EVALUATE THE EFFECT OF *CINNAMON ZEYLANICUM* 0IL EXTRACT IN INHIBITION OF BACTERIA IN LABORATORY BISCUIT

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ABSTRACT

This study was aimed to investigate effect of *cinnamon zeylnicum* oil extract in inhibition of bacteria in laboratory biscuit.revealed chemical research on cinnamon oil extract has the presence of alkaloids, glycosides, saponins, resins, coumarins, terpenes, flavonoids, and stimulants. Using GC-MS and FT-IR analysis, it was found that the hot water extract contains terpenes, glycosides, resins, saponins, and phenols, indicating the effectiveness of these beneficial components in cinnamon oil extract. Biscuits were manufactured in the laboratory and fortified with different concentrations (0.125%, 0.25%, 0.50%, and 0.75%) of *C. Zeylanicum* oil extract and stored for 10 days at room temperature. The results showed that 0.125% and 0.25% showed no inhibitory activity against bacterial counts, while those of 0.50% and 0.75% inhibited bacterial counts until the eighth day of storage. Regarding the sensory evaluation of the laboratory-manufactured biscuits fortified with cinnamon oil extract, treatment A5 (control) received the highest score of 6 for the texture property. In contrast, treatment A4 (0.75%) received the lowest score of 4.80. In the same property, for the smoothness attribute, treatment A2 (0.25%) received the highest score of 5.70, while treatment A4 (0.75%) received the lowest score of 4.10.

Keywords: antmicrobial activity, storage, alkaloids, saponins, GC-MS

تقيم تاثير مستخلص زيت *cinnamon zeylanicum* في تثبيط الاحياء المجهرية للبسكويت المختبري

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المستخلص

هدفت هذه الدراسة إلى التحقق من تأثير مستخلص زيت القرفة zeylnicum في تثبيط البكتيريا في البسكويت المختبري . كشفت الأبحاث الكيميائية في مستخلص زيت القرفه عن وجود قلويدات، وجليكوسيدات، وصابونين، وراتنجات، وكومارين، وتربنس، وفلافونويدات، ومنشطات ، وباستخدام تحليل GC–MS وFT–IR، تبين أن المستخلص المائي الحار يحتوي على التربينات والجليكوسيدات والراتنجات والصابونين والفينبلس مما يدل على فعالية المكونات المفيدة في مستخلص زيت القرفة تم تصنيع البسكويت والمضاف الية المستخلص الزيتي *C. Zeylanicm* مختبريا وبتراكيز مختلفة0.125 و 0.25 و 050 و0.75 % وحفظه مدة 10 أيام في درجة حرارة الغرفة أظهرت النتائج بان التركيزين 0.125% ,0.25 لم يظهر أي فعالية تثبيطية لاعداد البكتيرية في حين التركيزين 0.50%,0.75.ثبط نمو الاعداد البكتيرية لغاية اليوم الثامن من فترة الخزن .اما التقيم الحسي للبسكويت المصنع مختبريا والمضاف الية المستخلص الزيتي فقد حصلت المعاملة A5 (control) على اعلى قيمة بلغت 6 في خاصية النسجة في حين حصلت المعاملة A4(0.75%)على اقل قيمة بلغت 4.80 في نفس الخاصية .اما في خاصية الطراوة فقد حصلت A2(0.25%) على اعلى قيمة بلغت 5.70 في حين حصلت المعاملة A4(0.75%) على اقل قيمة بلغت 4.10.

الكلمات المفتاحية: الفعالية الضد الميكروبية، الخزن، القلويدات، الصابونين، GC–MS.

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INTRODUCTION

Essential oils derived from medicinal plants and possessing aromatic properties have been employed for their biological activity since ancient times(11,19). They provide a diverse array of applications. A significant number of them are now being utilised. Perfumes find use in several industries, such as perfumery, food additives, beverages, and the cosmetic sector, to mitigate the potential for allergic reactions. The pharmaceutical industry is an illustrative example of this phenomenon. Throughout millennia, medicinal applications have been attributed to them in traditional healing practices (41). Recently, there has been a surge in interest in natural goods due to their ease of availability, few side effects or low toxicity, and enhanced biocomposition compared to chemical treatments or compounds associated with chemotherapy. Medicinal plants provide a viable alternative to drugs (23 ,40). Medicinal plants may cure many illnesses (3, 39) due to their various biological actions. Essential oils are considered promising natural chemicals (e.g., antifungal, antibacterial, antiviral, anticancer, antioxidant, analgesic, anti-inflammatory properties, and immunomodulatory) (11). It is used for oral health, toothaches, oral infections, and to remove bad breath. It is also antiemetic, anti-diarrheal, and anti-flatulen (17). The Cinnamon plant is "considered one of the important medicinal plants because it is rich with many active compounds" (17) as was found ''the extract of *Zingiber officinale* rhizomes with concentration 500 mg /L can be used to disinfect'' (9). The investigation of the antiproliferative and cytotoxic activities of various essential oils on different tumour cells has been conducted, considering their composition (29). These essential oils have demonstrated effectiveness in the management and prevention of inflammation-related disorders, as well as in the generation of reactive oxygen species (ROS) (38). *Cinnamomum zeylanicum* is a botanical species belonging to the family Cinnamomum. The botanical collection known as Blume has over 300 species of perennial, fragrant trees and shrubs. The bark of the specimen has a reddish-brown hue, while an oval form and diagonal arrangement characterise its leaves. Furthermore, the blooms of this entity are of diminutive size. The concentration of volatile oil in *cinnamon* is 2%. The user's text does not contain any information to rewrite academically. The native regions of origin for this particular species include India, South America, and Southeast Asia. The oil derived from the bark of *cinnamon* consists predominantly of Trans-Cinnamaldehyde, ranging from 65% to 85%, with Eugnol content ranging from 5% to 10% (28). The remaining oil constituents include a combination of hydrocarbons and oxygen molecules. The compounds under consideration include β-caryophyllene, benzyl benzoate, linalool, eugenyl, cinnamic acid, and cinnamyl acetate. The flavour profile of cinnamon can be attributed to the presence of cinnamaldehyde. Additionally, cinnamon oil comprises several active ingredients contributing to its biological effects. These include Eugenol, methyl eugenol, phellandrene, and cinnamaldehyde. Among these components, cinnamaldehyde is the predominant compound, accounting for over 60% of the oil's composition and is pivotal in its observed activity and in treating antifungal and inflammatory disorders (47). Used for embalming by the ancient Egyptian people (36*). C. zeylanicum* and *C. aromaticum* (3) prepare different types of chocolate, beverages, spicy candies and liquors. Moreover, cinnamon is used in various savoury dishes, pickles, soups, and Persian sweets. It works as an antibacterial potential of the antibacterial''effect of *Cinnamomum zealynicum* bark aqueous , methanol, and chloroform extracts against some gram positive and gram negative" (8). It has been used (7). *''Thymus vulgaris* the used part (Leaves, Steams and Flowers) and *Cinnamomum zeylanicum*'' This study investigates the qualitative characteristics and effective ingredients of cinnamon bark oil. Additionally, it seeks to examine the impact of incorporating different concentrations of the oil extract into laboratory-made biscuits, as well as the potential for extending the shelf life of these biscuits. The primary focus of this research is to explore the inhibitory effect of cinnamon bark oil on the growth of harmful microorganisms. Furthermore, the

investigation involves the examination of the sensory attributes of the laboratory-produced biscuit item, as well as the implementation of a sensory assessment process.

MATERIALS AND METHODS

The six-well flat-bottom plates were provided by Iwaki SciTech Co., a company based in Japan. The reagents used in this study were obtained from Sigma-Aldrich (St. Louis, MO, USA). These reagents included Folin–Ciocalteu reagent, linoleic acid, gallic acid, β-carotene, Tween 40, and (2, 2-diphenyl-1-picrylhydrazyl) DPPH. Merck Co. supplied the microbiological medium, Mueller Hinton Broth (MHB), and Mueller Hinton Agar (MHA) from Darmstadt, Germany.

Isolation of essential Oil

Hydrodistillation extraction was employed to separate the essential oil from the bark of *Cinnamomum zeylanicum*, as conducted by researchers from the University of Baghdad in the field of biology. The extraction process lasted for three hours and involved dried bark weighing 20 grame along with a Clevenger-type apparatus. The essential oil was collected using glass vials that had been cleaned well. After collection, the vials were dried using anhydrous sodium sulfate and stored at a temperature of 4°C until they were ready for analysis (43). and 25 ml of extract was obtained

Characterisation of essential Oil (GC-MS). gas chromatography-mass spectrometry

The essential oil derived from cinnamon bark encompasses a distinct chemical constituent that has been meticulously extracted. Utilising the Gas Chromatography-Mass Spectrometry (GC-MS) technique, *Cinnamomum zeylanicum* was accurately identified and characterised. This advanced analytical procedure was conducted at the Gas Chromatography-Mass Spectrometry Laboratory, situated within the prestigious College of Agriculture at Basrah University, Iraq. A rigorous analysis of 1µL (0.01m) (samples of essential oil extracts procured from cinnamon barks was performed. For this purpose, a state-of-the-art GC SHIMADZU QP2010 ultra gas chromatograph, interfaced with a Mass Spectrometer (GC-MS), was employed. The system operated with a DbB5ms capillary column, utilising a split injection mode under pressure of 49.5kPa. To quantify the relative percentage abundance of each chemical constituent present in the essential oil, a comparative analysis was conducted. This involved assessing the average peak area of individual chemical compounds against the cumulative sizes of all detected components. The meticulous execution of this procedure facilitated a comprehensive understanding of the chemical profile of the cinnamon bark essential oil, contributing to the broader academic knowledge on the subject. To elucidate the chemical composition of *cinnamomum zeylanicum* essential oil, a comprehensive Gas Chromatography-Mass Spectrometry (GC-MS) analysis was conducted. The instrumental configuration comprised a TRACE MS mass spectrometer by Thermo Finnigan, paired with a DB-5 capillary column (30 m x 0.25 mm, possessing a stationary phase thickness of 0.25 µm). The thermal gradient program executed during the analysis involved an initial column temperature of 40°C, which was subsequently elevated to 250°C at a progressive rate of 2.5°C per minute. Helium was utilised as the carrier gas throughout the process, maintaining an ionisation energy of 70 eV. To accurately identify and quantify the constituents of the essential oil, the mass spectra of the oil components, as well as their respective retention indices, were meticulously compared against pre-existing database entries. Additionally, the retention indices were calculated with precision using a homologous series of n-alkanes (ranging from C8 to C22), which served as reference compounds (10, ,35).

(FTIR) Analysis fourier transform infrared spectroscopy: An FTIR spectrometer was used to investigate the functional groups of essential oil constituents. Potassium bromide and essential oil were mixed. Before being crushed into a pellet. The oil's FTIR spectra were measured with a scanning resolution of 4 cm^{-1} between 500 and 4000 cm^{-1}. using 0.5g (KBr)

(TPCs) Total phenolic contents

To ascertain the Total Phenolic Content (TPC) of the essential oil derived from *Cinnamomum zeylanicum* bark, the Folin–Ciocalteu assay was employed, adhering to the methodology

delineated by Abeysekera et al. (3). In this assay, a volume of 20 liters of the essential oil was meticulously combined with 110 liters of freshly prepared Folin–Ciocalteu reagent, which had been subjected to a 10-fold dilution before use. After this initial mixing, 70 litres of a sodium carbonate solution were introduced. The concoction was then incubated at room temperature for 30 minutes, facilitating the requisite chemical reactions. Following the incubation period, the absorbance of the mixture was quantitatively measured at a wavelength of 765nm. To accurately estimate the TPC of the essential oil, a standard curve was constructed utilising gallic acid, with concentrations ranging from 0.06 to 1 mg/mL(Figure 1) . The results gleaned from the absorbance measurements were then extrapolated against this standard curve, enabling the TPC of the essential oil to be expressed in terms of mg gallic acid equivalent (GAE) per g dry weight of the *C. zeylanicum* bark. This precise and standardised analytical procedure thus facilitated a rigorous quantification of the total phenolic content within the essential oil, contributing to the broader academic understanding of the phytochemical properties inherent to *Cinnamomum zeylanicum* bark.

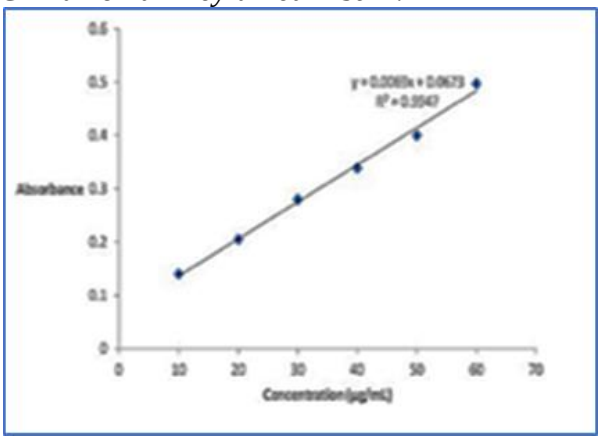

Fig.1.calibration curve for standard gallic Acid

Activity of antioxidant assays

radical scavenging activity 1،1-Diphenyl-2-picrylhydrazyl(DPPH)

2 mL DPPH methanolic solution was charged and combined with a 38.5 mL aliquot of (control sample) essential oil or methanol (104 M). For a total of 20 minutes, the pieces were kept in the dark. Before being detected against methanol at 517 nm. The DPPH radical scavenging activity of the essential oil was calculated using the equation below.

B-Carotene bleaching assay

This experiment used the Ribeiro-Santos et al. (35) technique. To 1 mL of -carotene solution, linoleic acid and tween 40 (200 mg) (20 mg) were added (2 mg -carotene in chloroform). After drying the solution at 40°C and vacuuming to remove the chloroform, 50 mL of oxygenated high-purity water was added. Then, the answer was forcefully agitated to create a carotene emulsion / linoleic acid, which was then combined with the addition of essential oil (200 mL). In a water bath, 120 minutes were spent heating the resulting combination, and its absorbance was measured at 470 nm (AS120). The sample control was made in the same way as the test sample, except instead of essential oil, methanol was employed. Before (AC0) and after (AC120) 120 minutes of incubation at 470 nm, the absorbance of the control sample was measured (AC120). Using the formula below, th oil's antioxidant activity was estimated (18, 37).

Activity of antimicrobial

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC): The minimum essential oil concentration and minimum necessary oil intensity (MBC) are attributed to the low critical oil concentration that kills bacteria in the first inoculum (no colony formation) (40). The lowest possible essential oil concentration (MIC) is defined as the least expensive average of a concentration of essential oils that causes no observable (turbidity) bacterial growth. The MIC and MBC of C were determined using Behbahani et al (2017) techniques. To begin, MHB medium was used to prepare the essential oil concentrations (100, 50, 25, 12.5, 6.25, 3.125, 1.562, 0.78, and 0.39 mg/mL), which were then 0.45 mg/ml syringe filters were used to sterilise the syringes. The oil was poured into the wells (96-well plates) previously filled with 20 μL of microbial suspensions at each concentration (20 μL). After a 24-hour incubation period at 37°C, the vessel was re-incubated with a 5-percent (20μL) triphenyl tetrazolium chloride solution. The MIC was established to be the lowest oil concentration that inhibited

microbial development, as seen by the lack of dark red colour in the wells. On MHA medium plates, a hundred microlitres of fluid was cultivated in the wells without microbial growth. The MBC was established as the smallest amount of oil that killed the bacterial strains, as demonstrated by the absence of discernible colonies on the medium's surface after the plates were held at 37°C for 24 hours.

Manufacture of laboratory biscuits

The biscuit was made in a lab by using the following ingredients: Materials:

White flour 100 g
Solid fat 22.7 g
salt 2.7 g
Baking powder 4.9 g
Milk 73.6 g

cinnamon oil extract was added to the laboratory biscuits with different concentration after that an (aqueous cinnamon)extract was added. Spicy 85% and aromatic oily) both separately in biscuit dough with concentrations 100m g /ml and equivalent to 0,125 , 0..25 0. 50, 0.75%. (21) 10 ml of each concentration.

The method of work:

The biscuits were prepared in the laboratory (with some modifications in the weights of the materials used) according to the following steps:

1- Sift the flour, salt, and baking powder together into a mixing bowl. The oven temperature is regulated at 218°C.

2- add the fat to the dry ingredients.

3- Then the liquid milk was added to the dry ingredients, then the ingredients were mixed well with the fork and several times (about 30 times) until the dough became homogeneous.

4- Spraying the wooden board with flour and spread the dough with a thickness of 0.5 pieces in a circular biscuit mold with a diameter of 5 cm.

5- Put the biscuits in the not greased mold using a special spatula knife and leave a distance of 1-1.5 cm between the biscuit pieces until the color (becomes golden(21).

How to store the biscuit

The biscuit was placed in sterile polyethene bags and kept at 30 degrees Celsius for ten days. Then, the microbiology examinations were performed every two days until the end of the storage period.

Preparing the sample for counting bacteria and moulds:- Total count of bacteria:

Counting the bacteria was done by pouring plates method and using nutrient agar and two repeaters for each dilution, where dilutions of 1×10^3 - 8×10^3cfu were 1ml was taken of each dilution was taken and placed in a sterilised dish; then, the nutrient medium was added to it with simple stirring and incubated at a temperature of 37C for 24 - 48 hours. The plates containing 30-300 colonies were selected for the same dilution.

Bacterial diagnosis

The types of bacteria that appeared during colony count were diagnosed, and the catalase and the indole tests were performed. The bacteria were diagnosed as mentioned in

Sensory evaluation

A sensory evaluation process was conducted for samples from the biscuit of the Department/ home economicse of the University of Baghdad. Their number was 10 assessors according to the evaluation form approved by the and the results were statistically analysed using the test (1).

Essential oil's antibacterial mechanism of action

The findings support the theory that essential oils extracted from *C. zeylanicum* harm *E. coli* and *L. innocua* cell walls. Membrane lysis and transformations caused by the essential oil's damage to membrane integrity and permeability may be responsible for the identical changes in bacterial cell walls (29*). C. zeylanicum* essential oil is a kind of essential oil that appears to have a membrane-related effect. It can also penetrate deeper into cells, speeding up the pace of cell death (20).

Statistical Analysis

The statistical program (36) was used in data analysis to study the effect of different treatments on the study's traits according to a complete random design (CRD), and the significant differences between the means were compared with the Least Significant Difference (LSD test).

RESULTS AND DISCUSSION

Chemic compositions of the essential oil

The antioxidant and antibacterial effects of essential oils and their physiologically active ingredients are exceptional. As a result, they've seen a lot of use in the food business,

including Surface packaging and active packaging cleaning of meat and fresh products (47). As a result, the chemical components, antioxidant impact, and This study looked at the antibacterial properties of *C. zeylanicum* essential oil. As shown in Table 1, GC-MS analysis revealed the presence of 17 chemical components in *C. zeylanicum* essential oil. The primary components of the essential oil were (E)-cinnamaldehyde (71.7%), linalool (7.1 %), -caryophyllene (6.45%), eucalyptol (5.42%), and eugenol (4.61%). p-cymene (1.91%), -humulene (1.71 %), -cadinene (1.42%), -pinene (1.33%) and limonene (1.23%). Were the other significant elements? Several investigations have revealed that cinnamaldehyde is the predominant chemical constituent in essential oil from the bark of *C. zeylanicum* (22, 43), which supports our findings (45).

Table 1. *Zeylanicum* essential oil chemical compounds

Comps.	Retention time. (min)	KI	Percentage %
Cinnamic alcohol	4.6	1212	0.15
3-phenyl, acetate	**6.5**	**1125**	**2.97**
6- octadecenoic acidcyclohexane	**15.5**	**1521**	**9.29**
carboxylic acid	**12.2**	**1280**	**8.41**
Benzaldehyde	**6.4**	**962**	**0.31**
β-Caryophyllene	**18.5**	**1519**	**6.45**
E)-cinnamaldehyde	**15.2**	**1413**	**71.7**
Eucalyptol	**8.0**	**1085**	**5.42**
Benzyl benzoate	**26.8**	**1711**	
Linalool	**9.8**	**1189**	**7.1**
Isoborneol	**11.6**	**1274**	**0.83**
γ-Terpinene	**8.6**	**1122**	**0.41**
Eugenol	**16.9**	**1468**	**4.61**
δ-Cadinene	**20.9**	**1582**	**1.42**
α-Humulene	**19.4**	**1542**	**1.71**
Acetic acid, cinnamyl ester	**19.2**	**1537**	**0.55**
Caryophyllene oxide	**22.6**	**1622**	**0.51**
			0.52
trans-Calamenene	**21.1**	**1586**	**0.72**
Limonene	**7.9**	**1075**	**1.23**

Cinnamon oil's chemical composition

Cinnamon essential oil yielded 5% of the total. The essential oil of *C. zeylanicum* included 30 components, according to GC-MS analysis. Cinnamaldehyde was the main ingredient in cinnamon essential oil. It accounted for 57.83 per cent of the total area of the other components. Cinnamic alcohol was 0.15 per cent, and 3-phenyl acetate was 2.97 per cent of the total area (Figure 2) and 9.29 and 8.41 per cent for 6- 6-octadecenoic acid and cyclohexane carboxylic acid, respectively (Table 1). Due to variances in agriculture dates, origin, plant, vegetable state, market storage circumstances, and growing season, the essential oils component may vary.

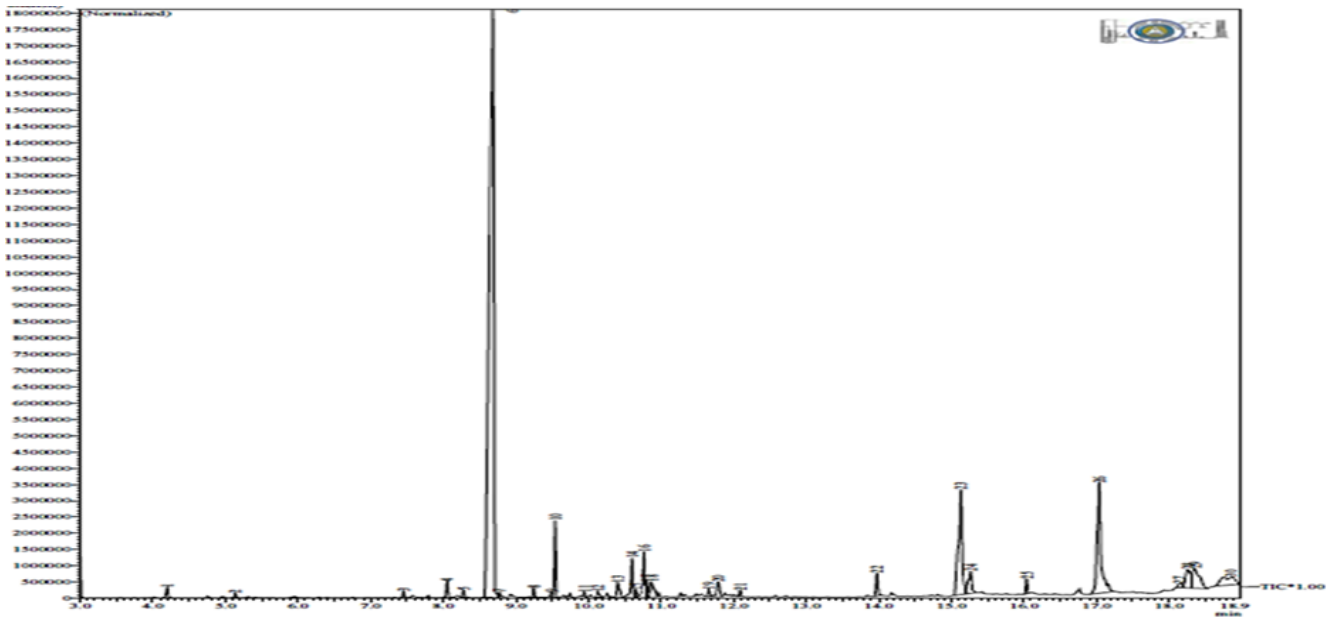

Fig. 2. Chromatogram of essential oils extracted from cinnamon barks

FTIR Analysis: Essential oils are thought to be complicated mixtures. The functional groups of C. zeylanicum were identified using FTIR spectroscopy. Essential oil. *C. zeylanicum* (Figure 3). The vibration stretching of aldehyde carbonyl (C=O) groups is responsible for the peaks at roughly 1680 cm1 and 1630 cm1, indicating *C. zeylanicum* essential oil contains a high proportion of cinnamaldehyde and aldehydes .Other notable peaks were found at 689 cm1 and 689 cm2 (vibration absorption of alkanes),970 cm−1 (C-H bond), 750 cm−1 (benzene rings = CH), 1126 cm−1 1235 cm−1 (C-O-C bond of aromatic acid ester (C-O and C-OH bonds), and C-OH groups of phenolic compounds), 1290 cm−1 (alkanes CH2), 1450 cm−1 (alcohol C-OH bond), 1455–1626 cm−1 (C=C bond), 1575 cm−1 (aromatic C=C bond) 1626–1732 cm−1 (C=O bond of carbonyl groups), 2932 cm−1 (=C-H bond), 2817 cm−1 (C-H bond of carbonyl groups), and 3028 cm−1 (aromatic C-H bond) (31, 42) All of these peaks indicate that the essential oil is high in phenolic and aromatic chemicals, particularly cinnamaldehydeThis is similar to what he found (6)‘‘The MIC method was used to investigate antibacterial activity by measuring the lowest inhibitory concentration’’

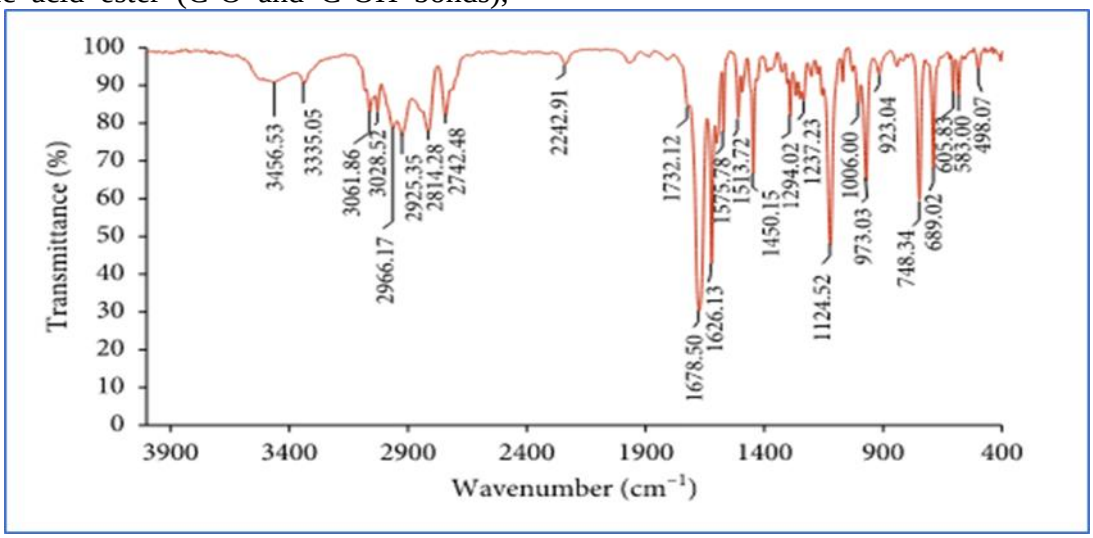

Fig. 3. Essential oil of *C. zeylanicum* groups

Antioxidant Activity

The antioxidant action of essential oils is most likely owing to a synergy between their components, and the primary features are principally the cause of this favourable biological effect (15). This study used DPPH-RS activity and -carotene bleaching assays to investigate the antioxidant activity *of C.*

zeylanicum essential oil. The DPPH-RS activity test is based on the interaction of powerful, stable free radicals and antioxidants with DPPH that produce a bright violet colour and then convert them to a colourless molecule; consequently, the degree of discolouration determines the antioxidant agent's free radical scavenging capacity (42). The essential oil of *C. zeylanicum* has a DPPH-RS activity of 71.12 0.77 percent, indicating that it has a remarkable ability to DPPH free radicals can be neutralised by using hydrogen atoms or electron donation (14)Table 2.

Table 2. Ascorbic acid (vitamin C): Similar concentrations were prepared

Comp	25(mg/ml)	50(mg/ml)	100(mg/ml)
Alcoholic extract	**76.21**	**75.81**	**88.17**
Aqueous extract	**42.18**	**55.12**	**79.57**
Ascorbic acid	**88.95**	**90.25**	**95.54**

Antibacterial Activity

Bacterial inhibition : Cinnamon essential oils have been shown to have antibacterial activity against six different bacterial species (Table 3). With increasing concentrations of essential oil, the inhibitory effect grew stronger. The addition of 18μl litres of cinnamon essential oil resulted in the Gram-positive and Gram-negative bacteria being inhibited by creating a broad inhibition zone. They were 30 and 31. 85, 28. 29. 24, 28, 21 and 26 mm of *L. monocytogenes, S. aureus, E. aerogenes, E. coli, and P. erogenous*. Essential oils might provide novel antibacterial chemicals, particularly for microorganisms that cause food deterioration. The presence of a broad-spectrum antibiotic compound may have antibacterial effects against both Gram-positive and Gram-negative bacteria, according to studies. These findings are consistent with previous research in the field, which found that the bark of cinnamon oil inhibited the growth of Gram-negative and Gram-positive bacteria. (13).

Table 3. Antibacterial activity of C. zeylanicum bark essential oil in vitro

	Antimicrobial assays		
Microbial isolates	**Disc diffusion agar (mm)**	**Well diffusion agar (mm)**	**MIC (mg/Ml)**
Escherichia coli	**18.00 ± 0.40**	**19.00±0.50**	**6.25**
Pseudomonas aeruginosa	**24.00 ± 0.32**	**27.00±0.67**	**3.13**
Salmonella typhimurium	**19.00 ± 0.70**	**22.00±0.48**	**6.25**
Listeria innocua	**30.00 ± 0.50**	**34.00 ±0.46**	**0.78**
Staphylococcus aureus	**26.00 ± 0.44**	**29.00±0.45**	**0.78**
Bacillus cereus	**27.00 ± 0.61**	**28.00±0.81**	**1.56**

Table 3. also includes the MBC and MIC *C. zeylanicum* essential oil, which has antimicrobial properties. Gram-positive bacteria, as can be shown, were growth-suppressed or killed in the presence of lower amounts of the essential oil than Gram-negative bacteria, mainly because their cell membranes have a single mucopeptide layer, which renders them more vulnerable to antimicrobial drugs. Gram-negative bacteria, on the other hand, have more cell membranes, a phospholipid layer and complicated lipopolysaccharide, with a significantly reduced diffusion rate to lipophilic-based antimicrobial chemicals in essential oils (19, 41). Researchers have made similar discoveries in the past (43). The antibacterial action of cinnamaldehyde, the primary chemical ingredient of *C. zeylanicum* essential oil, has been linked to preventing the synthesis of critical bacterial enzymes and causing severe damage to bacterial cell walls in Gram-negative/positive bacterial and fungal species (13, 20). As a result, the high cinnamaldehyde content of *C. zeylanicum* essential oil might be related to its antibacterial properties. There are similar studies when using the oil extract of lemongrass leaves against bacteria. The effectiveness and concentration were tested. *MIC* and *MBC"* were evaluated against some gram-positive and gram-negative bacteria. *Bacillus cereus, Staphylococcus aureus* and Micrococcus spp'' recorded high sensitivity to essential oil with inhibition zone reached mm (25), respectively.(31) *Antimicrobial activity of Laurus nobilis leavesWater extract* Results revealed that concentration (50, 100, 200) mg/ml showed high antibacterial activity

against *Staph aureus*"(34). activity against Pseudomonas aeruginosa

Effect on proliferation (Antiproliferative) The antiproliferative activity of *C. zeylanicum* essential oil on AT-MSCs was investigated using the MTT assay. MSCs may develop into a range of cell/tissue lineages and produce growth-promoting secretomes since they are multipotent self-renewing cells, including antioxidants and anti-inflammatory compounds, making them a good choice for regenerative medicine (23). The cells were given rising doses of the essential oil over 24 hours, ranging from 1 to 200 mgmL1. When the concentration of the essential oil was raised to 200 mgmL1, the antiproliferative activity of AT-MSCs increased considerably (Figure 4). The antiproliferative impact of the oil on AT-MSCs was determined to have an IC50 value of 83.51 mgmL1. Low quantities of *C. zeylanicum* essential oil seemed to drive cell proliferation and promote AT-MSC development, in addition to possessing excellent antioxidant activity and a significant bactericidal effect (< 50 mg·mL−1 at MBC) against all pathogenic and spoilage bacteria evaluated in this work (Table 4).

Table 4.Cinnamon bark essential oil concentrations (SD±: Standard division). And their antimicrobial zones for some microorganisms

Concertation of essential oil cinnamon 20mg/L	Bacteria Isolates inhibition Zones (mm)				
	Escherichia coli ATCC 25922	*Enterobacter aerogenes* ATCC 35029	*Listeria Monocytogenes* ATCC 9525	*Pseudomonas Erogenous* ATCC 10145	*Staphylococcus Aureus* ATCC 25923
6 µL	18.00±0.30	19.00 ±0.10	21.30 ±0.31	17.00 ±0.10	20.50 ±0.20
12 µL	22.20±0.39	22.10 ±0.48	26.70 ±0.52	22.23 ±0.21	27.03 ±0.22
18 µL	28.22 ±0.31	27.20 ±0.39	29.86±0.40	26.56 ±0.21	30.30 ±0.61

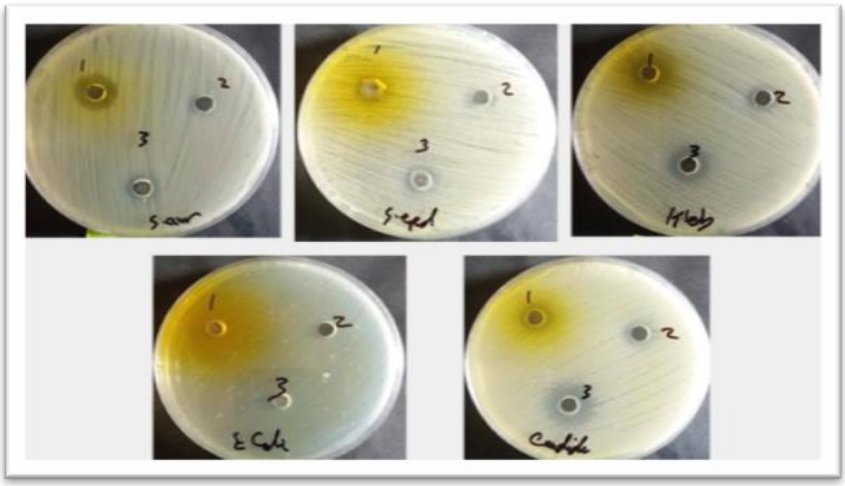

Fig. 4. Cinnamon essential oil antimicrobial zones for some microorganism

Bacterial isolaty kill time.

The effect of essential oils from *C. zeylanicum* barks at 10, 20, and 30 L.mL-1 on pathogenic bacteria cell viability (kill time) is shown in. (Figures 5, 6, 7, 8 and 9). Compared to the control sample, in the test, the essential oil decreased the viability of bacteria.

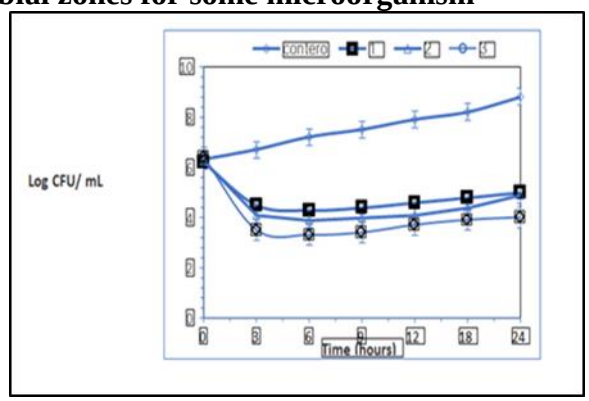

Fig. 5. *E. coli* viability cell in nutritive broth at 37 °C with varied concentrations of *C. zeylanicum* barks essential oil

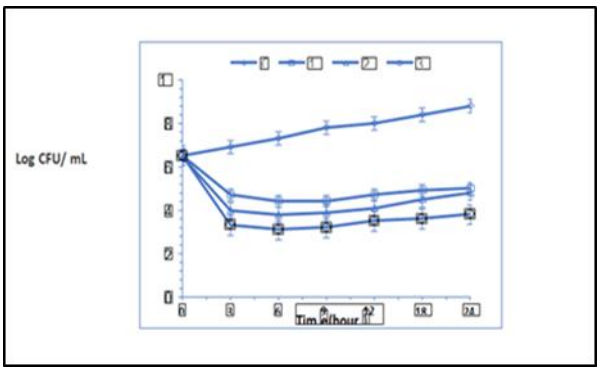

Fig.6. *E. aerogenes* viability cell in nutritive broth at 37°C with varying concentrations of *C. zeylanicum* bark essential oil

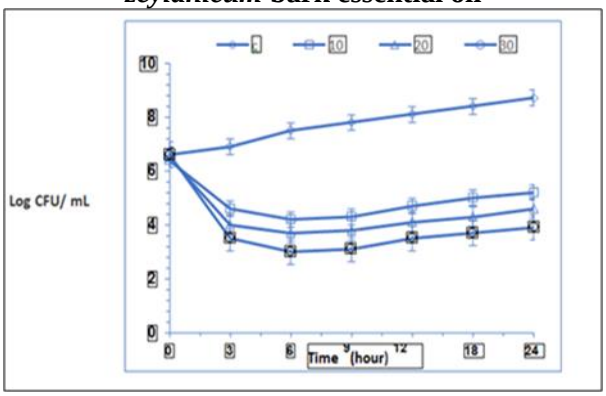

Fig. 7 .The viability cell of *L. monocytogenes* concentrations of C in nutritional broth At 37°*C, zeylanicum* bark essential oil is extracted

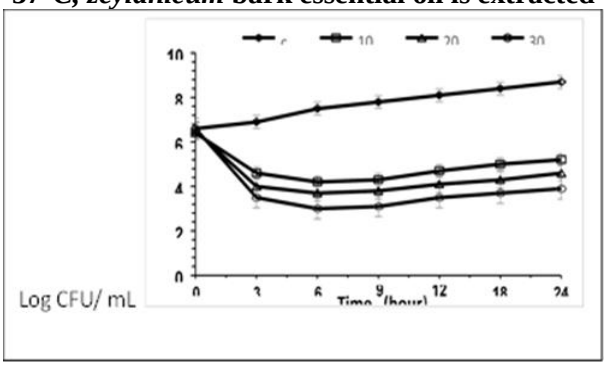

Fig.8 .*P. erogenous* viability cell in nutritional broth with various concentrations of *C. zeylanicum* barks essential oil at 37°C

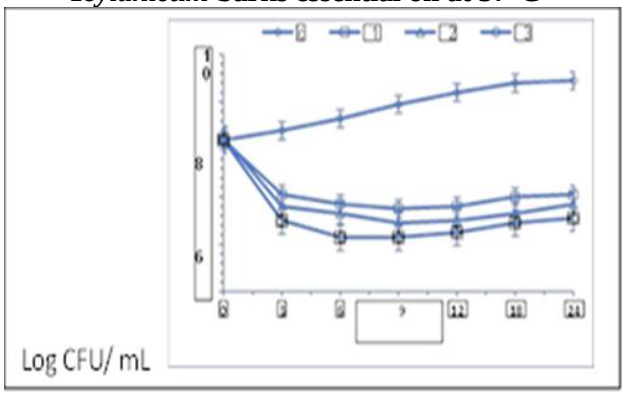

Fig.9. *S. aureus* viability cell in nutritive broth at 37°C with varied concentrations *of C. zeylanicum* barks essential oil

The antibacterial efficacy of essential oil was determined by killing all bacteria strains in a static concentration exposure (2, 31). Compared to the control sample, the essential oil of cinnamon caused a decline in the viable cell count of bacteria, ranging from 2 to 5 Logs. Cycles across the periods studied. The essential oil of C. zeylanicum can accumulate in the bacterial cell wall, causing membrane cell damage, cytoplasmic infiltration, proton motive force elimination, cell death, and cell lysis.(3, 30). Based on specific research, blending the critical components with the complete essential oil has a more potent antibacterial activity, showing that the crucial elements are vital to antioxidant activity and collaborative action (30). Cinnamon's action mechanism caused bacterial membrane structure to break down and protein denaturation. Cinnamaldehyde, the primary component of cinnamon essential oil, is an organic molecule with the formula $C_6H_5CH=CHCHO$ that is hypothesised to have antibacterial characteristics by blocking the formation of cell enzymes. Cinnamon's action mechanism disintegrated bacterial denaturation of proteins and the structure of membranes. Cinnamaldehyde is an organic compound with the formula C6H5CH=CHCHO that alters cell wall integrity, produces cytoplasmic acidity, cytoplasmic granulation, intracellular ATP depletion, and cytoplasm scarcity, and is thought to have antibacterial properties.

Effect of adding the extract (oil) on the number of bacteria during the storage period of the manufactured biscuit.

The results in Table 5 indicated the effectiveness of the essential oil extract *C. Z.ceylaicum* in reducing the inhibition of bacterial numbers in the manufactured biscuits, as the bacterial growth did not appear at the beginning of storage. In contrast, bacterial growth appeared during the first two days of the storage period when using the two concentrations 0.125% ,0.25% Where the number of colonies of bacterial cells amounted to $0.7x10^3$cfu $0.3x10^3$ cfu compared to the control treatment in which the number of bacterial colonies reached (2.4×10^3cfu) and the number of bacteria gradually increased in the control treatment until it reached on the

sixth day of storage (4.6×10³cfu) but when using the two concentrations (0.50%),(0.75%), it led to the prevention of the appearance of bacteria until the tenth day of storage, while the number of bacteria reached (7×10³cfu) (4.8×10³cfu) at the two concentrations (0,125%) (0.25%) compared to the control treatment in which the number of bacterial cells reached (8×10³ cfu)The result of the study agreed with the findings (27) (45) And when used Two Gram-negative bacterial isolates were selected, *Escherichia coli and Salmonella typh*i, that the alcoholic extract for cinnamon has better effect than the aqueous extract against these two bacterial isolates .When using clove hexane extract, Cinnamon and Datura ethanol extracts against bacteria. The use of (26) ‘‘revealed that there are a number of compounds that can produce mycelium with ability to inhibit microorganisms ’’ activities that cause food poisoning and some problems caused by bacteria.

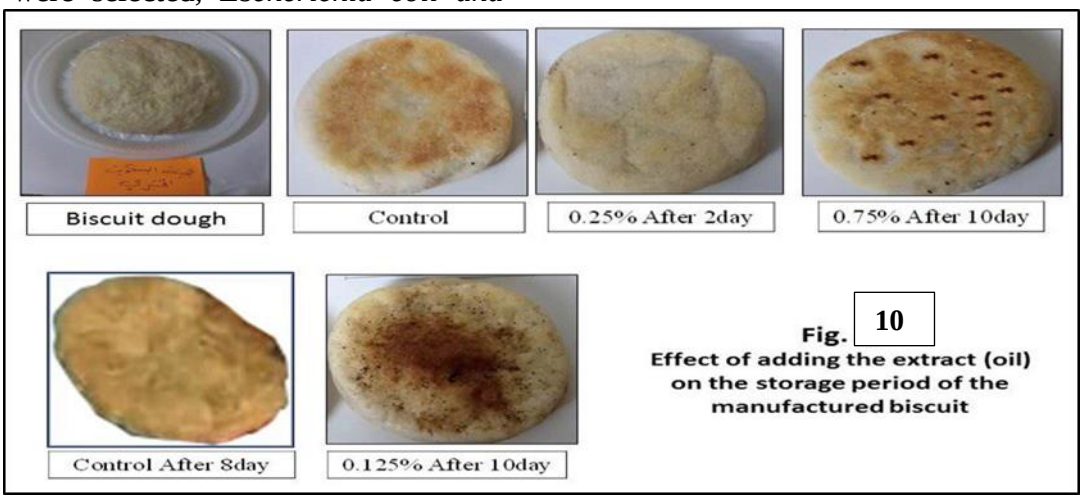

Fig. 10
Effect of adding the extract (oil) on the storage period of the manufactured biscuit

Table 5. Effect of extracts on bacterial numbers

Transactions	Concentrations%	0	2	4	6	8	10	day
A1	0.125	-	0.7	3	4.2	5.6	7	
A1	0.25	-	0.3	1.5	2	3.4	4.8	
A3	0 .50	-	-	-	-	-	-	
A4	0.75	-	-	-	-	-	-	
A5	Control	-	2 .4	3.5	4.6	5	8	

Sensory evaluation of the biscuit

In Table (6), the results of sensory evaluation of biscuits made by adding cinnamon bark oil extract in different concentrations showed that there were no significant at p>0.05 levels between treatments A1(0.125%), A2(0,25%), A3(0.50%) andA4(0.75%) andA5 treatment (control). Respectively, there was a slight increase in some coefficientsA1 (0.125%), A4 (0.75%), and A5 (control) as for the tissue traits, where significant differences appeared between the control treatment and the rest of the treatments, treatment (A5) obtained the highest value of (6.00). In comparison, treatment A4 (0.75%) received the lowest value of (4.80), and no significant differences appeared between them. Regarding the characteristic of freshness, significant differences emerged between the treatment A2(0.25%) and the treatments A1(0.125%), A3(0.50%), A4(0.75%), in addition to the control treatment (A5) where the treatment A2(0,25%)outperformed, as its value (5.70) followed by the two treatments A3(0.50%), A1 (0.125%) where their values reached (4.80)(4.70) respectively. As for the flavour, no significant differences appeared between the treatments. Treatment A1 (0.125%) got the lowest value, which amounted to (4.30), while the two treatments, A3 (0.50%) and A5 (control), called the same deal, reaching (4.90). There were significant differences between the two treatments. A3 (0.50%) A5 (control) in the laminated class, where the treatmentA3 (o.50%) outperformed, as its value (5.50) while the treatment (control) got the lowest value of (4.10) and there were no significant differences between the treatmentsA1 (0.125%), A3 (0.50%), A4 (0.75%).

Table 6. The effect of the studied parameters on the results of sensory evaluation

Transactions	Concentrations	Sensory qualities						
		appearance	texture	freshness	flavor	thickness	colour	general acceptance
A1	0.125	5.70± 0.41	4.90 ±0.33 b	4.70 ±0.52 b	4.30 ±0.35	4.20 ±0.29b	4.50 ±0.39	4.50 ±0.48 ab
A2	0.25	±5.00 0.26	4.90 ±0.27 b	5.70 ±0.36 a	4.70 ±0.56	4.50 ±0.50 ab	5.20 ±0.38	4.40 ±0.45 ab
A3	0.50	5.20±0.46	5.80 ±0.35 ab	4.80 ±0.20 b	4.90 ±0.43	5.50 ±0.40 a	5.00 ±0.21	4.40 ±0.42 ab
A4	0.75	5.70± 0.38	4.80 ±0.46 b	4.10 0.23±b	4.50 ±0.34	4.50 ±0.45 ab	5.30 ±0.47	5.60 ±0.56 a
A0	Control	5.70± 0.36	6.00 ±0.29 a	4.60 ±0.31 b	4.90 ±0.31	4.10 ±0.54 b	5.20 ±0.38	4.20 ±0.46 b
LSD value		1. 087NS	* 1.023	* 0.819	1.213 NS	* 1.380	0.982 NS	* 1.374

***P<0.05.(Averages carrying different letters within the same column differ significantly within the same column differ significantly among themselves**

excellent 7, Very good 6, good 5, average 4, acceptable 3, poor 2, very poor 1 Regarding the attribute of color, no statistically significant differences were observed across the various treatments. Examining the data presented in the corresponding table, it is evident that the treatment with 0.75% concentration manifested superior outcomes in terms of overall acceptability, achieving the highest score of 4.60. This was closely followed by treatment A1, with a 0.125% concentration, garnering a score of 4.50. Treatments A2 (0.25%) and A3 (0.50%) yielded comparable results, each achieving a score of 4.40. The spicy and distinct flavour of cinnamon prevalent in the biscuits can be attributed to the presence of cinnamaldehyde, as suggested by existing literature .The study elucidates that the decomposition of proteinaceous substances within the biscuits is facilitated by the action of proteolytic enzymes, which are secreted by aerobic and anaerobic bacteria. This enzymatic activity culminates in a series of biochemical transformations, leading to the degradation of amino acids and peptides. Consequently, compounds such as indole, sulfur dioxide, fatty acids, and ammonia are formed alongside the emission of undesirable odours, particularly from anaerobic bacteria. These chemical and microbial interactions not only impact the flavour profile of the biscuits unfavourably but also influence their texture and structural integrity, including the formation of carbon dioxide. Comprehending these processes and their implications is crucial for optimising product quality, necessitating a careful balance and consideration of the concentrations of ingredients like cinnamon essential oil in food products.

Conclusions

The essential oil derived from Cinnamomum zeylanicum is enriched with a plethora of bioactive constituents, a prominent example being (E)-cinnamaldehyde, which comprises an impressive 71.5% of the total oil composition, as elucidated by the current study. The bioactivity of this essential oil is further highlighted by its remarkable antioxidant and antibacterial properties, particularly its pronounced efficacy against Gram-positive bacterial strains. A noteworthy observation from this research is the dose-dependent antiproliferative effect exerted by the essential oil on adipose tissue-derived mesenchymal stem cells (AT-MSCs). Upon exposure to the essential oil for 24 hours across a concentration gradient spanning from 1 to 200 mg/mL, a marked antiproliferative response was recorded. These findings indicate the essential oil's significant potential for integration as a natural bioactive ingredient within food products. Despite these promising results, there is a paramount need for further investigative efforts to unravel the mechanistic underpinnings of the antiproliferative action elicited by C. zeylanicum essential oil. Additionally, expanding its application across a diverse array of food items is warranted,

thereby harnessing its bioactive properties to their full potential while ensuring safe and productive use.

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