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Extraction, characterization and antibacterial activity of medicinal plants for the control of food pathogens

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***Abstract*---**Preservatives are substances that are added to food or used in cooking in order to stop it from going bad or growing bacteria that could make people sick. On the other hand, although these compounds are designed to prevent microbial spoilage of food, they frequently have unintended consequences for human health, the distribution network for food, and the evolution of microbial resistance. As a result of these challenges, it is becoming increasingly vital to find a natural preservative that is both safe and healthy for human consumption. In some circumstances, plant extracts are utilised both to treat and prevent food-borne illnesses. In the present study, *Tinospora Cordifolia*, *Vitex Negundo*, and *Syzgium Cumini* were used as a medicinal plants. Among the 3 different plant extracts, it

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was discovered that only one of them, the *Vitex Negundo* extract 400μg/ml plant extract, was shown high inhibition activity against the food pathogens. For the purpose of preventing food poisoning and preserving food, natural alternatives based on these potentially useful plant extracts can serve as an effective replacement for antibacterial agents that are generated using chemical processes.

***Keywords*---**pathogen bacterial strains, food poisoning diseases, control, medicinal plants.

Introduction

Even in highly industrialised societies, bacterial food spoilage continues to be a concern that can damage any type of food and result in food waste and loss. An estimated 40 percent of the global food supply is wasted annually due to factors such as microorganisms that cause spoiling. Due to the fact that bacteria, yeast, and mould are three of the most common types of microorganisms, their presence can cause numerous types of food to spoil (1). When these microbes get into touch with food, they begin to proliferate and release metabolites that are poisonous to the food, resulting in food deterioration. Foodborne disease, which has become a major public health concern due to the prevalence of contaminated food products, is another pervasive problem with food safety. Foodborne infections are transmitted by consuming contaminated foods (2).

Microbes are easily accessible when crops are harvested, animals are slaughtered, items are processed, and packaging are sealed because they are naturally present in the environment. These bacteria can withstand the standard pasteurisation procedure as well as various methods of sterilisation, including low temperatures, altered packing atmospheres, and vacuum packaging (3). This has resulted in a decrease in the use of chemical additives in the food preservation process, as an increasing number of individuals are concerned about the potential health risks these synthetic substances represent. To prevent the development of hazardous microorganisms and extend the shelf life of food products without the use of artificial chemical preservatives, new environmentally acceptable strategies are necessary (4).

Tinospora cordifolia, a huge deciduous climbing shrub with striking greenish yellow flowers, is a member of the Menispermaceae family and is also known in Sanskrit as "Guduchi." It can only be found at greater altitudes. Male flowers that are found individually frequently form racemes, also known as racemose panicles. The length of time that flowers are in bloom increases throughout the year (5). Alkaloids, steroids, diterpenoid lactones, aliphatics, and glycosides have been isolated from the root, the stem, and the entire plant, in addition to other anatomical components. Anti-diabetic properties and properties against periods, spasms, inflammation, and arthritis; anti-oxidant properties; anti-allergic properties; anti-stress; anti-leprotic; anti-malarial; hepatoprotective; immunomodulatory; and anti-neoplastic activities; and properties against periods; and properties against inflammation; and properties against arthritis; and anti-oxidant properties; anti-allergic properties; anti-stress; anti-leprotic; anti- (6).

Several bioactive chemicals present in natural goods have the ability to target the complex network of proteins implicated in a range of diseases. The "chaste tree," commonly known as Vitex negundo or VN, is an exceptionally significant ethnobotanical plant with numerous medical purposes. Different species of Vitex have distinct chemical compositions, resulting in a broad spectrum of phytochemicals. Several plants' leaves, seeds, and roots have been identified to contain beneficial substances such as volatile oils, flavonoids, lignans, iridoids, terpenes, and steroids. The therapeutic benefits of these bioactive chemicals include antibacterial, anticancer, anti-inflammatory, and anti-oxidative properties (7).

Due to its antihyperglycemic, hypolipidemic, anti-inflammatory, cardioprotective, and antioxidant properties, Syzygium cumini (Myrtaceae) has been utilised for centuries in traditional herbal medicine. However, little is known about the mechanisms of action of the plant-wide bioactive compounds, such as flavonoids and tannins. Although these molecules are believed to be responsible for these actions, little is known about how they really function (8). Recently, numerous scientists have begun investigating the possibilities of employing plant extracts as natural preservatives. In old societies, numerous plant components, such as roots, stems, blooms, and branches, were harvested from the soil and used to treat a wide variety of human ailments. The flavonoids, alkaloids, tannins, and terpenoids present in medicinal plants all have antibacterial and antioxidant characteristics (9).

Material and methods

Plants extraction preparation

Materials comprising of three distinct plant species were acquired from a local market in Hyderabad, India, for this study. The names of these plant species are *Tinospora Cordifolia*, Vitex Negundo, and Syzgium Cumini. The final steps of the process were disinfecting, washing, rinsing with distilled water, and drying the item in the shade. In order for each plant species' dried plant material to pass through a 100 mm filter, it must first be ground into a fine powder. By first suspending fifty grams of the fine powder in ethanol for forty-eight hours while stirring, then filtering the solution through two layers of muslin, spinning it at nine thousand revolutions per minute for ten minutes, and finally filtering it once more through Whatman filter paper No. 1, a clear filtrate of the resulting solution was obtained. After lowering the temperature to 40 degrees Celsius, the filtrates were dehydrated and evaporated using a rotating vacuum evaporator. It was then weighed, packaged in extremely small vials, and stored at 5 degrees Celsius in the refrigerator.

Antibacterial activity of the plant extracts

The effectiveness of each plant extract's antibacterial qualities was evaluated using one of four bacterial strains known to cause food poisoning. There were found to be a total of four different bacterial strains, two of which were Gram positive (*Staphylococcus aureus* and *Bacillus Subtilis*), in addition to two Gram negative strains (*Escherichia coli* and *P. aeruginosa*). This collection of bacterial strains was obtained from the Mahatma Gandhi University in Nalgonda in India's Botany and Biotechnology collection. Each bacterial strain was subcultured on Mueller-Hilton agar slants for one night at a temperature of 35 degrees Celsius. The bacteria were collected using five millilitres of sterile water that did not contain any sodium chloride. After collection, the bacteria were diluted using a spectrophotometer with the wavelength set to 600 nm in order to achieve a viable cell count of 10^7 CFU/ml.

Antibacterial activity of plants extract

Using a technique called as disc diffusion, each plant extract is evaluated to determine if it exhibits antibacterial properties. The plant extract residues (50 mg) were re-dissolved in 2.5 ml of ethanol, sterilised via a Millipore filter (0.25 mm), and then deposited on sterile filter paper discs to achieve the necessary final concentration of 10 mg/disc (8 mm in diameter). Before adding Mueller-Hilton agar media, sterile Petri dishes had to be inoculated with a bacterial solution to achieve 10^5 CFU/ml medium concentrations. This was done in order to promote the growth of bacteria. As a loading control, sterile filter paper discs were placed atop Mueller-Hilton agar plates containing a plant extract concentration of 10 mg/ml. Positive controls were the filter paper discs treated with gentamycin. The plant extracts on the plates were allowed to diffuse for two hours before being placed in an incubator at 35 degrees Celsius for two days. Observed, measured, and recorded inhibitory zones are a reliable indicator of whether or not a drug possesses antibacterial capabilities.

Determination of minimum inhibitory concentrations (MIC's) of the effective plants extract

Antibiotic medication is stated to have a minimum inhibitory concentration when it can inhibit the development of germs after 24 hours of incubation (MIC). Extracts with a potent antibacterial activity at 10 mg/ml were tested for their ability to inhibit food poisoning-causing bacterial strains and their minimal inhibitory concentration (MIC) was determined by disc diffusion. Before being analysed, the plant extracts were sterilised using Millipore filters and placed onto sterile filter paper discs at the specified concentrations (100µg, 200µg, 300µg and 400µg /ml) (8 mm in diameter). After sterilising Petri dishes and filling them with Mueller-Hilton agar, pathogenic bacterial suspensions were introduced. Coated on Mueller-Hilton agar plates were filter paper discs holding various quantities of the plant extract. Two hours at 5 degrees Celsius in the refrigerator are followed by twenty-four hours at 35 degrees Celsius in the incubator. Comparisons were made between the inhibition zones and concentrations of the most effective plant extracts.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

In the following sections, the results of the GC-MS analysis of the entire plant extract (*Vitex Negundo*) are provided in further detail. The column was heated to 35 degrees Celsius for three minutes before the experiment began. The target temperature of 280 degrees Celsius was to be reached at a rate of 8 degrees Celsius per minute. One microliter of the sample was injected into the port, and it was then rapidly vaporised and transported down a column containing one millilitre per minute of helium carrier gas. It was captured at a frequency of 70 eV for the MS Spectrum. After separation in the column, the components were identified and then subjected to FID analysis. To identify the unknown compounds, we compared their spectra to those of recognised compounds using the database of the NIST MS 2.0 structural library.

Results and Discussion

Two Gram-positive (*B. cereus* and *S. aureus*) and two Gram-negative (*E. coli* and *P. aeruginosa*) strains of food poisoning bacteria (B. cereus and S. aureus) were evaluated for their antibacterial activity using disc diffusion. The research findings on the antibacterial properties of these plant extracts are displayed in Table 1 and Figure 1, respectively. The results revealed that each plant extract had the capacity to inhibit the microbiological growth of food-poisoning-causing bacteria; however, the results also demonstrated that each plant extract possessed a distinct degree of efficacy. At a dosage of 10 mg/ml, P. granatum extract was able to inhibit all potentially harmful bacteria, whereas C. cyminum extract was solely effective against *S. aureus* (10 &11). The antibacterial effectiveness of plant extracts from various plant species against the microorganisms that cause foodborne illness varies. S. aromaticum was capable of inhibiting the growth of *B. cereus*, *S. aureus*, *E. coli*, and *P. aeruginosa*. Z. officinales and T. vulgaris, on the other hand, were effective against three of the pathogens (B. cereus, S. aureus, and P. aeruginosa) (12&13).

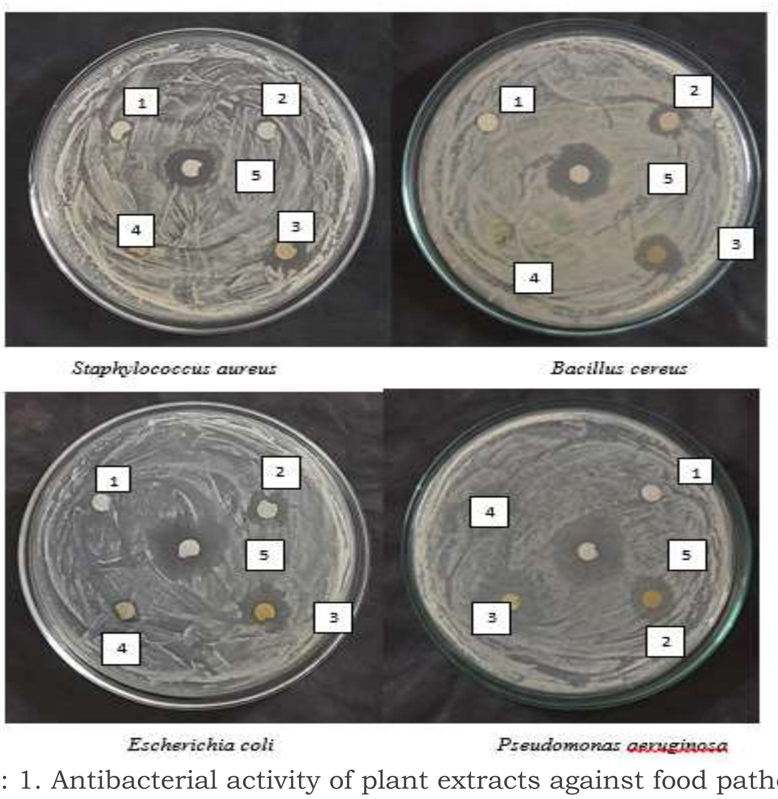

Figure: 1. Antibacterial activity of plant extracts against food pathogens

Here 1 indicates solvent; 2 indicates plant extract of *Tinospora Cordifolia;* 3 indicates *Vitex Negundo;* 4 indicates *Syzgium Cumini; 5* indicates antibiotic

Table: 1. Antibacterial activity of plant extracts

Bacteria	*Tinospora Cordifolia*	*Vitex Negundo*	*Syzgium Cumini*	Antibiotic
Staphylococcus aureus	2.3±0.1	5.8±0.2	1.8±0.1	3.5±0.2
Bacillus Subtilis	6.8±0.2	6.9±0.1	1.2±0.2	7.5±0.1
Escherichia coli	7.2±0.1	9.8±0.1	3.8±0.2	7.3±0.2
Pseudomonas aeruginosa	6.2±0.2	7.5±0.2	1.5±0.1	6.5±0.1

Inhibition zones (mm); Data are means of three replicates (n = 3) ± standard error

According to the findings of this study on the antibacterial activity, Staphylococcus aureus was the most resistant to the plant extracts, followed by other bacteria that were more susceptible to the extracted plants. The antibacterial activity against the bacteria that cause food poisoning was found to be highest in the extracts of P. granatum and S. aromaticum, which were determined to be the most potent. As a consequence of this, research was carried out using *Vitex Negundo* to determine their minimal inhibitory concentration (MIC).

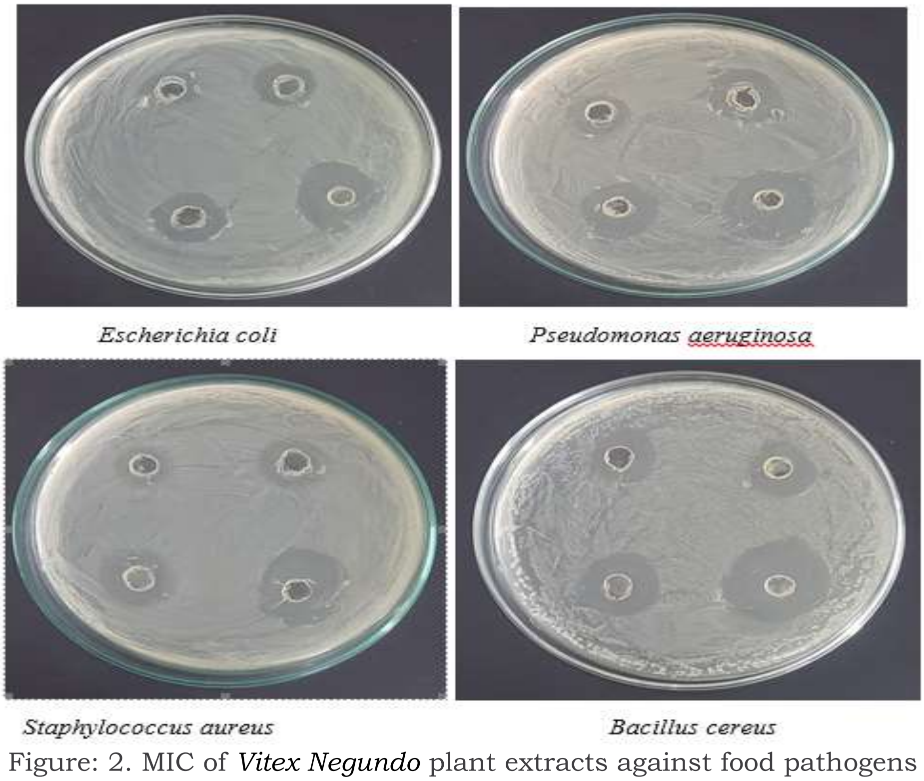

Figure: 2. MIC of *Vitex Negundo* plant extracts against food pathogens

Table: 2. MIC of *Vitex Negundo* plant extracts against food pathogens

	E. coli	*Bacillus*	*Staphylococcus*	*Pseudomonas*
100µg	0.6±0.1	0.7±0.1	0.7±0.1	0.9±0.1
200µg	0.9±0.2	1.1±0.1	1±0.1	1±0.2
300µg	1.1±0.1	1.3±0.2	1.1±0.4	1.2±0.1
400µg	1.3±0.1	1.4±0.1	1.3±0.1	1.5±0.4

Inhibition zones (mm); Data are means of three replicates (n = 3) ± standard error

These findings are consistent with (14, 15& 16). Extraction techniques, constituents, and bacterial strains used in the investigations that demonstrated a wide variation in the MIC of P. granatum extract have varied considerably. This diversity can be due to the ample freedom for experimentation that has existed. The volatile nature of the chemical components of the various plant extracts is an additional factor that may influence MIC. S. aromaticum extract at a dosage of 10 mg/ml inhibited the growth of B. cereus, S. aureus, E. coli, and P. aeruginosa; the respective inhibition zones for these bacteria were 14.6, 15.4, 11.9.3, and 13.4. These results are consistent with the findings of the study (17& 18). Despite the fact that cumin was ineffective against the other bacterial strains, the findings of (19), who stated that cumin had a MIC range of 6.25 to 12.5 mg/ml, contradicted these findings. Cumin extract at concentrations up to 60 mg/ml was found to be effective against the pathogens that cause food to spoil, and these results were consistent with those reported by other studies in the past (20). Research has shown that plant extracts and the beneficial substances they contain can be used to suppress the growth of germs that cause food poisoning or food rotting. Antimicrobial plant extracts (terpenoid, alkaloid, and phenolic compounds) are capable of interfering with proton transport from microbial cells to the outside environment. According to the findings of some researchers, this disturbance has the potential to trigger cell death or block enzymes important for the creation of amino acids. Other research suggests that the inhibitory impact of these plant extracts is a result of their hydrophobicity, which allows them to react with proteins in the microbial cell membrane and mitochondria, thereby altering their structures and permeability. The hydrophobic characteristics of these plant extracts make this possible (21). Using plant extracts that were shown to be beneficial in a recently published study may enable for the prevention of food poisoning and the preservation of food without the use of potentially hazardous chemical preservatives. In this experiment, a 400-gram concentration of Vitex Negundo was found to be particularly effective against all food-borne viruses (Table.2 & figure 2).

GC-MS Analysis

Below is displayed the chromatogram of the GC-MS spectrum analysis of the methanol extract from the entire plant of Vitex Negundo. This study depicts the peaks of various chemicals detected in the GC fractions. Table 2 outlines the pharmacological and biological activity of the bio-compounds that have been reported. It has been discovered in the scientific literature that virtually all phytocompounds possess antimicrobial, anti-inflammatory, antioxidant, and hepatoprotective properties.

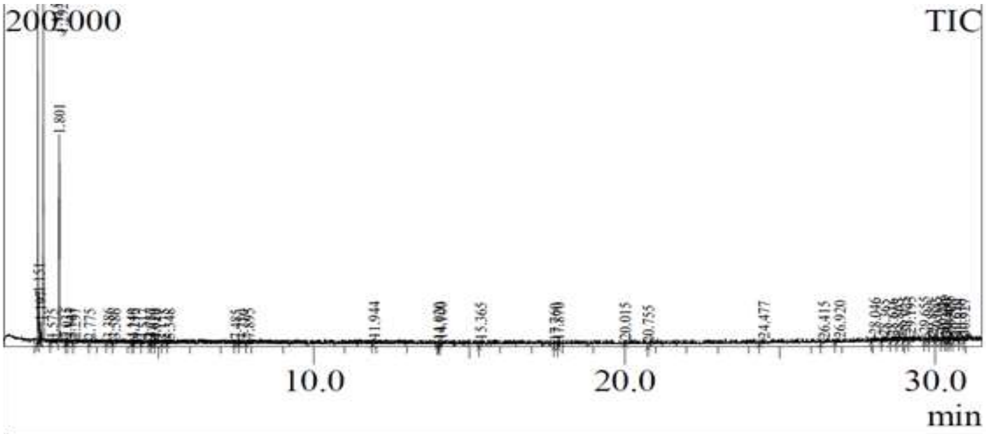

Figure.3: Chromatogram of Vitex Negundo

Table: 3. Compounds identified from *Vitex Negundo* extract

Peak	R#. Time	Area	Area%	Height	A/H	Base m/z	Name
1	1.115	128056	13.75	237850	0.54	46.90	Ethane, 1-chloro-1-fluoro-
2	1.151	20291	2.18	28277	0.72	44.95	Ethanol
3	1.197	14102	1.51	11939	1.18	42.95	Hydrogen azide
4	1.292	236959	25.44	255507	0.93	43.00	Ethyl Acetate
5	1.575	7723	0.83	2077	3.72	39.95	Argon
6	1.801	112018	12.03	119928	0.93	42.95	Acetic acid, 2-methylpropyl ester
7	2.035	7075	0.76	2830	2.50	39.95	Argon
8	2.141	6584	0.71	2519	2.61	40.00	Argon
9	2.297	11587	1.24	2502	4.63	40.00	Argon
10	2.775	7500	0.81	2292	3.27	40.00	Argon
11	3.386	9035	0.97	2622	3.45	40.00	2-Butynoic acid
12	3.586	9891	1.06	2511	3.94	39.95	2-Butynoic acid
13	4.140	6400	0.69	2661	2.41	40.00	Argon
14	4.215	11351	1.22	2703	4.20	40.05	Argon
15	4.517	6529	0.70	2469	2.64	39.90	Argon
16	4.737	7181	0.77	2938	2.44	40.00	Argon
17	4.839	8894	0.95	2376	3.74	39.95	Argon
18	4.910	12050	1.29	2306	5.23	39.85	Argon
19	5.175	9403	1.01	2352	4.00	39.95	Argon

20	5.348	7206	0.77	2425	2.97	39.95	Argon
21	7.485	6340	0.68	2239	2.83	39.90	2-Butynoic acid
22	7.710	9851	1.06	2214	4.45	43.90	2-Butynoic acid
23	7.895	6844	0.73	2590	2.64	39.90	Argon
24	11.944	6461	0.69	2643	2.44	40.00	Argon
25	14.020	8419	0.90	3086	2.73	39.80	Argon
26	14.100	8219	0.88	3930	2.09	43.90	2-Butynoic acid
27	15.365	10776	1.16	3300	3.27	43.95	Nitrous Oxide
28	17.760	16598	1.78	3252	5.10	44.00	2-Butynoic acid
29	17.870	6649	0.71	3563	1.87	43.85	2-Butynoic acid
30	20.015	6554	0.70	1831	3.58	40.00	Argon
31	20.755	12341	1.33	2050	6.02	44.00	Nitrous Oxide
32	24.477	9368	1.01	2700	3.47	39.90	Argon
33	26.415	7094	0.76	2408	2.95	44.00	2-Butynoic acid
34	26.920	7876	0.85	2271	3.47	40.00	2-Butynoic acid
35	28.046	7731	0.83	3678	2.10	39.90	Argon
36	28.365	10567	1.13	2814	3.76	206.95	2,5-Dimethoxy-4-(methylsulfone)amphetamine
37	28.616	10836	1.16	3154	3.44	206.90	2-Butynoic acid
38	28.805	7116	0.76	2985	2.38	206.95	5-Methyl-2-trimethylsilyloxy-acetophenone
39	29.025	8263	0.89	4355	1.90	206.85	2-Butynoic acid
40	29.195	16478	1.77	4287	3.84	206.85	5-Methyl-2-trimethylsilyloxy-acetophenone
41	29.655	11434	1.23	4497	2.54	206.90	5-

							Methyl-2-trimethylsilyloxy-acetophenone
42	29.885	22684	2.44	2519	9.01	206.90	5-Methyl-2-trimethylsilyloxy-acetophenone
43	30.045	7626	0.82	4180	1.82	44.00	2-Butynoic acid
44	30.226	7528	0.81	5103	1.48	206.80	5-Methyl-2-trimethylsilyloxy-acetophenone
45	30.330	9016	0.97	2573	3.50	206.85	5-Methyl-2-trimethylsilyloxy-acetophenone
46	30.400	6783	0.73	3154	2.15	206.85	2,5-Dimethoxy-4-(methylsulfone)amphetamine
47	30.455	8145	0.87	4791	1.70	206.85	2-Butynoic acid
48	30.650	18935	2.03	2617	7.24	207.00	5-Methyl-2-trimethylsilyloxy-acetophenone
49	30.816	6951	0.75	2394	2.90	39.95	Argon
50	30.927	8058	0.87	2504	3.22	206.90	1,2,4-Benzenetricarboxylic acid, 1,2-dimethyl ester

Conclusion

Food spoilage typically occurs as a direct consequence of the expansion of several pathogenic species of bacteria. Chemical preservatives are the most common type of additive used in the food manufacturing business and in consumer goods related to the food industry. Because of the adverse effects these chemical preservatives have on people's health, there is a growing demand for finding alternatives that are not only safe but also as effective as possible. Natural alternatives to the traditional antimicrobial agents that are used to prevent food poisoning and preserve food are available. These natural alternatives include extracts from plants that have been shown to be useful.

Conflict of interest

The authors declare that they have no conflict of interest for this study.

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