Al-Mukhtar Journal of Bacis Sciences

Voulme 23
Issue 1

APR 2025

ISSN : 3006 - 8649

PUBLISHED BY OMU

Al-Mukhtar Journal of Basic Sciences

Peer-reviewed scientific journal, Volume Twenty- Three, Issue One, 2025

Published by Omar Al-Mukhtar University, Al-Bayda, Libya

A peer-reviewed journal published by Omar Al-Mukhtar University,

Al Bayda, Libya

Peer-reviewed scientific journal, Volume Twenty- Three, Issue One, 2025

Email: omu.j.bas@omu.edu.ly

EDITORS & STAFF

Al-Mukhtar Journal of Basic Sciences 23: (1), 2025

Al-Mukhtar Journal of Basic Sciences 23 (1): 01-09, 2025
Doi: https://doi.org/10.54172/hs62y750

Research Article

Open Access

Study of Corrosion Inhibition of a Corrosion Inhibitor Prepared from Duomeen-T®, Ethylene Oxide and Benzyl Chloride for Carbon Steel in Acidic Water

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Received:
18 February 2025

Accepted:
25 April 2025

Publish online:
30 April 2025

Abstract

The research presents the effect of corrosion inhibition of a corrosion inhibitor formulated by blending Duomeen-T®, ethylene oxide (EO), and benzyl chloride on carbon steel in oilfield production facilities. The experiments were conducted in an environment with Sharara oilfield formation waters saturated with CO_2. Duomeen-T® acted as a hydrophobic agent, while EO enhanced the performance of the inhibitor by improving the stability of the protective film on the metal surface. The effectiveness of the inhibitor was assessed using the ACM linear polarization bubble test corrosion system. Findings reveal remarkable inhibition efficiency, even at lower concentrations, achieving over 95% inhibition in some cases at just 15 ppm of corrosion inhibitor.

Keywords: Duomeen-T®, Ethylene Oxide (EO), LPR, Formation Water, Benzyl Chloride.

INTRODUCTION

Corrosion is the degradation of materials, usually metal, owing to chemical reaction with the environment, which results in a functional failure of a component. It poses significant challenges in oil and gas production facilities. Ongoing research aims to develop new corrosion inhibitors to address corrosion problems faced in the petroleum industry (Samuel, Etim, Nweke-Maraizu, Bako, & Shinggu, 2023). When process equipment and other facilities in oil production and processing are made of steel exposed to a corrosive environment, studies estimate that around 2.2 pounds of steel are lost for every 7.46 barrels of processed oil. This shows the need for the implementation of effective corrosion mitigation strategies for maintaining mechanical integrity management of the assets. The losses due to corrosion in the petroleum industry are substantial, necessitating the implementation of preventive measures. Among the various approaches available for corrosion prevention, the addition of corrosion inhibitors is a cost-effective and efficient solution (Al-Otaibi et al., 2014). A

corrosion inhibitor is a chemical agent that diminishes or prevents the interaction between metals and their environment, even when added in small quantities. These inhibitors are commonly used in various systems, such as oil and gas production, cooling, and refining operations. Compared to other corrosion protection techniques, corrosion inhibitors are regarded as one of the most practical and economical options available (Hassan, Abdelghani, & Amin, 2007)

Classification of Corrosion Inhibitors:

The corrosion inhibitors are classified in different ways. The primary classification of corrosion inhibitors is based on their chemical composition and their mode of action. The classification is given below:

- Organic or inorganic corrosion inhibitors
- Anodic or cathodic corrosion inhibitors.
- Oxidants or non-oxidant corrosion inhibitors

Inorganic corrosion inhibitors typically exhibit either anodic or cathodic behaviors, while organic corrosion inhibitors function as protective film formers that envelop the entire metal surface, exhibiting both anodic and cathodic characteristics. Compounds such as amines, imidazolines, and quaternary ammonium substances act as organic film formers, mitigating corrosion through adsorption on the metal surface and effectively blocking corrosive agents from contacting the metal. These types of inhibitors are commonly used in the oil and gas sector (Al-Amiery, Yousif, Isahak, & Al-Azzawi, 2023; Alamiery, 2021; Dawood et al., 2021). The classification of organic inhibitors is generally influenced by their interactions with the metal surface and their impact on the potential of that surface. The chemical structure of the inhibitor molecule plays a crucial role in determining its effectiveness (Obot, Obi-Egbedi, & Umoren, 2009)

The efficacy of organic inhibitors is contingent upon various factors, including:

- The molecular shape and functional groups.
- The presence of aromatic structures and bonding types.
- The length of the carbon chain.
- The adhesion strength to the metal surface.
- The density and cross-linking characteristics of the protective layer. Organic corrosion inhibitors can be categorized into two main types: (a) water-soluble and oil-dispersible, and (b) oil-soluble and water-dispersible corrosion inhibitors. Their use depends on which liquid phase is predominant within the production system.

The mechanism by which organic corrosion inhibitors function involves the adsorption of electrically charged organic groups at the interface between the metal and the solution. The adsorption process is influenced by several factors, including the chemical structure of the molecules, the type of solution, the characteristics of the metal substrate, and the nature of the electrical double layer, whether it is positively or negatively polarized. There are various modes of adsorption, including:

- Pie-bond orbital adsorption.
- Electrostatic adsorption.
- Chemisorption.

Organic inhibitors generally function by their adsorption onto the metal surface, and their effectiveness can depend on how well they interact with active locations (Trabanelli, 2020)

Inhibition Efficiency Measurements:

The effectiveness of corrosion inhibitors is monitored through methods such as mass loss corrosion coupons and various electrochemical techniques. These electrochemical techniques include linear

polarization resistance (LPR) tests, electrical resistance (ER) probes, potential measurements, potentiodynamic tests, and electrochemical impedance spectroscopy.

Linear polarization resistance technique:

The linear polarization resistance method is widely recognized as the most utilized technique in electrochemical testing. In this approach, the working electrode is subjected to positive and negative polarization, typically set at ±20 mV from the open circuit potential (E_{cor}). Within this voltage range, the polarization curve is regarded as linear. This testing can be performed in laboratory settings and in the field. The total current density (i) at the metal electrode interface, which is the difference between the anode and cathode current densities, can be expressed as follows:

$$i_{net} = i_a - i_c$$

Where:

The anodic and cathodic current densities are represented by i_a and i_c, respectively, while i_{net} signifies the total current density at the interface. As described below, both the anodic and cathodic current densities depend on the potential difference and the slope of the polarization curve.

$$i_a = i_0 \exp\left[{(E - E^0)}/{\beta_a}\right]$$
$$i_c = i_0 \exp\left[{(E^0 - E)}/{\beta_c}\right]$$

The final form of the equation can be expressed as:

$$i_{net} = i_{cor}\left[\exp\left({\eta}/{\beta_a}\right) - \exp\left({\eta}/{\beta_c}\right)\right], \quad \eta \text{ is the over potential}$$

The equation outlined earlier indicates that the overall current density (i) has an exponential relationship with the overpotential, based on the following assumptions:

- The movement of charge at the boundary between the electrode and the electrolyte influences the process of electrochemical corrosion.
- The solution resistance is considered negligible.
- Concentration polarization is ignored.
- There are no secondary electrode reactions.

Under these assumptions, the corrosion current density can be represented as follows: (Esmaael & Alkathafi, 2019)

$$i_{cor} = {b_a b_c}/{2.303(b_a + b_c)\left({\delta i}/{\delta E}\right)} = {B}/{R_p}$$

B is a constant derived from calibrating measurements of mass loss due to corrosion, while b_a and b_c are referred to as Tafel slopes. To measure polarization resistance R_p, the working electrode is polarized ± 20 mV in both anodic and cathodic directions, starting from the open circuit potential (E_{cor}) (Yıldırım & Cetin, 2008).

MATERIALS AND METHODS

Chemicals Used:

All chemicals used in this study are reagent grade. The following chemicals are used in the formulation of corrosion inhibitors:

Duomeen-T® - It is a commercial product manufactured by the Nouryon company. The general chemical formula of Duomeen-T® is alkyldiamine (R-NH-$(CH_2)_3$- NH_2), where R is an alkyl group. The main function of this compound in the formulation of corrosion inhibitor is a dispersant agent.

Ethylene oxide - Ethylene oxide is a reactive compound often used in the synthesis of surfactants, emulsifiers, and other chemicals. In the formulation of the present corrosion inhibitor to create ethoxylated compounds are created to improve the solubility and effectiveness of the inhibitor in aqueous systems. The other benefit of ethylene oxide is to modify metal surface absorption and make the surface less susceptible to corrosion. It is also slightly buffers the organic acids.

Benzyl chloride – Benzyl chloride-derived compounds can adsorb onto metal surfaces, forming a hydrophobic layer that blocks corrosive agents such as water and oxygen from reaching the metal surface. It has been widely accepted that Benzyl Chloride shows an outstanding corrosion protection performance.

Formulation of Corrosion Inhibitor

The chemical formulation of the corrosion inhibitor used in this study is shown in Table 1.

Table 1: The chemical composition of corrosion inhibitor

Component	Percentage in (wt.%)
Deionized water	75
Duomeen-T® plus 9.5 mol Ethylene oxide	15
Benzyl chloride	10

Water Samples to Prepare Test Solution

The water samples were collected from the Sharara oilfield from the following locations:

a) Water from the discharge of the test separator, and
b) Water from the water source well (WSW 05).

To ensure accurate results, pH, CO_2, temperature, and alkalinity measurements of water samples were recorded at the site during sampling to prevent any alterations in the water characteristics during transportation to the laboratory. A comprehensive analysis of the water was conducted in a laboratory located in Benghazi city using ASTM standard methods. The results of water analysis are presented in Table 2.

Table 2: Results of chemical analysis of water samples

Property	Units	Water from the Outlet of the Test Separator	Water from Water Source Well (WSW 05)
pH	-	5.3	6.8
Sodium ion (Na^+)	mg/L	64241	640
Calcium ion (Ca^{2+})	mg/L	12000	128
Magnesium ion (Mg^{2+})	mg/L	1507	90
Barium ion (Ba^{2+})	mg/L	0	0
Strontium ion (Sr^{2+})	mg/L	0	1
Iron (Fe^{2+})	mg/L	109	0.04
Chloride ion (Cl^-)	mg/L	140500	925
Bicarbonate ion (HCO_3^-)	mg/L	124	155
Sulfate ion (SO_4^{2-})	mg/L	550	625

Test Solution

The test solution was prepared by bubbling carbon dioxide (CO_2) gas in the water samples collected from the Sharara oilfield before starting the study of corrosion measurement to ensure saturation and acidification of the solution. Bubbling of CO_2 in water for one hour in the water samples drops pH to 5. The temperature of the thermostatic bath was maintained at 70°C. This temperature is slightly higher than the field application temperature.

The concentrations of the corrosion inhibitors tested were 0.5, 10, 20, 50, and 100 ppm.

Corrosion Rate Measurement

ACM Gill-12 linear polarization corrosion rig, consisting of twelve capped glass cells of having capacity of one liter each, was used for corrosion measurement in this study. All these cells were dipped in a thermostatic water bath capable of maintaining a stable temperature. Each corrosion cell contains three SAE 2018 carbon steel electrodes that serve as the working, reference, and auxiliary electrodes. A computerized potentiostatic system is connected to each cell for precise measurements. To ensure saturation of the solution, CO_2 is introduced into the system via a gas regulator and rubber tubing. Data collected from the measurements are automatically transmitted to the software for continuous processing and calculation of corrosion rates. Fig.1 shows a complete overview of the ACM corrosion bubble test rig.

Figure: (1). LPR Gill-12 corrosion test system

RESULTS AND DISCUSSION

The test solutions were tested with addition of different concentrations of corrosion inhibitor. The results are presented in Figure 2. The figure displays the corrosion behavior over time, measured in hours, for the test solution prepared from the water obtained from the outlet of the test separator at various inhibitor concentrations ranging from 0 to 100 ppm. The introduction of the inhibitor leads to a notable reduction in the corrosion rate. As the concentration of the inhibitor increases, the corrosion rate of the test solution decreases. The value of approximately 1 mpy corrosion is achieved at the addition of 10 ppm of corrosion inhibitor. For concentrations above 20 ppm, the corrosion rate drops below 1 mpy after 15 hours of immersion. This corrosion rate is also lower than that of the reference sample, as shown in Fig. 2

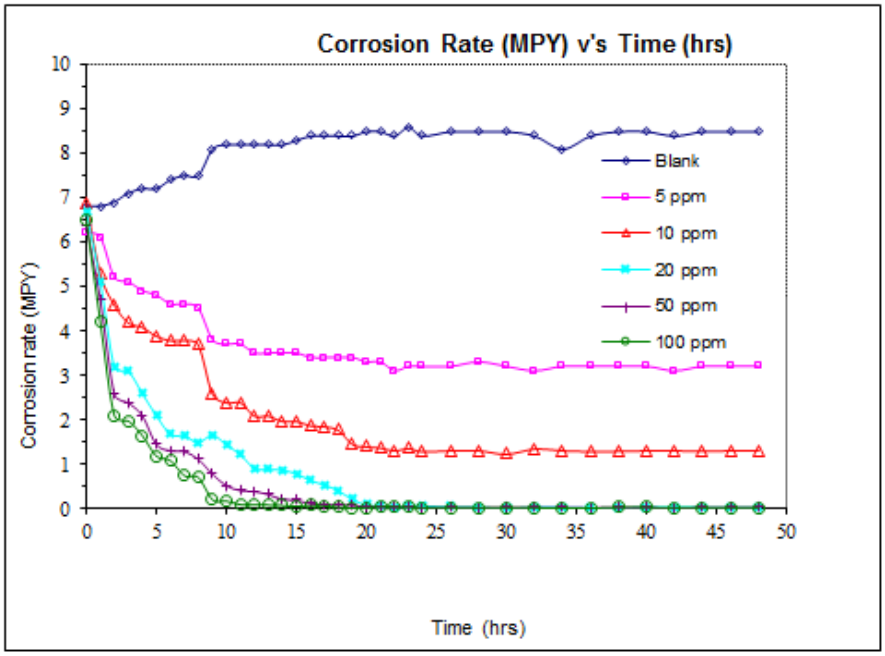

Figure:(2). Corrosion rate vs time for test separator outlet

Fig. 3 depicts the relationship between inhibition efficiency and inhibitor concentration, illustrating that 90% inhibition is achieved at a concentration of 15 ppm.

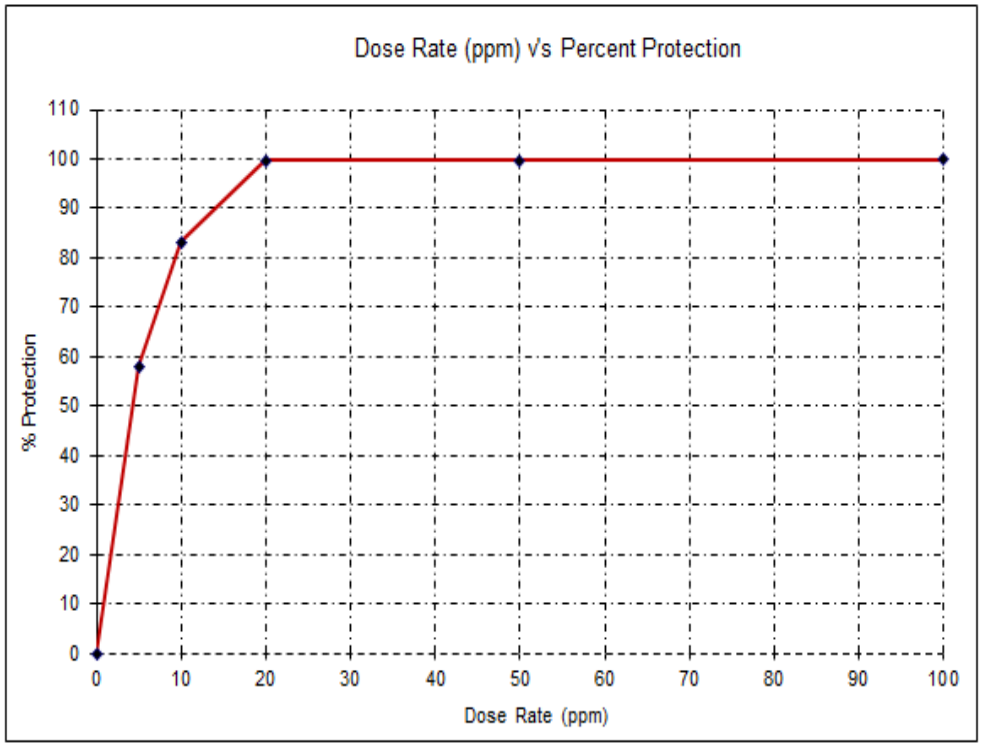

Figure: (3). Corrosion inhibitor dosage rate vs percent inhibition for test separator outlet

The rate of corrosion inhibitor was also evaluated using a test solution prepared from the water obtained from the water source well (WSW05). The results are presented in Figure 4. Linear polariza-

tion tests indicate that the corrosion rate decreased from 54 mpy to 13 mpy for 9 hours with an inhibitor concentration of 10 ppm. It was observed that as the concentration of the inhibitor increased, the time required for inhibition also decreased significantly.

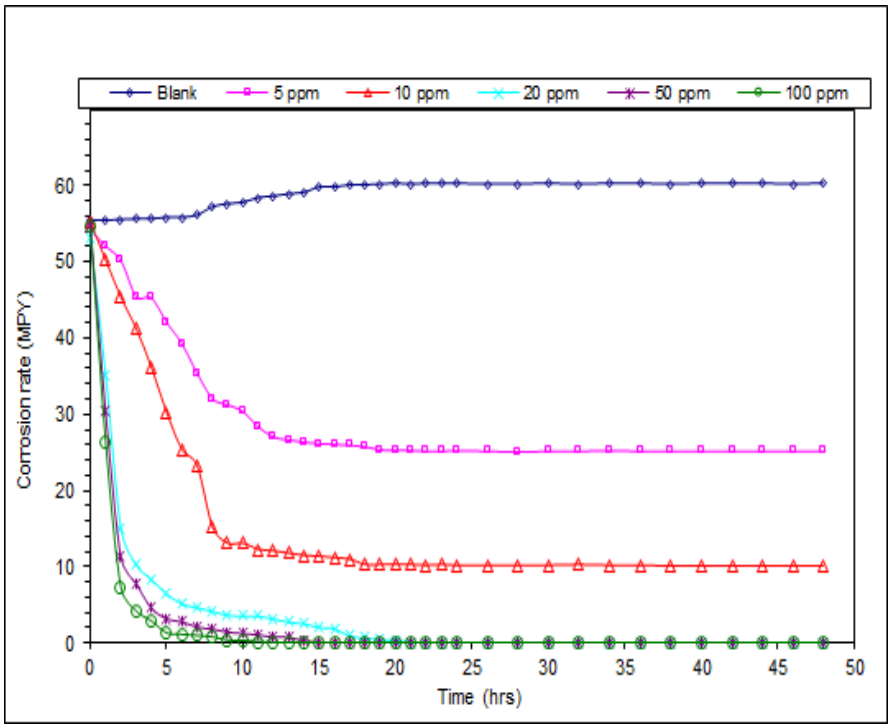

Figure: (4). Corrosion rate vs time for test solution prepared from water source well WSW 05

Figure 5 presents the results of inhibition efficiency with the inhibitor concentration for test sample prepared from the water obtained from WSW05 water source well, where approximately 98 % inhibition was reached at an inhibitor concentration of around 20 ppm.

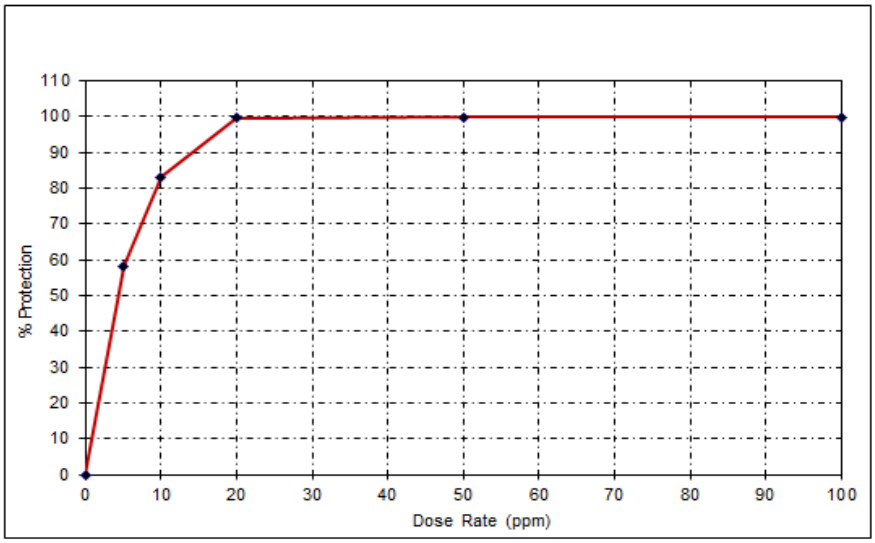

Figure: (5). dosage rate of corrosion inhibitor vs percent corrosion inhibition for the test solution prepared from water obtained from the WSW05 water source well

CONCLUSION

The findings of this study indicate that the corrosion inhibitor formulated with mixing duomeen-T®, ethylene oxide, and benzyl chloride offers exceptional corrosion inhibition for carbon steel in harsh oilfield production environments that are saturated with CO_2. Each component in the formulation and application plays a distinct role: they enhance the dispersion of the inhibitor in the corrosive medium, modify the metal surface, improve the adsorption of the inhibitor, and increase the durability and adhesion of the protective film on the metal surface**.**

Duality of interest: The authors declare no conflict of interest

Author contributions: Contribution is equal between authors.

Funding: The authors did not receive any funding (institutional, private, and/or corporate financial support)

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Al-Mukhtar Journal of Basic Sciences 23 (1): 10-21, 2025
Doi: https://doi.org/10.54172/31ke1w34

Research Article **Open Access**

Some Approximation Spaces via Supra Topology

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Received:
11 April 2025

Accepted:
28 April 2025

Publish online:
30 April 2025

Abstract

This paper introduces a new space based on a generalized neighbourhood system using the concept of a supra topology, termed a supra approximation space (briefly S^nAS). We investigate several properties of S^nAS and compare its advantages with those of the classical neighbourhood approximation space. Furthermore, we define and study a novel class of separation axioms using S^n-open in a supra approximation space (S^nAS). Finally, we explore some of their properties in the context of information system, particularly in approximation process of approximation and definability.

Keywords: Neighbourhood System; Rough Set Theory; Approximation Space; Supra Topology; Topological Space; T_0 ; T_1 And T_2.

INTRODUCTION

In 1983 (Mashhour, 1983) introduced supra topological spaces and studied S-continuous functions and S^*-continuous functions. They introduced the notion of S -open and S-closed sets and characterized these sets using S -closure and S-interior operators respectively.

Rough set theory, proposed by Pawlak in 1998 (Pawlak & Systems, 1998) extends classical set theory. Pawlak introduced the notion of an approximation space (U, R), where U is a universe set and R is an equivalence relation. Within this framework, he defined the lower approximation ($\underline{X}$), upper approximation ($\overline{X}$), and the boundary region ($bnd(X)$) of any subset $X \subseteq U$. A fundamental result establishes the relationship between these concepts: $(\underline{X}) \subseteq X \subseteq (\overline{X})$ and $bnd(X) = (\overline{X}) - (\underline{X})$.

In (Császár, 2004), the author further explored separation axioms using generalized topology notions. This paper introduces a novel structure called a supra approximation space (briefly S^nAS). We investigate key definitions and properties of approximations within S^n**AS**, including the formulation of lower and upper approximations. Moreover, we define and analyze new separation axioms based S^n-open sets, examining their fundamental properties. This work lays the foundation for future applications of separation axioms in related fields.

Definition 1.1. (Lashin et al., 2005)
consider (U,R) is a generalized approximation space. Then:

1. $N(x)=\{y\in U: xRy\}$ is called the right neighborhood of an element x,
2. $N(U)=\{N(x): x\in U\}$ is the collection of all neighborhoods in (U,R).

2- A Supra Approximation Space
We use a neighborhood to construct a supra approximation space (S^nAS). This space relies on a supra topological structure. Additionally, we investigate some definitions, including:

1. supra lower approximation,
2. supra upper approximation,
3. S^n- undefinable (rough) set in S^nAS,
4. S^n-internally, S^n-externally, and S^n-totally definable sets.

Definition 2.1.
consider (U,R) is a generalized approximation space. We define the class of a supra on (U,R) as:
$S^n(U)=\{\phi,U,\{X\subseteq U: X=\cup_{x\in U}N(x)\}\}$,
where $N(x)$ is the right neighborhood of x for all $x\in U$.

1. The members of the supra set $S^n(U)$ are called S^n-open sets,
2. The pair (U,R,S^n) is called a supra approximation space (in short, S^nAS),
3. The complement of an S^n-open set is called an S^n-closed set,
4. The class of all S^n-closed sets is denoted by $(S^n(U))^c$.

Example2.1.
Let (U,R,S^n) be an supra approximation space S^nAS, where $U=\{a,b,c,d\}$, R be a binary relation on U
$R=\{(a,b),(a,d),(b,b),(b,c),(c,d),(d,a)\}$.
The neighborhoods are defined as:
$N(a)=\{b,d\}$,
$N(b)=\{b,c\}$,
$N(c)=\{d\}$,
$N(d)=\{a\}$.
The supra set is:

$S^n(U)=\{\emptyset,\{a\},\{d\},\{b,c\},\{b,d\},\{a,d\},\{a,b,c\},\{a,b,d\},\{b,c,d\},U\}$,
and the class of S^n-closed sets is:
$(S^n(U))^c=\{\emptyset,\{a\},\{c\},\{d\},\{b,c\},\{a,c\},\{a,d\},\{a,b,c\},\{b,c,d\},U\}$.

Definition 2.2.
consider (U,R,S^n) is S^nAS, $X\subseteq U$. Then, X is called S^n-definable (or exact) if $S^n(X)=(S^n(X))^c$.

Definition 2.3.
Let U be a nonempty set and $N(U)$ be a nonempty collection of subsets of U. $N(U)$ is called supra topology (briefly$S^n T$) on U if:

1. $\emptyset, U \in N(U)$, and
2. For any collection $\{N(x)\}_{x\in U} \subseteq N(U)$, the union $\cup_{x\in U} N(x) \in N(U)$ where $N(x)$ is the right neighborhood of x for all $x \in U$.

The pair$\big(X, N(U)\big)$ is called a supra topological space (briefly S^nTS).The members of $N(U)$ are called supra- open sets, and a subset of a supra topological space $\big(U, N(U)\big)$is called supra-closed set if its complement is a supra- open set.

Definition 2.4.
Consider (U, R, S^n) is S^nAS, $X \subseteq U$. We define:

1. The supra lower approximation of X(in short $\underline{S^n}(X)$) as:

$$\underline{S^n}(X) = \cup\{G : G \in S^n(X),\ G \subseteq X\};$$

2. The supra upper approximation of X (in short $\overline{S^n}(X)$) as:

$$\overline{S^n}(X) = \cap\Big\{F : F \in \big(S^n(X)\big)^c,\ X \subseteq F\Big\}.$$

Theorem 2.1.
Let (U, R, S^n)be S^nAS on U and $B \subseteq U$.Then, $x \in \overline{S^n}(B)$ if and only if $G \cap B \neq \emptyset$ for every S^n-open set G such that $x \in G$.

Proof:
This follows directly from Definition 2.4.

Definition2.5.
Let (U, R, S^n) be an S^nAS. We say that the union of any family of elements of $S^n(U)$ is in $S^n(U)$.

Theorem 2.2.
Let $(U, R. S^n)$ be an S^nAS on U, and let $X \subseteq U$. Then:

1. $\underline{S^n}(X)$ is a supra set.
2. $\underline{S^n}(X)$ is the largest supra set contained in X.
3. X is a supra set if and only if $\underline{S^n}(X) = X$.

Proof:
This follows from the definitions of S^n. set and $\underline{S^n}(X)$.

Definition 2.6.
Let (U, R, S^n) be an S^nAS, and let $X \subseteq U$.Then:

1. The supra boundary of X (denoted byS^n- $bnd(X)$) is:

$$S^n\text{-}bnd(X) = \overline{S^n}(X) - \underline{S^n}(X).$$

2. The supra internal edge of X (denoted by $\underline{S^n}$- $edg(X)$) is:

$$\underline{S^n}\text{-}\, edg(X) = X - \underline{S^n}(X).$$

3. The supra external edge of X(denoted by$\overline{S^n} - edg(X)$) is:

$$\overline{S^n}\text{-}\, edg(X) = \overline{S^n}(X) - X.$$

Example 2.2.

Let (U, R, S^n) be an supra approximation space S^n*AS*, where $U = \{a, b, c, d\}$, R be a binary relation on U with the neighborhoods defined as $N(a) = \{a, b\}$, $N(b) = \{b, c\}$,$N(c) = \{d\}$, and $N(d) = \{c\}$.

The supra topology$S^n(U)$ is given by:

$S^n(U) = \{\emptyset, \{c\}, \{d\}, \{a, b\}, \{b, c\}, \{c, d\}, \{a, b, c\}, \{a, b, d\}, \{b, c, d\}U\}$.

The complement of$S^n(U)$,denoted as $\left(S^n(U)\right)^c$, is:

$\left(S^n(U)\right)^c = \{\emptyset, \{a\}, \{c\}, \{d\}, \{a, b\}\{c, d\}, \{a, d\}\{a, b, d\}, \{a, b, c\}, U\}$.

Now, consider the sets $X = \{b, c\}$, $Y = \{a, b\}$, and $Z = \{b, c, d\}$.

1. For X

   The lower approximation $\underline{S^n}(X) = X$.

   The upper approximation $\overline{S^n}(X) = \{a, b, c\}$.

   The supra boundary $S^n\text{-}bnd(X) = \{a\}$.

   The supra internal $\underline{S^n} - edg(X) = Y - \underline{S^n}(X) = \emptyset$.

   The supra external $\overline{S^n} - edg(X) = \overline{S^n}(X) - X = \{a\}$.

2. For Y

   The lower and upper approximation are equal $\underline{S^n}(Y) = \{a, b\} = Y = \overline{S^n}(Y)$.

   Thus, supra boundary $S^n\text{-}bnd(Y) = \emptyset$, and Y is S^n –definable.

3. For Z

   The lower approximation $\underline{S^n}(Z) = \{c, d\}$.

   The upper approximation $\overline{S^n}(Z) = U$.

   The supra boundary $S^n\text{-}bnd(Z) = \{a, b\}$.

   The supra internal $\underline{S^n} - edg(Z) = Z - \underline{S^n}(Z) = \{a\}$.

   The supra external $\overline{S^n} - edg(Z) = \overline{S^n}(Z) - Z = \{b\}$.

Definition 2.7.

Let (U, R, S^n) be a supra approximation space S^nAS and $X \subseteq U$. Then, X is called:

1. S^n -internally definable if and only if $\underline{S^n}(X) = X$.

2. S^n -externally definable if and only if $\overline{S^n}(X) = X$.

From a topological perspective, $\underline{S^n}(X)$ and $\overline{S^n}(X)$, can be reinterpreted using topological concepts.

Definition 2.8. (El-Shafei et al., 2016; Mashhour, 1983; Talabeigi & Computing, 2022)
A subfamily μ of U is said to be a supra topological on U, if:

1. $\emptyset, U \in \mu$ and
2. If $B_i \in \mu$ for all $i \in j$, then $\cup B_i \in \mu$.

The pair (U, μ) is called a supra topological space.The elements of μ are called supra- open sets in (U, μ) and a subset of a supra topological space (U, μ) is called supra-closed set if its complement is a supra- open set.

Definition 2.9.
Let (U, R, S^n) be a supra approximation space S^nAS and a supra topological space.
Then, for every $X \subseteq U$ we have:

1. X is said to be S^n-internally (S^n-externally,S^n-totally) definable if and only if X is supra open (supra closed, supra clopen) set in a supra topological space,
2. X is said to be S^n –undefinable (rough) set if and only if X is neither supra open nor supra closed in the supra topological space. clarification.

Proposition 2.2.
Let (U, R, S^n) be a supra approximation space S^nAS, $X \subseteq U$,and R be an equivalence relation. Then:

1. $\underline{S^n}\left(\underline{S^n}(X)\right) = \overline{S^n}\left(\underline{S^n}(X)\right)$;
2. $\overline{S^n}\left(\overline{S^n}(X)\right) = \underline{S^n}\left(\overline{S^n}(X)\right)$.

3- Separation Axioms in Supra Approximation Spaces
The main purpose in this section, we define and study some new Separation axioms by define S^n-open set which play an important role in distinguishing between sets and points in a topological space, and we study some separation properties in a supra approximation space.

Definition 3.1.
A supra approximation space (U, R, S^n) is:

1. T_0 -space if and only if for every two distinct points x and y on U, there exists an S^n-open set Gsuch that $x \in G$ and $y \notin G$,
2. T_1 -space if and only if for every two distinct points x and y on U, there exist two S^n-open sets G and H such that $x \in G, y \notin G$, and $y \in H, x \notin H$.

3. T_2 -space if and only if for every two distinct points x and y on U,there exist two disjoint S^n-open sets G and H such that $x \in G$ and $y \in H$.

Clearly $T_2 \Longrightarrow T_1 \Longrightarrow T_0$.

Proposition 3.1.
A supra approximation space$(U, R, S^n)$is T_0 if and only if $\overline{S^n}(\{x\}) \neq \overline{S^n}(\{y\})$ for all $x \neq y$,where $x, y \in U$.

Proof:
Consider $x \neq y$. Then, there exists $G \in S^n(U)$ such that $x \in G$ and $y \notin G$.

1. There exists $U - G \in \left(S^n(U)\right)^c$, where $x \notin U - G$ and $y \in U - G$ if and only if $x \notin \cap \left\{U - G : U - G \in \left(S^n(U)\right)^c : \{y\} \subseteq G\right\}$ if and only if $x \notin \overline{S^n}(\{y\})$ but $x \in \overline{S^n}(\{x\})$.
   Thus, $\overline{S^n}(\{x\}) \neq \overline{S^n}(\{y\})$.
2. Consider $\overline{S^n}(\{x\}) \neq \overline{S^n}(\{y\})$, i.e., there exists $z \in U$ such that $z \in \overline{S^n}(\{x\})$ and $z \notin \overline{S^n}(\{y\})$.

   Suppose $x \in \overline{S^n}(\{y\})$. This implies $\overline{S^n}(\{x\}) \subseteq \overline{S^n}(\{y\})$, which further implies $z \in \overline{S^n}(\{y\})$.
   This leads to a contradiction. Therefore, $x \notin \overline{S^n}(\{y\})$.
   Hence, $U - \overline{S^n}(\{y\})$ is a supra set containing x but not y.
   Thus, S^n is a T_0 space.

Proposition 3.2.
A supra approximation space (U, R, S^n) is T_1-space if and only if $\{x\}$ is S^n-externaly definable, for all $x \in U$.

Proof:

1. Consider $x \in U$, S^nAS(U, R, S^n) be a T_1-space. To prove that $\{x\}$ is S^n-externally definable,
   we show that $\{x\}^c$ is S^n-internally definable (a supra set).
   Let $y \in \{x\}^c (i.e., y \neq x)$.Since S^nAS is a T_1-space, there exists $G, H \in S^n(U)$ such that $y \in G, x \notin G$ and $x \in H, y \notin H$.
   Thus, for all $y \in \{x\}^c$ there exists $G \in S^n(U) : y \in G \subseteq \{x\}$.
   Since y is arbitrary, $\{x\}^c = \cup \{G \in S^n(U), G \subseteq \{x\}^c\}$.
   Therefore, $\{x\}^c$ is S^n-open, and $\{x\}$ is S^n- externally definable.
2. For the inversions consequence, assume $\{x\}$ be S^n-externally definable i.e., $\{x\}^c$ be a S^n-open set.

Consider $x, y \in U$ such that $x \neq y$. This means:
1. $x \in \{y\}^c, y \notin \{y\}^c$;
2. $y \in \{x\}^c, x \notin \{x\}^c$;
since $\{x\}^c$ and $\{y\}^c$ are S^n-open sets, a S^nAS (U, R, S^n) is a T_1- Space.

Proposition 3.3.
Consider (U, R, S^n) is S^nAS where R is preordering relation, if (U, R, S^n) is a T_1-Space then X is S^n- definable for all $X \subseteq U$.

Proof:
Consider $X \subseteq U$ to prove X is S^n-definable, we show that X is both S^n-internally and S^n-externally definable.

1. Since S^nAS is aT_1- Space,$\{x\}$ is S^n- externally definable for all $X \subseteq U$ (from Proposition3.2).
2. Both $X$and $U - X$ can be written as:
$$X = \cup_{x \in X} \{x\},$$
$$U - X = \cup_{x \in U - X} \{x\}.$$
3. from Proposition 3.7, X and $U - X$ are S^n-externally definable sets.
4. Since $U - X$ are S^n-externally definable, X is S^n-internally definable.
5. Thus,X is both S^n-internally and S^n-externally definable, making it S^n- definable.

Proposition 3.4.
A S^nAS that is a T_1- Space with preordering relation is a discrete approximation space.

Proof:
This follows directly from part 2 of Definition 3.1.

Definition 3.2.
Let (U, R, S^n)be S^nAS. The relation T_2 on U is defined by:
xT_2y if and only if there exists $G, H \in S^n(U)$ such that $x \in G, y \in H$ and $G \cap H = \emptyset$.
This relation is renamed (a separating relation).

Definition 3.3.
Let (U, R, S^n)be an S^nAS, and let T_2 be a separating relation on U. Then
$X^{T_2} = \{ y \in U : yT_2x, \forall x \in X\}$ is called the separating set of X.

Definition 3.4.
Consider (U, R, S^n)be an S^nAS and let $X, Y \subseteq U$. Then,
XT_2Y if xT_2y for all $x \in X$, $y \in Y$.

Remark 3.2.
We observe that the separating relationT_2 is has the following properties:

1. Irreflexive relation: xT_2y if and only if $x \neq y$.

2. Symmetric relation: xT_2y if and only if yT_2x.

For the $\Psi \subseteq S^n(U)$, where Ψ be collection of all neighborhoods, the relation T_2 can be redefined as follow:
xT_2y if and only if there exists $N_1, N_2 \in \Psi,\ x \in N_1, y \in N_2, N_1 \cap N_2 = \emptyset$.

Proposition 3.5.
Let (U, R, S^n) be an S^nAS, and let $X \subseteq U$.Then:

1. $X^{T_2} \subseteq X^c$.
2. $X^{T_2} \cap X = \emptyset$

Proof:

1. $y \in X^{T_2}$ if and only if yT_2x for all $x \in X$, which implies $y \neq x$ for all $x \in X$. if and only if $y \in X^c$.
2. Assume $X^{T_2} \cap X \neq \emptyset$ if and only if there exist $y \in U$ such that $y \in X^{T_2}$ and $y \in X$ implies $y \in X^c$ if and only if $X^c \cap X \neq \emptyset$ (contradiction).Then $X^{T_2} \cap X = \emptyset$.

Proposition 3.6.
Consider (U, R, S^n) be an S^nAS and $X \subseteq U$.Then:

1. $X^{cT_2} \subseteq X$.
2. $X \subseteq X^{T_2c}$.

Proof:
This follows directly from Proposition3.5.

Proposition 3.7.(Lin, 1988, 1989; Lin & computing, 1997)
Consider (U, R, S^n) be an S^nAS and $X \subseteq U$.Then, X^{T_2} is a supra set.

Proof:
To prove that X^{T_2} is a supra set, it is suffices to show that X^{T_2} can be written as a union of supra set.

1. Let $y_1 \in X^{T_2}$. By Definition 3.3, this means $y_1 T_2 x,$ for all $x \in X$.
2. From Definition 3.2, there exists $G_1, H_1 \in S^n(U)$ such that $y_1 \in G_1, x \in H_1$ and $G_1 \cap H_1 = \emptyset$.
3. Since $G_1 \cap H_1 = \emptyset$, for all $x \in X$ (where $x \in H_1$), it follows that $G_1 \subseteq X^{T_2}$.
4. Thus $y_1 \in X^{T_2}$ implies there exists $G_1 \in S^n(U)$ such that $y_1 \in G_1 \subseteq X^{T_2}$.

similarity, for any $y_2 \in X^{T_2}$, there exists $G_2 \in S^n(U)$ such that $y_2 \in G_2 \subseteq X^{T_2}$.
Finally, $y_i \in X^{T_2}$ implies $\exists G_i \in S^n(U)$ such that $y_i \in G_i \subseteq X^{T_2}$. Hence

$\cup_{i=1}^{n}\{y_i\} \subseteq \cup_{i=1}^{n} G_i \subseteq X^{T_2} = \cup_{i=1}^{n}\{y_i\}$ i.e., $X^{T_2} = \cup_{i=1}^{n} G_i$.Then X^{T_2} is a supra set.

Example 3.1.

Let (U, R, S^n) be an supra approximation space $S^n AS$, where $U = \{a, b, c, d\}$ and the neighborhoods are defined as:

$N(a) = \{a, b\}$,
$N(b) = \{b\}$,
$N(c) = \{d\}$,
$N(d) = \{a, c\}$.

The supra set and its complement are:

$$S^n(U) = \{\emptyset, \{b\}, \{d\}, \{a, b\}, \{a, c\}, \{b, d\}, \{a, b, d\}, \{a, b, c\}, \{a, c, d\}, U\},$$
$$\left(S^n(U)\right)^c = \{\emptyset, \{b\}, \{d\}, \{c\}, \{a, c\}, \{b, d\}, \{c, d\}, \{a, b, c\}, \{a, c, d\}, U\}.$$

Table (1). Table of operators.

X	X^{T_2}	$(X^{T_2})^c$	X^{cT_2}	$\underline{S^n}(X)$	$\overline{S^n}(X)$
$\emptyset$	U	$\emptyset$	$\emptyset$	$\emptyset$	$\emptyset$
$\{a\}$	{b, d}	$\{a, c\}$	$\emptyset$	$\emptyset$	{a, c}
{b}	$\{a, c, d\}$	{b}	$\{b\}$	{b}	{b}
{c}	$\{b, d\}$	$\{a, c\}$	$\emptyset$	$\emptyset$	{c}
{d}	{a, b, c}	{d}	$\{d\}$	{d}	{d}
$\{a, b\}$	$\{d\}$	$\{a, b, c\}$	$\{b\}$	$\{a, b\}$	$\{a, b, c\}$
{a, c}	{b, d}	$\{a, c\}$	$\{a, c\}$	{a, c}	$\{a, c\}$
{a, d}	$\{b\}$	$\{a, c, d\}$	$\{d\}$	{d}	$\{a, c, d\}$
$\{b, c\}$	$\{d\}$	$\{a, b, c\}$	{b}	{b}	$\{a, b, c\}$
{b, d}	$\{a, c\}$	$\{b, d\}$	$\{b, d\}$	{b, d}	{b, d}
$\{c, d\}$	$\{b\}$	$\{a, c, d\}$	{d}	{d}	$\{c, d\}$
$\{a, b, c\}$	$\{d\}$	$\{a, b, c\}$	{a, b, c}	$\{a, b, c\}$	$\{a, b, c\}$
$\{a, b, d\}$	$\emptyset$	U	$\{b, d\}$	$\{a, b, d\}$	U
$\{a, c, d\}$	$\{b\}$	$\{a, c, d\}$	{a, c, d}	$\{a, c, d\}$	$\{a, c, d\}$
{b, c, d}	$\emptyset$	U	{b, d}	{b, d}	U
U	$\emptyset$	U	U	U	U

Remark 3.3.

By comparing the lower approximation operation $\underline{S^n}(X)$ and X^{cT_2}, it follows that $\underline{S^n}(X)$ provides the best lower approximation. This can be observed in the sets$\{a, b\}, , \{a, b, d\}$ from the previous example. Similarly, by comparing the upper approximation $\overline{S^n}(X)$ and $(X^{T_2})^c$,it follows that $\overline{S^n}(X)$ provides the best upper approximation, as seen in the sets $\{c\}$ and $\{c, d\}$.

Proposition 3.8.

A (U, R, S^n) be a supra approximation space (S^nAS). Then, (U, R, S^n) is a T_2-Space if and only if $X^{T_2} = X^c$, for all $X \subseteq U$.

Proof:

From Proposition 3.5 we have $X^{T_2} \subseteq X^c$ for all $X \subseteq U$. Now, let $y \in X^c$. Then, $y \neq x$, for all $x \in X$,which implies there existence of $G, H \in S^n(U)$ such that $x \in G, y \in H$, and$G \cap H = \emptyset$ This means yT_2x for all $x \in X$, which is equivalent to $y \in X^{T_2}$. Therefore, ,$X^c \subseteq X^{T_2}$, and we conclude that $X^{T_2} = X^c$.

Proposition 3.9.(Al-Shami, 2016, 2017)
Let (U, R, S^n) be a supra approximation space (S^nAS). and $X \subseteq U$.Then, X is S^n-definible if and only if $(X^{T_2})^c = (X^c)^{T_2}$.

Proof:

1. **First part:** Assume $X \subseteq U$ is S^n-definible. Let $y \neq x$ where $x \in X$ and$y \in X^c$. Since X and X^c are supra sets, xT_2y for all $x \in X, y \in X^c$. By Definition 4.3, we have $y \in X^{T_2}$and $x \in (X^c)^{T_2}$ which implies $X^{T_2} = X^c$. Therefore $(X^{T_2})^c = X = (X^c)^{T_2}$.
2. **Second part:** Assume $(X^{T_2})^c = X = (X^c)^{T_2}$. By proposition3.7, if $(X^c)^{T_2} = X$. Then X is a supra set .Additionally, if $(X^{T_2})^c = X$ and X^c be a supra set, then X be S^n-definable set.

Proposition 3.10.
Let (U, R, S^n) be a S^nAS and $X \subseteq U$. The following statements are equivalent:

1. S^nAS is T_2-Space;
2. X is S^n-definable for all $X \subseteq U$;
3. S^nAS is discrete approximation space.

Proof:

1. $1 \Rightarrow 2$: Since (U, R, S^n) is a T_2-Space, from Proposition 3.8, we have $(X^c)^{T_2} = X$ and $X^{T_2} = X^c$, i.e., $X^{T_2 c} = X$ and $X^{cT_2} = X$. Then, X is an S^n-definible set from Proposition 3.9.
2. $2 \Rightarrow 3$: This is Obvious.
3. $3 \Rightarrow 1$: Let $\{x\}$ and $\{y\}$ are disjoint S^n-definable sets such that $\{x\} \cap \{y\} = \emptyset$, with $x \in \{x\}$ and $y \in \{y\}$ for all $x, y \in U$ Then S^nAS is T_2-Space.

Proposition 3.11.(Yao, 1999)
Let (U, R, S^n) be a S^nAS and $X \subseteq U$. If R is an equivalence relation, then $X^{T_2} = \left(\overline{S^n}(X)\right)^c$.

Proof:
Ago R is an equivalence relation, xT_2y if and only if $N_x \cap N_y = \emptyset$ (from Proposition 3.9).

1. $y \in X^{T_2}$ if and only if yT_2x, for all $x \in X$,

2. if and only if $N_x \cap N_y = \emptyset$,
3. if and only if $x \notin N_y$for $x \in X$,
4. if and only if $X \cap N_y = \emptyset$,
5. if and only if $y \in \left(\overline{S^n}(X)\right)^c$.

Thus, $X^{\tau_2} = \left(\overline{S^n}(X)\right)^c$.

CONCLUSION

In this work, we introduced and studied a new space based on a generalized neighbourhood system by using the concept of supra topology, called a supra approximation space (briefly S^nAS). We investigated key properties of S^nAS and compared its advantages with those of the neighbourhood approximation space.

Additionally, we defined and analyzed new Separation axioms using S^n-open sets, which play a crucial role in distinguishing between sets and points in a topological space. We also explored separation properties within the supra approximation space. In future work, we aim to extend the application of S^nAS to near- open sets and near- closed sets, further expanding its theoretical and practical implications.

Duality of interest: The authors declare that they have no duality of interest associated with this manuscript.

Author contributions : Contribution is equal between authors.

Funding: No specific funding was received for this work.

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Al-Mukhtar Journal of Basic Sciences 23 (1): 22-28, 2025
Doi: https://doi.org/10.54172/tdg2b571

Research Article

Open Access

Antimicrobial and Antioxidant Activities and Phytochemical Profiling of Crude Extracts from *Senna alexandrina*

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Received:
05 April 2025

Accepted:
27 April 2025

Publish online:
30 April 2025

Abstract

This study aimed to evaluate the biological activities and chemical characterization of crude extracts from *Senna alexandrina*. The plant extracts were prepared by sequential maceration of dried leaf powder using solvents of increasing polarity. Their antimicrobial activity was tested against two Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*), two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), and two fungi (*Candida albicans* and *Aspergillus niger*) utilizing the disc diffusion method. Antioxidant activity was measured by evaluating the scavenging of the stable 2,2-diphenyl-1-picrylhydrazyl free radical. Chemical characteristics such as total polyphenolic, flavonoid, and tannin contents were determined through spectrophotometric assays. Overall, the extracts showed stronger antifungal than antibacterial properties. Methanol and n-hexane extracts demonstrated significant antifungal activity with zones of inhibition measuring 25mm for *C. albicans* and 18mm for *A. niger*, respectively. The methanolic extract showed the highest antibacterial activity against *E. coli* with a 13mm zone of inhibition. It also exhibited the highest scavenging radical activity at 56%. Total polyphenolics were predominantly found in the ethyl acetate extract, reaching 136.8 ± 0.03 mg gallic acid equivalent per gram. Flavonoids were most abundant in the ethyl acetate extract with 499.33 mg quercetin equivalent per gram. Except for the methanolic extract of *S. alexandrina*, all extracts lacked tannins. In conclusion, this plant has potential as a valuable source of natural bioactive compounds.

Keywords: *Senna Alexandrina.*, Antimicrobial Activity, Antioxidant Activity; Total Phenolics.

INTRODUCTION

Natural products derived from plants have long been the mainstay for antibiotic development. With the growing acceptance of herbal medicines, the exploration of medicinal plants for novel active compounds has become a crucial avenue for discovering new antibiotic leads (Roy & Dutta, 2021). Senna species, part of the Fabaceae family, are found across the globe (NPGS, 2008). Notably, the tinnevelly senna (*Senna alexandrina* Mill.) is widely used in various laxatives. However, the agronomic characteristics of Senna species are not well-documented, as they have often been regarded as weeds. These species contain several phytochemicals with the potential for use in human medicine (Morris, 2009).

Senna alexandrina, commonly referred to as Senna, is naturally found across a range extending from Mali to Somalia and Kenya in Africa, as well as parts of Asia, including the Arabian Peninsu-

la, India, and Sri Lanka. This plant is also widely cultivated for commercial purposes in countries such as India, Sudan, Egypt, Pakistan, China, and Korea. Renowned for its high-quality and valuable varieties, this small shrub reaches a height of approximately 2 feet. It features an upright, smooth, pale green stem with long, spreading branches that support leaflets arranged in clusters of four or five pairs. These leaflets are typically around an inch long, lanceolate or obovate in shape, and mucilaginous with a sweetish taste. The distinguishing feature of Senna leaves is their form at the base and the lack of bitterness, setting them apart from angel leaves, which tend to be thicker and stiffer. The plant produces small yellow flowers, with broadly oblong pods measuring approximately 2 inches in length and 7/8 inch in width, each holding around six seeds (Viswanathan & Nallamuthu, 2012).

Senna alexandrina, known for its pods and leaves, has been used as a laxative for centuries due to its strong cleansing properties. Beyond its laxative effect, the leaves are traditionally applied in the treatment of a wide range of ailments including anemia, anorexia, bile disorders, bronchial issues, burns, cancer, cholera, constipation, cramps, skin disorders, dysentery, indigestion, intestinal problems, fever, fungal infections, stomach issues, gonorrhea, gout, bad breath, hemorrhoids, liver problems, herpes, hiccups, infections, jaundice, leprosy, leukemia, fungal diseases, nausea, neural disorders, acne, ringworm, spleen disorders, syphilis, typhoid fever, sexually transmitted infections, viral diseases, as an anti-parasitic agent, and for healing wounds (El-Morsy, 2013). This herb has been used in traditional medicine to treat cholera, liver diseases, constipation, typhoid, and a variety of other ailments (Ahmed et al., 2016). This study aimed to evaluate the biological activities and chemical makeup of crude extracts from *Senna alexandrina.*

MATERIALS AND METHODS

Plant Material

Plant material

Fresh leaves of *Senna alexandrina* were sourced from Khartoum, Khartoum State, in October 2022. The plant underwent authentication by a taxonomist at the Department of Botany, Faculty of Science, University of Khartoum in Sudan. The leaves were carefully washed and dried in the shade to prevent any adverse effects on the phytochemical properties of the desired components. They were then stored in airtight containers at room temperature, ready for future use.

Preparation of extracts

In a separate procedure, 20 grams of dried powdered leaves of *Senna alexandrina* were extracted consecutively through maceration using hexane, chloroform, ethyl acetate, and methanol, with 400 mL of each solvent. This process involved a shaker apparatus for approximately 24 hours at room temperature. Following extraction, the mixture was filtered, and the solvents were evaporated under vacuum with a rotary evaporator. The dry extracts obtained from each sample were weighed and stored at 4°C until needed.

Biological activity

Antimicrobial activity

The bacterial cultures utilized in the study included *Bacillus subtilis* NCTC 8236, *Staphylococcus aureus.*, ATCC 25923, *Escherichia coli.*, ATCC 25922, and *Pseudomonas aeruginosa.*, ATCC 10145 were tested alongside fungal strains *Aspergillus niger.*, ATCC 9763 and *Candida albicans.*, ATCC 7596. Each extract, loaded at 10 mg per disc, was assessed using the disc diffusion method as described (Ahmed et al., 2024b).

Antioxidant activity

The antioxidant activity of the extracts was assessed through the in vitro DPPH radical scavenging method, as described (Omer et al., 2024). Concentrations (1, 5, 10, 20, 40, 60, 80, and 100 µg/ml) were prepared by diluting the stock solution with methanol. The assay was conducted using 96-well microtiter plates. Each well received 70 µl of the sample solution, followed by the addition of 140 µl of 0.6×10^{6} mol/l DPPH. The mixture was gently shaken and left to incubate for 30 minutes in the dark at room temperature. Absorbance was then measured spectrophotometrically at 517 nm using a microtiter plate reader. Propyl gallate served as the positive control. The DPPH radical-scavenging activity was calculated using the formula: DPPH radical scavenging (%) = [1 - (Ablank – Asample) / Ablank] × 100 Here, Ablank represents the absorbance of the control reaction (with all components except the test sample), and Asample refers to the absorbance of the extract or reference sample.

Quantitative Analysis of Total Polyphenol, Flavonoid, and Tannin Contents

Analysis of Total Polyphenol Content

The total polyphenolic content was measured using the method outlined by (Wolfe et al. 2003).

Analysis of Total Flavonoid Content

The total flavonoid content was assessed by following the procedure described by (Ordonez et al. 2006).

Analysis of Total Tannin Content

The total tannin content was evaluated according to the procedure provided by (Sun et al. 1998)

Statistical analysis

All the procedures for extraction, antimicrobial analysis, and antioxidant studies were repeated in triplicate. The descriptive analysis (mean and standard deviation) was used to discuss the results, assuming the normal distribution of the studied variables.

RESULTS AND DISCUSSION

Yields of crude extracts

The yield of n-hexane, chloroform, ethyl acetate, and methanolic extracts from the leaves of *Senna alexandrina* was assessed. The study involved sequentially macerating dried leaf powder with hexane, chloroform, ethyl acetate, and methanol. In general, the methanol extract showed the highest yield at 6.39%, whereas the ethyl acetate extract had a notably lower yield of 0.49%, as shown in Table 1. According to (Feudjio et al., 2020), methanol's ability to readily penetrate plant cells and dissolve a wide range of bioactive compounds, both polar and many nonpolar, may explain the superior extraction yields observed for the plant. Additionally, (Stalikas, 2007), highlighted that factors such as the type of plant parts used, storage duration, and temperature can also influence yield percentages.

Biological activities

Antimicrobial activities

The plant demonstrated stronger antifungal activity than antibacterial activity. The methanol and chloroform extracts showed the highest antifungal effects, inhibiting *C. albicans* with a 25mm zone of inhibition and *Aspergillus niger* with 18mm. In terms of antibacterial action, the n-hexane and methanolic extracts were most effective, both influencing *Staphylococcus aureus* and *Escherichia coli* with a 13mm zone of inhibition. Comparing these antimicrobial findings for *S. alexandrina* to

earlier studies, it is consistent with (Vijaya Sekhar et al. 2016), which also reported that the methanolic extract exhibited strong antifungal properties.

Antioxidant activity

The antioxidant activity of extracts from the plant was assessed by examining their ability to scavenge DPPH free radicals, with results detailed in Table 3. The findings indicated that the methanolic extract exhibited the highest antioxidant activity at 56%, while ethyl acetate and hexane extracts showed lower activities at 8% and 3%, respectively, and the chloroform extract was inactive. Antioxidant activity is associated with phenolic (Thouri et al., 2017) and flavonoid (Kobus-Cisowska et al., 2019) compounds. Prior studies, including those by Ahmed et al. (2016), have also emphasized differences in antioxidant activity levels. The findings from this experiment align with and are supported by earlier research, such as the work of Akloul et al. (2014). Typically, a substance dissolves more easily in a solvent that shares a similar polarity (Wibisono et al., 2020).

Assessment of Total Polyphenolic, Flavonoid, and Tannin Content

The total polyphenolic content in the hexane, chloroform, ethyl acetate, and methanol extracts of *Senna alexandrina* was evaluated. The findings indicated that ethyl acetate extract had the highest concentration of polyphenolics, with 136.8 mg gallic acid equivalent (GAE) per gram. In contrast, most extracts showed a higher concentration of flavonoids, measured at 499.33 mg quercetin equivalent per gram. Notably, all extracts, except for the methanol extract from *Senna alexandrina*, were free of tannins.

Variations in the polyphenolic and flavonoid content of this studied species compared to values reported in previous literature could be linked to factors such as geographical locations and the climate conditions under which the plant grows (Khurm et al., 2020). Several researchers have noted a significant correlation between phenolic content and the antioxidant activity of extracts (Roy & Dutta, 2021). Despite their high phenolic content, these extracts exhibited little to no significant antiradical activity, indicating that the phytoconstituents present may lack potent antiradical properties. The study's calculated results for total flavonoid content in the solvents ethanol 96%, methanol, and ethyl acetate were 3.741%, 5.629%, and 7.492%, respectively. Similarly, the total phenolic content in ethanol 96%, methanol, and ethyl acetate solvents was recorded at 14.084%, 13.257%, and 12.007%, respectively (Ahmad et al., 2024a).

Table (1). Yields of crude extracts:

Plant	Yield (%)			
	N-Hexane	Chloroform	Ethyl acetate	Methanol
S. alexandrina	1.77	3.43	0.49	6.39

Table (2). Antimicrobial activity of extracts of *Senna alexandrina*

Botanical name	Extract	Inhibition zones diameter(IZD) in (mm)					
		B. subtitles.	*S.aureus*	*E.coli*	*P.aeruginosa*	*A.niger*	*C.albicans.*
S. alexandrina	N-hexane	NA	13 ± 0.1	11 ± 0.1	11 ± 0.4	17 ± 0.6	12 ± 0.8
	Chloroform	11 ± 0.3	NA	NA	NA	18 ± 0.6	NA
	Ethyl acetate	NA	NA	NA	NA	NA	NA
	Methanol	NA	11 ± 0.1	13 ± 0.8	11 ± 0.4	17 ± 0.8	25 ± 1.4
Gentamicin*	10µg/disc	23±0.01	28±0.01	23±0.02	23±0.0	-	-
Nystatin*	10µg/disc	-	-	-	-	22±03	20±.00

NA: not active,*positive control (10µg/disc). IZD (mm): >18mm:Sensitive: 14- 18mm: intermediate: <14mm: Resistant.

Table (3). Antioxidant Activities of *Senna alexandrina:*

Plant	Extract	RSA±SD(DPPH)
Senna alexandrina	N-h	3±0.04
	C-h	IA
	E-a	8±0.04
	MetH	56±0.01
Stander	PG	96±0.01

Key: IA= inactive, N-h: N- hexane, C-h: Chloroform, E-a: Ethyl-acetate, MetH: Methanol, PG: Propyl gallate.
SD: Standard Division,

Table (4). Total polyphenolic, flavonoids, and tannins contents in extracts of *Senna alexandrina.,*

Plant	Phenols (Y=0.005X+0.001) (mg GAE/g), R_2= 0.998	Flavonoids (Y=0.0012X+0.0958) (mg QE/g), R_2=0.0992	Tannin (Y=0.002X+0.590) (mg TAE/g), R_2=0.998
Hexane	0.00	262.33 ± 0.01	0.00
Chloroform	0.00	152.66 ± 0.11	0.00
Ethyl acetate	136.8 ± 0.03	499.33 ± 0.34	0.00
Methanol	78.0 ± 0.03	431.33 ± 0.06	14 ± 0.03

GAE: Gallic acid equivalent; QE: Quercitin equivalent; TAE: Tannic acid equivalent.

CONCLUSIONS

The extracts from *Senna alexandrina.,* displaying varying polarities, demonstrated diverse antimicrobial and antioxidant capabilities. The inhibitory zones varied depending on the type of microorganism being tested, with the extracts generally showing stronger antifungal activity than antibacterial. It is recommended to pursue further research to identify the specific phytochemicals responsible for these antimicrobial and antioxidant effects, alongside understanding their pharmacological mechanisms. Additionally, the potential for other beneficial biological activities such as anticancer, antimalarial, antiviral, and anti-inflammatory properties should be explored.

Duality of interest: The authors declare that they have no duality of interest associated with this manuscript.

Author contributions : Contribution is equal between authors.

Funding: No specific funding was received for this work.

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Al-Mukhtar Journal of Basic Sciences 23 (1): 29-36, 2025
Doi: https://doi.org/10.54172/h20j9r95

Research Article **Open Access**

Investigation of Pollen Morphology of *Malva* L. (Malvaceae) in Libya

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Received:
07 April 2025

Accepted:
28 April 2025

Publish online:
30 April 2025

Abstract

The *Malva* L. genus belongs to the *Malvaceae* Juss., which comprises about 40 species. This genus is represented by only six taxa in Libya, namely, *Malva aegyptia* L., *M. sylvestris* L., *M. verticillata* L., *M. nicaeensis* All., *M. parviflora* L. var. *parviflora* and *M. parviflora* L. var. *microcarpa* (Pers.) Loscos. In this study, the *Malva* L. species have been studied and investigated in terms of pollen morphology. Pollen morphology grains have been examined and studied in all taxa using a light microscope. Measurements of pollen diameter, pore diameter and arrangement, pollen shape, and spine features are recorded and comprehensively studied. Palynological results have revealed that pollen grains of *Malva* species are apolar, spheroidal, and polypantoporate. Pollen grains are characterized by spines, which are long, slender and pointed and/or short and pointed. Inter-spinal distance between apices was a remarkable feature that could be utilized with other features to demarcate species belonging to *Malva* L.

Keywords: Malva; Malvaceae; Pollen grains; Morphology; Taxonomy, Libya.

INTRODUCTION

The study of pollen morphology has gained importance owing to the crucial role it plays in the field of plant systematics. Many researchers have asserted the significance of using pollen morphology to delimit genera within different families (Bibi et al., 2010). Generally, when studying and investigating pollen grains, several characteristics must be examined and measured (Halbritter et al., 2018). These characteristics include polarity, size, shape, structure, and ornamentation. Pollen grains can be isopolar, heteropolar, or apolar. In terms of size, grains range between very small (< 10 µm), small (10-25 µm), medium (26-50 µm), large (51-100 µm) and very large (> 100 µm). The shapes of the pollen grains were determined based on the ratio between the length of the polar axis (P) and the equatorial diameter (E). Pollen grains can take several shapes such as preoblate, oblate, suboblate, oblate-spheroidal, spheroidal, prolate-spheroidal, prolate, and preprolate. The structure of pollen grains is distinguished by apertures (colpus and pores) and exine sculptures (Halbritter et al., 2018).

In plant systematics, pollen morphology has been regarded as a significant measure in plant taxonomy, as reported by systems such as Lindley, Fischer, and Erdtman (Perveen et al., 2007). The pollen morphology of different species belonging to the Malvaceae species has also been studied and investigated in several systems (El Naggar, 2004; Perveen et al., 2007; Arabameri et al., 2023;

Abdel khalik et al., 2021). According to(Culhane & Blackmore, 1988), Malvaceae can be divided into six pollen types based on pollen grain diameter, the number of apertures, and spinular morphology (Bibi et al., 2010). According to the results obtained in (Mallick, 2020), pollen grains of *M. alcea* are periporate, circular, with echinate exine ornamentation. On the other hand, pollen grains of *M. sida* are circular, large, polyporate, and have echinate exine ornamentation. However, no exact measurements were presented in this paper for pollen grain characteristics.In this study, six *Malva* taxa represented in Libya (Jafri & Ali, 1977) were investigated in terms of their pollen morphology. This study aims to provide a thorough understanding of pollen characteristics and point out their taxonomical implications in the field of plant systematics.

MATERIALS AND METHODS

Study area

This study was conducted in the north-eastern region of Libya. Localities and their specific locations are listed in Table 1.

Collection of plants

Malva was collected during the spring seasons of 2018 and 2019. Of the 40 *Malva* species, only six taxa were present in Libya. The collected and identified *Malvas* are *M. aegyptia*, *M. sylvestris*, *M. nicaeensis*, *M. verticillata*, and *M. parviflora* (var. *parviflora* and var. *microcarpa*). All species were preserved and deposited in the Silphium herbarium, at the Department of Botany, Faculty of Science, Omar AL-Mukhtar University, Albayda, Libya. Pollen grains of all the species under investigation were obtained from the preserved dry samples.

Preparation of Glycerin Jelly

The glycerin jelly technique is one of the most common techniques used for pollen investigation. Glycerin jelly was obtained following procedures described in the literature (Erdtman, 1952; Shaheen *et al.,* 2009). It consisted of 10 g of gelatin, distilled water (35 ml) placed taken into a beaker and heated up to 70-80˚ C at which point 10 g of gelatin was added. When the temperature increased, the solution became viscous, and 30 ml of glycerol was added and mixed with the solution. Finally, 0.1% safranin stain was added and it was approximately $1/8^{th}$ of the volume of the glycerin jelly solution. The whole solution was stirred up until a uniform dark pink color was obtained.

Mounting of pollens

To make it possible to examine pollen grains of the collected species, pollen grains were embedded in glycerin jelly and mounted on microscopic slides. Firstly, the pollen grains of each species were placed on a clean slide by gently tapping the anthers. Drops of the prepared glycerin jelly with 0.1% safranin were placed on pollen grains, which were then mixed together. A cover slip was carefully placed to avoid the formation of air bubbles. The slides were then left to cool to room temperature. After cooling, the cover slip edges were sealed with nail polish.

An OPTECH (Optical Technology) light microscope, (Model B4, EXACTA+ by EXACT+OPTECH Germany), was used to investigate the prepared pollen grains. All measurements were taken for 10-15 pollen grains for each species. Images of pollen grains were captured using a Samsung Galaxy A6+ smartphone camera. Microscope magnification of 40x and smartphone camera magnifications of 4x, 5x, and 6x were used according to pollen grain sizes and clarity of samples.

Table (1). Localities and coordinates of *Malva* samples used in this study

Locality	Latitude	Longitude	*M. aegyptia*	*M. sylvestris*	*M. verticillata*	*M. nicaeensis*	*M. parviflora* var. *parviflora*	*M. parviflora* var. *microcarpa*	*M. parviflora*
Tobruk	32° 4' 29.982" N	23° 53' 6.9498" E		X			X		X
Al-Tamimi	32° 20' 0.528" N	23° 3' 42.1308" E					X		X
	32° 23' 26.7324" N	23° 3' 24.1380" E		X					
Martoba	32° 36' 10.0008" N	22° 45' 53.4954" E		X			X		
Shahat	32° 49' 23.2386" N	21° 51' 12.8376" E		X	X				
	32° 49' 57.4176" N	21° 51' 47.8326" E	X						
Alfaydiah	32° 42' 0.561" N	21° 54' 40.3092" E					X		
	32° 37' 19.2822" N	21° 54' 58.7658" E					X	X	
	32° 34' 11.0814" N	21° 54' 31.2042" E	X	X		X			
Solonta	32° 35' 29.4462" N	21° 43' 5.6274" E		X					X
Gandola	32° 32' 22.6026" N	21° 34' 36.8724" E		X			X		X
Albayda	32° 46' 7.8888" N	21° 44' 57.1668" E			X	X	X		X
	32° 45' 54.162" N	21° 45' 7.6032" E			X				
	32° 46' 13.4724" N	21° 47' 3.8214" E					X		
	32° 46' 28.4586" N	21° 44' 23.2686" E			X	X			
	32° 45' 48.1104" N	21° 43' 49.2666" E				X	X		X
	32° 45' 34.5636" N	21° 42' 38.2314" E		X					
Massa	32° 44' 55.1466" N	21° 37' 34.1394" E				X	X		X
	32° 44' 55.8162" N	21° 37' 34.8846" E					X		X
	32° 45' 8.3052" N	21° 37' 39.2298" E					X		X
Wadi Alkuf	32° 41' 41.6286" N	21° 33' 44.1498" E			X	X	X		
Marawa	32° 29' 48.069" N	21° 25' 21.1866" E	X	X					
Taknis	32° 27' 51.069" N	21° 8' 36.0312" E					X		
	32° 28' 47.7546" N	21° 7' 25.7988" E		X		X	X		X
Algarieb	32° 34' 11.2296" N	21° 10' 24.693" E		X		X	X		X
Almarj	32° 29' 34.5726" N	20° 49' 58.0038" E			X				
	32° 29' 36.6648" N	20° 49' 19.2432" E					X		X
	32° 29' 55.323" N	20° 49' 36.4728" E			X				
Tocara	32° 31' 55.9446" N	20° 35' 24.1872" E					X		X
	32° 29' 4.0878" N	20° 30' 40.8666" E		X			X		
Deriyana	32° 21' 38.6424" N	20° 18' 56.4078" E					X		X
Benghazi	32° 5' 8.9838" N	20° 3' 56.0046" E					X		X
	32° 3' 50.8458" N	20° 5' 45.7074" E					X		
Qaminis	31° 39' 18.7956" N	20° 1' 6.7152" E					X		X
	31° 39' 39.4128" N	20° 1' 8.9106" E					X		X

RESULTS

Light microscopy investigations of pollen grains of all studied species have shown similar structures with some differences in size and arrangement, as shown in Figures 1 to 6. According to quantitative and qualitative measurements summarized in Table 2, pollen grains are 58.7-133.4 μm in diameter, spheroidal, and polypantoporate. Pores are 1.4-4 μm in diameter and are sometimes in a spiral pattern. The exine thickness is 4.3-6.4 μm, whereas nexine is about two times thicker than sexine. Spines of all studied species were long, slender and pointed (6.8-11.2 μm) and/or short and pointed (2.5-4.1 μm), regularly and densely distributed. Under a light microscope, the pollen sexine sculpture was difficult to distinguish. However, in some pollen grains the sculpture appeared rugulose to perforate.

Table (2). Summary of pollen grain, shapes, and sizes

	M. aegyptia	*M. sylvestris*	*M. verticillata*	*M. nicaeensis*	*M. parviflora* var. *parviflora*	*M. parviflora* var. *micro-carpa*
Shape	Spheroidal	Spheroidal	Spheroidal	Spheroidal	Spheroidal	Spheroidal
Pollen Diameter (µm)	74.2(82.8)93.3	98.4(108.3)111.6	93.6(101.6)133.4	78.5(99.2)116.6	66.8(71.3)78.1	58.7(67.9)73.9
Pollen Class	Pantoporate	Pantoporate	Pantoporate	Pantoporate	Pantoporate	Pantoporate
Spine height (µm)	2.8(5.5)8.6	2.5(6.3)11.2	3.2(7.2)9.5	4.1(6.8)9.1	3.7(6)7.5	3.1(5.1)6.8
Spine width (µm)	2(3.1)4.2	2.4(3.3)4.6	2.2(3.2)4	2(3)4.1	1.8(2.4)3.1	1.8(2.4)3.4
Interspinal distance between apices (µm)	5.1(7.5)10.9	5.7(9.2)13.3	6.3(9.7)12.5	6.2(10.4)16	4.3(6.5)9.3	5(6.6)8.1
Pore diameter (µm)	2(2.9)3.8	1.4(2.4)3.3	2(3.1)4	2.1(2.8)4	2(2.6)3.4	1.5(2.3)3.2
Sexine thickness (µm)	1.5	2.0	2.0	2.1	1.8	1.7
Nexine thickness (µm)	3.0	4.4	3.5	2.5	2.5	3.1
Intine thickness (µm)	1.4	2.1	1.7	2.1	1.6	1.7

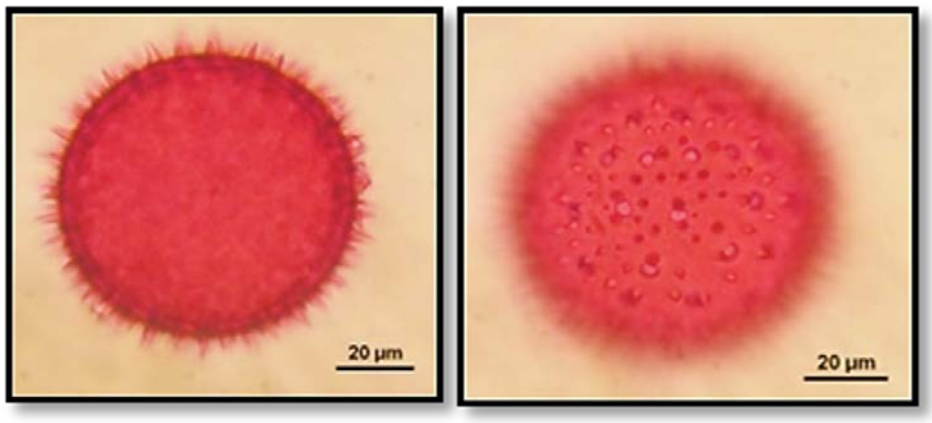
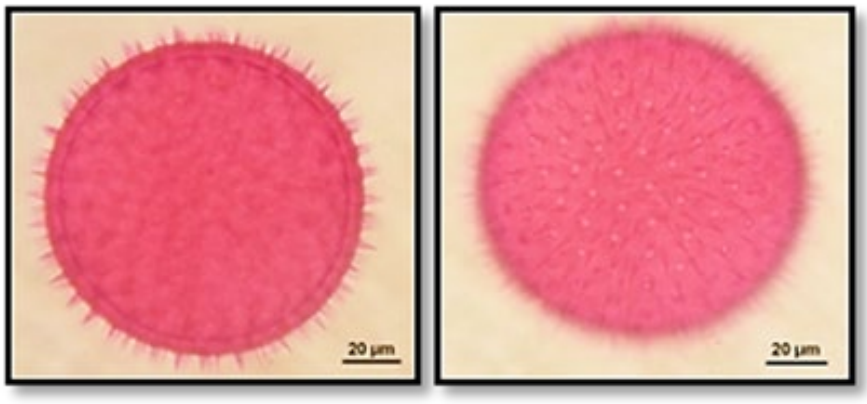


Figure (1). Pollen grains of M. aegyptia.,



F**igure (2).** Pollen grains of *M. sylvestris.*,

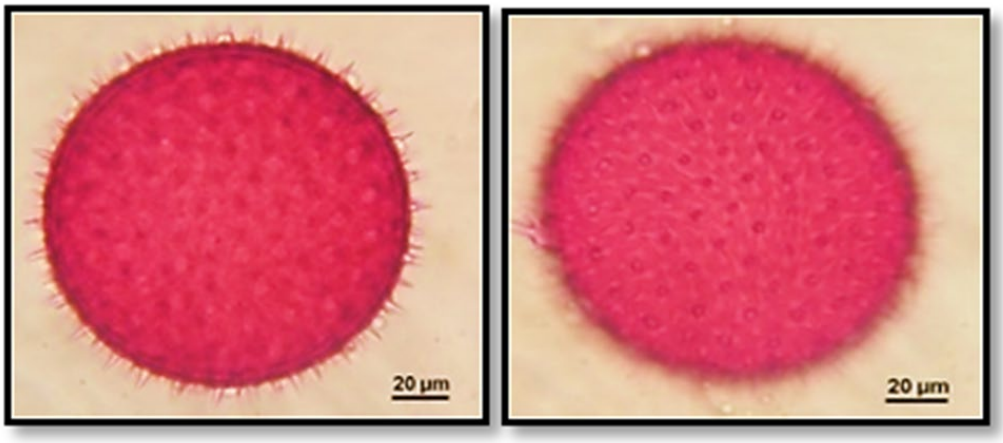


Figure (3). Pollen grains of *M. verticillate.*,

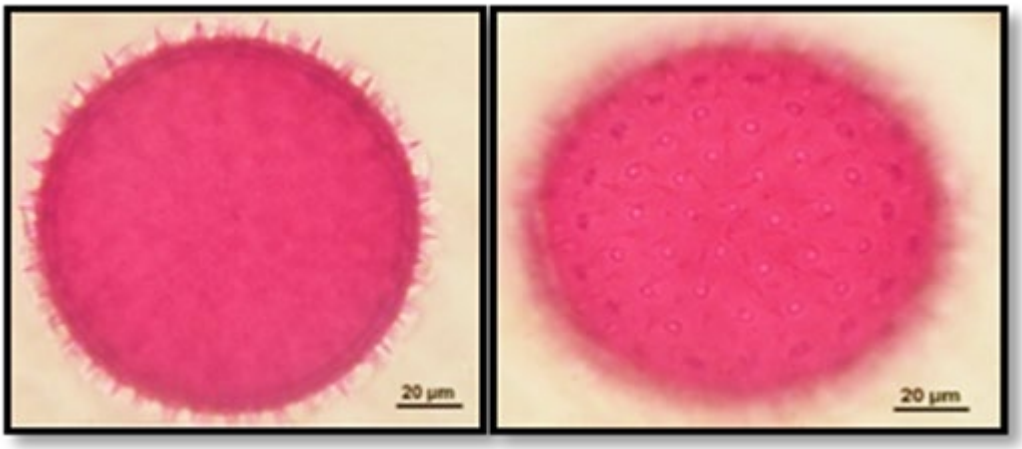


Figure (4). Pollen grains of *M. nicaeensis.*,

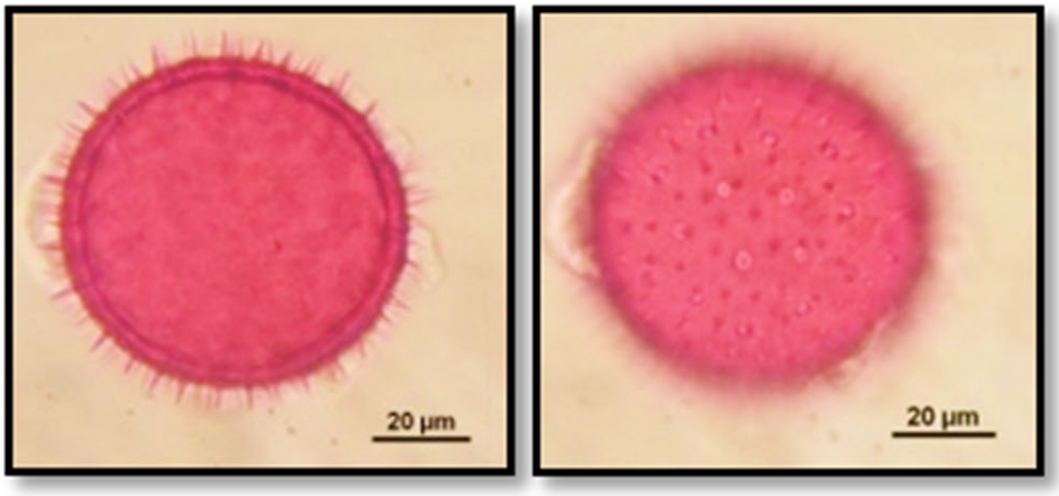


Figure (5). Pollen grains of *M. parviflora* var. *parviflora.*,

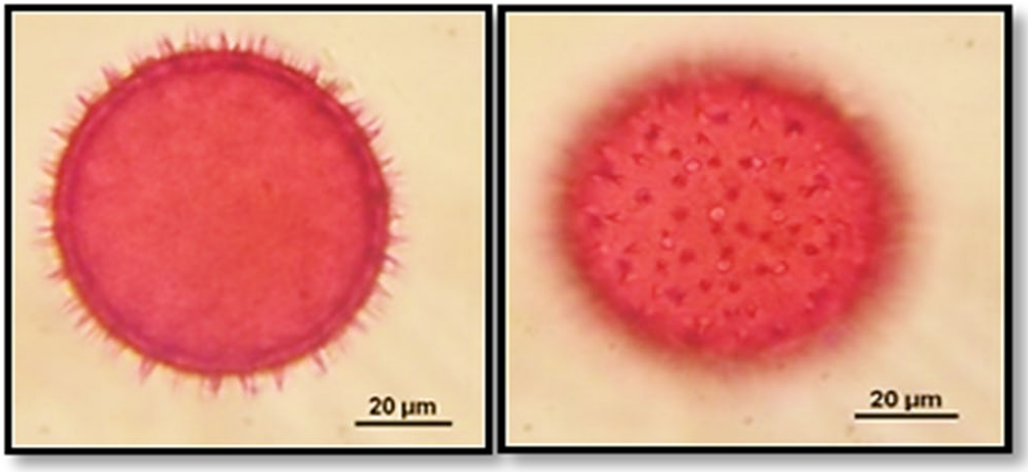


Figure (6). Pollen grains of *M. parviflora* var. *microcarpa.*,

DISCUSSION

The qualitative and quantitative data obtained for pollen morphology studies of all studied species are summarized in Table 2. Representative pollen grains are shown in Figures 1-6. These palynomorphological studies have confirmed that *Malva* is stenopalynous making the delimitation of different species quite difficult. The pollen grains are shown to be apolar, polypantoporate, and spheroidal. The size of the pollen varies considerably among pollen grains of the same species, as well as among pollen grains of different species. The smallest pollen size was reported in *M. microcarpa* of approximately 58.6 µm in diameter, whereas the largest size was reported in *M. verticillata* of approximately 133.4 µm in diameter. In agreement with (Shaheen et al., 2009 ; Abdel khalik et al., 2021), and despite the variations in pollen sizes, the present findings disagree with (Bibi et al., 2010) who state that pollen size is a reliable taxonomic tool for delimiting species. However, pollen size characteristics can be of taxonomic importance at the tribal level as reported by (El Naggar, 2004). Disagreement is extended to the study presented in (Arabameri et al., 2023), where the pollen size of *M. verticellata* was reported to be the smallest among the studied *Malva* taxa.

Spines of pollen grains are regarded as remarkable characteristics of malvaceous pollens (El Naggar, 2004; Shaheen et al., 2009). The spines show reliable variations in size, shape, and surface distribution (El Naggar, 2004). Spine height and width in the present study showed variations among pollens of the studied species. Spine height ranges from 2.5-11.2 µm in species of interest, which agrees with measurements reported in (Arabameri *et al.*, 2023; Abdel khalik et al., 2021; Shaheen et al., 2009). These values of spine height in our results disagree with those of (Perveen et al. 1994), who emphasized that spine height is strictly less than 7µm, which is considered as a significant delimiting feature of pollens of *Malva* species. In addition to spine height, the interspinal distance between apices is found to be a distinguishing feature of pollen grains of *Malva* species. It ranges between 4.3 µm and 16 µm. According to the data in Table 2 the studied species can be categorized into two main groups. Group I: species with an interspinal distance more than 12 µm, and Group II: species with an interspinal distance less than 12 µm. Group I contained *M. nicaeensis*, *M. sylvestris*, and *M. verticillata*, whereas Group II comprised *M. aegyptia*, *M. parviflora*, and *M. microcarpa*. Thus, the interspinal distance between apices features combined with spine height characteristics can be useful in delimiting species of *Malva*.

Pollen exine thickness varies greatly in the studied taxa and ranges between 4.3µm and 6.4 µm. This is due to variations in both sexine and nexine, which disagrees with (Christensen, 1986) who

states that sexine is usually of constant thickness in Malvaceae whereas nexine is of variable thickness. Based on present results, sexine is variable in thickness and ranges between 1.5 μm and 2.1 μm and nexine ranges between 2.5 μm and 4.4 μm. The present findings support (El Naggar, 2004; Shaheen et al., 2009) who both reported the variations in exine thickness owing to variations in sexine and nexine thicknesses. Furthermore, sexine thickness was almost the same as the intine thickness for all studied species.

CONCLUSION

The pollen morphology of the studied taxa was thoroughly investigated, and the study concluded that these taxa were stenopalynous. This makes the demarcation of species belonging to this genus difficult. Based on palynological studies, pollen grains of the studied taxa are apolar, spheroidal in shape, and polypantoporate in arrangement. Pollen sizes vary within the same species as well as among different species. Spine height and interspinal distance between apices are of the most importance according to the conducted study. They can be used to delimit between *Malva* species. Pollen exine thickness varied remarkably in the studied plants. This variation is related to the variations in sexine and nexine thicknesses.

Duality of interest: The authors declare that they have no duality of interest associated with this manuscript.

Author contributions : Contribution is equal between authors.

Funding: No specific funding was received for this work.

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Al-Mukhtar Journal of Basic Sciences 23 (1): 37-42, 2025
Doi: https://doi.org/10.54172/kzzfqt07

Research Article **Open Access**

Phytochemical and antioxidant Analysis of the five genus *Mentha* in AL-Jabal AL-Akhder – Libya

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Received:
10 April 2025

Accepted:
29 April 2025

Publish online:
30 April 2025

Abstract

In this study, significant differences were observed between the antioxidant values statistically analyzed by the standard curve obtained using different concentrations of the Prussian blue method to the extent that the five species can be divided into four groups and it confirmed by the statistical method used (standard deviation). The first group with the highest antioxidant value is *M. pulegium* followed by *M.spicata*, which occupied the second position (second group). The third group *M .piperita*, The last position with the lowest value was represented by *M. aquatic* and *M .longifolia* (the fourth group). Chemical tests for the presence of some secondary metabolites revealed that. The presence of Tannins, Sterols and Flavonoids in different amounts in the five species.*M. aquatic* and *M. longifolia* lack the presence of alkaloids, while there is a negative result for saponins in *M. longifolia*.

Keywords: Antioxidant; Chemical Taxonomy ; *Mentha*; Phytochemical.

INTRODUCTION

Mentha L. is the most important genus of the Lamiaceae family because there are many species that produce economically valuable essential oils. This genus is the second most oil-producing species after *Citrus* L (Mucciarelli, et al., 2001), and is of great medicinal and commercial importance. Typically, mint plants' leaves, flowers, and stems are utilized. These parts are commonly employed in herbal teas or as an addition to spice blends for various foods to enhance their flavor. Furthermore, mint is widely recognized as a popular remedy for conditions such as anorexia, flatulence, bronchitis, nausea, ulcerative colitis, and liver diseases. Among the numerous mint species, *M. piperita*, *M. spicata* and *M. Canadensis* are commercially the most significant. (Bhat, et al., 2002) Among these species, they are cultivated only for oil production. Pepper mint essential oil is one of the most popular and used essential oils, especially for its menthol, menthone and Carvone content. Wild mint is the best source of natural menthol. It is used to flavor pharmaceutical and oral preparations, such as toothpastes, creams, and mouthwashes. Its pleasant taste makes it an excellent gastric stimulant. Fresh and dried mint plants are widely used in various applications. Since ancient times, Eastern and Western cultures have used mint as a medicinal and aromatic plant. In terms of biological uses, mint is also used as an antimicrobial and antioxidant agent. Due to its antioxidant, antiradical, and chelating properties, the inclusion of mint in foods can help maintain the balance of the

redox state in the body and improve the safety and the effect on human welfare. Mint is widely used in cosmetics and soaps (Hajlaoui, et al., 2009). In addition, essential oil is used for its carminative, hepatoprotective, antiviral, and anticancer properties.

The antimicrobial properties of essential oils have found applications in various fields, including food preservation, pharmaceuticals, alternative medicine, and natural therapies. This is particularly significant due to the increasing prevalence of antibiotic-resistant bacteria and the limitations of traditional food preservation. Essential oils have demonstrated potent activity against a wide range of microorganisms, including both gram-positive and Gram-negative bacteria, as well as fungi and human pathogens. For instance, they have shown effectiveness against highly resistant strains of bacteria such as *Escherichia coli, Streptococcus pneumoniae, Salmonel laenteritidis* and *Salmonella typhi, which* often exhibit resistance to synthetic drugs. Furthermore, essential oils have exhibited significant antifungal properties, particularly against *Candida albicans* and dermatomycetes. Numerous laboratory studies have confirmed the antimicrobial activity of various *Mentha* species, both cultivated and wild.

MATERIALS AND METHODS

The plant species used in this research were collected from different areas in Al-Jabal Al-Akhder of Libya. They were identified in the Silphium Herbarium Department of Botany, Faculty of Science, Omer AL-Mukhtar University.

Table: (1). Location and details of the populations of *Mentha* Species studied

Scientific name	Common name	Collection site	Collection date
M. aquatica L.	Water mint	Wadi Darna	2022-5-24
		Al-Wasita	2021-12-29
M. pulegium L.	Wild mint	Ain Al-dabosia	2022-6-11
M. spicata L.	Nanaa	Wadi Darna	2022-4-28
M. piperita L.	Magriby mint	Wadi Darna	2022-4-28
		Al-Wasita	2021-12-29
M. longifolia L.	Saudi mint	Al-Bayda	2022-5-26

The fresh samples were stretched between newspaper sheets and pressed inside a herbarium press, and allowed to dry. To avoid rotting of the plant material, the sheets were changed after two days from the time of collection. The samples were then mounted, labeled, and deposited with the other species at the Herbarium of the Botany Department.

Plant specimens were identified by comparison with the description mentioned in Libyan flora books under the supervision of the Silphium Herbarium team at Omar Al-Mukhtar University.

Determination of Antioxidant Capacity by Prussian Blue Method

One gram of the powdered sample was dissolved in ether oil. The dissolved powder is then extracted by mixing twice with 10 ml of methanol, then with 10 ml of 1% hydrochloric.

Acid: methanol (v/v). The three combined extracts were subjected to evaporation under reduced pressure. The resulting residue was subsequently dissolved in 10 ml of methanol. Half a milliliter of the solution was diluted with 3 ml of distilled water, 3 ml (0.008 M) $K_3Fe(CN)_6$ (potassium ferric cyanide), 3 ml of 0.1 M HCl, and 1 ml of 1% $FeCl_3$ (ferric chloride). The blue dye was allowed to develop for five mined the absorbance is measured at 720 nm against the blank (Wangensteen, et al., 2004). A standard curve was prepared using different concentrations of ascorbic acid. Samples dilution was required in some samples before treatment with the Prussian blue reagent.

Phytochemical Analysis

Preparation of Crude Plant Extracts

The leaves of the studied plants were separated and washed several times with distilled water. The samples were then dried in a dark and dry place. Then the samples were ground by mortar and stored in polyethylene bottles until analysis.

The crude plant extracts were prepared by dissolving 100g each of the samples separately in 500 ml of successive solvents with increasing polarities (chloroform, ethanol, and ethyl acetate).

The plant materials were soaked overnight in the solvent, filtered and evaporated to dryness under reduced pressure in a rotary evaporator. The extracts were then evaporated and weighed. Three replicates were used for each sample.

Phytochemical Screening of Extracts

All the phytochemical screening tests were carried out according to standard methods. The methods are described as follows:

Chemical Tests

Preliminary screening for the major classes of secondary metabolites was conducted according to the techniques described by (Harborne, 1973).

1) Tannins

One ml of the reagent 1% $FeCl_3$ (ferric chloride solution) was added two ml of the ethanol extract were taken in a test tube. Blue color develops in cases of the presence of tannins.

2) Saponins:

Five ml of tap water is added to 1 ml of each extract, then shaken vigorously for five minutes, a froth develops having 1cm high and persists for 15 minutes indicating the presence of Saponin.

3) Sterols

One ml of the chloroform extract of each sample with 0.3 ml of acetic anhydride was mixed, and then a few drops of concentrated sulfuric acid were added along the side of the dry test tube. A reddish-violet color is produced at the junction of the two layers, and chloroform solution acquires a green color in case of the presence of sterols.

4) Flavonoid glycosides

The extracts (alcohol and aqueous) of the tested herbal plants were further extracted with 1% hydrochloric acid. Each extract was subjected to the following test: 10 mL of each extract was rendered alkaline, where a faint yellow color is produced in the case of the presence of flavonoids.

Two ml of the extract is mixed with 0.2 ml ethanolic-naphthol (2%), and 2 ml of concentrated sulfuric acid is added to the side of the dry test tube. A violet ring is observed at the junction of the two layers, indicating the presence of glycosides.

5) Alkaloids

The extracts of the tested herbal plants were further extracted with 20 ml of dilute hydrochloric acid, cooled and rendered alkaline with dilute ammonium hydroxide solution, then extracted with chloroform. The chloroform extract is subjected to the following tests:

Dragendorff: The preparation of the reagent:

Solution a. Solution a. Basic bismuth nitrate was dissolved in a mixture of 10 ml of acetic acid and 40 ml of distilled water.

Solution b. About (8g) of potassium iodide was dissolved in 20ml water.

Stock solution: Equal volumes of solutions (a) and (b) are mixed.

A few drops of chloroform extract were applied to filter paper, allowed to dry, and sprayed with the reagent orange color is observed in cases of the presence of alkaloids.

RESULTS

Antioxidants Determination

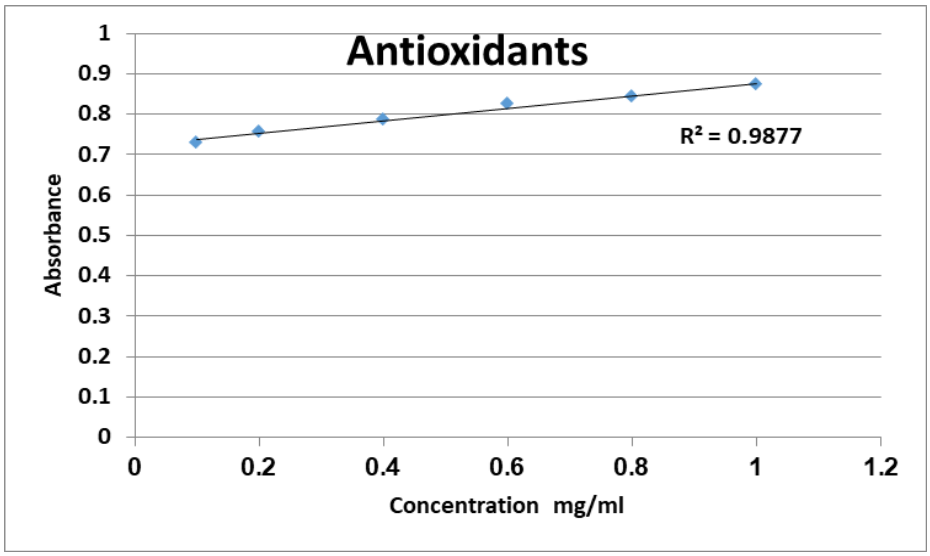

Fig:(1) . Standard Curve for the Estimation of Aantioxidant (Conc.mg/ml aginst Absorbance nm)

Significant differences were observed between the antioxidant values statistically analyzed by the standard curve obtained using different concentrations of the Prussian blue method (fig10) , (Table7), to the extent that the five species can be divided into four groups as and it is confirmed by the statistical method used (standard deviation).

1-The first group with the highest antioxidant value is *M. pulegium*
2-Followed by *M. spicata* which occupied the second position (second group).
3-The third group *M. piperita*.
4-The last position with the lowest value was represented by *M. aquatic* and *M. longifolia* (the fourth group).

Table: (2). Levels of Antioxidants in Samples of *Mentha* Species (Mean ±SD)

Samples	Mean ±SD
M. aquatica	2.13400 ± 0.01493 ^{C}
M. piperita	2.2223 ± 0.0252 ^{BC}
M. pulegium	2.8443 ± 0.0403 ^{A}
M. longifolia	2.1477 ± 0.0573 ^{C}
M. spicata	2.3363 ± 0.0677 ^{B}
Standerd	0.80200 ± 0.00784 ^{D}

Values are expressed as means ± SD; each sample group consisted of three replicates. Mean values within the same column that do not share the same superscript letter (a, b, c, d) were statistically significantly different from each other with $p<0.05$.

Phytochemical Analysis:

Chemical tests for the presence of some secondary metabolites revealed that:
The presence of Tannins, Sterols and Flavonoids in different amounts in the five species.

M. aquatic and *M. longifolia* lack the presence of alkaloids, while a negative result for saponines is found in *M. longifolia*.

Table: (3). Levels of Chemical Tests in samples of *Mentha* Species

Mentha Spp	Tannins	Saponines	Sterols	Flavonoids	Alkaloids
M. aquatic	+	+ +	+ +	+ + +	-
M. piperita	+ +	+ +	+ + +	+ +	+
M. pulegium	+ + +	+ + +	+ +	+	+
M. longifolia	+ +	-	+ + +	+	-
M. spicata	+	+	+ + +	+ + +	+ + +

DISCUSSION

In this study, all essential oils derived from peppermint exhibited strong antioxidant properties. This high antioxidant activity can be attributed to the elevated levels of oxygenated mono terpenes present in all peppermint species. The difference in antioxidant activity found between species reflects differences in oil composition. The substance that played a major role in changing the antioxidant activity among the students was menthol. However, the lack of a significant relationship between the amount of other components and the antioxidant activity does not eliminate the antioxidant capacity, since its biological activities are well established. Therefore, it is often the secondary components, rather than the primary components of essential oils, that contribute significantly to their antioxidant activity. Furthermore, the synergistic interaction between small and large components within the oil can enhance its overall antioxidant potential. (Benabdallah, et al., 2018).Many medicinal plants, such as those belonging to the genus *Mentha*, are rich in antioxidants like ascorbic acid, phenolic compounds, and carotenoids. These compounds have the ability to delay or prevent cellular aging processes (Park et al., 2019). Phenolic compounds, in particular, function as free radical scavengers and inhibit lipid peroxidation (Rice-Evans et al., 1997).

The results obtained for the chemical presence of secondary metabolites provided different points of support for both the similarities and differences among the species within the group. Tannins are present in the species. Identified a variety of plant sources for traditional tanning materials, including oak, willow, chestnut, sumac, alder, birch, hemlock, barberry, heather, bloodroot, alfalfa ,tea, sweet galls, and certain ferns. Saponins, however, were not found in *M. longifolia*. In contrast, saponins are prevalent in many desert plants as reported by (Sparg et al., 2004; Alaila et al., ;2017) further confirmed the presence of saponins in three species of Lamiaceae. (Kambouche.et al., 2009) specifically analyzed Anabasis articulata and concluded that the saponins play a crucial role in blood sugar regulation.

The absence of detectable saponins in the two species examined in this study may be attributed to their low concentration. Flavonoids are present in different concentrations. The highest concentration was observed in *M. aquatica* and *M. spicata*; followed by *M. piperita*, while the lowest concentrations were detected in *M. pulegium* and *M. longifolia*. It has also been reported that, in general, a specific group of flavonoids (Malkin & Rabinowitz 1967) characterizes each of the species.

CONCLUSION

According to the results recorded in this study for the antioxidants, Phytochemicals in five species of Mentha, the difference in antioxidant activity found between species reflects differences in oil composition. Also, the results obtained for the chemical presence of secondary metabolites

provided different points of support for the similarities and differences regarding the species of the group, for example, Flavonoids are present in different concentrations. The highest concentration was observed in *M. aquatica* and *M. spicata*; followed by *M. piperita*, while the lowest. Concentrations were detected in *M. pulegium* and *M. longifolia*.

Duality of interest: The authors declare that they have no duality of interest associated with this manuscript.

Author contributions : Contribution is equal between authors.

Funding: No specific funding was received for this work.

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