Antioxidant Activity and Inhibitory Effects of Ginger, Green tea, and Cinnamon, Alone and in Combination, on *Staphylococcus aureus* and *Escherichia coli*

Heba F.A. Yacoub; Gaber A.H. El-Baradei; Sameh A. Awad; Ibrahim M. El-Sayed[1]

ABSTRACT

Herbs and spices were used by the ancient Egyptian and have been used for centuries in India and China. Ginger, Green tea and Cinnamon were extracted and pH value of Ginger, Green tea and Cinnamon were (4.92, 5.25, 4.6) respectively, while % titratable acidity were (0.21%, 0.33%, 0.11%) respectively. Maximum value of Ferric Reducing Antioxidant Power (FRAP) observed in (Cinnamon combined with Green tea) extracts (0.25 mg GAE/g) and maximum value observed in (Cinnamon combined with Green tea) oils (0.39 mg GAE/g). Maximum total phenolic content value observed in (Cinnamon combined with Green tea) extracts (C+T) was (20.23 mg GAE/g) and maximum value observed in (Cinnamon combined with Green tea) oil (C+T) was (37.01 mg GAE/g). The antioxidants activity (DPPH) of oils are higher of extracts. The Cinnamon and Green tea oil in combination (C+T) showed the higher antioxidant activity (86.20%) comparing to the other oils. By GC-MS analysis, Zingiberene is the main constituent of essential oil of Ginger (20.52%), 1.8-Cineole is the main constituent of essential oil of Green tea (31.46%) and Cinnamaldehyde is the main constituent of essential oil of Cinnamon (30.83%). By increasing concentrations of cinnamon, Green tea and Ginger (oils and extracts) inhibition clear zone on *Escherichia coli* (ATTC 25922) and *Staphylococcus aureus* (ATTC6538) increased.

The impact of (T+C) extract on the growth of *Staphylococcus aureus* (ATTC6538) was lower than *Escherichia coli* (ATTC 25922). There is no effect of Green tea, Ginger and Cinnamon extracts or oils separately or in-combination on the growth of yogurt starter and karish cheese starter cultures.

Keywords: Ginger, Green tea, Cinnamon, antioxidant activity, GC-MS, Antibacterial activity, *Escherichia coli* (ATTC 25922), *Staphylococcus aureus* (ATTC6538).

INTRODUCTION

Herbs and spices have been utilized since ancient times, with evidence of their use in ancient Egypt, as well as their long-standing applications in India and China. In the modern era, herbs and spices are recognized not only for enhancing the sensory acceptability of food products but also for contributing to consumer health (Suliman *et al.,* 2019). Specifically, they are derived from various parts of plants and are commonly employed to impart characteristic flavor and aroma to foods, while at the same time improving the visual appeal and overall attractiveness of fortified products (El-Sayed and Youssef, 2019). Moreover, dietary catechins, which are polyphenolic compounds predominantly found in plants such as tea-particularly Green tea has been shown to exhibit strong antioxidant activities and demonstrate significant potential in supporting and promoting human health (Johnson *et al.,* 2012).

Studies suggest that drinking tea can significantly reduce the likelihood of developing various serious long-term illnesses such as cancer, coronary heart disease (CHD), and stroke (Oze *et al.,* 2014).

Cinnamaldehyde was identified as the predominant constituent of the isolated oil. Therefore, considering the diverse chemical components present in cinnamon essential oil, further investigations into its properties, biological activities, and potential applications are recommended, particularly regarding its use as a medicinal alternative or as a supplementary agent in the management of various diseases, certain types of cancer, and during chemotherapy (Kamaliroosta *et al.,* 2012).

Ginger (*Zingiber officinale*) has been extensively used as a spice in a wide variety of foods and beverages. It is also regarded as an important medicinal plant, as its rhizomes contain several biologically active compounds (Hanou *et al*., 2016). Both Ginger and Cinnamon have been reported to serve as potential natural sources of antioxidants in foods (El-Ghorab *et al.,* 2010). Furthermore, the essential oil and fatty acid composition of stored *Zingiber officinale* Roscoe (ginger) has been characterized using Gas Chromatography–Mass Spectrometry (GC/MS) analysis (Oforma *et al*., 2019).

Ünal *et al.* (2018), research was conducted to evaluate the impact of adding Green and black tea extracts on the microbial characteristics, as well as the antimicrobial and antioxidant efficacy, of drinking yogurt over a 21-day storage period. Results indicated that samples fortified with 2% of either Green or black tea maintained higher viable counts of the yogurt starter cultures compared to those infused with a 4% ratio.

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Furthermore, both tea types demonstrated antimicrobial effects against common pathogens, including *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, and *Candida albicans*, though the Green tea samples exhibited a stronger inhibitory action. Throughout the storage period, the samples containing Green tea extract consistently demonstrated the greatest DPPH scavenging activity compared to the counterparts that were fortified with black tea extract.

The aim of this work studied the functionality of Green tea, Ginger and Cinnamon independently or in combination in relevant to anti-oxidant and antimicrobial activities.

MATERIALS AND METHODS

1. Chemicals:

2,2 Diphenyl-1-picrylhydrazyl (DPPH) was picked up from Merk, Darmstadt, Germany. Folin- Coagulant reagent was picked up from LOBA CHEMIE PVT. LTD. Phosphate buffer was purchased from Biomark laboratories, Pune 411041. India. Ferric chloride, Potassium ferric cyanide and Gallic Acid were purchased from TECHNO PHARMCHEM, BAHADURARH, HARYANA (INDIA) AN ISO 9001. Tricholoro acetic acid was purchased from LOBA CHEMIE PVT. LTD.

Methanol- phenolphthalein- Sodium hydroxide- Ammonia- Sodium carbonate- Sodium citrate 2% – Sulphuric acid – Hydrochloric acid were purchased from New-Lab Co. for laboratories supplies, Alex., Egypt.

HPLC chemical used (benzene, methanol, sulfuric acid) were grade of Merck.

Standard fatty acid methyl ester mixture (Part number: CRM47801, Sigma-Aldrich Co LLC, Merck KGaA, Darmstadt, Germany).

2. Oils and Extracts:

Oils (Ginger oil, Cinnamon oil, Green tea oil), Cinnamon Bark, Ginger Roots and Green Tea leaves acquisition of the items took place at a local marketplace.

3. Preparation of Ginger, Green tea and Cinnamon extracts:

3.1. Preparation of Ginger extract:

Ginger extract was ready as pointed out by Sasi Kumar *et al.* (2013) (Fig. 1).

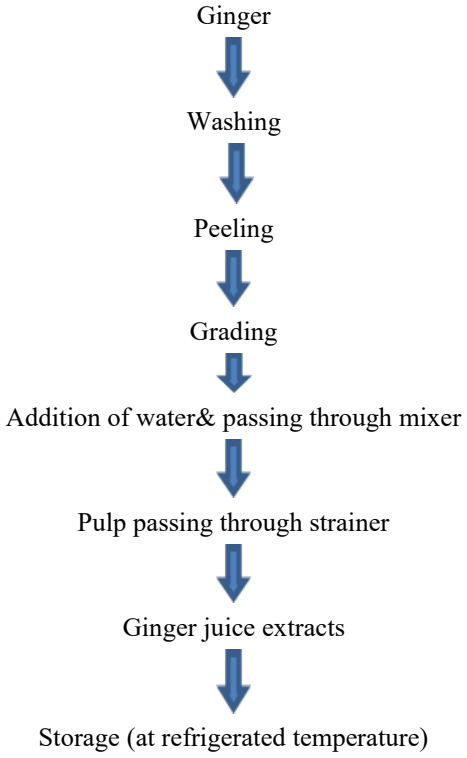

Fig. 1. Flow chart of Ginger extract

3.2. Preparation of Green tea extract:

We followed the Tea Extract Preparation Protocol as outlined by Jaziri *et al.* (2009). 2 grams of tea infusion was prepared by removing the contents of each tea bag and steeping them in 100 mL of hot water (87–90°C) for 10 minutes at a concentration of 2.0% (w/v), which corresponds to the strength of a merit teacup. The infusion was then filtered through Whatman No. 4 filter paper and used for subsequent analyses.

3.4. Preparation of Cinnamon extract:

Cinnamon extract was prepared According to Suliman *et al.* (2019). Cinnamon bark powders (5g) were extracted with 50 ml of water at room temperature (24°C) for 12h using shaker. The tear out was distilled and centrifuged at 4000 rpm for 10 minutes and the supernatant was obtained.

4. Chemical Analysis of Ginger, Green tea and Cinnamon extracts:

4.1. The pH value of Ginger, Green tea and Cinnamon:

The pH value was measured in extracts using a digital electrode pH meter (AD1030)- Adwa.

4.2. Determination of % Titratable acidity of Ginger, Green tea and Cinnamon:

It was determined as lactic acid % according to the A.O.A.C. (2003).

5. Determination of antioxidant activity of Ginger, Green tea and Cinnamon extracts separately or in combination:

5.1. Determination of Total phenolic content of Ginger, Green tea and Cinnamon extracts and oils separately or in combination:

The total phenolic content (TPC) of water-soluble extracts was analyzed in triplicate using the Folin-Ciocalteu method as described by Abirami *et al.* (2014). A volume of 1.5 mL of Folin-Ciocalteu reagent (diluted 10-fold) and 1.2 mL of sodium carbonate solution (7.5% w/v) were added to 300 µL of the water-soluble extract. The mixture was thoroughly shaken and incubated at room temperature for 30 minutes. Absorbance was then measured at 765 nm using a UV-2100 spectrophotometer (Unico, USA). The TPC was expressed as milligrams of gallic acid equivalents (GAE) per milliliter of extract.

5.2. Determination of antioxidant activity by (FRAP):

Ferric ion reducing antioxidant power (FRAP) was determined according to Oyaizu (1986). One milliliter of the extract was blended with 2.5 mL of phosphate buffer (0.1 M, pH 6.6) and 2.5 mL of potassium ferricyanide solution (1% w/v). The blend was incubated in a bath of water at 50°C for 20 minutes, then cooled to room temperature. Subsequently, 2.5 mL of trichloroacetic acid (10% w/v) was added, and the mixture was centrifuged at 10,000 × g at 4°C for 10 minutes. From the resulting supernatant, 2.5 mL was collected and combined with 2.5 mL of distilled water and 0.5 mL of ferric chloride solution (0.1% w/v). The mixtures were left to stand for 30 minutes, after which the absorbance was measured at 700 nm using a UV/Visible spectrophotometer (UV-2100, Unico, USA). In triplicate, the assay was executed, and the FRAP values were calculated as milligrams of gallic acid equivalents (GAE) per milliliter of extract, based on a standard curve.

5.3. Assessment of antioxidant activity by DPPH Assay (2,2 Diphenyl-1-picrylhydrazyl) free radical scavenging Activity:

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was determined in triplicate following the method of Lim and Quah (2007).

Briefly, 1 mL of extract of the water-soluble was blended thoroughly with 2 mL of a 0.15 mM DPPH solution in methanol. In the dark the blend was brewed at room temperature for 30 minutes.

The absorbance (Abs) was measured at 517 nm against distilled water (used as a blank) using a spectrophotometer (UV-2100, Unico, U.S.A). A control sample was prepared by substituting the extract with 1 mL of methanol and adding it to 2 mL of the DPPH solution.

The radical scavenging activity of the extracts was subsequently employed the equation:

$$\text{Radical scavenging activity\%} = (1 - \text{Abs}_{\text{sample}}/\text{Abs}_{\text{control}}) \times 100$$

6. Gas chromatography–mass spectrometry (GC-MS) analysis:

The chemical composition of the samples was analyzed using a GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) equipped with a direct capillary column TG–1MS (30 m × 0.32 mm × 0.25 µm film thickness). The oven temperature program was initiated at 60°C, followed by an increase of 5°C/min up to 280°C with a 1-min hold, and subsequently raised to 300°C at a rate of 30°C/min. The injector temperature was maintained at 270°C. Helium served as the carrier gas at a constant flow rate of 1 mL/min. A solvent delay of 4 min was applied, and 1 µL of diluted sample was automatically injected in split mode using an AS3000 autosampler coupled with the GC. Compounds were identified by comparing their mass spectra against the WILEY 09 and NIST14 spectral databases. The Electron Ionization (EI) mass spectra were collected in

full-scan mode across an m/z range of 50–650, utilizing an ionization energy of 70 eV. To ensure accurate readings, the transfer line and ion source temperatures were precisely maintained at their respective 280°C and 200°C (Abd El-Kareem *et al.,* 2016).

7. Determination of the antibacterial activity of oils and extracts of Ginger, Green tea and Cinnamon separately or in combination:

The antibacterial activity of Ginger, Green tea and Cinnamon extracts or oils was conducted against *Staphylococcus aureus* (ATTC 6538) and *Escherishia coli* (ATTC 25922).

The antibacterial activity was achieved using a direct method (Tagg *et al.,* 1976), and a well method of diffusion (Obeidat *et al*., 2012 and Balouiri *et al*., 2016) which involves scaling the diameter of the clear zone of inhibition that forms around the well.

Concerning the direct method, the indicator strains (18hr) were diluted to 1/10 and 0.25 ml of 1/10 dilution from an (18hr), culture was added to 10 ml of suitable soft agar medium (Nutrient agar 0.75 % agar). After gentle blending, the contents from each tube were dispensed onto the surface of appropriately prepared agar medium plates. After incubation the plates at 37°C for 3 hr. 10 µl of the sterilized extract (by using Millipore filter 0.45µm) of Ginger, Green tea and Cinnamon was spotted on the surface of agar medium. The plates were incubated for 24 hr at 37°C. After incubation period the plates were examined for inhibition zones.

Concerning the well diffusion method after solidification agar wells were prepared using Cork borer.

8. Statistical Analysis:

To scrutinize the data, use IBM SPSS statistics software, version 25.0 (IBM Corp., Armonk, NY, USA) (IBM Corp., 2017). All outcomes were exhibited as mean ± standard error (SE). One-way analysis of variance (ANOVA) was performed, and then Student–Newman–Keuls (SNK) method is a stepwise multiple comparisons procedure used to identify sample means that are significantly different from each other. The criteria for statistical significance were set at $p \leq 0.05$.

RESULTS AND DISSCUSSION

1. Chemical Analysis of Ginger, Green tea and Cinnamon extracts (pH value and % Titratable acidity):

The results in Table (1) show that pH value of Cinnamon, Green tea and Ginger extracts were 4.6, 5.25 and 4.92 respectively. Also, % Titratable acidity were 0.11%, 0.33 % and 0.21% respectively.

Table 1. Chemical Analysis of Ginger, Green tea and Cinnamon extracts (pH and % Titratable acidity)

Extracts	pH value	% Titratable acidity
Cinnamon (C)	4.6	0.11
Green tea (T)	5.25	0.33
Ginger (G)	4.92	0.21

2. Ferric Reducing Antioxidant Power (FRAP) analysis of Cinnamon, Ginger, Green tea (oils and extracts) and in-combination between them:

The results in Table (2) and Figure (2) reveal the Ferric Reducing Antioxidant Power (FRAP) analysis of Cinnamon, Ginger, Green tea (oils and extracts) separately and in-combination between them. For extracts, maximum value was observed in (Cinnamon combined with Green tea) extracts (0.25 mg GAE/g) while minimum value was observed in Ginger extract (0.13 mg GAE/g). For oils, maximum value was observed in (Cinnamon combined with Green tea) oils (0.39 mg GAE/g) while minimum value observed in Ginger oil (0.23 mg GAE/g). Mustafa and Chin (2023) the study revealed that sun-drying using ethanol as a solvent produced dried Ginger with a high flavonoid content and demonstrated the highest FRAP values, total antioxidant activity, as well as the strongest $ABTS^{+}$ radical cation and DPPH radical scavenging activities. The FRAP values of the essential oils were found to be nearly comparable to those obtained from the DPPH assay. Moreover, Ginger essential oil exhibited the greatest efficiency in reducing Fe^{3+}ions. The data aligns with the earlier by El-Ghorab *et al.* (2010).

Table 2. Ferric Reducing Antioxidant Power (FRAP) analysis of Cinnamon, Ginger, Green tea (oils and extracts) separately or in-combination between them.

Treatments	Oils (mg GAE/g) Mean ± SD	Extracts (mg GAE/g) Mean ± SD
G	0.23 ± 0.18^{cd}	0.13 ± 0.05^{c}
C	0.37 ± 0.11^{b}	0.23 ± 0.01^{ab}
T	0.35 ± 0.06^{bc}	0.22 ± 0.02^{b}
G+C	0.36 ± 0.04^{bc}	0.20 ± 0.02^{ab}
G+T	0.29 ± 0.11^{c}	0.18 ± 0.02^{b}
C+T	0.39 ± 0.02^{a}	0.25 ± 0.07^{a}
G+T+C	0.33 ± 0.08^{c}	0.22 ± 0.06^{ab}

G: Ginger C: Cinnamon T: Green tea

3. Total phenolic content (TPC) of Cinnamon, Ginger, Green tea (oils and extracts) separately or in-combination between them:

The results in Table (3) and Figure (3) show a TPC of Cinnamon, Ginger, Green tea (oils and extracts) separately or in-combination between them. The high

content of TPC was recorded when extracts of Cinnamon and Green tea are in-combination (20.23 mg GAE/g). Regarding the oils of Ginger, Cinnamon, and Green tea, Cinnamon and Green tea oils combined showed the highest total phenolic content at (37.01 mg GAE/g), compared to the other oils. Ünal *et al.* (2018) revealed that, he influences of Green and Black tea supplementation on the microbiological characteristics, antimicrobial properties, and antioxidant capacity of drinking yoghurt was systematically evaluated over a 21-day storage period. The results indicated that both types of tea contributed positively to the functional properties of yoghurt; however, the impact of Green tea was more pronounced. In particular, supplementation with Green tea significantly enhanced the total phenolic content compared to Black tea, which is consistent with the well-documented higher phenolic and catechin concentrations in Green tea. These findings suggest that incorporating Green tea into fermented dairy products may provide superior health-promoting properties, while also improving their functional value and consumer appeal.

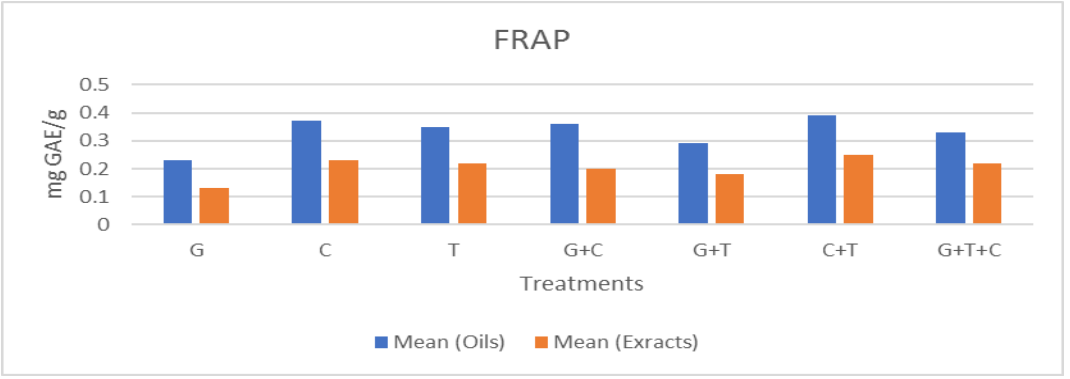

Fig. 2. Changes in Ferric Reducing Antioxidant Power (FRAP) analysis of Cinnamon, Ginger, Green tea (oils and extracts) separately or in-combination between them.

Table 3. Total Phenolic Content (TPC) of Cinnamon, Ginger, Green tea (oils and extracts) and combination between them.

Treatments	Oils (mg GAE/g) Mean ± SD	Extracts (mg GAE/g) Mean± SD
G	25.51 ± 0.04^{f}	15.31 ± 0.006^{d}
C	18.01± 0.01^{g}	19.42±0.003^{b}
T	30.41± 0.02^{d}	18.32±0.003^{c}
G+C	33.63± 0.04^{c}	19.51±0.004^{b}
G+T	28.01± 0.03^{e}	19.01±0.001^{b}
C+T	37.01± 0.02^{a}	20.23±0.006^{a}
G+T+C	34.31± 0.03^{b}	19.67±0.006^{b}

G: Ginger C: Cinnamon T: Green tea

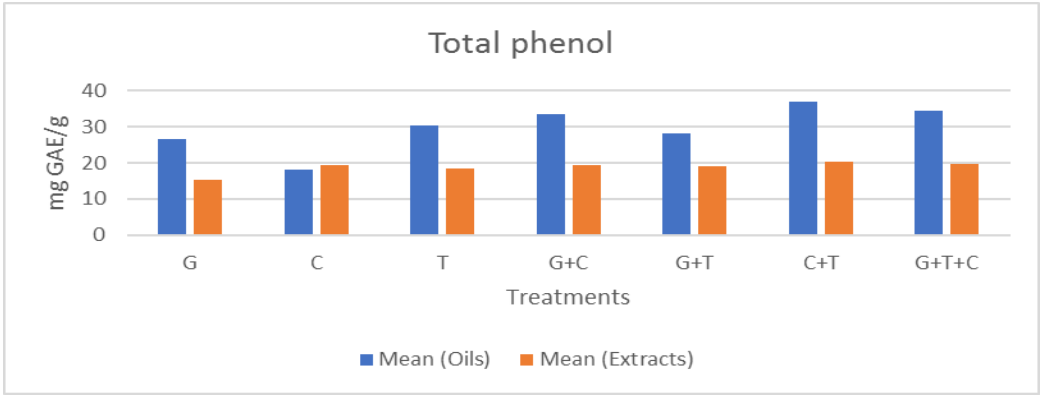

Fig.3. Total Phenolic Content (TPC) of Cinnamon, Ginger, Green tea (oils and extracts) and combination between them.

4. Antioxidant activity (DPPH) inhibition activity % of Cinnamon, Ginger, Green tea (oils and extracts) and in-combination between them.

Results in Table (4) and Figure (4), show the antioxidant activity of Ginger, Cinnamon and Green tea extracts and oils when they are separately or in-combination. From Table (5), it is clear that the antioxidants activity (DPPH) of oils are higher that than of extracts. The Cinnamon and Green tea oil in combination (C+T) oils. Among the tested samples, demonstrated superior antioxidant activity, reaching 86.20%, which was higher than that of the other compounds. Rahman *et al.* (2021), showed that the Black and Green teas from different brands were compared in terms of their bioactive compounds and antioxidant activity. Green tea exhibited substantially higher antioxidant capacity than standard ascorbic acid in both ABTS and DPPH free radical scavenging assays. Moreover, Green tea consistently demonstrated superior antioxidant activity compared to black tea. In agreement with Singh and Ahmad (2015), resulted that, the essential oil extracted from the leaves of *Cinnamomum zeylanicum* exhibited notable antioxidant activity, as evaluated using DPPH and superoxide anion scavenging assays, with values of 70.55 and 32.34 µg/mL, respectively. Afdal *et al.* (2023), among the tested samples, demonstrated the highest antioxidant activity, based on its DPPH radical scavenging capacity. Ali *et al.* (2018) proposed that ginger's rhizome and callus serve as a promising source of phenolic compounds possessing antioxidant properties. Specifically, 6-gingerol and 6-shogaol demonstrated similar levels of antioxidant effectiveness.

According to Ashfaq *et al.* (2021), *Cinnamomum zeylanicum*, a member of the Lauraceae family, is one of the well-known species of the cinnamon plant. Its antioxidant activity has been widely evaluated through different antioxidant assays, alongside investigations of its chemical constituents. Moreover, C. *zeylanicum* has been reported to exert multiple beneficial effects on human health and is extensively applied across various industries. Singh and Ahmad (2015), pointed out that the essential oil extracted from the leaves of *Cinnamomum zeylanicum* exhibited significant antioxidant activity. This was confirmed through DPPH and superoxide anion scavenging assays, with respective values of 70.55 and 32.34 µg/mL.

Table 4. Antioxidant activity (DPPH) inhibition activity % of Cinnamon, Ginger, Green tea (oils and extracts) and combination between them.

Treatments	Oils	Extracts
	DPPH inhibition activity % (A.A)%	DPPH inhibition activity %(A.A%)
G	71.15	17.00
C	83.11	26.32
T	84.22	23.96
G+C	80.41	26.51
G+T	79.20	24.01
C+T	86.20	28.44
G+T+C	80.75	24.51

G: Ginger C: Cinnamon T: Green tea

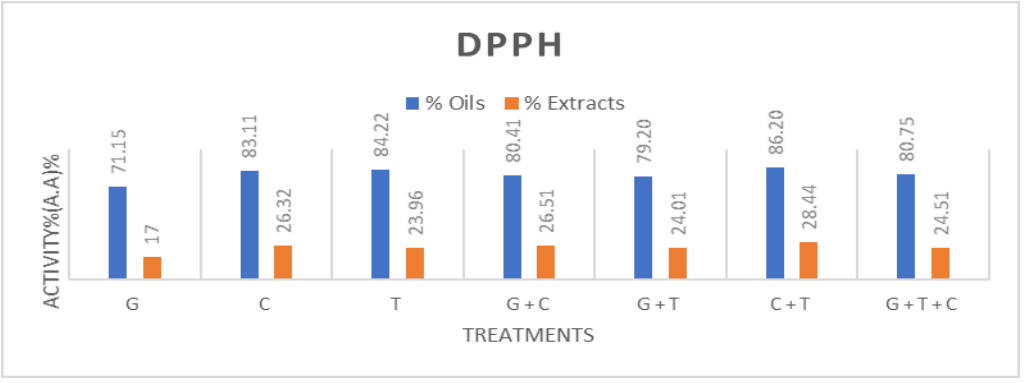

Fig. 4. Antioxidant activity (DPPH) inhibition activity % of Cinnamon, Ginger, Green tea (oils and extracts) and combination between them.

5. GC-MS Analysis of components of Ginger oil:

A total number of compounds were identified in Ginger essential oil through GC–MS analysis, as presented in Table (5) and Figure (5), which illustrate the compounds along with their retention times and relative concentrations (%). The dominant constituents of Ginger oil were identified as zingiberene (20.52%), camphor (11.88%), and α-curcumene (9.22%), with zingiberene being the principal component of Ginger essential oil. These findings are in agreement with those of Thao *et al.* (2023), who reported zingiberene (18.95%) and camphene (15.27%) as the two predominant compounds in Ginger oil. The presence of various bioactive constituents in Ginger essential oil is likely responsible for its antioxidant potential. For example, Barros Gomes *et al.* (2019) evaluated the molluscicidal activity of *Zingiber officinale* Roscoe rhizome oil against *Biomphalaria glabrata* and identified 18 compounds, with zingiberene, geranial, and nerolidol as the main constituents. Similarly, Al-Dhahli *et al.* (2020), reported zingiberene as the predominant volatile compound in both Chinese and Saudi Ginger oils.

Table 5. GC-MS Analysis of components of Ginger oil:

Component of Ginger oil	RT(min)	(Area%)
β-Myrcene	4.52	4.85
Sabinene	4.76	3.11
β-pinene	5.24	1.87
Camphene	5.31	3.50
α-pinene	6.07	2.20
D- Limonene	6.47	3.31
Broneol	7.89	7.97
Bornylacetate	15.74	0.97
Camphor	16.38	11.88
Geranial	17.17	1.62
Citral	17.85	8.48
Neral	18.06	1.12
Zingerberene	18.89	20.52
α-curcumene	18.26	9.22
Azulene	18.58	6.55
Naphthalene	19.34	1.42
α-bisabolene	19.50	3.86
Cedrene	19.96	2.70
β-Elemene	30.02	2.96
Phellandrene	32.83	1.87
	Total	100

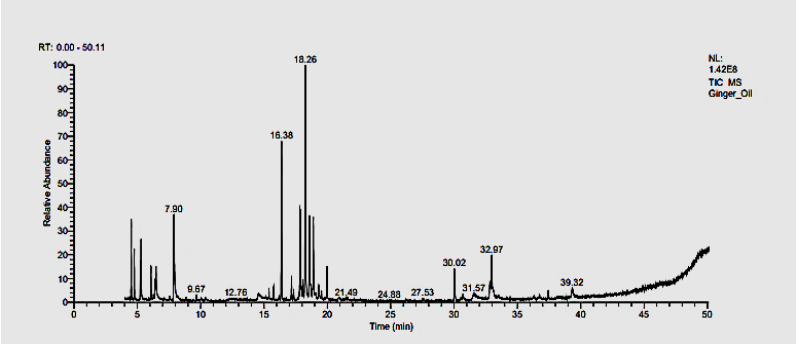

Fig.5. GC-MS Analysis of components of Ginger oil

Khalil *et al.* (2022), using GC–MS, analyzed fresh Ginger rhizomes and reported an essential oil yield of 0.168% by weight, with zingiberene (18.61%), α-curcumene (9.91%), β-sesquiphellandrene (9.25%), naphthalene (9.09%), and 2-oxabicyclo[3.3.0]octane, 1,3,3-trimethyl (7.17%) as the major constituents. Furthermore, El-Baroty *et al.* (2010) characterized the essential oils from *Cinnamomum zeylanicum* bark and *Zingiber officinale* rhizomes using TLC and GC–MS, and identified antimicrobial and antioxidant components through TLC-bioautography. Their results showed that Ginger oil was rich in sesquiterpene hydrocarbons, particularly β-sesquiphellandrene (27.16%), caryophyllene (15.29%), zingiberene (13.97%), and ar-curcumene (6.62%).

6. GC-MS Analysis of components of Green tea oil:

The total number of compounds identified in Green Tea essential oil using GC–MS analysis are presented in Table (6) and Figure (6), showing the retention times and relative concentrations (%). The dominant constituents were 1,8-cineole (31.46%), terpinen-4-ol (13.09%), and α-phellandrene (8.04%), with 1,8-cineole being the principal component of Green Tea essential oil. In comparison, Ahmed (2022) reported the chemical composition of Tea tree essential oil analyzed by GC–MS, where the most abundant compounds included cyclohexane (2.80%), α-pinene (2.27%), (+)-4-carene (7.31%), p-cymene (3.80%), D-limonene (1.10%), eucalyptol (4.98%), terpinene (17.74%), terpinolene (2.78%), terpinen-4-ol (43.94%), and α-terpineol (3.61%). Similarly, Malik and Upadhyay (2022) identified a total of 34 chemical constituents in Tea tree oil through gas chromatographic analysis, with the majority of the oil composed of 1,8-cineole (4.51%), cyclohexanol (8.78%), terpineol (2.65%), terpinen-4-ol (12.11%), α-pinene (10.5%), β-pinene (5.81%), and terpinolene.

Table 6. GC-MS Analysis of components of Green Tea oil:

Components of Green Tea oil	RT (min)	(Area%)
Cyclohexane	4.40	1.46
α-Thujene	4.52	4.48
β-Pinene	5.30	1.02
β-Myrcene	6.18	4.17
α-Pellandrene	6.26	8.04
D- Limonene	6.41	5.00
Terpinen-4-ol	7.15	13.09
Camphene	7.87	2.00
1.8 Cineole	32.97	31.46
Sabinene	10.07	4.70
Limolene	10.34	1.17
β-Ocimene	16.20	1.14
α-Pinene	16.37	4.18
α-Terpinene	16.86	1.77
Cymene	17.34	3.11
Carene	18.20	3.22
γ-Terpinene	18.83	2.44
Terpinolene	30.02	2.13
α-Thujene	32.82	5.40
	Total	100

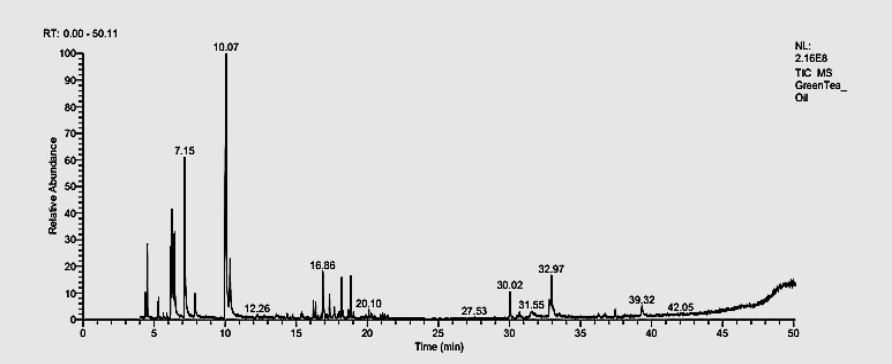

Fig. 6. GC-MS Analysis of components of Green tea oil

7. GC-MS Analysis of Cinnamon oil:

In Table (7) and Figure (7) illustrate the compounds identified in Cinnamon essential oil through GC–MS analysis. The dominant constituents were cinnamaldehyde (30.83%), limonene (8.26%), and β-pinene (8.05%), with cinnamaldehyde being the principal component of Cinnamon oil. These outcomes are suitable with Alizadeh Behbahani *et al.* (2020), that 17 chemical constituents in Cinnamon oil, with (E)-cinnamaldehyde (71.5%), linalool (7.00%), β-caryophyllene (6.40%), eucalyptol (5.40%), and eugenol (4.60%) as the major components. Several studies have similarly confirmed cinnamaldehyde as the predominant compound in Cinnamon bark essential oil. For instance, Chairunnisa *et al.* (2017) identified four major constituents of Cinnamon oil-trans-cinnamaldehyde (56.10%), 1,8-cineole (16.53%), α-pinene (3.44%), and α-terpineol (3.05%)- based on GC–MS analysis.

Table 7. GC-MS Analysis of components of Cinnamon oil:

Components of Cinnamon oil	RT (min)	(Area%)
α-Pinene	4.38	1.15
Limonene	4.49	8.26
β-Pinene	4.73	8.05
Cymene	5.28	1.15
β-Myrcene	6.29	3.18
Eucalyptol	6.38	6.43
Carene	9.99	5.10
Thymol	12.92	7.51
Cinnamaldehyde	12.99	30.83
Benzylbenzoate	13.22	1.93
γ-Terpinene	13.31	1.07
α-Bisabolol	15.60	3.62
Sabinene	15.68	2.27
β –Caryphyllene	16.31	2.02
α-Thujene	17.80	4.54
Camphene	18.00	0.99
Linalool	18.17	3.24
Bornylene	18.54	2.59
Copaene	19.89	1.92
Naphthalene	30.68	4.15
	Total	100

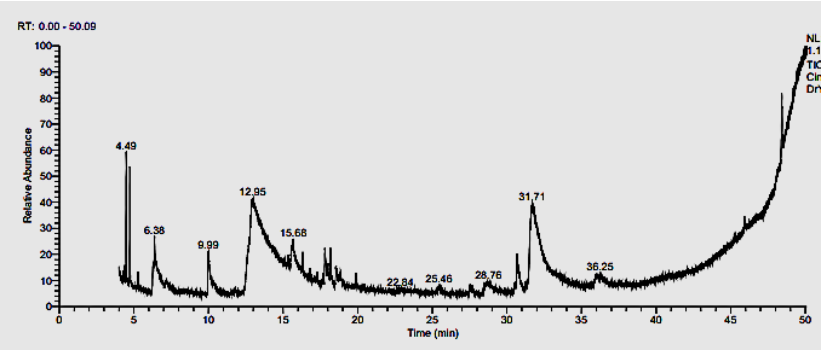

Fig. 7. GC-MS Analysis of components of Cinnamon oil

8. Antibacterial activity of Cinnamon extracts and Cinnamon oils against *Escherichia coli* (ATTC 25922) and *Staphylococcus aureus* (ATTC6538)

The results presented in Table (8) and Figures (8, 9), demonstrate the impacts of various concentrations of Cinnamon oil and extract on *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 6538), compared with the undiluted samples of Cinnamon oil and extract. The findings revealed that increasing concentrations of both Cinnamon extract and oil led to larger inhibition zones against *E. coli* and *S. aureus*. Similarly, Chowdhury *et al.* (2019) evaluated the comparative antimicrobial potential of ethanol extracts of Ginger (*Zingiber officinale*) and Cinnamon (*Cinnamomum zeylanicum*), and reported that Cinnamon extract exhibited significant activity against *E. coli*, which was the most sensitive organism among those tested. Wong *et al.* (2014) further reported that Cinnamon extracts contained high levels of cinnamaldehyde, which contributed to their antimicrobial activity, although the tests were limited to only four bacterial strains. They suggested that expanding the range of bacterial species tested would provide more comprehensive insights, given the variability in antibiotic resistance. Beyond antimicrobial evaluation, other assays such as antioxidant activity assessments may also provide a broader understanding of the bioactivity of Cinnamon compounds. In line with this, Parasthi *et al.* (2020) demonstrated significant antibacterial activity of Cinnamon against *E. coli*, reporting an inhibition zone diameter of 6.77 mm, which confirmed the potential effectiveness of Cinnamon extract.

Table 8. Antibacterial activity of Cinnamon extracts and Cinnamon oils against *Escherichia coli* (ATTC 25922) and *Staphylococcus aureus* (ATTC6538)

Concentrations	Cinnamon extract		Cinnamon oil	
	***E. coli* (mm)** Mean ±SD	***Staph. aureus* (mm)** Mean ±SD	***E. coli* (mm)** Mean ±SD	***Staph. aureus* (mm)** Mean ±SD
Non-dilution	11±0.04[a]	11±0.19[a]	34±1.03[a]	15±1.03[a]
Dilution ½	6 ±0.07[a]	7 ±0.08[a]	20±1.18[a]	10±1.72[a]
Dilution ¼	3 ±0.02[b]	5± 0.09[a]	13±1.07[b]	8±0.96[b]
Dilution 1/8	1 ±0.02[b]	3 ±0.04[b]	9±1.23[b]	5±0.88[c]
Dilution 1/16	0.0±0.0	1±0.06[b]	5±1.16[b]	3±1.65[d]
Dilution 1/32	0.0±0.0	0.0±0.0	2±1.12[b]	1±1.09[d]

mm: diameter millimeter

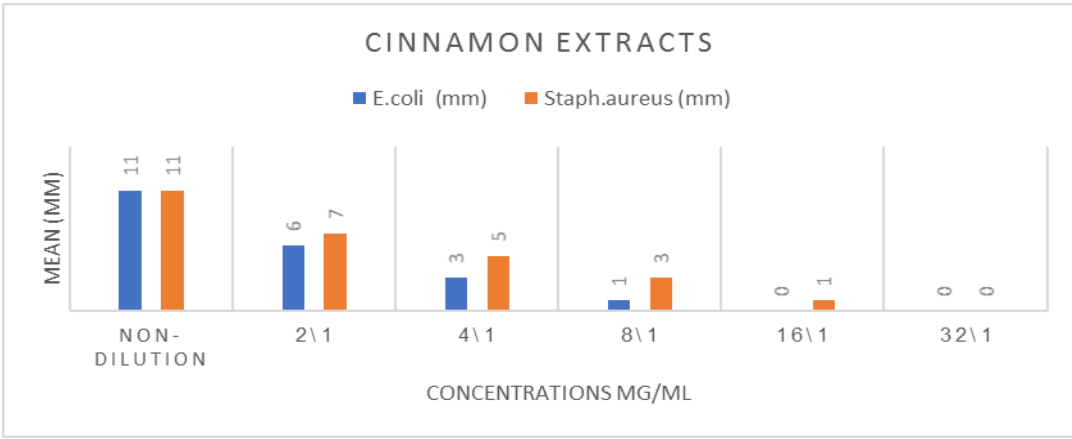

Fig.8. Antibacterial activity of Cinnamon extract against *Escherichia coli* (ATTC 25922) and *Staphylococcus aureus* (ATTC6538)

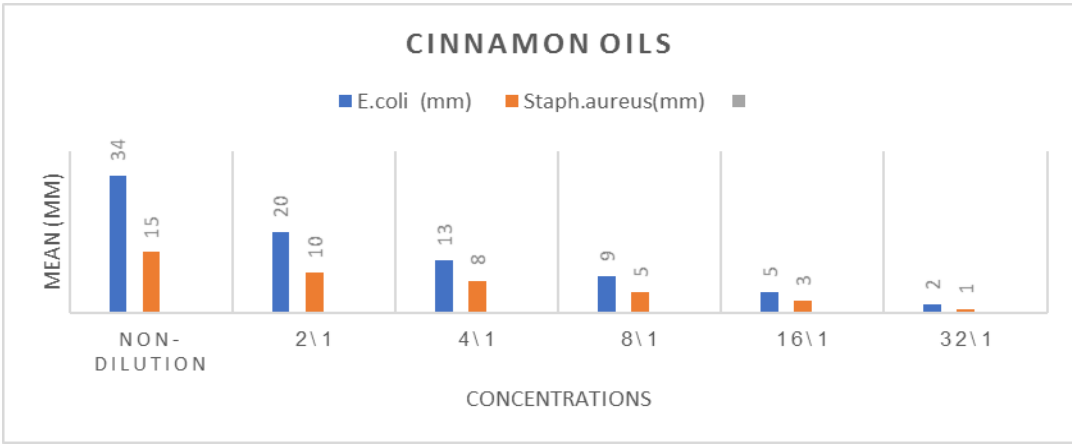

Fig.9. Antibacterial activity of Cinnamon oil against *Escherichia coli* (ATTC 25922) and *Staphylococcus aureus* (ATTC6538)

9. Antibacterial activity of Green tea extract and oil against *Escherichia coli* (ATTC 25922) and *Staphylococcus aureus* (ATTC6538):

Results in Table (9) and Figures (10, 11), show the effect of different concentrations of Green tea extract and oil against *Escherichia coli* (ATTC 25922) and *Staphylococcus aureus* (ATTC6538) compared with non-dilution of Green tea oil and extract. Results revealed that by increasing the dilution of Green tea extract and oil inhibition clear zone on *E. coli* and *Staph. aureus* decreased. Thao *et al.* (2023), mentioned that, terpinen-4-ol exhibited the highest antibacterial activity against *Staphylococcus aureus*.

Table 9. Antibacterial activity of Green tea extract and oil against *Escherichia coli* (ATTC 25922) and *Staphylococcus aureus* (ATTC6538)

Concentrations	Green tea extract		Green tea oil	
	***E. coli* (mm)** Mean ±SD	***Staph. aureus* (mm)** Mean ±SD	***E. coli* (mm)** Mean ±SD	***Staph. aureus* (mm)** Mean ±SD
Non- dilution	19±0.52^{a}	14±0.96^{a}	31±1.18^{a}	20±0.95^{a}
Dilution ½	11±0.43^{a}	9±0.72^{ab}	20±1.64^{a}	11±0.84^{a}
Dilution 1/4	6±0.32^{b}	6±0.66^{ab}	11±1.45^{a}	0.0±0.0
Dilution 1/8	2±0.07^{b}	4±0.63^{ab}	5±1.54^{a}	0.0±0.0
Dilution 1/16	0.0±0.0	2±0.64^{ab}	0.0±0.0	0.0±0.0
Dilution 1/32	0.0±0.0	1±0.19^{b}	0.0±0.0	0.0±0.0

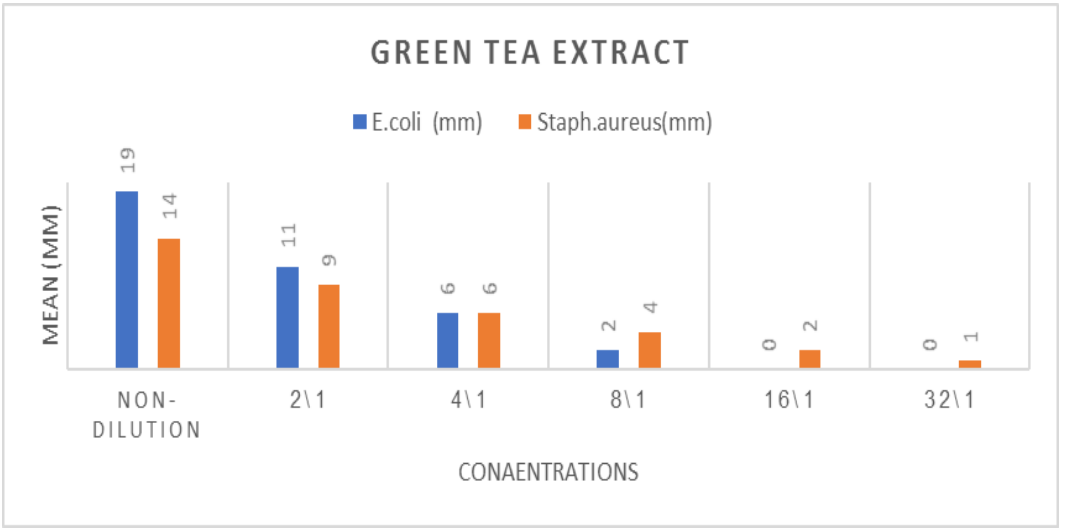

Fig. 10. Antibacterial activity of Green tea extract against *Escherichia coli* (ATTC 25922) and *Staphylococcus aureus* (ATTC6538)

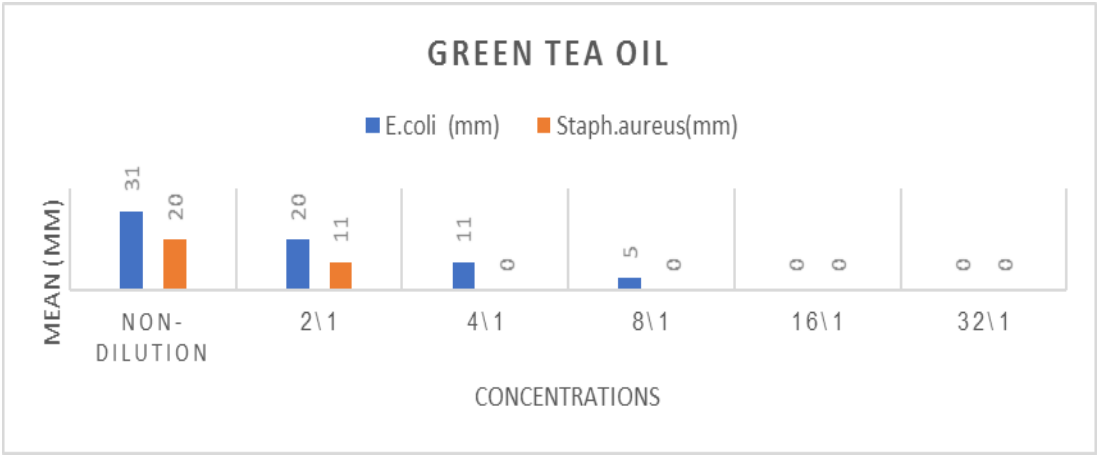

Fig. 11. Antibacterial activity of Green tea oil against *Escherichia coli* (ATTC 25922) and *Staphylococcus aureus* (ATTC6538)

10. Antibacterial activity of Ginger oil on *Staphylococcus aureus* (ATTC6538)

The results presented in Table (10) and Figure (12), illustrate the effect of different concentrations of Ginger oil on *Staphylococcus aureus* (ATCC 6538), compared with the undiluted oil. The findings showed that increasing concentrations of Ginger oil resulted in larger inhibition zones against *S. aureus*. These observations are in line with Al-Dhahli *et al.* (2020), observed that Ginger essential oil demonstrated greater antibacterial efficacy against Gram-positive bacteria in comparison with its activity against Gram-negative strains. This antimicrobial capacity is thought to stem from key bioactive components, including ar-curcumene and α-zingiberene. These compounds collectively establish Ginger oil as an effective candidate for a natural antimicrobial substance.

Table 10. Antibacterial activity of Ginger oil against *Staphylococcus aureus* (ATTC6538)

Concentrations	Ginger oil
	***Staph. aureus* (mm)**
	Mean ±SD
Non- dilution	5±0.003[a]
Dilution 1/2	3±0.007[a]
Dilution 1/4	1±0.004[a]
Dilution 1/8	0.0±0.0
Dilution 1/16	0.0±0.0
Dilution 1/32	0.0±0.0

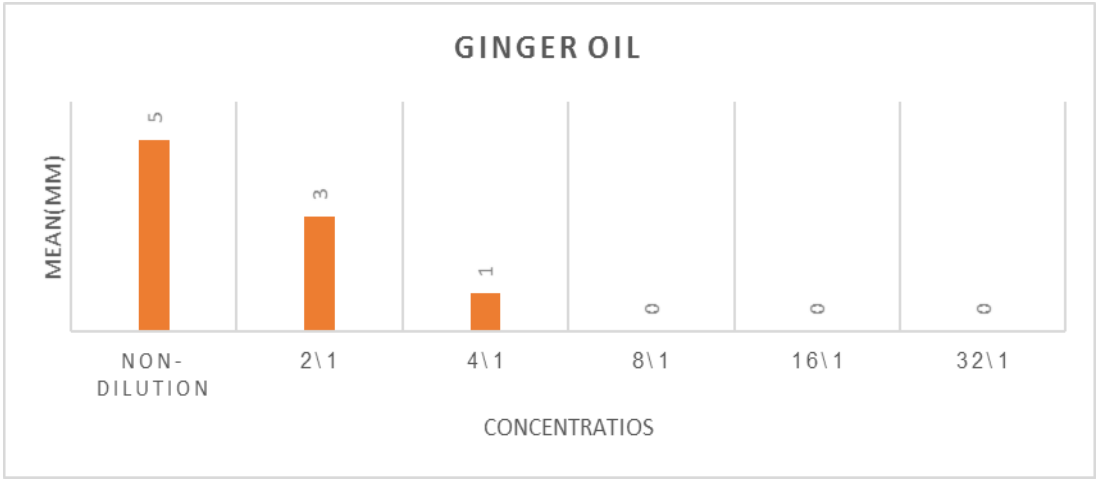

Fig. 12. Antibacterial activity of Ginger oil against *Staphylococcus aureus* (ATTC6538).

11. Antibacterial activity of combination between oils and between extracts against *Escherichia coli* (ATTC 25922) and *Staphylococcus aureus* (ATTC6538):-

From the results presented in Table (11) and Figures (13–14 (a–h)), it was observed that the highest inhibition of *Escherichia coli* (ATCC 25922) was achieved with the combination of Green tea oil and Cinnamon oil (T+C), where the inhibition zone reached 35 mm. In contrast, the greatest inhibition of *Staphylococcus aureus* (ATCC 6538) was obtained with the combination of Green tea oil and Ginger oil (T+G), producing an inhibition zone of 21 mm. Interestingly, the effect of the (T+C) extract on *S. aureus* was higher than on *E. coli*, suggesting differential susceptibility between the two bacterial strains.

These findings are in agreement with previous studies. Baljeet *et al.* (2015) reported that spices are rich sources of bioactive antimicrobial compounds, with combined extracts producing inhibition zones ranging from 12.3 to 19.6 mm against different bacteria (*Bacillus subtilus*, *Pseudomonas fluroscens*, *Salmonella Typhi*). Similarly, García-Díez *et al.* (2017) demonstrated that combining 13 essential oils (EOs) of herbs and spices exerted antimicrobial activity against both *E. coli* and *S. aureus*, highlighting that the synergistic effects of EOs depend on their antimicrobial potency and minimum inhibitory concentration (MIC) against foodborne pathogens. Likewise, Bag and Chattopadhyay (2015) evaluated synergistic interactions of selected spice and herb essential oils, such as Cumin, Ginger, and Turmeric, and Confirmed that their combinations enhanced antibacterial activity against *S. aureus* and *E. coli*.

Table 11. Antibacterial activity of combination between oils and between extracts against *Escherichia coli* (ATTC 25922) and *Staphylococcus aureus* (ATTC6538):-

Treatments	Strain	Diameter (mm) Mean ±SD
(C+G) oil	*E.coli*	33 ± 0.97^{ab}
(C+G) oil	*Staph. aureus*	15 ± 0.71^{f}
(T+G)oil	*E.coli*	30 ± 0.67^{b}
(T+G)oil	*Staph. aureus*	21 ± 0.59^{c}
(T+C) oil	*E.coli*	35 ± 0.87^{a}
(T+C) oil	*Staph. aureus*	18 ± 0.64^{d}
(T+C+G)oil	*E.coli*	18 ± 0.63^{de}
(T+C+G)oil	*Staph. aureus*	17 ± 0.58^{e}
(T+C) extract	*E.coli*	20 ± 0.62^{c}
(T+C) extract	*Staph. aureus*	15 ± 0.89^{f}

G: Ginger C: Cinnamon T: Green tea

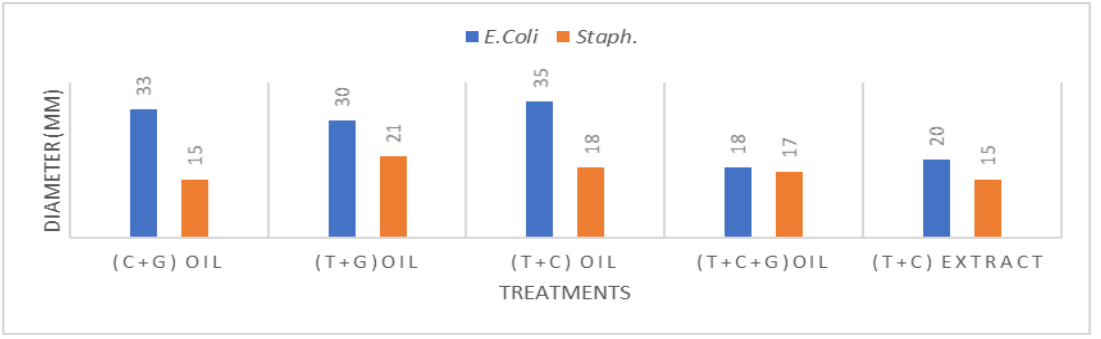

Fig. 13. Antibacterial activity of combination between oils and between extracts against *Escherichia coli* (ATTC 25922) *and Staphylococcus aureus* (ATTC6538)

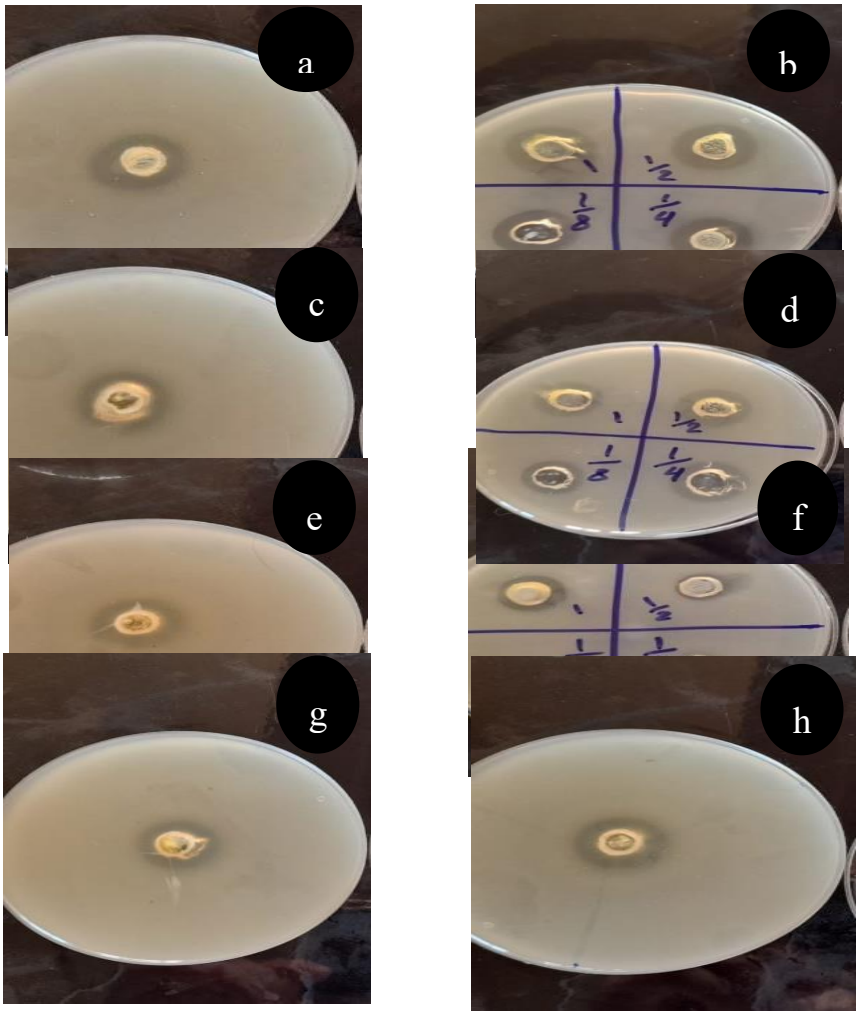

Fig. 14. Inhibition of *Escherichia coli* (ATTC 25922) and *Staphylococcus aureus* (ATTC6538) by Ginger, Green tea and Cinnamon oils.

(a and b): inhibition of *Escherichia coli* (ATTC 25922) by combination between Green tea and Cinnamon oil (T+C)
(c and d): inhibition of *Staphylococcus aureus* (ATTC6538) by combination between Green tea and Cinnamon oil (T+C)
(d and f): inhibition of *Staphylococcus aureus* (ATTC6538) by combination between Cinnamon and Ginger oil (C+G)
(g): inhibition of *Staphylococcus aureus* (ATTC6538) by combination between Green tea, Cinnamon and Ginger oil (T+C+G).
(h): inhibition of *Escherichia coli* (ATTC 25922) by combination between Green tea, Cinnamon and Ginger oil (T+C+G).

Table 12. Antibacterial activity of oils, extracts and their combination on yogurt starter and karish cheese starter cultures:

Treatments	yogurt starter (Z)	karish cheese starter (K)
C oil	0.0±0.0	0.0±0.0
G oil	0.0±0.0	0.0±0.0
T oil	0.0±0.0	0.0±0.0
(T+C) oil	0.0±0.0	0.0±0.0
(T+G)oil	0.0±0.0	0.0±0.0
(C+G) oil	0.0±0.0	0.0±0.0
(T+C+G)oil	0.0±0.0	0.0±0.0
C extract	0.0±0.0	0.0±0.0
T extract	0.0±0.0	0.0±0.0
(T+C) extract	0.0±0.0	0.0±0.0

G: Ginger C: Cinnamon T: Green tea

12. Antibacterial activity of oils, extracts and their combination on yogurt starter and karish cheese starter cultures:

The results obtained from Table (12), show that there is no effect of Green tea, Ginger and Cinnamon extracts or oils separately or in-combination on the growth of yogurt starter and karish cheese starter cultures.

CONCLUSIONS

The present study demonstrated that extracts and essential oils of Ginger, Green tea, and Cinnamon possess strong antimicrobial activity against both Gram-negative bacteria (*Escherichia coli*, ATCC 25922) and Gram-positive bacteria (*Staphylococcus aureus*, ATCC 6538). In addition, these extracts and oils exhibited high antioxidant potential, highlighting their dual role as natural antimicrobial and antioxidant agents. These findings suggest that such natural products may serve as promising alternatives or complementary approaches in food preservation and in the development of natural therapeutic agents.

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الملخص العربي

النشاط المضاد للأكسدة والتأثيرات المثبطة للزنجبيل والشاي الأخضر والقرفة، كلٌّ على حدة أو مجتمعةً، على بكتيريا المكورات العنقودية الذهبية والإشريكية القولونية

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استخدم المصريون القدماء الأعشاب والتوابل، كما استُخدمت لقرون في الهند والصين. استُخلص الزنجبيل والشاي الأخضر والقرفة، وكانت قيمة الرقم الهيدروجيني (pH)لكلٍّ من الزنجبيل والشاي الأخضر والقرفة (٤,٩٢، ٥,٢٥، ٤,٦) على التوالي، بينما كانت نسبة الحموضة القابلة للمعايرة (٠,٢١ %، ٠,٣٣ %، ٠,١١ %) على التوالي. تم تسجيل أقصى قيمة لقوة مضادات الأكسدة المختزلة للحديديك (FRAP)في مستخلصات(القرفة الممزوجة بالشاي الأخضر) (0.25ملغ/GAE جم) وأعلى قيمة في زيوت (القرفة الممزوجة بالشاي الأخضر) (0.39 ملغ/GAE جم). أما أقصى قيمة لمحتوى الفينول الكلي في مستخلصات (القرفة الممزوجة بالشاي الأخضر) (C+T) فكانت (20.23 ملغ/GAE جم) وأعلى قيمة في زيت (القرفة الممزوجة بالشاي الأخضر) (C+T)كانت (37.01 ملغ/GAE جم). كما أن نشاط مضادات الأكسدة (DPPH) في الزيوت أعلى من نشاط مضادات الأكسدة في المستخلصات. وقد أظهر زيت القرفة والشاي الأخضر الممزوج (C+T) نشاطًا مضادًا للأكسدة أعلى (٨٦,٢٠ %) مقارنةً بالزيوت الأخرى. بتحليل كروماتوغرافيا الغاز–مطياف الكتلة(GC-MS) ، يُمثل الزنجيبرين المكون الرئيسي للزيت العطري للزنجبيل (٢٠,٥٢ %)، و١.٨-السينول(٣١,٤٦ %)، والقرفة (٣٠,٨٣ %). بزيادة تركيزات القرفة والشاي الأخضر والزنجبيل (زيوت ومستخلصات)، ازدادت منطقة تثبيط بكتيريا الإشريكية القولونية ATTC) (25922والمكورات العنقودية الذهبية (ATTC6538). كان تأثير مستخلص (T+C) على نمو المكورات العنقودية الذهبية (ATTC6538)أقل منه على الإشريكية القولونية ATTC) . (25922لا يوجد تأثير لمستخلصات أو زيوت الشاي الأخضر والزنجبيل والقرفة، سواءً بشكل منفصل أو مجتمعة، على نمو بادئ الزبادي ومزارع بادئ جبن القريش.

الكلمات المفتاحية: الزنجبيل، الشاي الأخضر، القرفة، النشاط المضاد للأكسدة، كروماتوغرافيا الغاز–مطياف الكتلة، النشاط المضاد للبكتيريا، الإشريكية القولونية ATTC) (25922، المكورات العنقودية الذهبية(ATTC6538)