INSIGHTS-JOURNAL OF
LIFE AND SOCIAL SCIENCES

INSIGHTS-JLSS
Insights-Journal of Life and Social Sciences

ANTIOXIDATION AND PHYTOCHEMICAL ANALYSIS OF MINT, FENNEL SEEDS AND CARDAMOM; AN APPROACH TOWARDS SYNERGISTIC POTENTIAL

Original Article

Unaiza Tahir[1]*, Muhammad Asim Raza Basra[1]
[1]School of Chemistry, University of the Punjab Lahore, Pakistan.
Corresponding Author: Unaiza Tahir, School of Chemistry, University of the Punjab Lahore, Pakistan, unaizatahirchohan@gmail.com

Conflict of Interest: None Grant Support & Financial Support: None

Acknowledgment: We sincerely acknowledge the support and guidance of all contributors who made this research possible.

ABSTRACT

This research was conducted to evaluate the combined antioxidant potential of *Mentha piperita leaves, Foeniculum vulgare seeds, and Elettaria cardamomum cloves*. The study was designed to determine whether the mixture of these herbs would enhance or reduce their antioxidative effectiveness. Antioxidants are known to protect cells from oxidative stress caused by free radicals, which can damage cellular components. In this study, extracts of mint (*Mentha*), fennel (*Foeniculum*), and cardamom (*Cardamomum*) were prepared individually, and a combined extract was also made by boiling all three herbs together in the particular ratios as used in kehwa. These extracts were tested using standard antioxidant assays, including DPPH, ABTS, FRAP, and Phosphomolybdenum free radical scavenging methods, to measure their antioxidant capacities. It was found that the combined extract showed greater antioxidant activity than the individual extracts of mint leaves and fennel seeds but was less effective than cardamom alone. To further understand this result, FTIR (Fourier Transform Infrared Spectroscopy) analysis was carried out to identify chemical compounds present in the extracts. It was observed that certain compounds found in cardamom were missing in the combined extract. Additionally, phytochemical analysis was performed, and it suggested that alkaloids present in cardamom cloves may have degraded or lost their activity when combined with mint leaves and fennel seeds. Based on these findings, it was concluded that cardamom should not be mixed with mint and fennel if the goal is to maximize antioxidant potential, as its effectiveness may be reduced in combination.

Keywords: Antioxidant Potential, DPPH assay, ABTS assay, FRAP assay, Phosphomolybdenum assay, Mint, fennel, cardamom

© 2025 et al. -Health And Research Insights-Open access under CC BY License (Creative Commons). Freely distributable with appropriate citation

INTRODUCTION

The rise of photosynthetic organisms like blue-green algae and plants led to oxygen buildup in the atmosphere and oceans, shifting Earth's environment from reducing to oxidizing [1]. This transformation posed a challenge for early life to develop antioxidant defenses but also offered the opportunity to harness oxygen for energy and biosynthesis [2]. These changes drove evolutionary diversification and the emergence of aerobic life[3].Aging and many diseases are linked to reactive oxygen species (ROS) and free radicals, which are unstable molecules that can damage cells, proteins, and DNA. This oxidative stress disrupts normal cellular functions, contributing to conditions like cancer, and neurodegenerative disorders. Over time, the accumulation of this damage accelerates aging and disease progression [4, 5]. There is also large data showing that oxidative stress is linked to complex systemic pathologies such as endometriosis, where dysregulated neurotrophins cause damage to multiple organs, further highlighting the widespread effects of free radicals that extend beyond the classical systems of focus [6].

Understanding how free radicals function is essential for developing effective nutritional strategies to combat the harmful effects of space radiation. The body naturally generates free radicals—such as superoxide, nitric oxide, and hydroxyl radicals—as well as other reactive species like hydrogen peroxide, peroxynitrite, and hypochlorous acid, mainly through aerobic metabolism[7, 8]. Free radicals are extremely reactive chemicals that can cause considerable damage to biological components, including proteins, lipids, and DNA. Free radicals cause structural alterations in proteins, which results in the creation of carbonyl compounds [9]. These carbonyl compounds are well known for being accurate indicators of oxidative stress and protein oxidation in the body [10]. Recent observations indicated that oxidative imbalance, including that created by trace mineral deficiencies such as zinc, can also modify gut microbiota activity and antioxidant enzyme pathways, highlighting the metabolic dependencies that underlie the process of oxidative damage management [11]. Medical disorders include ischemia (limited blood flow) and brain damage have been closely linked to free radical activity. The abrupt return of blood flow during ischemic episodes may trigger a surge in the generation of free radicals, which can cause additional tissue damage [12]. This phenomenon is referred to as reperfusion injury[13]. Excessive oxidative stress in the brain has been connected to neurodegenerative conditions including Parkinson's and Alzheimer's[13].

Oxidation of proteins can disrupt cellular processes and play a role in the emergence of a number of illnesses [14]. *Mentha piperita, Foeniculum* vulgare, and *Elettaria cardamomum* are medicinal herbs widely recognized for their potent antioxidant properties and have been traditionally used in various cultures, especially in Asia. A notable example is a herbal infusion known as "Kehwa," which combines these three herbs to address gastrointestinal issues and promote overall wellness [15]. The phytochemical analysis of traditional medicines such as Caralluma tuberculata revealed that natural extracts elicit a variety of bioactive constituents including antioxidant and antibacterial properties [16].

Mentha piperita (commonly known as peppermint) is well-known for its refreshing flavor and therapeutic qualities. It contains bioactive compounds such as menthol, flavonoids, and rosmarinic acid, which contribute to its strong antioxidant capacity [17]. These constituents help enhance the body's defense mechanisms by neutralizing harmful free radicals, thereby protecting cells from oxidative stress and associated damage [18].*Foeniculum vulgare*, or fennel, is another valuable herb that has been used both in culinary and medicinal practices. Rich in phenolic compounds like anethole and flavonoids, fennel exhibits significant anti-inflammatory and antioxidant effects [19]. Its ability to scavenge free radicals contributes to reduced cellular inflammation and supports digestive health, making it a common ingredient in herbal remedies for bloating and indigestion [20, 21]. Recent developments in personalized medicine have demonstrated the role of biochemical profiling using salivary diagnostics and the importance of phytochemical compounds in safe-based therapeutics. These observations help clinical translation of classical herbal antioxidants [22].

Elettaria cardamomum, known as green cardamom, is an aromatic spice that also functions as a medicinal herb [23]. It contains essential oils such as 1,8-cineole and terpinene, along with polyphenols that exhibit antioxidant, antimicrobial, and anti-inflammatory propertie [24, 25]. Cardamom is often used to alleviate symptoms of nausea, improve metabolic function, and enhance oral and digestive health [26]. Moreover, the rising popularity of natural immunity and wellness, especially after COVID-19, has drawn more attention to herbal remedies, indicating an evolution in community health behavior and a necessity to develop and conduct more phytochemical validation work [27].

To evaluate the antioxidant potential of these herbs, several in vitro assays are employed. These include the Phosphomolybdenum assay, which measures the total antioxidant capacity by quantifying the reduction of molybdenum (VI) to molybdenum (V) and DPPH assay which measures antioxidant activity by quantifying the ability of a compound to scavenge or reduce the stable free radical DPP [27, 29]. The ABTS assay, is also subjected which assesses the ability of antioxidants to scavenge ABTS•+ radicals; and the FRAP (Ferric

© 2025 et al. -Health And Research Insights-Open access under CC BY License (Creative Commons). Freely distributable with appropriate citation.

Reducing Antioxidant Power) assay, which estimates the reducing potential of antioxidants by converting ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions [28, 30, 31]. These methods provide a quantitative basis to compare and validate the antioxidant activity of herbal extracts [28]. In summary, the combination of *Mentha piperita, Foeniculum vulgare*, and *Elettaria cardamomum* in traditional beverages like kehwa is rooted in their individual and synergistic antioxidant properties.

MATERIAL AND METHODS

Extract formation

Two grams of dried powder from each individual herb were accurately measured and taken separately. Each sample was added to 500 milliliters of distilled water in a clean beaker and subjected to boiling for a sufficient period to ensure thorough extraction of active constituents. Following the boiling process, the mixtures were allowed to cool slightly and then filtered carefully using appropriate filter paper to remove solid residues. The filtrates were further boiled to reduce their volume significantly, concentrating the extracts. The concentrated solutions were then transferred into clean, labeled Petri dishes and left to air dry at room temperature until solid residues were obtained. For the preparation of the combined extract, dried powders of Cardamom, Mint, and Fennel seeds were mixed thoroughly in a ratio of 2:1:0.5 by weight. This mixture underwent the same extraction process: boiling in 500 milliliters of distilled water, filtration, and volume reduction by boiling, and air drying in Petri dishes to yield the final solid extracts.

Control Formation

A 2000 mM stock solution of ascorbic acid was prepared by dissolving 3.52 g of ascorbic acid in 10 mL of distilled water. This solution served as the control standard in all subsequent assays [32]. From the stock, a series of ten two-fold serial dilutions were prepared by the half-dilution method, where each subsequent dilution was obtained by mixing equal volumes of the preceding solution with distilled water, thereby halving the concentration at each step. As a result we had a series of 2000mM, 1000mM, 500mM, 250mM and so on solutions of ascorbic acid [33]. These dilutions were used to evaluate the concentration-dependent effects of ascorbic acid and to serve as a reference for comparing the antioxidant or bioactive potential of the herbal extracts under study. All dilutions were freshly prepared before being used in antioxidation assay.

Preparation of Serial Dilutions of Herbal Extracts

To assess the bioactivity of herbal extracts, a series of ten serial dilutions were prepared for each extract. Initially, 10 g of the herbal extract was accurately weighed and dissolved in 5 mL of distilled water to obtain the stock solution with a concentration of 2 g/mL (2000 mg/mL). This solution was designated as the first dilution. Subsequent dilutions were prepared using a 2-fold serial dilution method, in which equal volumes (1:1 ratio) of the preceding dilution and distilled water were mixed to reduce the concentration by half at each step [34]. As a result, the second dilution had a concentration of 1 g/mL, the third was 0.5 g/mL, the fourth was 0.25 g/mL, and so on, continuing until the tenth dilution. All dilutions were freshly prepared prior to being used in antioxidant assays. As a result 4 dilution sets each having 10 dilutions of cardamom, fennel seeds, mint and combined extract were formed.

DPPH Assay

In this experiment, 0.02 g of DPPH (2,2-diphenyl-1-picrylhydrazyl) was dissolved in 30 mL of ethanol to prepare a 50 mM solution. Taking into account DPPH's sensitivity to light, to prevent photo degradation, the DPPH solution was stored in an aluminum foil-wrapped beaker and all the procedure was carried out under low-light conditions. Five sets of dilutions were prepared already. Each dilution, was in dark glass vials to minimize light exposure, received 0.1 mL of the prepared DPPH solution [35]. The mixtures in each set were subjected to the DPPH assay independently to assess the antioxidant activity of the extracts, as DPPH decolonization indicates free radical scavenging potential. The change in color of the DPPH solution in each dilution was measured using a Stalwart STX 900 Xenon Lamp UV-Visible spectrophotometer set at a wavelength of 520 nm, at 10, 25, 40 and 55 minutes interval [36].

ABTS Assay

Both a 2.45 mM potassium persulfate solution and a 7 mM ABTS solution were made in ethanol. After mixing the two solutions in equal parts, they were left to incubate for 12 to 16 hours at room temperature. Following incubation, the solution was diluted until a reading of 0.7±2 was read with a spectrophotometer at 734nm [37]. ABTS solution was added to each dilution, and measurements were made. Every set of dilutions was measured at 734 nm with a UV-visible spectrophotometer at intervals of 10, 25, 40, and 55 minutes [38].

© 2025 et al. -Health And Research Insights-Open access under CC BY License (Creative Commons). Freely distributable with appropriate citation.

FRAP Assay

40ml of distilled water was combined with 0.4g of ferricyanide to form 1% potassium ferricyanide solution. A 10% solution of trichloroacetic acid was prepared by agitating 3.6g of the acid in 36ml of distilled water. 20 milliliters of distilled water were used to dissolve 0.02 grams of ferric chloride, yielding a 0.1% $FeCl_3$ solution. Two milliliters of each dilution were taken, and 2.5 milliliters of 1% potassium ferricyanide were then added. 50°C was the temperature at which the combination was incubated for 20 minutes. After allowing the combined dilutions to reach room temperature, they were centrifuged for 30 minutes at 3000 rpm. From each combination of dilution, 2.5 ml of supernatant was placed into vials.
Then, 2.5 milliliters of distilled water and 1 milliliter of a FeCl3 0.1% solution were added to each vial [39]. Every set of dilutions had its UV-visible spectrophotometer readings collected at 593 nm at intervals of 10, 25, 40, and 55 minutes.

Phosphomolybdenum Antioxidation Potential Assay

0.048g and 0.275g of each component were dissolved in 60ml of distilled water to create a 0.48mM solution of ammonium molybdate and a 28mM solution of sodium phosphate, respectively [40]. The reagent having equal parts of sodium phosphate, ammonium molybdate, and diluted H2SO4 was added to each dilution one by one of all the dilution sets. Each set of dilutions was incubated for 1.5 hours at 95°C. Readings from the UV-visible spectrophotometer were taken at 695 nm at 10, 25, 40, and 55-minute intervals [39, 41].

Phytochemical Analysis

The investigation of phytochemicals involved the identification of several substances. Using the Salkowski test, which revealed a reddish-brown layer at the contact, terpenoids were detected [42]. The ferric chloride test revealed the presence of tannins, giving rise to a greenish-black color [43]. By producing a magenta color, the Zinc-HCl reduction test showed the presence of flavonoids [44]. Benedict's test was used to identify reducing sugars, which produced a brick-red precipitate. By adding H2SO4 to the chloroform-extract mixture and causing it to turn red, steroids were found [45]. Using Wagner's technique, alkaloids were identified as a brown precipitate [46]. Ultimately, Fehling's technique was used to identify glucosides, and following heating, color changes were seen [47].

RESULTS

For each assay a graphical representation was created with % inhibition, calculated as %inhibition = (At/Ac - 1) × 100, on the Y-axis and the $\log_{10}$ of the concentration of dilution on the X-axis for each [48]. At each time interval, a graph was plotted, from which the IC_{50} value was determined. This procedure was repeated across ten different dilutions for each extract, and the corresponding IC_{50} values were obtained for each assay.

IC_{50} for DPPH Assay

The IC_{50} values of ascorbic acid, used as the control, show its strong antioxidant capacity. These values gradually decrease over time, from 0.011006 g/ml at 10 minutes to 0.010417 g/ml at 55 minutes, reflecting consistent and efficient free radical scavenging activity. Ascorbic acid serves as a reliable standard for comparing the antioxidant strength of the plant extracts tested in the DPPH assay (Table 1).

Among the herbal extracts, *Elettaria cardamomum* (Cardamom) showed the highest antioxidant activity, with the lowest IC_{50} values across all time intervals. *Foeniculum vulgare* (Fennel) displayed moderate activity, while *Mentha piperita* (Mint) had the weakest antioxidant potential, as indicated by its highest IC_{50} values. The combined extract of Cardamom, Mint, and Fennel demonstrated intermediate activity, showing a beneficial but not superior effect compared to Cardamom alone. All extracts exhibited a gradual decrease in IC_{50} values with time, indicating that their ability to neutralize free radicals improved as the reaction progressed (Table 2).

© 2025 et al. -Health And Research Insights-Open access under CC BY License (Creative Commons). Freely distributable with appropriate citation.

Table 1: Table 1 shows how the effectiveness of ascorbic acid (vitamin C) in neutralizing free radicals changes slightly over time, based on DPPH assay results. The IC_{50} values represent the concentration required to reduce the DPPH free radical activity by half—a common measure of antioxidant strength

IC 50 0f Control for DPPH (Ascorbic Acid)				
Concentration	**10 Min**	**25 Min**	**40 Min**	**55 Min**
mM	62.515	60.123	59.964	59.127
g/ml	0.011006	0.010589	0.010558	0.010417

Table 2: IC_{50} values of four herbal extracts in the DPPH assay at different time intervals, indicating their antioxidant activity. *Mentha piperita* (peppermint leaves), *Foeniculum vulgare* (fennel seeds), *Elettaria cardamomum* (cardamom cloves), and a combination of all three—measured at four time intervals using the all samples DPPH assay.

IC 50 of 4 extracts for DPPH assay (g/ml)				
Extracts	10 Min	25 Min	40 Min	55 Min
Mentha Piperita (leaves)	0.604	0.581	0.573	0.543
Foeniculum vulgare (seeds)	0.289	0.265	0.247	0.219
Elettaria cardamomum (cloves)	0.177	0.157	0.137	0.111
Combination of former 3	0.342	0.327	0.312	0.301

IC_{50} for ABTS Assay

The IC_{50} values of ascorbic acid in the ABTS assay decrease steadily over time, ranging from 3.10×10^{-5} g/mL at 10 minutes to 2.32×10^{-5} g/mL at 55 minutes. This gradual decline reflects the high and time-dependent antioxidant potential of ascorbic acid, making it a reliable positive control for evaluating the free radical scavenging activity of plant extracts. The lower the IC_{50} value, the stronger the antioxidant activity, and ascorbic acid demonstrates consistent efficiency throughout the tested time intervals (Table 3).

Among the tested extracts, *Elettaria cardamomum* (Cardamom) showed the strongest antioxidant activity in the ABTS assay, with exceptionally low IC_{50} values at all time points, decreasing from 0.000423 g/ml to 0.000243 g/ml. The combined extract of Cardamom, Mint, and Fennel also displayed significant antioxidant capacity, though slightly less than Cardamom alone. *Foeniculum vulgare* (Fennel) exhibited moderate activity, but an unexpected rise in IC_{50} to 0.568 g/ml at 55 minutes suggests a possible anomaly or experimental variation. *Mentha piperita* (Mint) had the weakest activity among the samples, with relatively high IC_{50} values throughout. Overall, all extracts showed a general trend of decreasing IC_{50} values over time, indicating enhanced radical scavenging with prolonged exposure (Table 4).

© 2025 et al. -Health And Research Insights-Open access under CC BY License (Creative Commons). Freely distributable with appropriate citation.

Table 3: This table shows the decreasing IC_{50} values of ascorbic acid over time, indicating its increasing antioxidant activity in the ABTS radical scavenging assay.

IC 50 0f Control for ABTS Assay (Ascorbic Acid)				
Concentration	**10 Min**	**25 Min**	**40 Min**	**55 Min**
mM	0.176	0.165	0.147	0.132
g/ml	3.10×10^{-5}	2.91×10^{-5}	2.59×10^{-5}	2.32×10^{-5}

Table 4: This table presents the antioxidant activity of *Mentha piperita*, *Foeniculum vulgare, Elettaria cardamomum*, and their combination, measured as IC_{50} (g/mL) over 10 to 55 minutes.

IC 50 of 4 extracts for ABTS Assay (g/ml)				
Extracts	10 Min	25 Min	40 Min	55 Min
Mentha Piperita (leaves)	0.468	0.421	0.373	0.333
Foeniculum vulgare (seeds)	0.0744	0.0721	0.0675	0.568
Elettaria cardamomum (cloves)	0.000423	0.000312	0.000302	0.000243
Combination of former 3	0.0521	0.0456	0.0421	0.0407

IC_{50} for FRAP Assay

The IC_{50} values of ascorbic acid in the FRAP (Ferric Reducing Antioxidant Power) assay show a clear decreasing trend over time, starting from 3.22×10^{-6} g/mL at 10 minutes to 2.08×10^{-6} g/mL at 55 minutes. This consistent decline highlights the strong and time-dependent antioxidant capacity of ascorbic acid, making it a reliable positive control in this assay. The lower IC_{50} values indicate higher efficiency in reducing ferric ions, confirming ascorbic acid's potent reducing power as a standard antioxidant agent (Table 5).

In comparison, the plant extracts exhibit varying antioxidant activities. *Elettaria cardamomum* (Cardamom) shows the most consistent and potent antioxidant potential, with notably low IC_{50} values throughout the time intervals, particularly at 40 minutes (0.0128 g/mL). *Mentha piperita* (Mint) displays moderate activity with a slight increase in IC_{50} at later times, suggesting a reduction in antioxidant effectiveness. *Foeniculum vulgare* (Fennel) shows more variability, with fluctuating IC_{50} values and the highest at 40 minutes (0.19558 g/mL), indicating weaker performance. The combined extract of all three plants demonstrates intermediate activity, with relatively stable IC_{50} values across time, suggesting a moderate and consistent antioxidant response (Table 6).

© 2025 et al. -Health And Research Insights-Open access under CC BY License (Creative Commons). Freely distributable with appropriate citation.

Table 5: This table presents the decreasing IC_{50} values of ascorbic acid over time, indicating its strong and time-dependent antioxidant activity in reducing ferric ions.

IC 50 0f Control for FRAP Assay (Ascorbic Acid)				
Concentration	**10 Min**	**25 Min**	**40 Min**	**55 Min**
mM	0.0183	0.0157	0.0145	0.0118
g/ml	3.22×10^{-6}	2.77×10^{-6}	2.55×10^{-6}	2.08×10^{-6}

Table 6: This table presents the antioxidant activity of *Mentha piperita, Foeniculum vulgare, Elettaria cardamomum*, and their combination over time. *Elettaria cardamomum* showed the strongest activity with the lowest IC_{50} values, while *Foeniculum vulgare* showed the weakest. The combination extract and *Mentha piperita* exhibited moderate activity.

IC 50 of 4 extracts for FRAP Assay (g/ml)				
Extracts	10 Min	25 Min	40 Min	55 Min
Mentha Piperita (leaves)	0.0231	0.0236	0.0261	0.0415
Foeniculum vulgare (seeds)	0.16726	0.1113	0.19558	0.1834
Elettaria cardamomum (cloves)	0.0186	0.0229	0.0128	0.0264
Combination of former 3	0.0494	0.0478	0.0505	0.0505

IC_{50} for Phosphomolybdenum Assay

The IC_{50} values of ascorbic acid in the phosphomolybdenum assay decrease gradually from 1.99×10^{-5} g/mL at 10 minutes to 1.87×10^{-5} g/mL at 55 minutes. This steady reduction indicates strong and consistent antioxidant activity, confirming ascorbic acid as a reliable positive control in ferric ion reduction. The minimal variation in values across time points reflects its stability and efficient performance throughout the assay duration (Table 7).

In the phosphomolybdenum assay, *Elettaria cardamomum* showed the strongest antioxidant activity, with very low IC_{50} values at each time point, especially at 40 minutes. *Foeniculum vulgare* displayed moderate effectiveness, while *Mentha piperita* had the weakest activity, with IC_{50} values remaining high throughout. The combination of all three extracts demonstrated improved performance compared to Mint and Fennel individually, suggesting possible synergistic interactions. IC_{50} values for most extracts remained relatively stable over time, except for a slight increase in Cardamom at 55 minutes (Table 8).

© 2025 et al. -Health And Research Insights-Open access under CC BY License (Creative Commons). Freely distributable with appropriate citation.

Table 7: The table shows a gradual decrease in IC_{50} values over time, indicating increasing antioxidant activity of ascorbic acid. It serves as a standard for evaluating total antioxidant capacity.

IC 50 of Control for Phosphomolybdenum antioxidant Assay (Ascorbic Acid)				
Concentration	**10 Min**	**25 Min**	**40 Min**	**55 Min**
mM	0.1132	0.1112	0.1099	0.1062
g/ml	1.99×10^{-5}	1.96×10^{-5}	1.94×10^{-5}	1.87×10^{-5}

Table 8: This table presents the antioxidant capacity of *Mentha piperita, Foeniculum vulgare*, *Elettaria cardamomum*, and their combination over time. *Elettaria cardamomum* showed the strongest activity with the lowest IC_{50} values, while *Mentha piperita* exhibited the weakest. The combination and *Foeniculum vulgare* extracts showed moderate antioxidant effects.

IC 50 of 4 extracts for Phosphomolybdenum antioxidant assay (g/ml)				
Extracts	10 Min	25 Min	40 Min	55 Min
Mentha Piperita (leaves)	0.7686	0.7534	0.7601	0.7535
Foeniculum vulgare (seeds)	0.1455	0.1266	0.1371	0.1287
Elettaria cardamomum (cloves)	0.0046	0.0018	0.0015	0.0312
Combination of former 3	0.0510	0.0496	0.0521	0.0456

FTIR Graph of Mentha piperita leaves extract

The FTIR spectrum of ***Mentha piperita*** **(Mint) leaves extract** reveals the presence of various functional groups, indicating the presence of bioactive compounds. A broad and strong absorption band around **3945 cm^{-1}** corresponds to **O–H stretching vibrations**, typically found in alcohols and phenolic compounds, which are common in mint due to its antioxidant content [49]. Peaks near **2920–2850 cm^{-1}** may indicate **C–H stretching** from alkanes [50]. A prominent peak at **1630–1580 cm^{-1}** is observed, which represents **C=C stretching** vibrations from aromatic rings or **C=O stretching** of conjugated ketones or flavonoids [51]. This suggests the presence of aromatic compounds and possibly aldehydes or ketones. The multiple peaks between **1500–1000 cm^{-1}** (e.g., at 1398, 1260, 1097, and 1066 cm^{-1}) likely correspond to **C–O stretching vibrations**, often associated with ethers, esters, or phenolic compounds [52].Smaller peaks around **800–600 cm^{-1}** are typically linked to **C–H bending** out-of-plane, found in aromatic compounds [53] (Fig. 1).

© 2025 et al. -Health And Research Insights-Open access under CC BY License (Creative Commons). Freely distributable with appropriate citation.

Sample ID:Mint
Sample Scans:32
Background Scans:32
Resolution:4
System Status:Good
Method Name:Chemistry
User:Chemistry
Date/Time:08/07/2023 10:26:45 AM
Range:4000 - 650
Apodization:Happ-Genzel
File Location:C:\Users\Public\Documents\Agilent\MicroLab\Results\\ftir results\Mint_2023-08-07T10-26-45.a2r

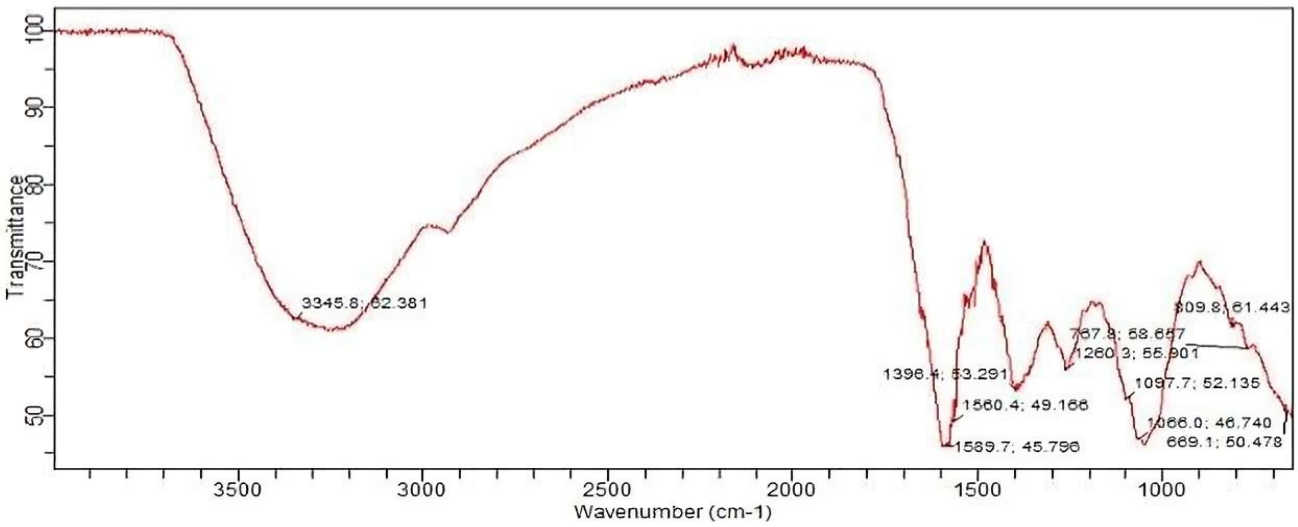

Figure 1: FTIR spectrum of Mentha piperita (Mint) leaves extract showing prominent peaks for O–H stretching, C=O stretching, and C–O bending, indicating the presence of phenolic compounds, flavonoids, and other oxygen-containing functional groups responsible for its antioxidant properties

FTIR Graph of Foeniculum vulgare seeds extract

The FTIR spectrum of Foeniculum vulgare (Fennel) seeds extract indicates the presence of various functional groups commonly found in antioxidant and phytochemical compounds. A broad absorption band around 3390.5 cm^{-1} corresponds to O–H stretching vibrations, suggesting the presence of hydroxyl groups typically found in alcohols and phenolic compounds [54]. The peak at 2924.1 cm^{-1} is attributed to C–H stretching, indicating the presence of aliphatic hydrocarbons such as fatty acids or essential oils [55]. In the fingerprint region, the peak at 1559.9 cm^{-1} may be due to C=C stretching vibrations in aromatic rings or N–H bending, which are common in flavonoids and other bioactive aromatic compounds [56, 57]. The peak at 1380.2 cm^{-1} corresponds to C–H bending, while those around 1103.3 cm^{-1} and 1046.5 cm^{-1} represent C–O stretching vibrations, often found in esters, ethers, or alcohols [52]. Additional peaks between 880 and 690 cm^{-1} indicate C–H out-of-plane bending, typically associated with substituted aromatic rings [58]. These spectral features collectively confirm the presence of functional groups that support the antioxidant and medicinal properties attributed to fennel seed extract (Fig. 2).

© 2025 et al. -Health And Research Insights-Open access under CC BY License (Creative Commons). Freely distributable with appropriate citation.

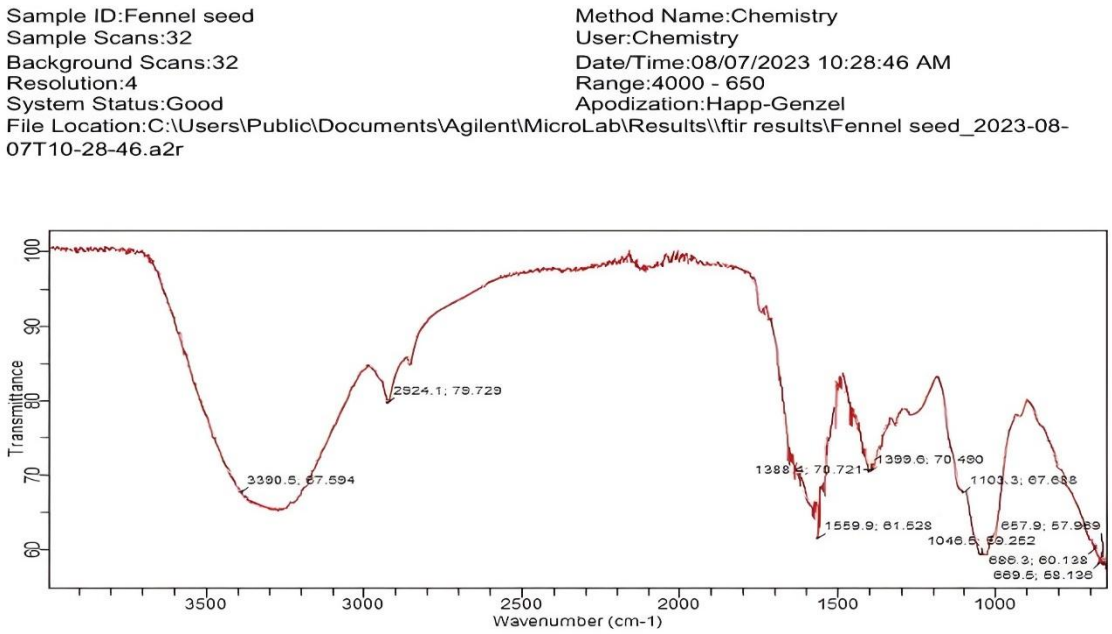

Figure 2: FTIR spectrum of *Foeniculum vulgare* (Fennel) seeds extract showing characteristic peaks for O–H, C–H, C=O, and C–O functional groups, indicating the presence of phenolic compounds, alkanes, and aromatic structures associated with antioxidant activity.

FTIR of Elettaria cardamomum cloves extract

The region near 2920 cm^{-1} usually corresponds to C–H stretching vibrations of alkanes, suggesting the presence of hydrocarbon chains, possibly from essential oils [59]. A peak at 1648 cm^{-1} corresponds to C=C stretching in aromatic rings or C=O stretching in conjugated systems, indicating the presence of compounds such as flavonoids or aldehydes [60]. Several peaks between 1500 and 1600 cm^{-1} suggest aromatic ring vibrations, further supporting the presence of polyphenolic compounds. The fingerprint region, especially between 1000 and 1100 cm^{-1}, shows strong absorptions attributed to C–O stretching, which can be related to alcohols, ethers, or esters [61] (Fig. 3).

© 2025 et al. -Health And Research Insights-Open access under CC BY License (Creative Commons). Freely distributable with appropriate citation.

Sample ID:2
Sample Scans:32
Background Scans:32
Resolution:4
System Status:Good
Method Name:Chemistry
User:Chemistry
Date/Time:08/07/2023 10:35:49 AM
Range:4000 - 650
Apodization:Happ-Genzel
File Location:C:\Users\Public\Documents\Agilent\MicroLab\Results\\ftir results\2_2023-08-07T10-35-49.a2r

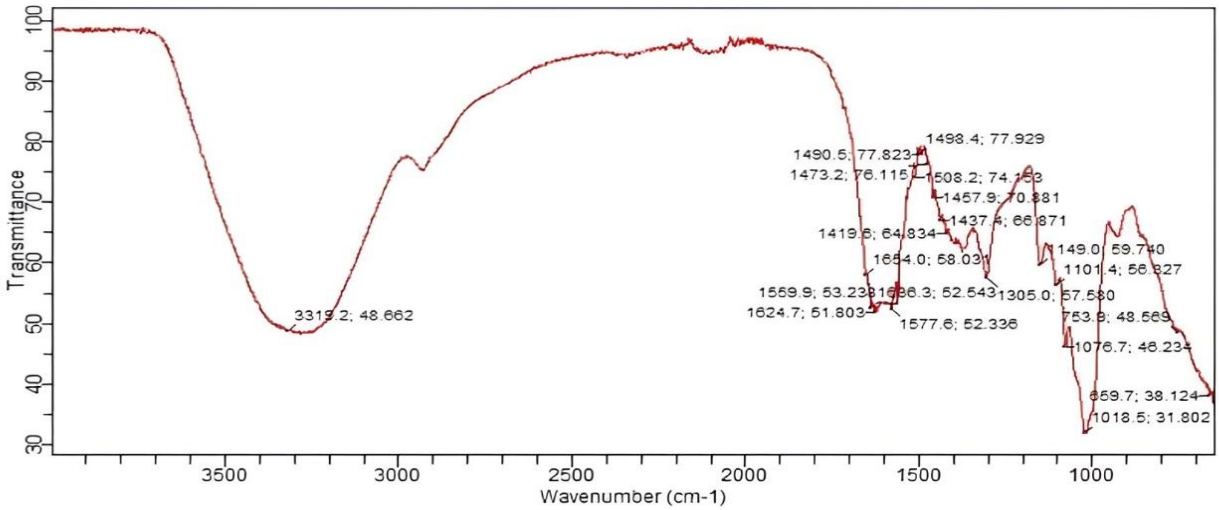

Figure 3: FTIR spectrum of *Elettaria cardamomum* (cardamom) clove extract showing characteristic peaks corresponding to O–H stretching (~3319 cm^{-1}), C=C or C=O stretching (~1648 cm^{-1}), aromatic ring vibrations (1500–1600 cm^{-1}), and C–O stretching in the fingerprint region (1000–1100 cm^{-1}), indicating the presence of phenols, flavonoids, essential oils, and other bioactive compounds

FTIR of Combined Extract

A broad absorption peak at 3292 cm^{-1} corresponds to O–H stretching vibrations, commonly associated with alcohols and phenolic compounds, which are prevalent in all three herbs and are known for antioxidant activity. The small shoulder near 2920 cm^{-1} suggests C–H stretching of alkanes, likely from essential oils such as menthol (from *Mentha*), anethole (from *Foeniculum*), and cineole (from *Elettaria*).The peak at 1654 cm^{-1} may indicate C=C stretching of aromatic rings or C=O stretching, pointing to the presence of flavonoids or aldehydes. Peaks around 1608 cm^{-1} and 1437 cm^{-1} further support the presence of aromatic and conjugated structures, which are common in plant secondary metabolites. The fingerprint region (1500–650 cm^{-1}) contains several sharp peaks, including those at 1101 cm^{-1}, 1025 cm^{-1}, and 669 cm^{-1}, attributed to C–O, C–N, and C–H bending vibrations. These suggest the presence of esters, ethers, and alkaloids [62] (Fig. 4).

© 2025 et al. -Health And Research Insights-Open access under CC BY License (Creative Commons). Freely distributable with appropriate citation.

Sample ID:Mix E
Sample Scans:32
Background Scans:32
Resolution:4
System Status:Good
Method Name:Chemistry
User:Chemistry
Date/Time:08/07/2023 10:27:53 AM
Range:4000 - 650
Apodization:Happ-Genzel
File Location:C:\Users\Public\Documents\Agilent\MicroLab\Results\\ftir results\Mix E_2023-08-07T10-27-53.a2r

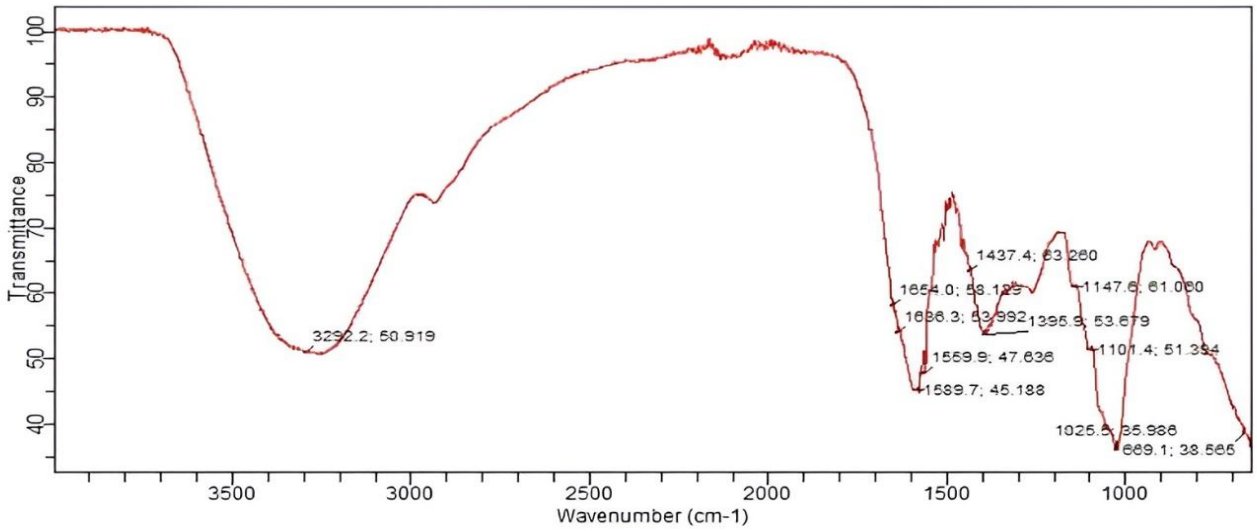

Figure 4: FTIR spectrum of the combined herbal extract containing *Mentha piperita* (leaves*), Foeniculum vulgare* (seeds), and *Elettaria cardamomum* (cloves), showing characteristic peaks for O–H stretching (~3292 cm^{-1}), C–H stretching (~2920 cm^{-1}), C=C/C=O stretching (~1654 cm^{-1}), aromatic ring vibrations (~1608–1437 cm^{-1}), and C–O/C–N stretching in the fingerprint region (~1101–1025 cm^{-1}), indicating the presence of phenolics, flavonoids, essential oils, and other bioactive phytochemicals.

Presence of phytochemicals

The phytochemical screening of the extracts reveals distinct profiles for each herb. Mint extract showed the presence of tannins, terpenoids, reducing sugars, flavonoids, and glucosides, indicating a broad range of bioactive compounds. Cardamom extract tested positive for terpenoids, reducing sugars, and alkaloids, while lacking tannins, flavonoids, and glucosides. Fennel seeds extract contained terpenoids, reducing sugars, and alkaloids but tested negative for other phytochemicals. The combined herb extract displayed the presence of tannins, terpenoids, reducing sugars, flavonoids, and glucosides, reflecting a synergistic composition. Notably, none of the extracts showed the presence of steroids, suggesting their absence across all samples (Table 9).

© 2025 et al. -Health And Research Insights-Open access under CC BY License (Creative Commons). Freely distributable with appropriate citation.

Table 9: Phytochemical screening of individual and combined herbal extracts indicating the presence (+) or absence (–) of key bioactive compounds such as tannins, terpenoids, flavonoids, alkaloids, and glucosides across mint, cardamom, fennel seeds, and their combination.

Phytochemicals	Mint Extract	Cardamom Extract	Fennel Seeds Extract	Combined Herb Extract
Tannins	+	-	-	+
Terpenoids	+	+	+	+
Reducing Sugars	+	+	+	+
Flavanoids	+	-	-	+
Alkaloids	-	+	+	-
Steroids	-	-	-	-
Glucosides	+	-	-	+

DISCUSSION

The antioxidant potential of *Mentha piperita* (mint), *Elettaria cardamomum* (cardamom), and *Foeniculum vulgare* (fennel seeds) was studied both individually and in a combined aqueous extract using various assays, including DPPH, ABTS, FRAP, and Phosphomolybdenum. Mint showed considerable antioxidant capacity due to its high content of polyphenols (19–23%) and flavonoids (12%), with compounds like eriocitrin, rosmarinic acid, and luteolin playing a significant role. Fennel seeds also exhibited strong antioxidant potential, with DPPH inhibition activity ranging from 75.26% to 95.69%. Cardamom, however, demonstrated the most pronounced antioxidant activity on its own, with phenolic content ranging from 0.317 to 1.66 g and flavonoids between 11.33 to 14.63 g per 100 g of sample, contributing to its 85–90% antioxidant capacity. This strong antioxidant potential of cardamom can be linked to its alkaloid composition, which has also been linked to a strong anti-inflammatory potential in pharmacological investigations [63]

When the three herbal extracts were mixed, a change in antioxidant behavior was observed. The IC-50 values of the combined extract were higher than those of cardamom alone in all assays. A higher IC-50 value indicates that a greater concentration of antioxidants is required to neutralize 50% of free radicals, suggesting a reduction in efficiency. This shows a negative mutualistic or anti-synergistic effect, especially for cardamom. However, the IC-50 values of the combined extract were lower than those of mint and fennel individually, suggesting a partial synergistic interaction for these two herbs. This supports the idea that mixtures of bioactive compounds could show non-linear behavior, in which the combined effect is not simply additive, and dominant components in the mixture may be less effective [64].

FTIR analysis provides insight into the molecular interactions behind this behavior. Cardamom's spectrum displayed distinct peaks, especially near 3319 cm^{-1} and in the region between 1600 to 1000 cm^{-1}, which are typical of phenolic and aromatic compounds. These peaks were less prominent or absent in the combined extract, implying that several of the active phenolic components in cardamom were either degraded or not well represented in the mixture. This loss of phenolic content may explain the drop in antioxidant activity when the herbs were combined. Equivalent impairment of active molecules during multi-input therapeutic strategies has been detailed, with interferences among other agents compromising the structural stability of important molecules [65].

Phytochemical screening revealed that terpenoids and reducing sugars were present in all extracts. Tannins and flavonoids were detected in mint and the combined extract but not in cardamom and fennel alone. Alkaloids were found in cardamom and fennel but absent in the combined sample, while glucosides were found in mint and the mixture. Steroids were not present in any of the extracts. The impairment

© 2025 et al. -Health And Research Insights-Open access under CC BY License (Creative Commons). Freely distributable with appropriate citation.

of the biological activity of the combined extract can be caused by the loss of alkaloids, since the structural stability of these molecules is crucial in maintaining the antioxidant activity [66].

Cardamom shows the highest antioxidant activity among the three herbs when used alone, largely due to its phenolic content. When combined with mint and fennel, its antioxidant effect is reduced, suggesting a negative interaction. While the mixture enhances the performance of mint and fennel, it diminishes the effectiveness of cardamom. This outcome highlights that combining herbs can alter their individual biochemical behavior, sometimes reducing the beneficial effects of stronger components. It is consistent with regulatory effects in molecular pathways where interactions with secondary agents may reduce the expression or action of primary functional compounds [67].

CONCLUSION

The four extracts' FTIR examination revealed that while many of the chemicals found in cardamom were absent from the combined extract, the phenolic compounds found in mint and fennel seeds were present in some amount. Cardamom contains alkaloids, according to phytochemical testing, yet the combined extract tested negative for them. Strong antioxidants are found in alkaloids. Consequently, it is plausible that the cardamom extract's alkaloids underwent degradation or transitioned into different forms due to the compounds derived from the fennel and mint seeds in the combined extract. As a result, one should consume the extracts of these three herbs separately to receive the greatest benefit in terms of anti-oxidation within the body.

AUTHOR CONTRIBUTION

Author	Contribution
Unaiza Tahir*	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Muhammad Asim Raza Basra	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published

REFERENCES

1. Sundaram Sanjay, S. and A.K. Shukla, *Free radicals versus antioxidants*, in *Potential Therapeutic Applications of Nano-antioxidants*. 2021, Springer. p. 1-17.

2. Datta, K., S. Sinha, and P. Chattopadhyay, *Reactive oxygen species in health and disease.* National Medical Journal of India, 2000. **13**(6): p. 304-310.

3. Beckman, K.B. and B.N. Ames, *The free radical theory of aging matures.* Physiological reviews, 1998.

4. Kogure, K., et al., *Free radical damage of the brain following ischemia.* Progress in brain research, 1985. **63**: p. 237-259.

5. Valenzuela, A., J. Sanhueza, and S. Nieto, *Natural antioxidants in functional foods: from food safety to health benefits.* Grasas y aceites, 2003. **54**(3): p. 295-303.

6. Qiblawi, S., et al., *Therapeutic interventions of cardamom in cancer and other human diseases.* Journal of Pharmaceutical Research International, 2020. **32**(22): p. 74-84.

© 2025 et al. -Health And Research Insights-Open access under CC BY License (Creative Commons). Freely distributable with appropriate citation.

7. Noreen, S., et al., *Pharmacological, nutraceutical, functional and therapeutic properties of fennel (Foeniculum vulgare).* International Journal of Food Properties, 2023. **26**(1): p. 915-927.

8. Balakrishnan, A., *Therapeutic uses of peppermint-a review.* Journal of pharmaceutical sciences and research, 2015. **7**(7): p. 474.

9. Pacifico, S., et al., *Winter wild fennel leaves as a source of anti-inflammatory and antioxidant polyphenols.* Arabian journal of chemistry, 2018. **11**(4): p. 513-524.

10. Wan, C., et al., *Antioxidant activity and free radical-scavenging capacity of Gynura divaricata leaf extracts at different temperatures.* Pharmacognosy magazine, 2011. **7**(25): p. 40.

11. Benzie, I.F. and Y. Szeto, *Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay.* Journal of agricultural and food chemistry, 1999. **47**(2): p. 633-636.

12. Brown, N., J.A. John, and F. Shahidi, *Polyphenol composition and antioxidant potential of mint leaves.* Food Production, Processing and Nutrition, 2019. **1**(1): p. 1-14.

13. Singh, R., R. Kaushik, and V. Jaglan, *Antibacterial and antioxidant activity of green cardamom and rosemary extract in food products: A brief review.* Pharma Innov J, 2018. **7**(6): p. 568-73.

14. Noreen, S., et al., *Antioxidant activity and phytochemical analysis of fennel seeds and flaxseed.* Food science & nutrition, 2023. **11**(3): p. 1309-1317.

1. Olson, J.M.J.S., *The Evolution of Photosynthesis: Hypothesis: Photosynthetic bacteria and blue-green algae shared a common photoheterotrophic ancestor.* 1970. **168**(3930): p. 438-446.

2. Metcalfe, N.B. and C.J.F.E. Alonso-Alvarez, *Oxidative stress as a life-history constraint: the role of reactive oxygen species in shaping phenotypes from conception to death.* 2010. **24**(5): p. 984-996.

3. Scandalios, J.G.J.T.i.b.s., *The rise of ROS.* 2002. **27**(9): p. 483-486.

4. Barja, G.J.T.i.N., *Free radicals and aging.* 2004. **27**(10): p. 595-600.

5. Valko, M., et al., *Free radicals, metals and antioxidants in oxidative stress-induced cancer.* 2006. **160**(1): p. 1-40.

6. Tahira S, Bibi F, Niaz M, Bukhari AA, Butt AI, Khan E. Multi-organ pathophysiology of endometriosis: Evaluating neurotrophin linked multifaceted health risks assessments. Official Journal of Pakistan Medical Association, Rawalpindi–Islamabad Rawalpindi–Islamabad Branch Apr–Jun 2025 Volume 50 Number 2. 2025 Apr;50(2):377.

7. Fang, Y.-Z., S. Yang, and G.J.N. Wu, *Free radicals, antioxidants, and nutrition.* 2002. **18**(10): p. 872-879.

8. Channon, K., T.J.J.o.P. Guzik, and Pharmacology, *MECHANISMS OF SUPEROXIDE PRODUCTION IN HUMAN BLOOD.* 2002. **53**(4): p. 515-524.

9. Phaniendra, A., D.B. Jestadi, and L.J.I.j.o.c.b. Periyasamy, *Free radicals: properties, sources, targets, and their implication in various diseases.* 2015. **30**: p. 11-26.

10. Dalle-Donne, I., et al., *Protein carbonyl groups as biomarkers of oxidative stress.* 2003. **329**(1-2): p. 23-38.

11. Durrani M, Bukhari AA, Abubakr M, Zaman MH, Khan F, Nasim S. THE EFFECT OF LOW ZINC ON THE FABRICATION OF SHORT CHAIN FATTY ACIDS BY COLONIC MICROBIOTA IN INFANTS. GRASAS Y ACEITES. 2022 Jul;16:2.

12. Rodrigo, J., et al., *The role of free radicals in cerebral hypoxia and ischemia.* 2005. **39**(1): p. 26-50.

13. Khalil, A.A., et al., *Reperfusion injury.* 2006. **117**(3): p. 1024-1033.

14. Winterbourn, C.C., et al., *Protein carbonyl measurements show evidence of early oxidative stress in critically ill patients.* 2000. **28**(1): p. 143-149.

15. Siddiqi, M., A. Tricker, and R.J.C.l. Preussmann, *Formation of N-nitroso compounds under simulated gastric conditions from Kashmir foodstuffs.* 1988. **39**(3): p. 259-265.

© 2025 et al. -Health And Research Insights-Open access under CC BY License (Creative Commons). Freely distributable with appropriate citation.

16. Bukhair SA, Behramand, Bukhari AA, Shah SS, Umm-e-Aiman, Zaman MH. Phytochemical Screening Of Different Fractions And Anti-Bacterial Activity Of Caralluma Tuberculata. J Adv Zo. 2024 Feb: 45(S2).

17. Nair, B.J.I.j.o.t., *Final report on the safety assessment of Mentha piperita (Peppermint) oil, Mentha piperita (Peppermint) leaf extract, Mentha piperita (Peppermint) leaf, and Mentha piperita (Peppermint) leaf water.* 2001. **20**: p. 61-73.

18. Singh, R., M.A. Shushni, and A.J.A.J.o.C. Belkheir, *Antibacterial and antioxidant activities of Mentha piperita L.* 2015. **8**(3): p. 322-328.

19. Kooti, W., et al., *Therapeutic and pharmacological potential of Foeniculum vulgare Mill: a review.* 2015. **4**(1): p. 1-9.

20. Oktay, M., et al., *Determination of in vitro antioxidant activity of fennel (Foeniculum vulgare) seed extracts.* 2003. **36**(2): p. 263-271.

21. Rather, M.A., et al., *Foeniculum vulgare: A comprehensive review of its traditional use, phytochemistry, pharmacology, and safety.* 2016. **9**: p. S1574-S1583.

22. Kausar R, Batool A, Bukhari AA, Shaikh AR, Aleem A, Minhas M, Hussain M. Crosslinking Salivary Diagnosis with Non-Invasive Insights to Oral Pathology: Novel Systematic Insights to Personalized Medicine in Disease Management. . Pak Armed Forces Med J 2025; 75(3): 611-616.

23. Ashokkumar, K., et al., *Botany, traditional uses, phytochemistry and biological activities of cardamom [Elettaria cardamomum (L.) Maton]–A critical review.* 2020. **246**: p. 112244.

24. Souissi, M., et al., *Antibacterial and anti-inflammatory activities of cardamom (Elettaria cardamomum) extracts: Potential therapeutic benefits for periodontal infections.* 2020. **61**: p. 102089.

25. Amma, K., et al., *Chemical composition, flavonoid-phenolic contents and radical scavenging activity of four major varieties of cardamom.* 2010. **1**(3): p. 20-24.

26. Singletary, K.J.N.T., *Cardamom: Potential health benefits.* 2022. **57**(1): p. 38-49.

27. Farid B, Bukhari AA, Tauqir S, Bibi N, Majid B, Jan M. Vaccine hesitancy in COVID-19: a major matter of concern. J Rehman Med Inst. 2022 Oct 8;8(3):20-3.

28. Untea, A., et al., *Comparison of ABTS, DPPH, phosphomolybdenum assays for estimating antioxidant activity and phenolic compounds in five different plant extracts.* 2018.

29. Gulcin, İ. and S.H.J.P. Alwasel, *DPPH radical scavenging assay.* 2023. **11**(8): p. 2248.

30. Nenadis, N., et al., *Estimation of scavenging activity of phenolic compounds using the ABTS•+ assay.* 2004. **52**(15): p. 4669-4674.

31. Benzie, I.F., et al., *The ferric reducing/antioxidant power (FRAP) assay for non-enzymatic antioxidant capacity: concepts, procedures, limitations and applications.* 2018: p. 77-106.

32. Griffiths, H., J.J.E.T. Lunec, and Pharmacology, *Ascorbic acid in the 21st century–more than a simple antioxidant.* 2001. **10**(4): p. 173-182.

33. Kitanidis, P.K.J.W.r.r., *The concept of the dilution index.* 1994. **30**(7): p. 2011-2026.

34. Lefkovits, I., *Limiting dilution analysis*, in *Immunological methods*. 1979, Elsevier. p. 355-370.

35. Cheng, Z., et al., *High-throughput relative DPPH radical scavenging capacity assay.* 2006. **54**(20): p. 7429-7436.

36. Picollo, M., M. Aceto, and T.J.P.s.r. Vitorino, *UV-Vis spectroscopy.* 2019. **4**(4): p. 20180008.

37. Re, R., et al., *Antioxidant activity applying an improved ABTS radical cation decolorization assay.* 1999. **26**(9-10): p. 1231-1237.

38. Karaçelik, A.A., et al., *Antioxidant components of Viburnum opulus L. determined by on-line HPLC–UV–ABTS radical scavenging and LC–UV–ESI-MS methods.* 2015. **175**: p. 106-114.

© 2025 et al. -Health And Research Insights-Open access under CC BY License (Creative Commons). Freely distributable with appropriate citation.

39. Langley-Evans, S.C.J.I.j.o.f.s. and nutrition, *Antioxidant potential of green and black tea determined using the ferric reducing power (FRAP) assay.* 2000. **51**(3): p. 181-188.

40. Aguilar Urbano, M., M. Pineda Priego, and P. Prieto, *Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E1.* 2013.

41. Tomovska, J. and I. Vllasaku, *Phosphomolybdate test method for antioxidant activity in extracts of animal feed.* 2021.

42. Rahman, A., et al., *Salkowski's reagent test as a primary screening index for functionalities of rhizobacteria isolated from wild dipterocarp saplings growing naturally on medium-strongly acidic tropical peat soil.* 2010. **74**(11): p. 2202-2208.

43. Pizzolato, P., R.J.J.o.H. Lillie, and Cytochemistry, *Mayer's tannic acid-ferric chloride stain for mucins.* 1973. **21**(1): p. 56-64.

44. Cierpiszewski, R., et al., *Removal of zinc (II) from spent hydrochloric acid solutions from zinc hot galvanizing plants.* 2002. **41**(3): p. 598-603.

45. Daniels, R., C.C. Rush, and L.J.J.o.c.e. Bauer, *The Fehling and Benedict tests.* 1960. **37**(4): p. 205.

46. Jha, D.K., et al., *Detection and confirmation of alkaloids in leaves of Justicia adhatoda and bioinformatics approach to elicit its anti-tuberculosis activity.* 2012. **168**(5): p. 980-990.

47. MacLean, H.J.B.J., *Observations on Fehling's test for dextrose in urine.* 1906. **1**(2): p. 111.

48. Suriyatem, R., et al., *Predictive mathematical modeling for EC50 calculation of antioxidant activity and antibacterial ability of Thai bee products.* 2017. **7**(9): p. 122-133.

49. Ebata, T., et al., *OH Stretching Vibrations of Phenol−(H2O) 1 and Phenol−(H2O) 3 in the S1 State.* 1996. **100**(2): p. 546-550.

50. Snyder, R.G., et al., *Isolated C–H stretching vibrations of n-alkanes: Assignments and relation to structure.* 1984. **81**(12): p. 5352-5361.

51. Gough, K.M., J.R. Dwyer, and R.J.C.J.o.C. Dawes, *Ab initio analysis of CH and CC stretching intensities in Raman spectra of hydrocarbons.* 2000. **78**(7): p. 1035-1043.

52. Edington, S.C., J.C. Flanagan, and C.R.J.T.J.o.P.C.A. Baiz, *An Empirical IR Frequency Map for Ester C□ O Stretching Vibrations.* 2016. **120**(22): p. 3888-3896.

53. Margoshes, M. and V.J.S.A. Fassel, *The infrared spectra of aromatic compounds: I. The out-of-plane CH bending vibrations in the region 625–900 cm− 1.* 1955. **7**: p. 14-24.

54. Gerhards, M. and K.J.T.J.o.c.p. Kleinermanns, *Structure and vibrations of phenol (H2O) 2.* 1995. **103**(17): p. 7392-7400.

55. Sinclair, R., A. McKay, and R.N.J.J.o.t.A.C.S. Jones, *The infrared absorption spectra of saturated fatty acids and esters1.* 1952. **74**(10): p. 2570-2575.

56. Shen, L., T. Doyle, and W.J.T.J.o.c.p. Graham, *Fourier transform spectroscopy of C4H (butadiynyl) in Ar at 10 K: C–H and C≡ C stretching modes.* 1990. **93**(3): p. 1597-1603.

57. Staufer, M., et al., *Density functional and infrared spectroscopy studies of bonding and vibrations of NH species adsorbed on the Ru (001) surface: a reassignment of the bending mode band.* 1996. **369**(1-3): p. 300-312.

58. Kross, R.D., V.A. Fassel, and M.J.J.o.t.A.C.S. Margoshes, *The Infrared Spectra of Aromatic Compounds. II. Evidence Concerning the Interaction of π-Electrons and σ-Bond Orbitals in CH Out-of-plane Bending Vibrations1.* 1956. **78**(7): p. 1332-1335.

59. Jiang, X., et al., *Determination of the acid values of edible oils via FTIR spectroscopy based on the OH stretching band.* 2016. **212**: p. 585-589.

60. Starsinic, M., et al., *FTIR studies of Saran chars.* 1983. **21**(1): p. 69-74.

61. Ramírez-Hernández, A., C. Aguilar-Flores, and A.J.D. Aparicio-Saguilán, *Fingerprint analysis of FTIR spectra of polymers containing vinyl acetate.* 2019. **86**(209): p. 198-205.

© 2025 et al. -Health And Research Insights-Open access under CC BY License (Creative Commons). Freely distributable with appropriate citation.

62. Dayton, D., M.D. Marshall, and R.J.T.J.o.c.p. Miller, *The fundamental C–H stretching vibration and associated intermolecular bending hot band of SCO–HCN.* 1991. **95**(2): p. 785-792.

63. Bukhari AA, Shaikh AR, Salman W, Bhatti FA, Malik W, Minhas M, Muddasser A, Khaliq H. Pathophysiological role of nerve growth factor (NGF) in asthma: insights into airway inflammation, remodeling, and neural regulation in intensive care settings. Anaesth. pain intensive care. 2025;29(3):681-689.

64. Zhang Y, Ilyas M, Yilin W, Su Y, Bukhari AA, Ahmad A. A Methodical Approach to Scrutinize the Role of Body Mass Index in Heart Rate Increment and Recovery. Journal of Gandhara Medical & Dental Sciences (JGMDS). 2022 Oct 2;9(4):31-6.

65. Deng X, Bukhari AA, Huang J, Liu K, Durrani M, Zaman MH. Evaluating binaural beats as a therapeutic tool for intervening visually-induced motion sickness in virtual reality environments. Journal of Rehman Medical Institute. 2022 Apr 8;8(1):03-11.

66. Gong J, Ali N, Abubakar M, Bukhari AA, Ilyas M, Bashir U, Ali M, Zaman MH. THE PREDOMINANT ROLE OF CD4+T CELL-DERIVED SMALL EXTRACELLULAR VESICLES IN IMMUNE ENHANCEMENT. Journal of Population Therapeutics and Clinical Pharmacology. 2023 Nov:30(18):1774-1783.

67. Zhang X, Ge Y, Bukhari AA, Zhu Q, Shen Y, Li M, Sun H, Su D, Liang X. Estrogen negatively regulates the renal epithelial sodium channel (ENaC) by promoting Derlin-1 expression and AMPK activation. Experimental & Molecular Medicine. 2019 May;51(5):1-2.

© 2025 et al. -Health And Research Insights-Open access under CC BY License (Creative Commons). Freely distributable with appropriate citation.