhibition % = A0- A1 / A0 * 100.

3.5 HPLC – PDA Detection of Phytochemicals

The following standards: gallic acid, 3,4-dihydroxybenzoic acid, 3,4-dihydroxyphenylacetic acid, chlorogenic acid, 4-hydroxyphenyl acetic acid, vanillic acid, caffeic acid, syringic acid, isovanillic acid, *p*-coumaric acid, ferulic acid, sinapic acid, rutin, verbascoside, quercetin, *trans*-cinnamic acid, and kaempferol were prepared using a solvent of 20% ethanol with a concentration of 25 mg/ 100 mL. A standard mixture was made by mixing 1.0 mL of each standard solution into a 25 mL volumetric flask that was made up to volume with the same solvent.
Table 1 shows the mobile phase composition and the gradient elution method used for the detection of main components, where (A) is 0.5% acetic acid and (B) is acetonitrile. RP BDS Hypersil C18 column (Thermo Scientific, 250 x 4.6 mm, 3 µm) was used, with a flow rate of 0.6 mL/minute. The PDA range was set from 210 to 400 nm, while the column temperature was set to 25°C. The injection volume was set to 20 µL. All samples were filtered through a 0.45 µm disposable filter.

Table 3.5.1. Mobile phase composition.

Time (minutes)	A%	B%
0	95	5
50	80	20
65	65	35
70	40	60
75	10	90
78	95	5
80	95	5

T_a

3.6 Antibacterial Activity by Disc diffusion method

The antibacterial activity of the Artemisia (leaves and seeds) extract was assessed using the agar disc diffusion method. The agar disc diffusion method was used to conduct the antibacterial test.

The identical solvents (absolute ethanol) used to dissolve the samples were also used to create negative controls. Penicillin (10 units) and Gentamicin (10 micrograms/disc), two common antibiotics, were employed as positive controls for the bacterium under test. The diameter of the zones of inhibition surrounding the disc against the tested microorganisms was used to gauge the antibacterial activity. the agar media Mueller-Hinton Agar (MHA) in the Petri dish is poured to a depth of approximately 4-6 mm The diameter of a standard Petri dish typically ranges between 90 to 100 millimeters (mm). The antibacterial activity was evaluated by measuring the diameter of the zones of inhibition around the discs against Staphylococcus aureus (gram-positive), Escherichia coli (gram-negative), and Pseudomonas aeruginosa (gram-negative). These bacterial cultures were taken from the Microbiology Labs at Al-Quds University.

To grow the bacteria, Mueller-Hinton Agar (MHA) was used as the solid culture medium in Petri dishes. The MHA was prepared and sterilized by autoclaving for 30 minutes, and then poured into sterile Petri plates and left to solidify for 10-15 minutes. The plates were stored upside down in a plastic bag until used to prevent moisture from condensing on the surface of the medium. The inoculums were then evenly dispersed on the surface of the MHA using a cotton swab.

For the antibacterial assay, a sterile disc with a diameter of 5mm was impregnated with 50 microliters of the artemisia extract at two different concentrations, while negative controls were prepared using 50µl of the solvent. The prepared bacterial species were seeded onto the MHA plates, and a reference antibiotic disc was placed on the surface of the MHA as a positive control. The plates were then incubated at 37°C for 24 hours, and the diameter of the zones of inhibition around the discs was measured to evaluate the antibacterial activity of the Artemisia (leaves and seeds) extract.

3.7 Phase behavior

The water titration method produced the pseudo-ternary phase diagrams of the mixture of oil, water, and surfactant.

A phase tetrahedron, with the apexes representing the pure components, was used to plot the phase behavior of the systems made up of artemisia extract, water phase, Span 20, and Tween 80 (Surfactants 1:1).

In glass test tubes with screw lids, 0.05g of a combination comprising artemisia extract dissolved in absolute ethanol and surfactant at various weight ratios—as indicated in the table (3.7.1) were prepared and vortexed at room temperature (25°C) (Abuhilal et al,2023).

The weight ratios of surfactants and an oil phase are shown in the table (3.7.1).

Wight ratio	1:9	2:8	3:7	4:6	5:5	6:4	7:3	8:2	9:1
Surfactant	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
Oil	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1

The table (3.7.2) shows that the samples were titrated with the water phase until the endpoint was achieved. Using a vortex mixer, vigorous mixing was carried out following each addition of the aqueous phase, and equilibration time was permitted in between. A centrifuge was used for five minutes if required (Abuhilal et al,2023).
The amount of water added at each titration is displayed in Table (3.7.2)

%	4%	8%	12%	16%	20%	24%	28%	32%	36%
Weight	0.0417	0.0453	0.0494	0.0541	0.0595	0.0658	0.0732	0.0817	0.0919
%	40%	44%	48%	52%	56%	60%	64%	68%	72%
Weight	0.1042	0.1191	0.1374	0.1603	0.1894	0.2273	0.2773	0.3473	0.4466
%	76%	80%	84%	88%	92%	96%			
Weight	0.5955	0.8337	1.2070	2.0833	4.1667	12.5			

25°C was the temperature at which the phase diagram was examined. identifying multiple phases with the unaided eye. Light can differentiate the single liquid solution formed by the microemulsion sample, which will be considered clear. Determine a single-phase boundary, then use Origin Pro 8.1 software to create the phase diagram (Abuhilal et al,2023).

3.8 The size and surface morphology methods

3.8.1 AFM

The size and surface morphology of the produced nanoparticles were assessed in this work using Atomic Force Microscopy (AFM). Using the SPM-9700HT instrument, the analysis was carried out at Palestine Technical University's Kadoorie Nanotechnology Research Laboratory. Sample preparation, tapping mode imaging, and data processing were all part of the experiment.

Accurate AFM measurements required a substrate surface that was smooth and clean. To get rid of surface impurities, silicon wafer substrates were thoroughly cleaned with distilled water and ethanol. Using a micropipette, a tiny amount (about 5–10 µL) of the nanoparticle suspension was

applied to the surface. To guarantee a uniform distribution of nanoparticles free from aggregation or deformation from rapid evaporation, the droplet was allowed to naturally dry at ambient temperature in a dust-free environment. The sample was mounted onto the AFM's sample stage once it had completely dried. Tapping mode, which reduces lateral stresses and stops soft or weakly bound nanoparticles from deforming or moving, was used for imaging. Selected areas ranging from 1 µm × 1 µm to 10 µm × 10 µm were scanned. To make sure the measured particle sizes were representative and statistically sound, several areas of the sample were scanned. For high-resolution imaging, the AFM system used a silicon tip with a radius of nanometers. The instrument's built-in software was used to process the obtained AFM pictures. Particle dimensions were determined using height and lateral profiles, and the particle size distribution was ascertained by analyzing topographical data. To determine the average particle diameter and evaluate the sample's dispersion and homogeneity, measurements were taken of at least 20 distinct nanoparticles. In addition to offering insights into the architecture and aggregation behavior of the produced nanoparticles, this research allowed for the accurate determination of particle size. (45)

3.9 Optical characterization methods

3.9.1 UV-Visible Spectroscopy

UV-Vis spectroscopy is a vital tool in the analysis of plant-based formulations, including Artemisia herba-alba microemulsions, playing a fundamental role in the characterization of phytochemicals. The absorbance bands observed in the UV-visible range are mainly due to electronic transitions within bioactive compounds such as flavonoids, phenolics, and other conjugated systems. These compounds typically exhibit strong absorption in the UV region, particularly between 200–400 nm, owing to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions in their molecular structures. The technique is widely used to monitor the presence, stability, and interaction of Artemisia extracts within microemulsion systems. Moreover, shifts or changes in the absorbance peaks can provide insights into the encapsulation efficiency, degradation behavior, or interactions between plant constituents and surfactants, making UV-Vis spectroscopy an indispensable method in phytochemical and formulation research. This tells us how much of a specific compound is present based on how much light was absorbed (Musa et al,2024).

Principle of UV-visible

[Light Source] → [Monochromator] → [Sample Cuvette with Artemisia Microemulsion] → [Detector] → [Absorbance Output]

3.9.2 Photoluminescence Spectroscopy

The foundation of optical characterization methods is photoluminescence spectroscopy (PL), which is widely utilized to comprehend photo-excitation processes since it is non-destructive and may yield valuable information about both intrinsic and extrinsic transitions. Due to their exceptional luminous qualities and very high biocompatibility, nanoparticles, microemulsions have lately become a distinct class of luminescent materials and are being used in various applications.

As a result, excitation and emission spectra are used to describe each compound's fluorescence characteristics (Musa et al,2024).

Principle of Photoluminescence spectroscopy

[Excitation Light Source] → [Sample (Artemisia Microemulsion)] → ↑ Electron Excitation

↓ Relaxation

[Emission of Light] → [Detector] → [Emission Spectrum]

3.10 Preparation of cream:

Disodium EDTA (Phase A) and water were measured. Phase B (glycerin and xanthan gum) was made into a slurry and mixed with phase A. At 75°C, phase A/B was heated. Phase C was heated to 75°C after being combined. Phase A/B was then supplemented with phase C [GSC: Glyceryl Stearate Citrate, Cetearyl Alcohol, Ceteareth-20 (Emulgade 1000 NI)], Ceteargl alcohol, Caprylic/Capric Triglycerides (Myritol 318), Isopropyl myristate (Crodamol IPM), and Shea butter] under high shear stirring and mixing until a smooth, glossy, homogenous emulsion formed. Low shear stirring was used to allow the liquid to cool until it was below 40°C. Phase D [Tocopherols, Ethylhexylglycerin (Euxyl PE9010), Phenoxyethanol, and Crud Extract Seeds and Leaves of Artemisia] was added and mixed until homogeneous using low shear. Less than 25°C was permitted to chill the mixture. The liquid was covered and stirred the following day before being poured off after the pH was checked and adjusted to 5-5.5.

Artemisia extract makes up around 0.15% of the cream.

In an incubator with 75% relative humidity (RH), the creams' stability was tested at 8°C+0.1°C in the refrigerator and at 25°C+1°C, 40°C+1°C, and 40°C+1°C (Tarayrah et al, 2021).

Chapter four

Results and Discussion

This chapter presents a detailed description of the research conducted on the formulation and characterization of microemulsions using Artemisia herba-alba extract. The study focuses on the extraction process, the preparation of microemulsions, and their physicochemical properties. In addition, the optimization of formulation parameters and the evaluation of the biological activities of the prepared microemulsions were discussed.

Previous chapters have covered the experimental procedures, materials, and techniques employed in the extraction of Artemisia herba-alba leaves and seeds, as well as the methods used to prepare and characterize the microemulsions. The effects of different formulation variables on the stability and properties of the microemulsion systems were systematically investigated.

4.1. Determination of Total Phenolic content (TPC)

Using a spectrophotometric technique, TPC was measured at 760nm. The results were expressed in milligrams of Gallic Acid per gram of artemisia extract.

TPC of two plant components, the leaves and the seeds, of Artemisia herba-alba extracts. This result makes it clear that different plant portions provide variable amounts of phenolic chemicals when extracted. The extract from the seeds had the highest TPC, followed by the extract from the leaves. According to the study's findings, Artemisia seeds had a higher concentration of phenolic compounds (881 mg GAE/g) than Artemisia leaves (302 mg GAE/g).

The multifunctional bioactivity of phenolic compounds is well-known, and it includes the capacity to quench singlet oxygen and scavenge free radicals. Thus, the promise of the plant as a natural antioxidant source is shown by the high concentration of these chemicals found in Palestinian Artemisia herba-alba seeds and leaves. This implies that, when taken properly, artemisia extracts may have health-promoting qualities and help lower oxidative stress. Comparing the TPC values of Palestinian Artemisia with those reported from different geographical sources in further research would be interesting.

4.2 Total Flavonoid content (TFC)

Using a spectrophotometric technique, TFC was measured at 510 nm. The results were expressed in milligrams of catechin per gram of artemisia extract.
The total flavonoid content (TFC) of Artemisia herba-alba leave extract was 371.59 mg/g, while the TFC of the seed extract was 314.23 mg/g. These results show that both plant parts are abundant in flavonoids. One kind of flavonoid that is well-known for its anti-inflammatory, antioxidant, and possibly medicinal properties is catechin.

An essential metric for assessing a plant extract's antioxidant capabilities and its health advantages is its TFC. Natural antioxidants called flavonoids have the ability to combat free radicals and lower oxidative stress, which is directly related to the development of chronic illnesses, including diabetes, cancer, and cardiovascular disease.

The comparatively high TFC values in this investigation for Artemisia seeds and leaves, particularly the leaf extract, point to a robust antioxidant potential. This makes Artemisia herba-alba a viable option for creating nutraceuticals or functional meals that promote human health and fend off degenerative illnesses.

It is crucial to understand that the TFC can change based on a number of variables, including the solvent type, plant portion examined, extraction technique, and the plant material's place of origin. When analyzing the TFC results and their implications for applications relating to health, these factors should be considered.

4.3. Antioxidant Assay

The following formula was used to compute the free radical scavenging activity:

A0-A1/A0 * 100 *A*0 is the percentage of radical scavenging from the DPPH assay: The control's absorbance at 517 nm is equal to 0.670

*A*1: Sample absorbance at 517 nm = 0.173 for seed extract and 0.172 for leave extract

According to the results, the artemisia seed extract had a 74.18% radical scavenging activity, whereas the leave extract had a 74.33% one. These results show that both extracts have potent antioxidant properties and are efficient in scavenging free radicals. This is advantageous since free radicals are known to induce oxidative stress, which damages cells and tissues and plays a role in the emergence of certain illnesses. It is crucial to remember that while the DPPH test is a practical and popular way to assess antioxidant activity, it only accounts for a portion of a compound's potential antioxidant capacity.

4.4. Antibacterial assay

For centuries, people have utilized plants as self-medication and herbal remedies to treat illnesses. Specifically, the use of plants in Palestine is a custom that mostly depends on observations rather than data from scientific experiments. However, because bacterial resistance and toxicity limit the effective life span of any antibiotic, humanity is constantly searching for new and emerging antimicrobial medicines. Thus, preliminary screening of the antibacterial activity of artemisia extract against several kinds was carried out in the subsequent investigation.

Three clinical pathogens (Escherichia coli, Staphylococcus aureus, and Klebsiella pneumoniae) were used to test the antibacterial activity of the extract of artemisia seeds and leaves. using the disc diffusion method in the presence of positive control (Gentamicin 10 μg/disc) and Penicillin 10 units. Measurements were made of the zones of inhibition (Fig.4.4.1) and the average outcomes of the inhibition zones in Tables (4.4.1) and (4.4.2).

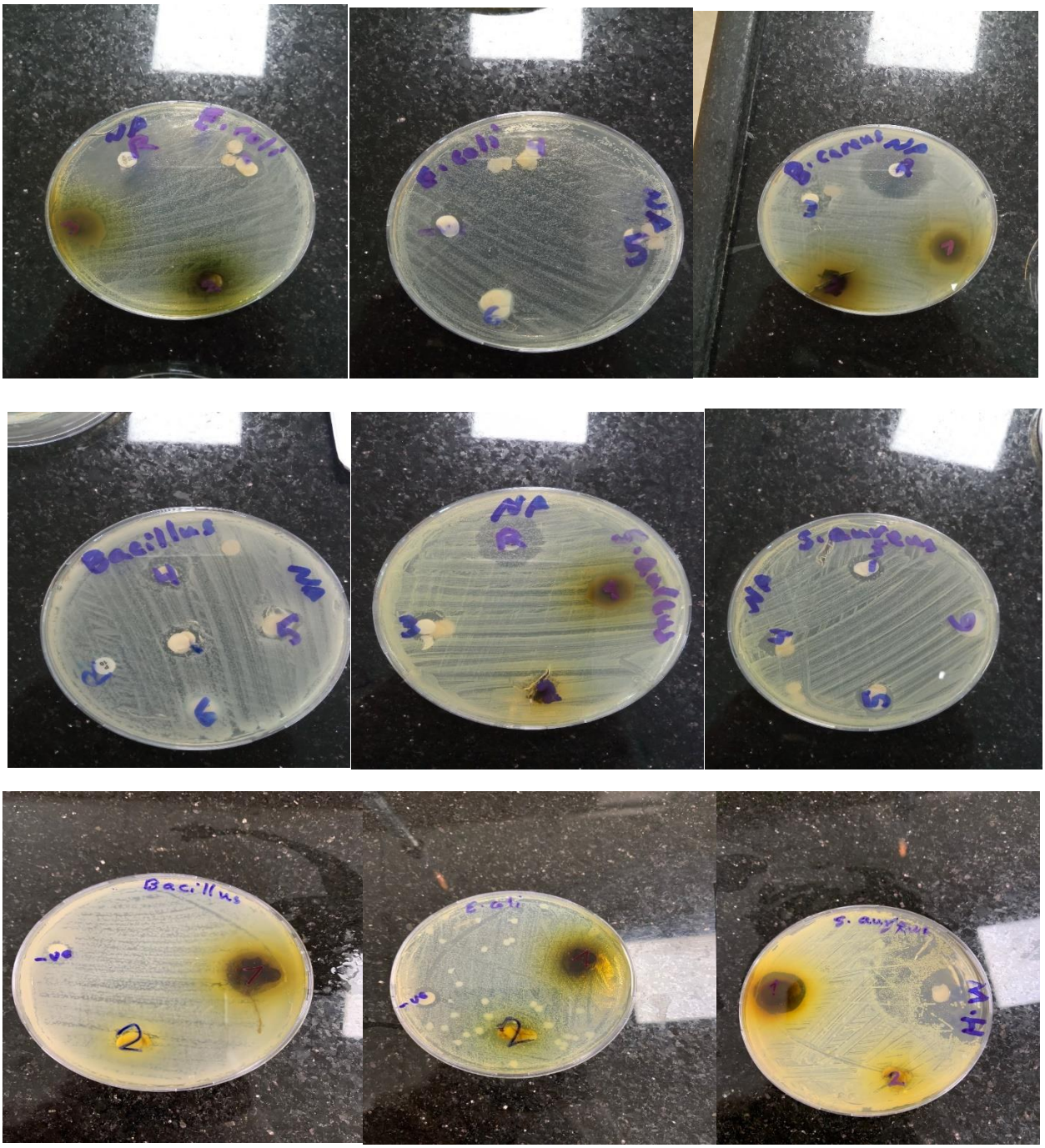


Figure 4.4.1: The zone of inhibition results for Artemisia herba-alba extracts (seeds: samples 1, 3, and 5; leaves: samples 2, 4, and 6) demonstrated variable antibacterial activity depending on the form and concentration used. The tested samples included crude extracts, ethanol-dissolved extracts, and microemulsion formulations, each exhibiting differing levels of inhibitory effects against the clinical.

Table 4.4.1. The diameter of the zone of inhibition of artemisia seeds against clinical pathogens.

Clinical pathogens	**Average zone of inhibition (mm)**			
	1(extract)	**3(Extract& ethanol)**	**5(microemulsion)**	**Seeds extract (1)**
Escherichia coli	14 mm	16 mm	11 mm	16 mm
Staphylococcus aureus	12 mm	10 mm	13 mm	18 mm
Klebsiella pneumonia	27 mm	12 mm	ND	18 mm

*ND: Not detected.

Table 4.4.2. The diameter of the zone of inhibition of artemisia leaves against clinical pathogens.

Clinical pathogens	**Average zone of inhibition (mm)**			
	2(Extract)	**4(Extract& ethanol)**	**6(microemulsion)**	**leaves extract (2)**
Escherichia coli	11 mm	14 mm	10 mm	ND
Staphylococcus aureus	ND	10 mm	12 mm	ND
Klebsiella pneumonia	27 mm	24 mm	ND	ND

*ND: Not detected.

4.5 HPLC – PDA Detection of Phytochemicals

Seventeen standards of flavonoids and phenolic compounds were separated in different retention times, and each has its corresponding number in Figure 1. This optimal chromatogram was chosen because it revealed all the standards at 280 nm, although each has a different maximum wavelength.

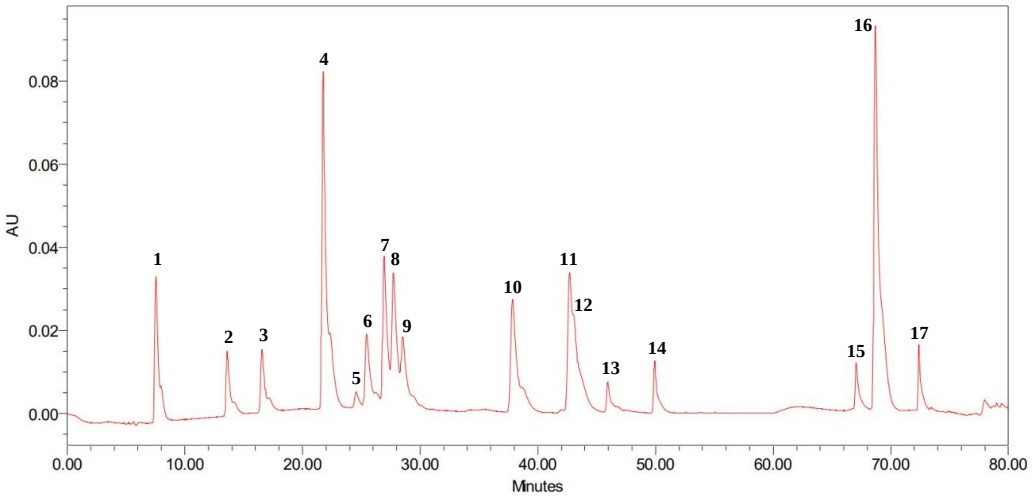
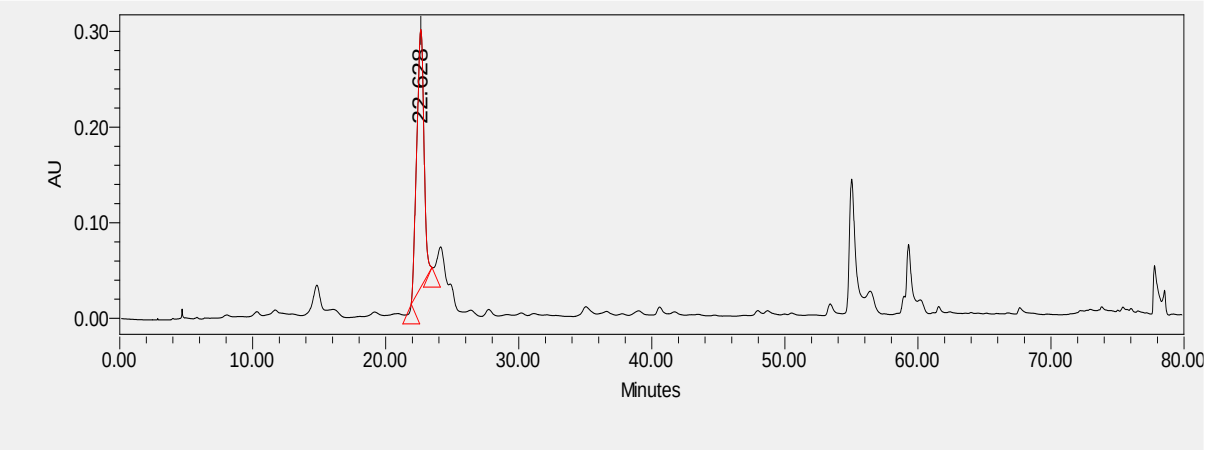


Figure (4.5.1) HPLC chromatogram for standards used at 280 nm: 1. Gallic acid, 2. 3,4-dihydroxybenzoic acid, 3. 3,4-dihydroxyphenylacetic acid, 4. Chlorogenic acid, 5. 4-hydroxyphenylacetic acid, 6. Vanillic acid, 7. Caffeic acid, 8. Syringic acid, 9. Isovanillic acid, 10. *p*-coumaric acid, 11. Ferulic acid, 12. Sinapic acid, 13. Rutin, 14. Verbascoside, 15. Quercetin, 16. *trans*-cinnamic acid, and 17. Kaempferol.

Artemisia Leave extract:

HPLC results were expressed in these selective chromatograms. **Figure 4.5.2 shows** the chromatogram for the Artemisia leave extract extracted with 95% methanol at 280–nm. The identification was done through the retention time and wavelengths for both standards and samples. Accordingly, the following compounds were identified: chlorogenic acid at 22.62 minutes in leaves.



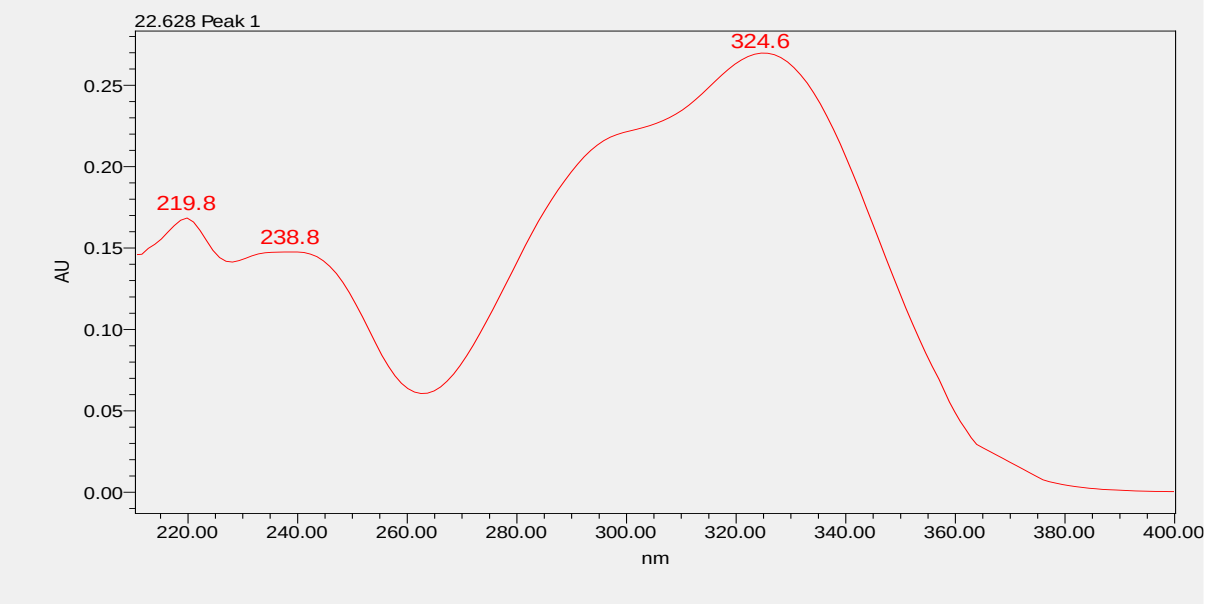
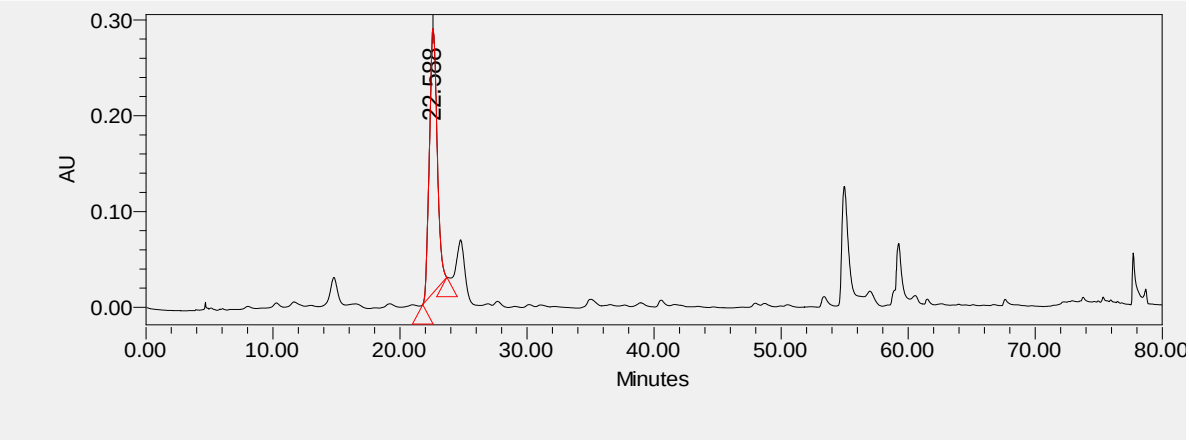


Figure (4.5.2) HPLC chromatogram for artemisia leave extract extracted with 95% methanol at 280 nm (a) and UV spectrum for the chlorogenic peak detected at 22.62 minutes.

Artemisia seeds extract: HPLC results were expressed in these selective chromatograms. **Figure 4.5.3.** shows the chromatogram for the Artemisia seed extract extracted with 95% methanol at 320 nm. The identification was done through the retention time and wavelengths for both standards and samples. Accordingly, the following compounds were identified: chlorogenic acid at 22.58 minutes in seeds.



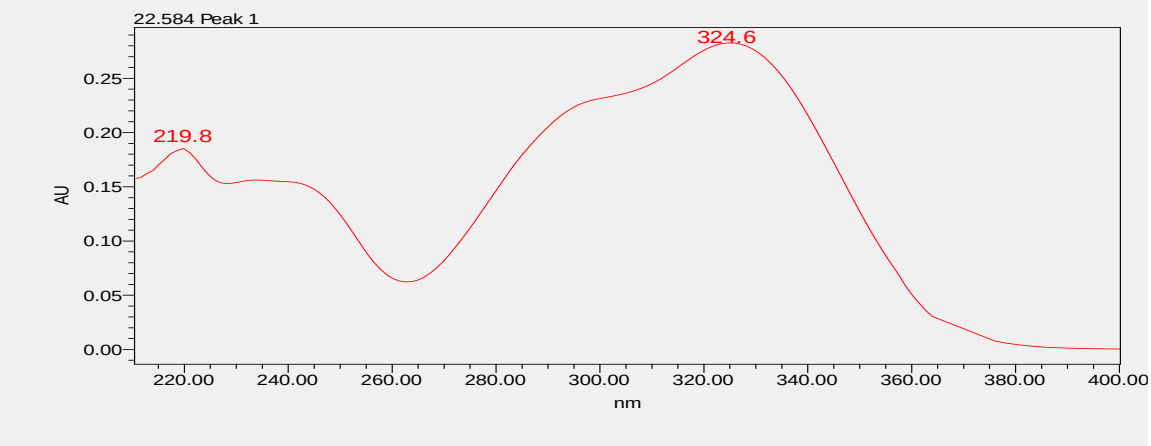
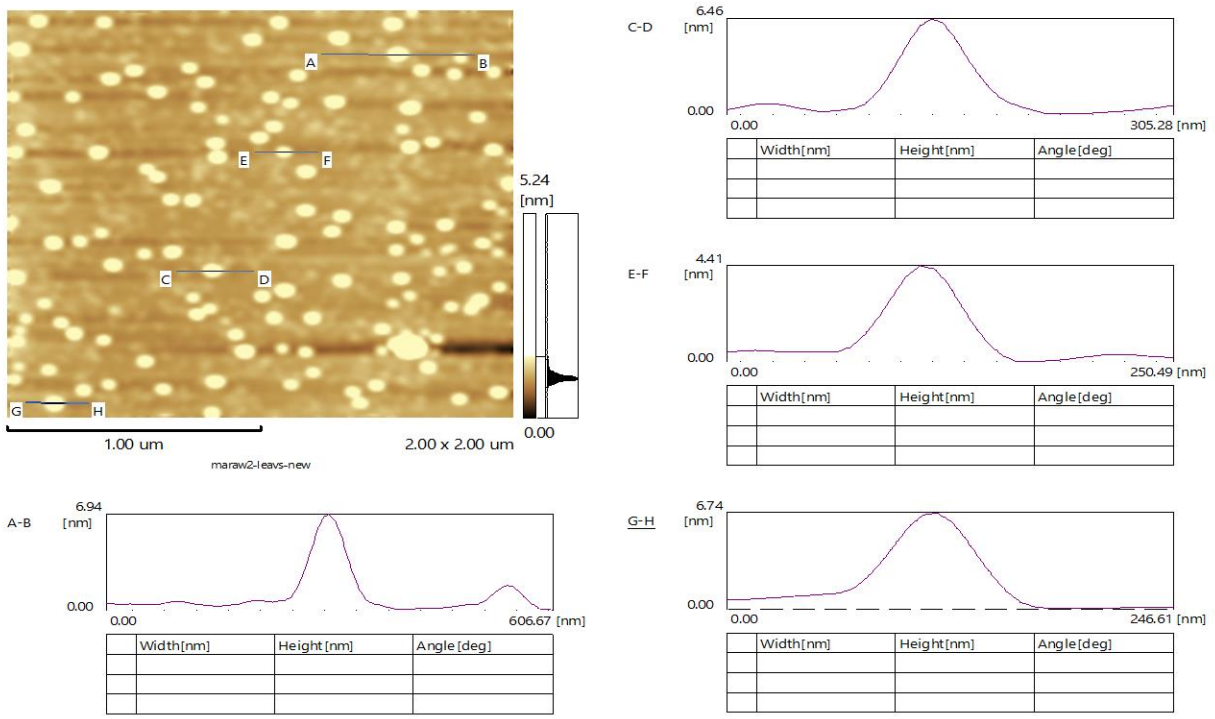


Figure 4.5.3. HPLC chromatogram for Artemisia seeds extract extracted with 95% methanol at 310 nm (a) and UV spectrum for the chlorogenic peak detected at 22.58 minutes.

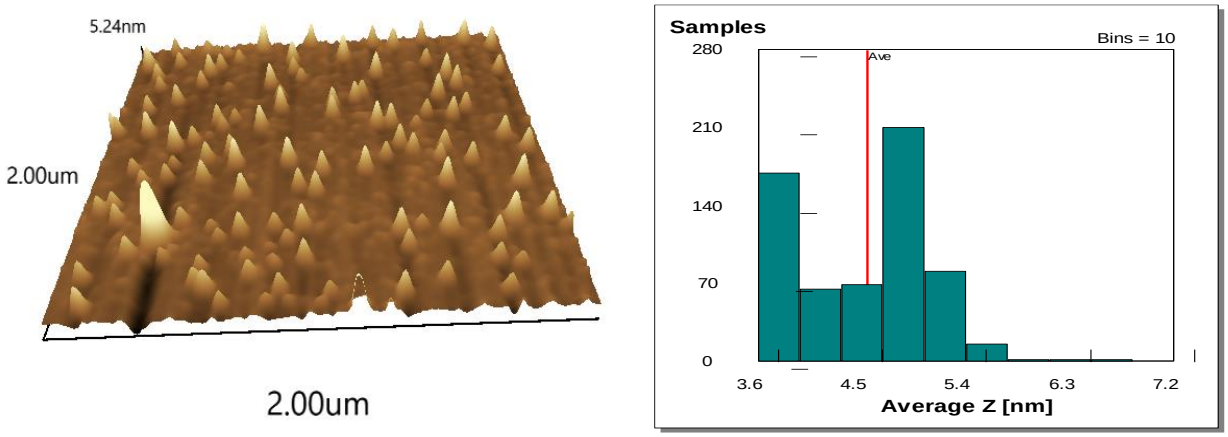
4.6 The size and surface morphology

4.6.1 AFM images and optical properties of Artemisia microemulsion (leaves and seeds) extract Nanoparticles (NP)

Images of NP using artemisia Leave extract in **Figure (4.6.1.1)**



(a)

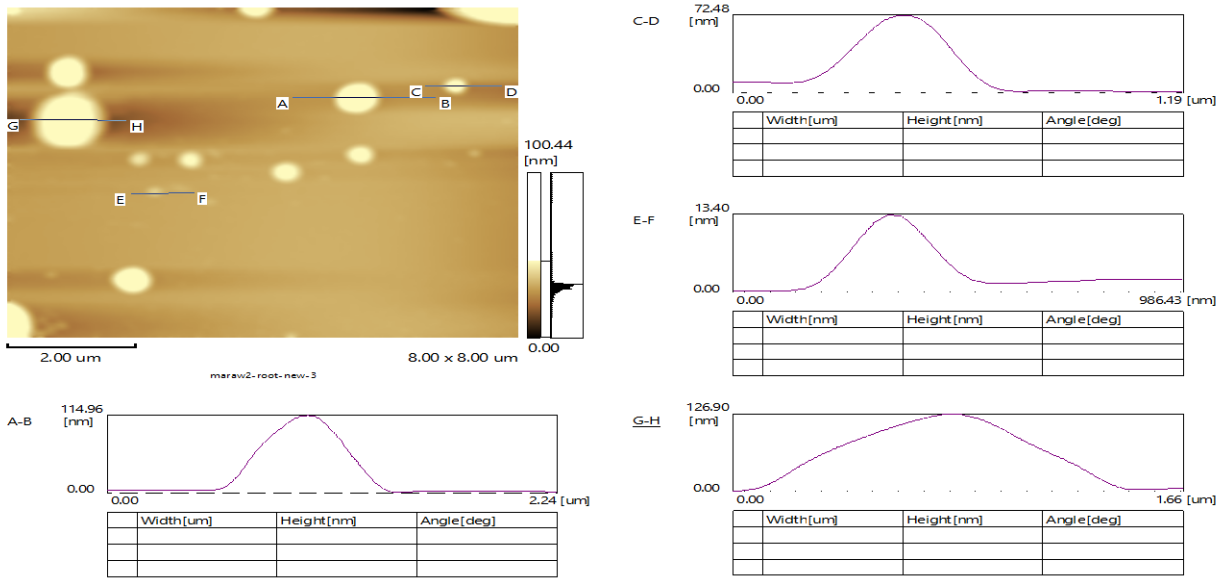


(b) (c)

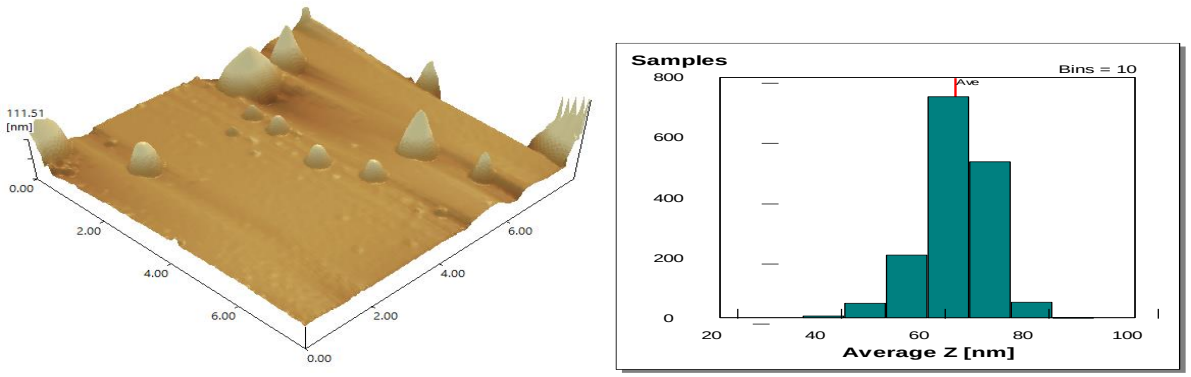
Figure (4.6.1.1) (a) Atomic force microscopy (AFM) topography images of Artemisia microemulsion extract leaves on a mica substrate, Scan sizes of 2 µm × 2 µm, (b)Three-dimensional projection of nanoparticles, (c) Particle size distribution histogram.

The results for artemisia microemulsion extract leaves nanoparticles reveal that the particle topography and three-dimensional projection of the nanoparticles also appear to be spherical, as shown in Figure (4.6.1) (a, b). Figure (4.6.1) (c) indicates the heights of artemisia microemulsion in the range of 3.5 and 5.4 nm; Histogram analysis in (c) revealed that the mean particle size was approximately 4.5 nm.

Images of NP using Artemisia microemulsion seeds extract in **Figure (4.6.1.2)**



(a)



(b) (c)

Figure (4.6.1.2) (a) Atomic force microscopy (AFM) topography images of Artemisia extract seeds on a mica substrate, Scan sizes of 2 µm × 2 µm, (b)Three-dimensional projection of nanoparticles, (c) Particle size distribution histogram.

The results for artemisia microemulsion extract seeds nanoparticles reveal that the particle topography and three-dimensional projection of the nanoparticles also appear to be spherical, as shown in Figure (4.6.2) (a, b). Figure (4.6.2) (c) indicates the heights of artemisia microemulsion in the range of 40 and 80 nm; Histogram analysis in (c) revealed that the mean particle size was approximately 65 nm.

4.7 Optical characterization:

In this section, the optical results attained with Artemisia herba alba microemulsion extract by UV-vis and photoluminescence are discussed.

4.7.1 UV-Vis

The absorption spectra of artemisia microemulsion leave extract are typically examined to analyze its biochemical composition, especially the presence of compounds such as flavonoids, phenolic acids, and terpenoids, as shown in Figure (4.7.1.1)

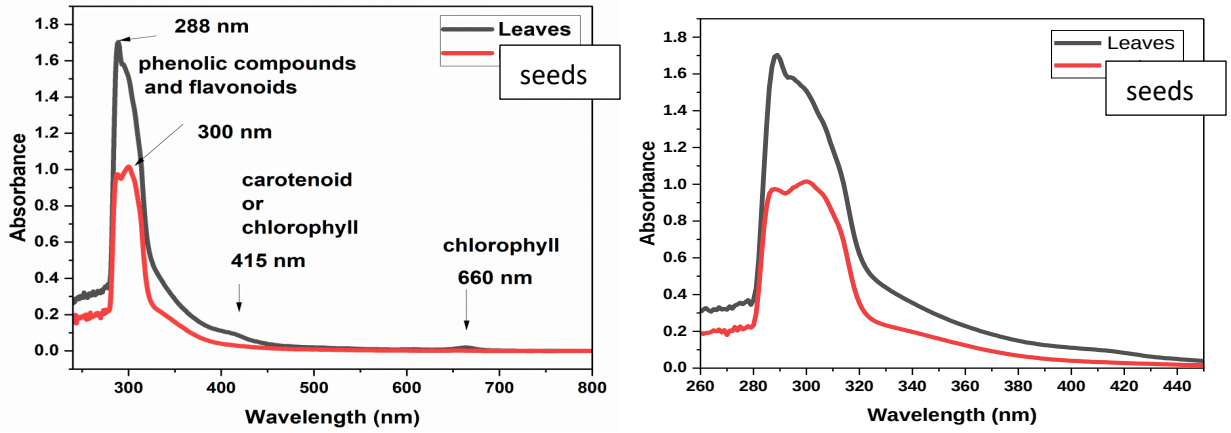


Figure (4.7.1.1) UV–vis spectrum of the artemisia microemulsion extract leave at wavelengths 200 to 800 nm**.**

Illustrates the absorption characteristics of Artemisia microemulsion leave extract, as measured by a UV-vis spectrophotometer. The absorption spectrum of P. Palaestina is distinguished by prominent bands with several peaks occurring at wavelengths of 288, 300, 415, and 660 nm. The initial two peaks, at 288 nm and 300 nm, are likely due to the presence of flavonoids, phenolic acids, and terpenoids in the leaves and seeds extract.

The peak at 415 nm is the presence of chlorophyll or carotenoid. Lastly, the peak at 660 nm is indicative of the presence of chlorophyll.

4.7.2 Fluorescence analysis

of Artemisia extract is a powerful technique for studying its biochemical constituents, particularly those with fluorescent properties like flavonoids, phenolic compounds, coumarins, and certain terpenoids. as shown in figure (4.7.2.1) These compounds can exhibit fluorescence when excited by specific wavelengths of light, making it possible to detect, identify, and quantify them.

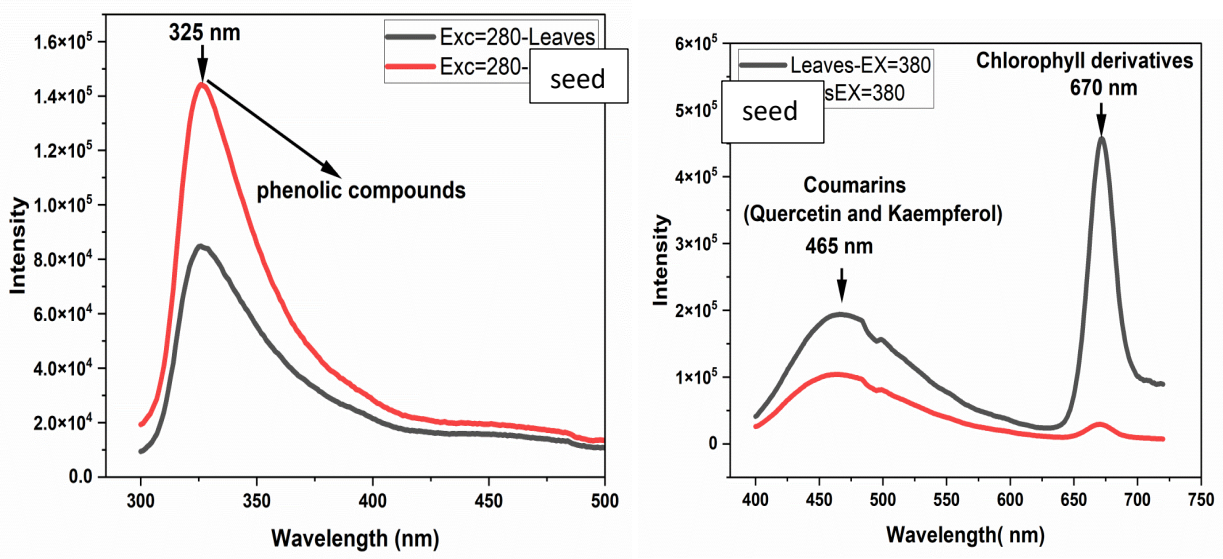


Figure (4.7.2.1): Emission spectrum of Artemisia microemulsion leaves and seeds extract.

Illustrates the fluorescence characteristics of Artemisia microemulsion leaf extract, as measured by a fluorescence spectrophotometer. The emission spectrum of Artemisia herba-alba revealed several prominent fluorescence peaks following excitation in the UV range (300–400 nm), corresponding to the presence of naturally fluorescent phytochemicals in the extract.

A distinct emission peak at approximately 450 nm is attributed to quercetin, a major flavonoid in Artemisia species. Another peak near 480 nm corresponds to kaempferol, indicating the presence of multiple flavonoid compounds. Broad emissions between 450–500 nm also suggest the presence of coumarins, which are known for their strong fluorescence activity.

In addition, a red emission band observed between 650–680 nm is characteristic of chlorophyll derivatives, further confirming the complex phytochemical composition of the extract. These fluorescence features reflect the presence of flavonoids, phenolic compounds, terpenoids, and chlorophyll-related constituents within the microemulsion system.

4.8 Phase behavior

The microemulsion was determined to be a low-viscosity, isotropic, transparent, and clear solution. With a different ratio system at 25°C (Fig. 4.8.1) (Fig. 4.8.2), when water was added, these characteristics indicated that the microemulsion had formed successfully. In formulations with

90% or less extract and 10% or more surfactant mixture, the microemulsion region started before the first 4% water addition. This area showed that the system could withstand a high-water content by extending continually until the water apex.

It was discovered that the microemulsion region, shown by the yellow zone, occupied a large portion of the ternary diagram. It covers a wide range of Artemisia herba-alba seed extract content and spans from moderate to high water concentrations and moderate amounts of surfactant mixture (Tween 80 and Span 20). This implies that stable microemulsions can be formed by the system across a range of compositions. The remaining areas outside the yellow area were categorized as non-microemulsion zones because they displayed turbidity or phase separation. OriginPro 2022 software was used to create the diagram.

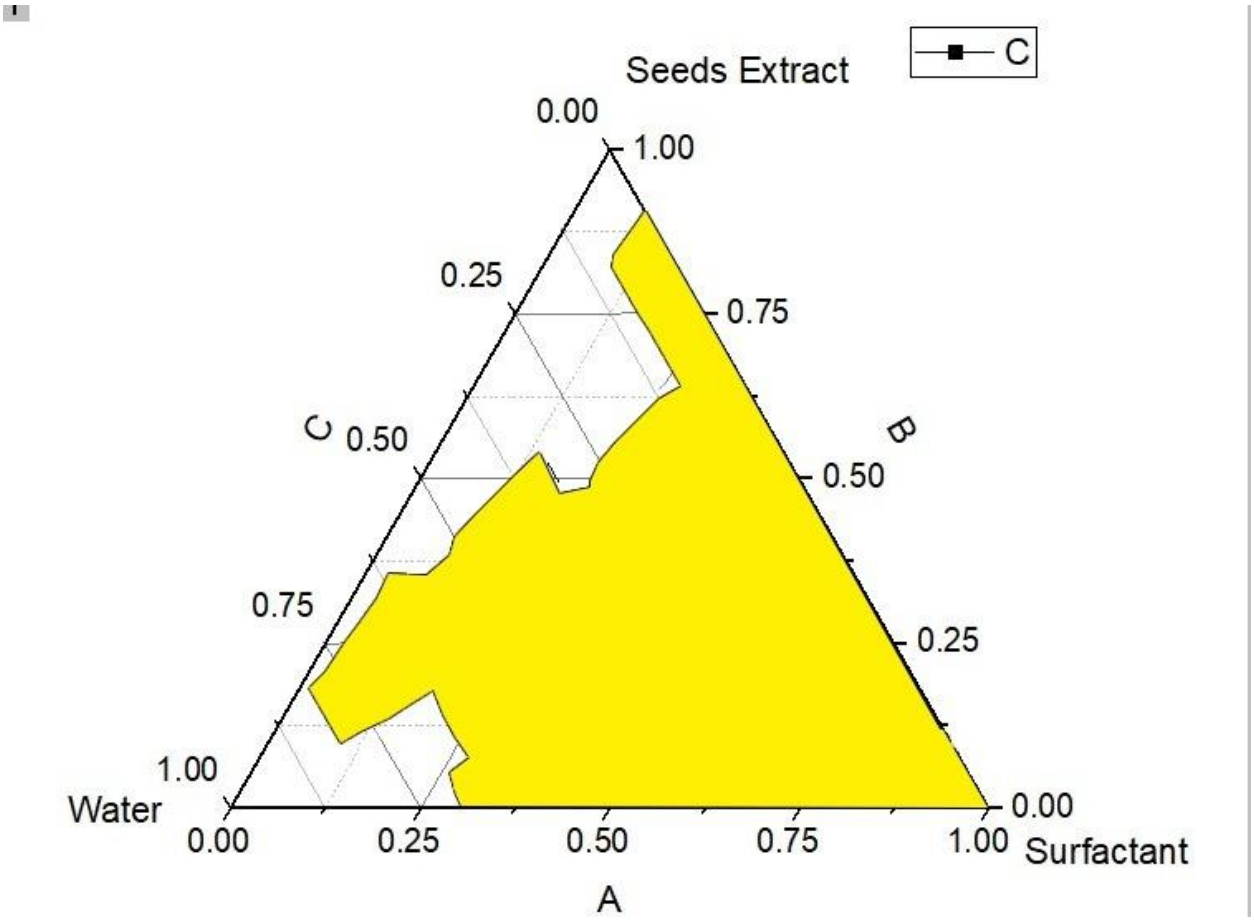


Figure 4.8.1: Pseudo-ternary phase diagram of microemulsions containing up to 90% Artemisia herba-alba seed extract and at least 10% surfactant mixture (Tween 80: Span 20) at 25°C. The yellow area represents stable, clear, and isotropic microemulsions across various water and extract ratios. Non-yellow zones indicate turbidity or phase separation. Created using OriginPro 2022.

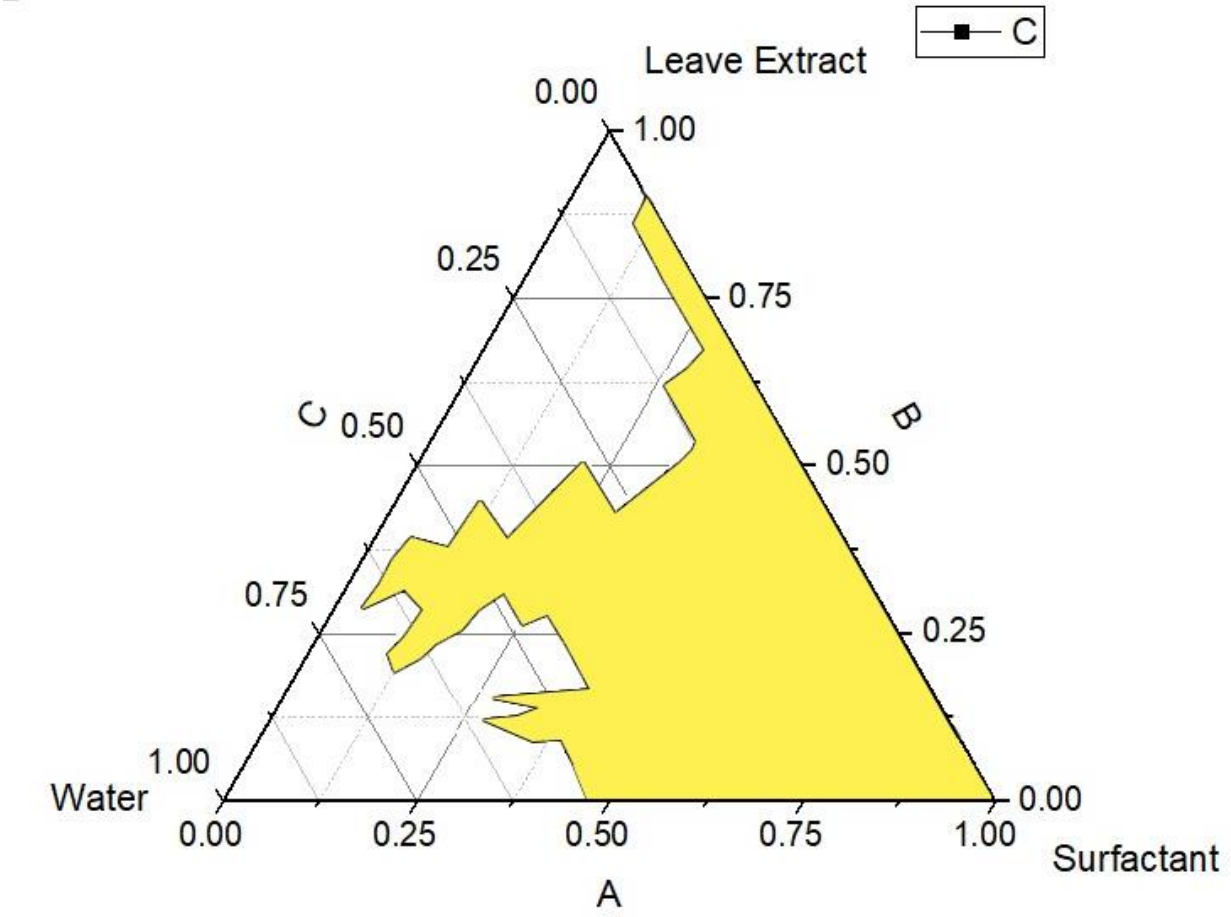


Figure 4.8.2: Pseudo-ternary phase diagram of microemulsions containing up to 90% Artemisia herba-alba seed extract and at least 10% surfactant mixture (Tween 80: Span 20) at 25°C. The yellow area represents stable, clear, and isotropic microemulsions across various water and extract ratios. Non-yellow zones indicate turbidity or phase separation. Created using OriginPro 2022.

4.9 Preparation of cream

When used topically, the formula has anti-aging, antiarrhythmic, anti-inflammatory, and depigmenting properties. It is also soothing, moisturizing, odor-free, absorbs well by the skin (it doesn't leave a film), doesn't irritate the skin (pH = 5-5.5), has a decent color, spreads smoothly (it's not too viscous, and applies rapidly), and doesn't irritate.

The samples stored at 8°C and 25°C did not liquefy. Up to the seventeenth day in the evaporimeter, the sample remained stable at 40°C and 40°C+75% RH. At various temperatures, the formulation remained stable. This might be because artemisia has antibacterial properties.

Chapter five

Conclusion

This study has successfully developed and validated a novel, biocompatible microemulsion-based delivery system incorporating Artemisia herba-alba extracts, offering promising applications in pharmaceutical and cosmetic formulations. The research presents a comprehensive investigation into the phytochemical composition, antioxidant potential, antibacterial efficacy, and emulsification behavior of Artemisia herba-alba from Palestinian origins.

5.1 Key Findings:

1. Innovative Formulation Development:
   Stable and transparent microemulsions were formulated using non-ionic surfactants (Tween 80 and Span 20) and characterized through pseudo-ternary phase diagrams. This novel formulation approach optimized the solubilization and delivery of bioactive compounds extracted from *Artemisia herba-alba*.
2. Phytochemical and Antioxidant Profiling:
   Spectrophotometric analyses revealed that seed extracts possessed the highest total phenolic content (881 mg GAE/g), while leaf extracts showed elevated flavonoid content (371.59 mg QE/g). DPPH assays confirmed potent antioxidant activity (>74% inhibition), highlighting the plant's potential as a natural antioxidant source.
3. Bioactive Compound Identification:
   HPLC-PDA analysis identified major bioactive constituents, including chlorogenic acid and rutin, further supporting the therapeutic potential of *Artemisia herba-alba* extracts.
4. Nanoscale Characterization:
   Atomic Force Microscopy (AFM) revealed the formation of nanoparticles with sizes as

small as 4.5 nm in leaf-based systems, demonstrating the ability of the extracts to produce ultra-small particles suitable for transdermal or topical applications.

5. Optical and Fluorescence Properties:
   UV-Vis and fluorescence spectroscopy confirmed the presence of key phytochemical classes (flavonoids, phenolic acids, terpenoids, and coumarins), with distinct emission profiles supportive of a rich metabolic fingerprint.
6. Antibacterial Activity:
   The formulated extracts exhibited moderate antibacterial effects against *S. aureus*, *E. coli*, and *K. pneumoniae*, with stronger activity observed from leaf oils, indicating their potential as natural antimicrobial agents.
7. Topical Cream Formulation:
   A stable topical cream was developed from the microemulsion and evaluated for physical stability, providing a user-friendly platform for future pharmaceutical or cosmeceutical deployment.

Recommendations for Future Work:
Future studies should explore in vivo pharmacological assessments, expand on the anti-inflammatory and anti-malarial activities of the formulations, and investigate potential enhancements through nanoparticle integration or drug loading. Furthermore, clinical evaluation of topical formulations may support the transition of *Artemisia*-based products into commercial therapeutic use.

In conclusion, this work provides the first scientific framework for the emulsification behavior and functional application of Artemisia herba-alba in microemulsion systems. The results affirm the extract's strong antioxidant and antibacterial properties, confirming its suitability as a natural therapeutic agent in modern drug delivery systems.

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صياغة وتوصيف مستحلب دقيق باستخدام مستخلص الشيح الفلسطيني

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ملخص

تقدم هذه الدراسة نهجًا جديدًا ومُثبتًا لتحضير وتوصيف مستحلبات دقيقة تحتوي على مستخلصات الشيح الأبيض العشبي، وذلك لاستخدامها في تطبيقات صيدلانية وتجميلية محتملة. طُوّرت هذه المستحلبات الدقيقة باستخدام مواد خافضة للتوتر السطحي ، وتُميّزها مخططات طور شبه بديل لتحديد سلوك الاستحلاب الأمثل. حُللت (Span 20وTween 80) متوافقة حيويًا مستخلصات الميثانول وأسيتات الإيثيل من أوراق وبذور الشيح لتحديد محتواها الكيميائي النباتي، وقدرتها المضادة للأكسدة، وفعاليتها المضادة للبكتيريا. حُدّدت كمية الفينول والفلافونويد الكلي باستخدام طرق القياس الطيفي الضوئي، حيث أظهرت ، بينما أظهرت (غم/GAE ملغ من 302) مقارنةً بالأوراق (غم/GAE ملغ من 881) مستخلصات البذور محتوى فينوليًا أعلى مستخلصات الأوراق مستويات أعلى من الفلافونويد (371.59 ملغ/غم). تم تأكيد النشاط المضاد للأكسدة من خلال اختبار DPPH HPLC-PDA. (تثبيط >74%)، وتم تحديد مواد كيميائية نباتية رئيسية مثل حمض الكلوروجينيك والروتين من خلال تم تحليل شكل وحجم الجسيمات النانوية باستخدام مجهر القوة الذرية، وكشفت عن جسيمات صغيرة تصل إلى 4.5 نانومتر. تم تأكيد الخصائص البصرية باستخدام مطيافية الأشعة فوق البنفسجية والمرئية والفلورسنت. أظهرت الاختبارات المضادة للبكتيريا ضد المكورات العنقودية الذهبية، والإشريكية القولونية، والكليبسيلا الرئوية فعالية متوسطة. تم بنجاح تطوير مستحلبات دقيقة مستقرة وشفافة، وتم تحضير تركيبة كريم موضعي وتقييم ثباتها الفيزيائي. يُعد هذا أول تقرير يُفصّل سلوك الاستحلاب ونظام التوصيل القائم على المستحلب الدقيق للشيح الفلسطيني العشبي الأبيض، مما يضع أساسًا لتطبيقه في العلاجات الطبيعية.

الكلمات المفتاحية: عشبة الشيح الأبيض، المستحلب الدقيق، التحليل الكيميائي النباتي، النشاط المضاد للأكسدة، النشاط المضاد للبكتيريا، مخطط الطور البديل الزائف، الفلافونويدات، المركبات الفينولية، كروماتوغرافيا السائل عالي الأداء، المجهر الذري للقوة، مطياف الأشعة فوق البنسفجية المرئية، مطياف الفلورسنت، تركيبة كريم موضعي للعلاجات الطبيعية.