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Original Article

Evaluation of Antioxidant and Antimicrobial Activity of Some Medicinal Plant Extracts on *Escherichia coli* Isolated from Poultry Feces

Bahman Fazeli-Nasab[1*], Moharam Valizadeh[2] and Maryam Beigomi[3]

[1]*Research Department of Agronomy and Plant Breeding, Agricultural Research Institute, University of Zabol, Zabol, Iran*
[2]*Research Center of Medicinal Plants, University of Sistan and Baluchestan, Zahedan, Iran*
[3]*Department of Food Science and Technology, Zahedan University of Medical Sciences, Zahedan, Iran*

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ABSTRACT

Continuous and indiscriminate use of chemical drugs causes an important phenomenon of resistance to microorganisms and consequently, the effect of the drugs is weakened or neutralized. On the other hand, it has been reported that many plant essential oils have a significant inhibitory effect on pathogenic microorganisms, so, this study aimed to evaluate the antioxidant and antimicrobial activity of several plant extracts on *Escherichia coli* isolated from poultry feces. *Cichorium intybus* L., *Hypericum perforatum* L., *Lavandula angustifolia* Mill., and *Thymus vulgaris* L. from Shahrekord were collected and determined in the botanical laboratory of University of Zabol. To prepare the ethanolic extract, 40 gr of dried leaves of plants used in 400 cc of ethanol were used. The various strains of *E. coli* used were isolated from poultry feces by biochemical, bacteriological, and growth tests as well as standard tests. Determination of the free radical trapping activity was perfomed bydiphenylpicryl hydroxyl, and then the antimicrobial effects were investigated by diffusion method in Müller Hinton agar medium using 6 mm paper discs according to Bauer and Kirby instructions. Statistx ver10 software was used for statistical calculations. Mean comparisons were performed using the least significant difference (LSD) test. The results showed an increasing trend of the antioxidant activity of the extracts with increasing the concentration of plant extracts. The interaction of plant extract and the amount of extract in trapping free radicals showed that the highest antioxidant activity at low concentrations of the extract (16 and 32 µg/ml) was observed in the chicory extract following by licorice extract, but it licorice extract showed the highest activity at high concentration (64 µg/ml). In general, *H. perforatum* L. was the most effective plant in trapping free radicals. The lowest MIC of *H. perforatum* L. was 3.1 ppm, which was unilaterally inhibited at this concentration. *H. perforatum* L. and chicory had the highest (5.38) and lowest (2.23) diameter halos of inhibition of *E. coli* growth. Considering the side effects of chemical drugs and antibiotics as well as the potential effect of medicinal plant extracts used, especially *H. perforatum* L. on *E. coli*, compared to Cefazolin, it is recommended to use *H. perforatum* L. in inhibiting growth of *E. coli.*

INTRODUCTION

Continuous and indiscriminate use of chemical drugs causes an important phenomenon of resistance to microorganisms, and by creating this phenomenon, the effect of drugs is weakened or neutralized, and finally, due to the increase in the amount of drug use and the tendency to use new compounds, it becomes stronger. Also, another disadvantage of using these drugs is the increase of their side effects, which leads to diseases that are more dangerous than the original disease [1-3].

Many plant extracts have been reported to have significant inhibitory effects on pathogenic microorganisms, and it has been found that most plant extracts taken out from pharmaceutical plants have antifungal, anti-parasitic, antibacterial, and

*Corresponding author: Department of Agronomy and Plant Breeding, Agricultural Research Institute, University of Zabol, Zabol, Iran
Email Address: bfazeli@uoz.ac.ir

antimicrobial properties; therefore, plant extracts have been severely screened and used in the fields of pharmacology, herbal pharmacology, medical and clinical microbiology, phytopathology, preservation of food, fruits and vegetables [4]. Consumption of these herbal medicines are more popular among the people. These reasons have led to an increase in new global research to introduce the antibacterial effects of various plants in recent years [5].

Plants play a very important role in maintaining human health and improving the quality of life for thousands of years. Pharmaceutical plants have beneficial properties, including their antibacterial, anti-parasitic, anti-fungal, and anti-oxidant properties. In recent years, plant products (secondary metabolites) have been used to treat most human and animal diseases due to their easy availability, ease of use, and fewer side effects compared to chemical products [6, 7]. On the other hand, plant-derived secondary metabolites such as phenol and flavonoids have a strong potential to scavenge free radicals that are present in all parts of plants such as leaves, fruits, seeds, roots, and skin. So, due to the high prevalence of chronic diseases and erosive, it is logical to use plants to provide the antioxidants needed by the body, especially plants that have high phenol and flavonoids. Thus, to provide the natural anti-oxidants needed by the body, the consumption of plants with high phenolic compounds is recommended [8].

Phenolic and flavonoids compounds have several biological properties such as anti-oxidant properties, trapping free radicals, and anti-inflammatory properties. These compounds prevent or delay oxidative damage to fats and other important molecules and prevent cancer and coronary heart disease [9]. Phenolic compounds are among the compounds that are present in all plants, including fruits, vegetables, grains, etc. Naturally, there are over 8,000 different phenolic compounds with effects such as involvement in cell wall construction, involvement in plant defense mechanisms, and involvement in fruit properties such as color, smell, and taste in plants. Phenolic compounds are also considered as indicators for physiological stages during fruit growth [4,10,11].

Research has shown that the source of phenols and flavonoids in different parts of the world depends on the type of diet of people in the region. For example, in countries such as Japan and China, the consumption of green tea provides these compounds needed by the body, while in western countries, these substances are supplied by consuming apples and onions, and in eastern countries by consuming fermented vegetables and foods [12]. In Iran, there is no specific type of use of substances containing anti-oxidants, but with various advertisements, such as consuming raw and cooked vegetables, leaves of various plants and trees (in the form of infusions, distillates, extracts, jams, syrups, pickles, ingredients detergents (including cedar) and even consumption in the form of curds, etc.) have been used, which according to various researches, different organs of plants that have a special type of anti-oxidants should be used.

Secondary metabolites of medicinal plants, such as plant extracts, have been studied for their anti-microbial effects and it is estimated that at least one-third of all medicinal products have plant origin or have been modified after extraction from the plant. In addition to preventing the growth of bacteria and mold contaminants in food, these substances are used to increase the shelf life of processed foods in the food system and also to extend the shelf life of fruits and vegetables[13,14].

So far, the anti-microbial properties of some plants have been evaluated on various bacteria. For example, inhibitory and anti-microbial properties of essential oil and thyme extract on *E. coli* , *Pseudomonas aeruginosa*, and *Staphylococcus aureus* [15], aqueous extract on *P. aeruginosa* strains [16], extracts of various parts of saffron, including leaves and saffron Epidermis, *S. aureus* , Micrococcus and fungi [17], Rosemary extract has been studied on *Loconocetus mesenteric*, *Listeria monocytogenes*, *S. aureus* , *Streptococcus mutans* and *Bacillus* [18] and yarrow extract on *Candida albicans* and *Bacillus* [19].

Chicory (*C. intybus* L.) belongs to the genus Asteraceae. Its roots, leaves, and seeds contain many medicinal compounds such as inulin, Sesquiterpene, Lacoten, Kumarin, flavonoids, and vitamins that are used as anti-hepatitis, anti-nephrotic, anti-cancer, anti-appetite, anti-cancer, and anti-inflammatory. Antibacterial properties have also been reported for chicory root extract [20].

Hypericum perforatum L. contains a wide range of secondary metabolites such as flavonoids, proanthocyanidins, naphthodiandrons that have many therapeutic properties such as anti-tuberculosis, anti-thyroid, anti-thyroid, anti-thyroid it

has bacteria but its greatest effect and application has been reported in the treatment of depression [21].

Lavandula angustifolia Mill. affects most organs and cells of the body. This plant has a broad effect on the central and peripheral nervous system such as sedative, anticonvulsant, anti-epileptic, anti-anxiety, anti-depressant, and nerve protection effects. It also has analgesic and anti-inflammatory effects and reduces morphine tolerance and dependence. *L. angustifolia* Mill. also affects cellular mechanisms such as oxidation reactions (reduction of oxidative reactions), programmed cell death (anti-apoptotic), and nitric oxide production (reduction of NO) and affects cell genetic health. The effects of *L. angustifolia* Mill. seem to be exerted by calmodulin calcium and its related kinases [22].

Thymus vulgaris L. is a perennial plant of the genus *Lamiaceae* (*Labiatae*) that has a cushion or clumpy structure and has a straight herbaceous or woody stem with a branch 10 to 30 cm in height and some cases up to 45 cm. *T. vulgaris* L. contains essential oils and compounds such as flavonoids, saponins, and bitter substances. The most important components of *T. vulgaris* L. essential oil are phenolic compounds such as thymol and carvacrol [5, 8].

Because different medicinal plants have different effects on microbes and the other hand, the use of different antibiotics is unfortunately common among people [23], so in the present study, we tried to try some of these medicinal plants (chicory (*C. intybus* L.), *H. perforatum* L., *Lavender* (*L. angustifolia* Mill.) and *T. vulgaris* L. were collected from Shahrekord and evaluated for the lack of growth of *E. coli* bacteria and finally compared with common antibiotics (Compare such as gentamicin (GM), azithromycin (AZM), amoxicillin (ACC), amikacin (AN), Cefazolin (CZ)).

MATERIAL AND METHODS

Herbal Materials

Medicinal plants of *C. intybus* L., *H. perforatum* L., *L. angustifolia* Mill., and *T. vulgaris* L. from Shahrekord (Coordinates: 32 ° 19′32 ″ N) 502 ′ 50 ° E) were collected and determined in the botanical laboratory of Zabol University.

Preparation of the Ethanolic Extract

About 40 g of dried leaves of plants used in the shade and the vicinity of air, mill (IKA company model A11 basic Germany) and then soaked in 400 ml of ethanol 96% for 48 hours at room temperature, and on a Shaker (UniEquip company model SKIR-601L Germany) was shaken. After the desired time, the extracts were filtered, then the solvent was evaporated at a temperature of less than 40 °C by a rotary device (Pars Azma Company, model RO02, Iran). The obtained extracts were weighed and 100 mg of the extract powder was dissolved in 1 ml of DMSO solvent. The extracts were stored in a refrigerator at 4 °C until used in antimicrobial experiments [5].

Bacterial strains

The various strains of *E. coli* used in this study were isolated from poultry feces and cultured on nutrient agar area. Bacterial strains isolated by a variety of biochemical, bacteriological, and growth tests (oxidase, catalase, bacterial motility, glucose tests such as lactose fermentation, sucrose, glucose) as well as standardized tests such as gram staining, acid staining, and phyla acid staining. Colonies are identifiable [25]. After observing colony growth, hot staining, observation of cocci and gram-negative diplococci, as well as oxidase test, were used for identification. In the next step, using biochemical subjects, culture on mechanical agar and incubation at 37 °C and 42 °C, citrate test, motion test and culture on OF medium (fermentation and oxidation) containing glucose, definitive diagnosis of bacteria took place.

Preparation of the Antibiotic Concentrations

Pure antibiotic powders were obtained from Mast Company. Solutions for each antibiotic powder were selected and stock antibiotic solution was prepared at a concentration of 10 mg/ml. For each antibiotic, weigh the desired amount of powder and dissolve it in a certain amount of solvent (until the powder is completely dissolved), and then, using a diluent suitable for each antibiotic, place it to the desired volume in a balloon. The selection of the concentration range of each antibiotic has been done according to testing different dilutions of antibiotics and their effect on bacterial growth. This was done to prepare serial antibiotic dilutions. First, 128 /l was taken from an antibiotic stock with a concentration of 10 ml/mg and added to tube A, and to tube A by adding 872 µl of sterile diluent to Müller Hinton broth 1280 Micrograms per milliliter was made.

Then, 125 µl of tube A solution was poured into tube B, and dilution of 160 µg/ml was obtained by adding 875 µl of Müller-Hinton broth to tube B. In the next step, 125 µl of solution of tube B was poured into tube C and by adding 875 µl of Müller Hinton broth to this tube, a dilution of 20 µg / ml was obtained. Antibiotic dilutions of tubes A, B, and C are used to prepare serial dilutions used in the minimum inhibitory concentration (MIC). Serial from 512 to 1 µg/ml was prepared for these antibiotics [26].

Evaluation of Antibiotic Activity

10 pure strains of *E. coli* were determined by the Kirby-Bauer method [27] and their susceptibility to antibiotics was evaluated. For this purpose, first under sterile conditions next to the flame and under the laminar hood, the prepared bacterial suspension was removed and spread on the surface of the plate containing Müller Hinton agar medium. In the next step, 4 wells with a diameter of 5 mm and a distance of 2 cm were created on the surface of the plate. Then the solutions of 1-2-3 and 4 extracts were prepared with a dilution of 100 ppm and added to each of the wells [27]. They were kept at 37 degrees Celsius for 24 hours. After this period, bacterial cultures were examined for the formation or non-formation of the non-growth zone and the diameter of the non-growth zones was measured in millimeters by a caliper. Determination of susceptibility of strains to antibiotics such as gentamicin (GM) (10 µg), azithromycin (AZM) (15 µg), amoxicillin colloid acid (AMC) (30 µg), Amikacin (AN) (30 µg), Cefazolin (CZ) (30 µg) (antibody of medicine-Iran) was performed [21]. After 24 hours of incubation at 37 ° C, the diameter of the growth inhibition zone was measured for each antibiotic and the results for each antibiotic were recorded as sensitive, intermediate, and resistant according to the relevant instructions, and the results were compared with the NCCS standard table [28,29].

Test of MIC by Micro Dilution Method

This test was performed to determine the minimum concentration of antibiotic that inhibits bacterial growth. In this study, micro broth dilution method of MIC was used to determine the MIC of antibiotics including imipenem, ampicillin and ciprofloxacin in *E. coli* isolates.

Determination of MIC and MBC of Essential Oils of Plants Used on *E. coli*

The MIC of essential oils of plants were perfurmed by the eye method, the first 100 microliters of Mueller Hinton Broth medium (manufactured by Merk-Germany) was added to each well with a microtiter plate[30, 31]. Then, in the first well, 100 µl of dilution of 20 mg/ml of essential oil was added, and after mixing, 100 µl of the first well was removed and added to the second well. Similarly, double dilutions were continued in other wells. It should be noted that in this case, the first well contains 20 mg per microliter of essential oil, and thus in subsequent wells, this amount is reduced to half of the previous well. Finally, 10 µl of each bacterial suspension (cfu = 108 1 1.5 per ml, half McFarland) was added to the wells. DMSO was added to the negative control well (without essential oil) and then the microtiter plate was incubated for 37 hours at 37 ° C.

The MIC was defined as the lowest concentration required to stop the growth of bacteria at the end of 24 hours of incubation. To determine the minimum bactericidal concentration (MBC), 10 microliters of the contents of the wells were cultured on nutrient agar medium (manufactured by Merk-Germany) at the end of 24 hours of incubation and the plates were studied for 24 hours to study bacterial growth. The lowest concentration of essential oil in which 99.9% of bacteria did not grow was considered as MBC[30, 31]. All antimicrobial tests were repeated 3 times.

Evaluation of the Ability to Trap Radicals or Measure Antioxidant Properties

DPPH was used to determine free radical scavenging activity. Weigh 40 mg of the extracts and dissolve in 25 ml of methanol. Three concentrations of this solution (16, 32 and 64 µl) were dissolved in DPPH (with a concentration of 0.1 mmol) to 4 ml. It was then placed at room temperature for one hour. Ultimate light absorption was performed with a wavelength of 517 nm. Ascorbic acid can be used for positive control (control) [32].

$$F = \frac{A_b - A_s}{A_b} * 100$$

F = DPPH radical trapping value; A_b = Blank absorption; A_s = sample or standard absorption

Data Analysis

Statistix ver 10 software [33] was used for statistical calculations. Mean comparison was performed using

the least significant difference (LSD) test at the level of one percent and Excel was also used to draw the shapes.

RESULTS

Evaluation of the Ability to Trap Free Radicals

The results of evaluating the ability to trap free radicals of hydroalcoholic extracts of *C. intybus* L., *H. perforatum* L., *L. angustifolia* Mill., and *T. vulgaris* L., showed that different extracts, as well as different concentrations of the extract, were signicificant on free radical trapping ($P<0.05$) (Table 1). Antioxidant properties of *C. intybus* L. was in the range of 84.53 to 97.21% with an average of 94.24%. Antioxidant properties of *H. perforatum* was in the range of 47.73 to 98.05% with an average of 83.69%. Antioxidant properties of *T. vulgaris* L. was in the range of 72.59 to 12 88. % with an average of 81.50% and also Antioxidant properties of *L. angustifolia* Mill. was in the range of 74.51 to 80.95% with an average of 63%. LSD post hoc test showed that among the different concentrations of the extract, the highest and lowest effective concentration were 64 µg/µl and 16 µg / µl, respectively, and the antioxidant activity of the extracts increased with increasing the concentration of the extract (Fig. 2). Also, an increasing trend for antioxidant activity of the extracts was observed with increasing the concentration of plant extracts The interaction of plant extract and the amount of extract in trapping free radicals also showed that at low concentrations of the extract (16 and 32 µg/ml), the highest antioxidant activity was for *C. intybus* L. extract following forlicorice extract, but at high concentration (64 µg in ml) the highest activity belonged to licorice extract (Fig. 3). In general, *H. perforatum* L. has been the most effective plant in trapping free radicals

Fig. 1 Appearance of plants used *T. vulgaris* L. (A), *C. intybus* L. (B), *H. perforatum* L. (C) and *L. angustifolia* Mill. (D) (Reference; Section A [http://iranbehlimo.com] and Section D [24])

MIC and MBC of Plant Extracts on *E. coli*

The lowest MIC of chicory (*C. intybus* L.) against *E. coli* samples was 6.25 ppm, with two strains inhibited at this concentration, while the highest MIC was 50 ppm, with three strains inhibited at this concentration. The lowest and highest MBC were equal to 12.5 ppm (two strains destroyed) and 100 ppm (three strains destroyed) (Table 2). The lowest MIC of *T. vulgaris* L. was 6.25 ppm, in which three strains were inhibited, while the highest MIC was 50

ppm, of which 4 strains were inhibited in this concentration. The lowest MBC was equal to 12.5 ppm and the highest MBC was equal to 100 ppm, in which 4 strains were destroyed. While one *E. coli* strain did not grow at any concentration of the extract (Table 2). The lowest MIC of *H. perforatum* L. was 3.1 ppm, which was unilaterally inhibited at this concentration. The maximum MIC was 50 ppm, which was unilaterally inhibited. The lowest MBC was equal to 6.25 ppm, in which one strain was lost. The highest number of samples was inhibited at a concentration of 12.5 ppm. The highest MBC of *H. perforatum* L. was 100 ppm, but one strain was lost in this concentration (Table 2).

The lowest MIC of *lavender* (*Nepeta binaludensis* Jamzad) was 25 ppm that with two strains inhibited at this concentration and the highest MIC was 50 ppm thnat 16 strains inhibited at this concentration (Table 2). The highest MBC MBC was 50 ppm that sixteen strains were killed. The lowest MBC was equal to 12.5 ppm and the highest MBC was equal to 100 ppm that four strains were destroyed. While one *E. coli* strain did not grow at any concentration of the extract.

Table 1 Analysis of variance result for ability to trap free radicals

Source	DF	SS	MS	F
DPPH[#]	2	114.17	57.08	4.33 [**]
Plant Extract	3	4553.8	1517.93	115.2 [**]
DPPH × Plant Extract	6	4634.05	772.34	58.61 [**]
Error	24	316.24	13.18	-
Total	35	9618.25	-	-

[**] Significant at the level of one percent [#]DPPH: 2,2-diphenyl-1-picrylhydrazyl

Table 2 MIC and MBC of four medicinal plant extracts

Strains	*N. binaludensis* Jamzad MIC-MBC	*H. perforatum* L. MIC-MBC	*T. vulgaris* L. MIC-MBC	*C. intybus* L. MIC-MBC
1	Not growth	25-50	12.5-25	25-50
2	25-50	25-50	12.5-25	25-50
3	25-50	50-100	12.5-25	25-50
4	12.5-25	25-50	6.25-12.5	25-50
5	12.5-25	25-50	12.5-25	25-50
6	25-50	25-50	12.5-25	25-50
7	25-50	25-50	12.5-25	25-50
8	25-50	25-50	6.25-12.5	25-50
9	25-50	25-50	6.25-12.5	50-100
10	25-50	25-50	-	25-50
11	25-50	12.5-25	25-50	25-50
12	25-50	12.5-25	25-50	25-50
13	25-50	12.5-25	25-50	25-50
14	25-50	3.1-6.25	50-100	25-50
15	25-50	25-50	25-50	25-50
16	-	-	50-100	50-100
17	25-50	12.5-25	50-100	50-100
18	25-50	12.5-25	25-50	25-50
19	25-50	12.5-25	50-100	25-50
20	-	12.5-25	12.5-25	6.25-12.5
21	25-50	12.5-25	12.5-25	6.25-12.5

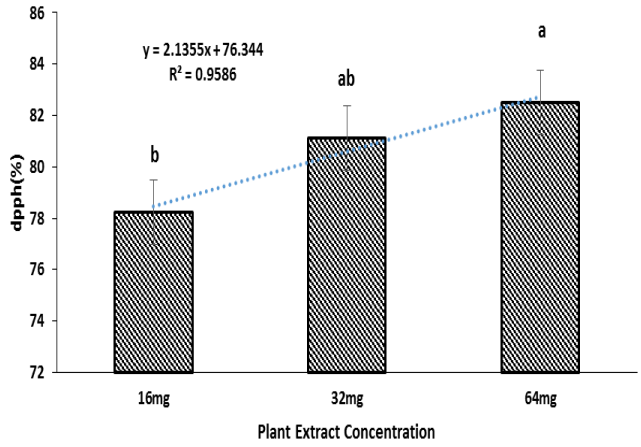

Fig. 2 The ability to trap free radicals in different concentrations of plant extracts, Similar letters indicate no significant difference.

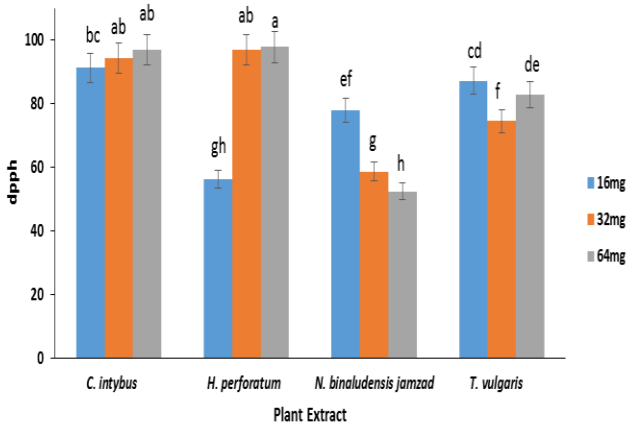

Fig. 3 Evaluation of the interaction of antioxidant activity of the extract and different concentrations of plant extracts, Similar letters indicate no significant difference.

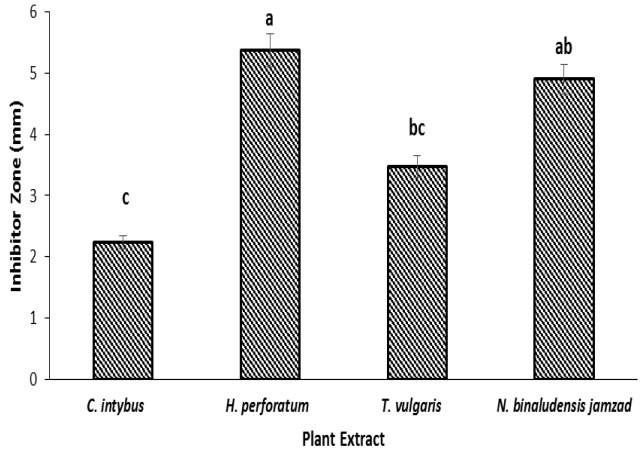

Fig. 4 Diameter of growth inhibition zone of plant extract against *E. coli* at a dilution of 100 ppm, Similar letters indicate no significant difference.

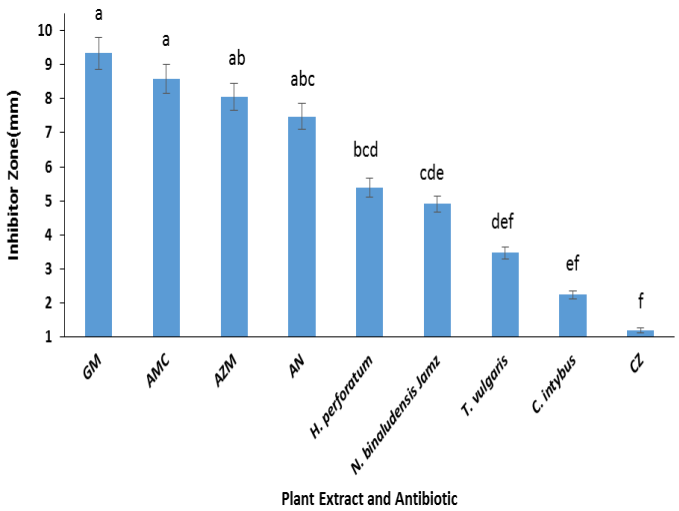

Fig. 5 Diameter diameter of growth inhibition zone of plant extracts and antibiotics used against *E. coli* at dilution of 100 ppm, Similar letters indicate no significant difference

Diameter of Inhibition Zone of Plant Extracts on *E. coli*

The diameter of growth inhibition zone of plant extracts against *E. coli* was diluted to 100 ppm and it was found that different extracts had different effects on the inhibition of *E. coli* growth ($p < 0.05$) (Table 3). LSD post hoc test showed that *T. vulgaris* L. and chicory had the highest (5.38) and lowest (2.23) diameter of the inhibition zone on inhibition of *E. coli* growth, respectively (Fig. 4).

The results of evaluating the effect of plant extracts and antibiotics on inhibition of *E. coli* growth showed that different extracts, as well as antibiotics, had significant effects on inhibition of *E. coli* growth ($p < 0.05$) (Table 4).

LSD post hoc test showed that gentamicin and Cefazolin antibiotics had the highest (9.3 mm) and lowest (1.19 mm) effects in inhibiting *E. coli* growth, respectively. All plants used had poorer performance in inhibiting *E. coli* growth than most antibiotics except Cefazolin (Fig. 5). Due to the side effects of chemical drugs and antibiotics as well as the potential effect of medicinal plant extracts used, especially herringbone on *E. coli*, compared to Cefazolin, it is recommended to use herringbone to inhibit *E. coli* growth.

Table 3 Analysis of variance diameter of growth inhibition zone of plant extract against *E. coli* at 100 ppm dilution

Source	DF	SS	MS	F
Plant Extract	3	128.90	42.730	6.98 **
Error	80	489.810	6.12	-
Total	83	618	-	-

** Significant at the level of one percent

Table 4 Analysis of variance of the effect of plant extracts and antibiotics on inhibition of *E. coli* growth

SOURCE	DF	SS	MS	F
SOV	8	1429.28	178.660	9.11**
ERROR	180	3529.05	19.606	
TOTAL	188	4958.33		

** Significant at the level of one percent

DISCUSSION

The results of the DPPH test showed that the ability of extracts of case species, rosemary, Hamdani cultivar, and other species to inhibit free radicals depends on the concentration of extracts and has increased with increasing concentration of their anti-radical activity. As it was the most effective in 40 mg concentration of Hamdani tulip extract; however, in evaluating the antioxidant properties, extracts that are more effective in foot concentrations are important. Among them, Myrtus extract was the most effective extract both in terms of antioxidant properties and antimicrobial activity [6] The antimicrobial property has a positive correlation and basically, the antioxidant properties of the extracts increase with increasing concentration of total phenol compounds [34] and this ability depends on the number of aromatic rings and the nature of the hydroxyl displacement groups. The hydroxyl ions in the reaction medium increase the probability of hydrogen transfer to free radicals and consequently the inhibitory power of the extract [8, 35]. In the present study, it was found that the antioxidant activity of the extracts increased with increasing the concentration of the extract, which was similar to the presented research. Also, the most effective plant on *E. coli* was the extract of the herb flower.

Although the methanolic extract of Myrtus did not show any effect on *E. coli* infiltration activity, the MBC for *E. coli* was reported to be higher than 40 mg/ml [36] and other studies have shown no effect of *E. coli* extract [37,38] but in the present study, ethanolic extracts of all plants used were effective on *E. coli* , which shows the ability of these plants to control *E. coli*.

Antibacterial effect of acidic methanol extract of stigma and petals of different species of saffron on two types of gram-positive bacteria (*Bacillus subtilis* and *S. aureus*) and two gram-negative bacteria (*E. coli* and *P. aeruginosa*) by measuring the diameter of the zone by inoculation, bacterium Determination of the minimum MIC was investigated and it was concluded that *Bacillus* subtilis was the most sensitive bacterium and *E. coli* was the most resistant bacterium to the extracts [39]. In a study, the antimicrobial effect of aqueous extracts of petals [40] and stigmas [41, 42] of saffron on some foodborne pathogenic bacteria were found that aqueous extract of saffron petals was effective on *Salmonella typhimurium* but it was not eefective on *S. aureus*, and *E. coli*; however, the results of the present study show the growth inhibition of ethanolic extracts of various plants, especially herring and chicory compared to saffron on *E. coli* .

Rosemary hydroalcoholic extract with an average diameter of 16 mm was the most effective bacterial growth inhibition zone and hydroalcoholic extract of the case plant with an average of 13 mm diameter growth inhibition zone was the least effective extract in inhibiting *E. coli* growth. Among the concentrations used in the extract, the most effective and least effective concentrations were 120 and 90 mg of hydroalcoholic extract, respectively. On the other hand, 120 mg of rosemary hydroalcoholic

extract with an average of 20 mm diameter of growth inhibition zone was the most effective, and the concentration of 60 mg of case hydroalcoholic extract with an average of 10 mm diameter of growth inhibition zone was the least effective [35]. Although all the plants used were effective in controlling *E. coli*, but the maximum mean diameter of the growth inhibition zone was related to sage with an average of 5.38 mm, which indicates the ineffectiveness of the plants used in this study (chicory, sage, *T. vulgaris* L.). Horticulture and *L. angustifolia* Mill. (Binaloodi) compared to the research plants presented (case plants, saffron, yarrow, *T. vulgaris* L.and rosemary). In a study, the antimicrobial effects of *T. vulgaris* L. ethanolic extract against human pathogenic bacteria, and the results showed that *T. vulgaris* L. leaf extract in all concentrations inhibited the bacteria. Also, the lowest MIC against *S. pneumoniae*, *H. alvei*, *P. mirabilis*, and *S. marcescens* was 6.25 ppm [43]. In the present study, the MIC of *T. vulgaris* L. on *E. coli* was 6.25 mg/ml.

In a study, the chemical composition and antibacterial activity of Iranian *L.* × hybrid were investigated and it was concluded that the diameter of the growth inhibition zone was obtained in a range from 9.36 mm against S. aureus to 23.3 mm against E. coli. They also reported that there was a significant relationship between the composition of essential oil and the level of antibacterial effect expressed as inhibition areas [44]. In the present study, the diameter of *N. binaludensis* Jamz growth inhibition zone on *E. Coli* inhibition was 4.9 mm, which indicates the less effective anti-*E. coli* property of *N. binaludensis* Jamz than *Lavandin*.

The antibacterial properties of essential oils and hydrosols and aqueous extracts of *L.* spp grown in Australia were investigated and it was concluded that the hydrosols and aqueous extracts of the leaves of the plant had no antibacterial activity; they have concluded that different species of *L.* spp may have different antibacterial properties [45]. In the present study, *N. binaludensis* Jamz was effective on *E. Coli* and its stunting zone diameter was 4.9 mm, which confirms the different effects of different species of *L.* against bacteria. The essential L. angustifolia has an interesting antimicrobial effect and maybe a new potential source for a natural antimicrobial drug as well as a new wound healing product [46] that confirms the effect of the plant species on antimicrobial properties.

Medicinal plants are one of the most prominent plants in the field of allochemicals due to their secondary metabolites. On the other hand, the demand for medicinal compounds has increased, but some of these plants have limited natural habitats, and depending on the environmental and geographical conditions of the plant, their collection is difficult. Low concentrations of these compounds in plants, limited natural resources, increasing degradation of forests, pastures, and green space, extinction of diverse plant and animal species, problems related to domestication and agronomy of these plants, researchers' attention to using biotechnological solutions to increase production and has focused on the productivity of medicinal plants. By using various sciences such as biology, biochemistry, genetics, etc., and by using cell culture, organ and porcine culture methods, genetic engineering, and molecular markers, it can increase the efficiency of plants as renewable sources for drug production [20].

CONCLUSIONS

The results of this study suggest that the essential oils of *H. perforatum* L.and then chicory, respectively, can be useful alone or in combination with other antimicrobial agents to treat infections caused by *E. coli*. However, testing in the living system is necessary to evaluate the possible toxicity of essential oils, especially *H. perforatum* L. essential oil, to examining of (*in vivo*) their properties and effects and to obtain appropriate concentrations of these essential oils for use in living organisms.

Abbreviation

DPPH: 2,2-diphenyl-1-picrylhydrazyl
MBC: Minimum Bactericidal Concentration
MIC: Minimum Inhibitory Concentration

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