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OPTIMIZATION OF COLD PLASMA TREATMENT FOR MICROBIAL DECONTAMINATION IN LICORICE (*GLYCYRRHIZA GLABRA* L.) AS A MEDICINAL PLANT

Peyman Yari[1,2], *Masoumeh Khanahmadi*[2], *Ahmad Tajehmiri*[1], *Saman Hosseini*[1,3], *Elnaz Hosseininezhadian koushki*[1], *Kheirollah Yari*[1]*

Address(es):
[1] Medical Biology Research Center, Health Technology Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran.
[2] Department of Medicinal Plants, Institute of Higher Education, Jahad-e-Daneshgahi, Kermanshah Unit, Iran.
[3] Department of Plant Protection, College of Agriculture and Natural Resources, Razi University, Kermanshah, Iran.

*Corresponding author: khirollah.yari@yahoo.com

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ABSTRACT

The cold plasma treatment is a novel, non-thermal, and environmentally safe technique to reduce the microbial population of pathogenic microorganisms in various food packaging materials and agriculture products. This research investigated the effect of cold plasma treatment on the microbial population of licorice (*Glycyrrhiza glabra* L.). Samples of licorice root were treated with cold plasma in different conditions. The effect of main variables such as energy generator, power, exposure time, pressure, temperature, injected gas, frequency, and the amount of injected gas was optimized. Also, the effect of cold plasma on the glycyrrhizic acid in samples at the highest cold plasma level was studied. The results illustrated that cold plasma technology reduced the undesirable biological contamination in the treated samples. In the current study, the lowest microbial population in treated samples was shown in conditions with cold-vacuumed plasma systems, pressure at 10^{-2} TORR, injected gas with oxygen and argon (60 L/min), radio frequency energy (RF) with a frequency of 13.56 MHz, exposure time of 3 min, the temperature at 30ºC, and power of 100w. This treatment had high efficiency in microbial population reduction and growth inhibition, with 69.6% and 97.38%, respectively. Also, the results of HPLC methods demonstrated that there were not any significant differences between the glycyrrhizic acid of the control sample and cold-plasma-treated samples. The results of this study indicate that cold plasma technology can be used as an important antibacterial method for medicinal plants to reduce microbial population and minimize food quality losses.

Keywords: Cold plasma, Non-thermal treatment, Decontamination, Licorice, Microbial population

INTRODUCTION

Herbal medicine is an age-old tradition of using natural products and therapeutic plants to avoid and treat ailments **(Firenzuoli & Gori, 2007; Idu, Omonigho, Erhabor, & Efijuemue, 2010)**. In all regions of the world, in both developing and developed countries, the use of medicinal plants due to their bioactive substances is gaining much attention in health practice **(Barnes, 2007; Rahmani, Khan, & Aldebasi, 2017; Smith-Hall, Larsen, & Pouliot, 2012)**.
Glycyrrhiza glabra L. (Licorice) is a native and pasture medicinal plant that belongs to the genus *Glycyrrhiza* and species *glabra* Linn **(A. M. Khan *et al.*, 2013; Ramsis *et al.*, 2024)**. Licorice is distributed in central and southwest Asia and the Mediterranean region **(Parvaiz *et al.*, 2014; Sharma & Agrawal, 2013)**. Licorice is one of the most commercial and valuable plants widely utilized in different industries, such as tobacco, cosmetics, food, and pharmaceutics **(Wahab *et al.*, 2021)**. More investigations on rhizomes and roots of *Glycyrrhiza glabra* illustrated the therapeutic qualities and pharmaceutical properties on health, such as memory improvement **(Dhingra, Parle, & Kulkarni, 2004)**, spatial memory **(Ravichandra, Devi, Adiga, & Rai, 2007)**, cerebral ischemia **(Zhan & Yang, 2006)**, anxiolytic activity **(Ambawade, Kasture, & Kasture, 2001)**, antioxidant, antiviral **(Adianti *et al.*, 2014)**, anti-inflammatory **(Chandrasekaran *et al.*, 2011)**, antitumor **(R. Khan *et al.*, 2013)**, antimicrobial **(Ahn *et al.*, 2012)**, and many other activities **(Belinky, Aviram, Mahmood, & Vaya, 1998)**.
Plant products can accept a high level of microbial contamination due to their growing environment **(Kneifel, Czech, & Kopp, 2002; Mahunu *et al.*, 2024)**. Deactivation of microorganisms in medicinal plants is usually carried out with several important disinfectants, including methyl bromide, ethylene oxide, thermal treatment, UV radiation, gamma rays or high-energy electrons, and ozone **(Leistritz, 1997)**. However, these treatment methods have limitations and disadvantages in reducing the microbial population. The use of ethylene oxide and methyl bromide is prohibited in Europe and many other countries due to the release of toxic by-products and sustained toxic compounds, environmental issues, and the variation in organoleptic properties of treated samples. On the other hand, the thermal treatment can change the color, nutritional, and effective substance of medicinal plants **(Farkas, 1998; Moreau, Orange, & Feuilloley, 2008; ElGamal *et al.*, 2023)**. Gamma irradiation is effectively used for microbial decontamination, but products treated with irradiation are unpopular in many countries due to public fear and legal regulations **(Kim, Lee, & Min, 2014)**. Also, UV radiation does not inhibit microbial growth effectively due to the lack of penetration results in nonhomogeneous microbial decontamination **(Kim *et al.*, 2014)**. Therefore, finding a new and appropriate method for the microbial decontamination of medicinal plants is important.
Today, cold plasma as an eco-friendly technology gaining rising attention with promising decontamination properties in many industries that can be used to make healthy food and vegetables and improve the quality of plant materials **(Banu *et al.*, 2012; Karkhanis & Singh, 2024; Liu, Zhao, Zhang, Gao, & Meng, 2021)**. Numerous investigations illustrate that cold plasma treatment might be used to protect food **(Moreau *et al.*, 2008)**. Plasma is the fourth state of matter in the universe and neutral ionized gas. It is usually divided into two types, based on the characteristics and situations they form, into thermal and non-thermal **(Banu *et al.*, 2012; Bora, Khan, & Mahnot, 2022; Moreau *et al.*, 2008)**. Plasma is generated by the selective transfer of electrons in a wide range of pressures and temperatures, which is provided by mechanical, thermal, nuclear, radiation, and magnetic energy **(Mizuno, 2009; Barjasteh *et al.*, 2023)**. Plasma generation produces active chemical species such as free radicals, electrons, and photons, positive and negative charge ions, and non-excited and excited atoms and molecules **(Karkhanis & Singh, 2024; Pogorzelska-Nowicka *et al.*, 2021)**. Plasma is utilized in several cases, such as clearing the air in closed environments, improving fuel quality and assisting in plasma fuel, sterilization of heat-sensitive materials, application of biological products in the medical and dental industry, water purification, food industry, inactivation of microorganisms and packaging industry for clearing surfaces, etc. **(Fernández & Thompson, 2012; Sainz-García & Alba-Elías, 2023)**. Cold plasma creates reactive compounds for the destruction of cell membranes, and those reactive compounds can denature the proteins and DNA by UV radiation and ion bombardment, which in turn effectively inactivates a wide range of microorganisms, including bacterial, fungal, and viruses **(Moisan *et al.*, 2002; Ulbin-Figlewicz, Brychcy, & Jarmoluk, 2015; Tasouji *et al.*, 2018)**. The purposes of the current study were to (a) investigate the effect of cold plasma on microbial population in Licorice powder, (b) study the effect of cold plasma on the active constituent of licorice, (c) optimize cold plasma treatment for microbial decontamination.

MATERIAL AND METHODS

Licorice powder preparation and Cold plasma treatment

Licorice (*Glycyrrhiza glabra* L.) powder was purchased from Zagros Bioidea Company (Iran). All chemicals and reagents were of analytical grade. The culture media were sterilized by autoclaving at 121°C for 20 min.
In 2020, *Glycyrrhiza glabra* was treated with cold plasma (CUTE Model) under different conditions (Table 1). For cold plasma treatment, eleven experiments were designed, and the effect of operational variables was optimized. The sample that was not treated was also considered negative control. The results obtained under different treatment conditions were compared with the results of the control experiment. The exposure time varied between 1 to 4 minutes. The used frequencies were between 0.05 and 13.56 MHz, and the reactive power was between 50-120 w. After cold plasma exposure, sampling was started immediately, and the treated samples were stored at 4°C until further analysis.

Table 1 Experimental parameters and their levels for optimization of cold plasma treatment.

Experiment	Cold plasma type	Generator of energy	Power (W)	Exposure time (min)	Gas type	Pressure (torr)	Temperature (ºC)	Gas injection (L/Min*)	Frequency (MHz)
A	Atmospheric	LF	50	1	Oxygen	atmospheric	30	10	0.05
B	Under vacuum	RF	100	2	Oxygen (80%)+Argon (20%)	10^{-3}	24	50	13.56
C	Cold Atmospheric	LF	50	1	Helium	atmospheric	30	50	0.05
D	Under vacuum	RF	120	2.5	Oxygen (50%)+Argon (40%)+Nitrogen(10%)	10^{-2}	30	50	13.56
E	Under vacuum	LF	100	3	Air	10^{-1}	30	70	0.04
F	Under vacuum	LF	100	3	Air (80%)+Argon(20%)	10^{-2}	30	60	0.04
G	Step1: Under vacuum	RF	100	3	Oxygen (80%)+Argon (20%)	10^{-2}	30	60	13.56
	Step 2: Under vacuum	RF	100	2	Plasma water vapor	10^{-2}	40	20	13.56
H	Step1: Under vacuum	RF	100	3	Air (80%)+Argon(20%)	10^{-2}	30	60	13.56
	Step2: Under vacuum	RF	100	1	Plasma water vapor	10^{-2}	40	20	13.56
I	Step1: Under vacuum	RF	100	3	Air (80%)+Argon(20%)	10^{-2}	30	60	0.04
	Step2: Under vacuum	LF	100	2	Plasma water vapor	10^{-2}	40	20	0.04
M	Step1: Under vacuum	RF	100	3	Air (80%)+Argon(20%)	10^{-2}	30	60	13.56
	Step2: Under vacuum	RF	100	3	Plasma water vapor	10^{-2}	40	20	13.56
N	Step1: Under vacuum	RF	100	3	Air (80%)+Argon(20%)	10^{-2}	30	60	13.56
	Step2: Under vacuum	RF	100	4	Plasma water vapor	10^{-2}	40	20	13.56

Antimicrobial activity assay

In vitro antimicrobial assay

The antimicrobial activity of cold plasma was determined from the difference between microbial growth and the population of non-treatment (control) and treatment. After each treatment, the initial sample suspension was prepared using the following method: 1 g of the sample (treated Licorice powder) was mixed with 9 mL of 0.1% sterile peptone water in sterile conditions. At room temperature, the mixture was homogenized. The dilution was done in five steps by transferring 1 mL of the solution to 9 mL of 0.1% sterile peptone water. 0.1 mL of each dilution was spread on the plate count agar medium (PCA) for all the samples and incubated at 37°C for 24 h. Three replications were conducted for all the microbial tests **(Viuda-Martos *et al.*, 2008)**. Furthermore, a similar method, Mueller Hinton Broth medium, was used to investigate the growth of bacteria. Additionally, Sabouraud dextrose agar (SDA) medium was used to examine the growth of fungi. The plates were incubated at 25°C for one week for fungi growth.

Measurement of microbial growth and population

After 24 hours, using a UV–vis spectrophotometer, the bacterial suspension's optical density (OD) was measured at a wavelength of 600 nm and compared with the control treatment. The inhibition rate of bacterial growth was calculated using formula 1. In this formula, GIR (%) is the percentage of inhibition of bacterial growth, OD control is the concentration of bacterial growth in the control treatment, and OD treatment is the concentration of bacterial growth in treatments **(Huang, Wu, He, Ye, & Li, 2017)**.

$$\text{Growth Inhibition Rate (\%)} = \frac{\text{OD control - OD treatment}}{\text{OD control}} \times 100 \qquad (1)$$

Following the incubation period (24 h at 37°C), colonies were visually counted using a magnifying glass. The microbial population reduction was expressed as a logarithmic cycle and calculated using formula 2. In this formula, MPR (%) is the percentage of reduction of microbial population, N_0 is the number of control sample colonies, and N_t is the number of colonies of samples after treatment with cold plasma **(Mascarenhas *et al.*, 2022)**.

$$\text{Microbial Population Reduction (\%)} = \frac{\text{Log } N_0 - \text{Log } N_t}{\text{Log } N_0} \times 100 \qquad (2)$$

Statistical analysis

All the tests were performed in a completely random design, and three repetitions were considered for each treatment. Analysis of variance (ANOVA) procedure in SAS (version 9.3) was used to analyze the data obtained from tests. The mean comparison was performed using Duncan's multiple range test at the probability level of 1%. GraphPad prism 8 software was used to draw the graphs.

Identification of Enterobacteriaceae by the API 20E system

API 20 E Kit was used to identify *Enterobacteriaceae* and other non-fastidious, Gram-negative rods. In API 20 E Kit, 21 miniaturized biochemical tests and a related database were used. The experiments were performed in duplicate, and the mean values were observed. Oxidase activity for all isolated strains was tested with an Oxidase kit (Padtan Teb Co.).

HPLC analysis of glycyrrhizic acid in cold plasma-treated samples

The *Glycyrrhiza glabra* treated with cold plasma were analyzed using a High-Performance Liquid Chromatography (HPLC). For this purpose, the licorice extract of selected treatments was dissolved in 20 mL of methanol and mixed thoroughly. The supernatant was centrifuged (20 min at 30000 rpm) and decanted. Then, it was filtered through a syringe filter (0.45 µm). The HPLC was analyzed using High-Performance Liquid Chromatography (HPLC, Knauer, V7603) with a reverse type Agilent RPC-18 column (250×6.4 mm ID, pore size 5µm) for quantitative analysis of Glycyrrhizic acid. The mobile phase comprised a mixture of acetic acid, water, and acetonitrile (1:61:38 volume ratio) and was delivered at a flow rate of 2 mL/min. The detector wavelength was 254 nm.

RESULTS

Effect of cold plasma on microbial growth and population

To measure the microbial growth in Mueller Hinton Broth, the optical densities (OD_{600}) of diluted culture were measured at 600 nm using a UV–vis spectrophotometer. All treatments significantly reduced the microbial growth and population compared to the control treatments at the probability level of 1%. As indicated in Figure 1, the highest inhibition rate of microbial growth was related to the treatment *N* and *M*, with 97.38% and 97.08%, respectively. The treated sample with cold plasma in conditions *N* and *M* (pressure at 10^{-2} TORR, injected gas 60

L/min, RF-13.56 MHz, exposure time of 3 min, 30ºC, and 100 w power) had the lowest, and the control sample had the highest growth (Figure 1). As demonstrated in Figure 2, the highest microbial population reduction was related to the treatment *N* and *M*, with 69.6% and 67.47%, respectively. The usage of plasma treatment for licorice herb samples results that counting the microbial population in the control (untreated) of licorice being the highest. Still, the treatment samples of *H, G, M,* and *N* had the lowest microbial population. It was also observed that cold plasma had the lower antimicrobial efficiency of *C, F, A,* and *E* treatments. Additionally, the bacterial growth in the Broth medium and microbial population had a high correlation with each other (Pearson correlation coefficient of 0.950 and probability of significant difference 0.001). Our results indicated that conditions with cold-vacuumed plasma systems, pressure at 10^{-2} TORR, injected gas with oxygen and argon (60 L/min), radio frequency energy (RF) with a frequency of 13.56 MHz, exposure time of 3 min, the temperature at 30ºC, and power of 100 watts were the most effective condition for microbial inactivation.

For further investigation of the presence of fungus in licorice, samples were cultured in the Sabouraud dextrose agar. Results showed that treated samples of cold plasma prevented fungus growth completely; however, the fungi had grown in control samples. Our results indicated that cold plasma can be considered a disinfestation method for plant materials due to its strong potential for fungal and microbial inactivation. Staining with Ketone Blue was utilized to specify the type of growing fungus in the control sample of the licorice. Based on staining with Ketone Blue, the species of grown fungus in the control sample was *Aspergillus flavus*.

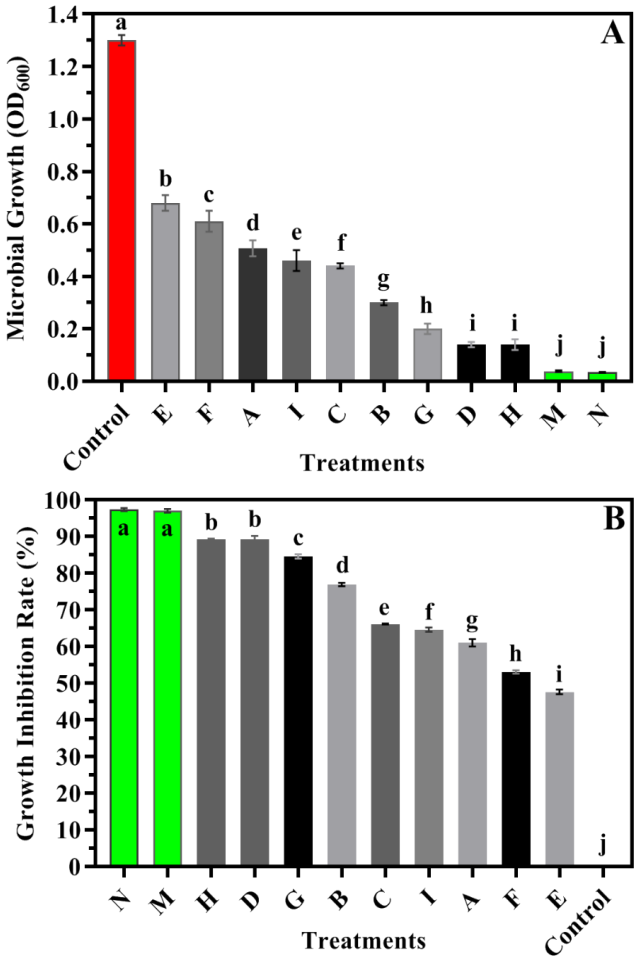

Figure 1 The effect of different cold plasma treatments (see Table 1 for treatment conditions) on microbial growth. The means were compared at the level of 1% probability using Duncan's multiple range test. The difference between means with common letters are not statistically significant. A) Optical densities of microbial growth. B) Microbial growth inhibition rate**.**

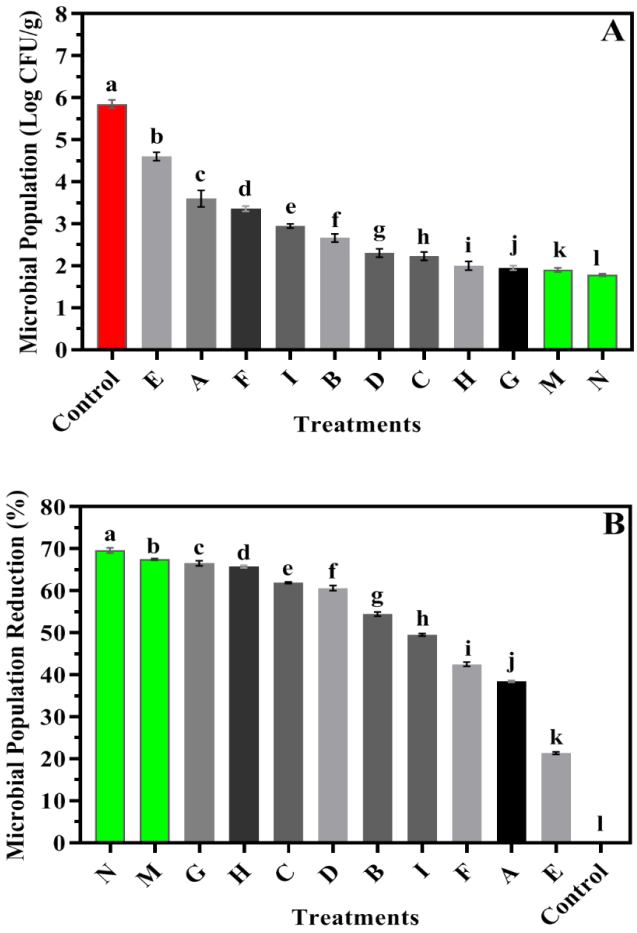

Figure 2 The effect of cold plasma treatments on the Microbial population in Licorice. The means were compared at the level of 1% probability using Duncan's multiple range test. The difference between means with common letters are not statistically significant. A) Logarithmic microbial population. B) Microbial population reduction.

Results of the API Diagnostic Kit for Licorice

The results gained using the API 20 E Diagnostic Kit for licorice samples illustrated that the Gram-negative bacilli in treatment and control samples of licorice herb were *Escherichia coli* and *Serratia ficaria*. Gram-positive bacilli, *Streptococcus,* and Gram-positive cocci were *Lactococcus lactis*, *Streptococcus uberis, and Enterococcus faecium, respectively.* The Gram-staining method was used to determine the effect of the cold plasma treatment samples on the Gram-positive and negative microorganisms. According to Table 2, it was found that *B, F,* and *E* samples had a high bacterial variation, and *M* and *N* with a type of bacteria (gram-negative bacilli) had the lowest diversity among the samples.

Table 2 Identification of grown bacteria in the treated licorice samples by Gram-staining and API 20 E Kit

licorice Samples	Stained Samples
Control (Untreated)	Gram negative bacilli, Gram-positive cocci, Streptococcus, Gram positive bacilli, Gram-positive coccobacilli
Treatment A	Gram negative bacilli, Streptococcus, Gram positive bacilli
Treatment B	Gram negative bacilli, Streptococcus, Gram-positive cocci
Treatment C	Streptococcus, Gram-negative bacilli
Treatment D	Gram negative bacilli, Gram negative bacilli
Treatment E	Gram negative bacilli, Gram-positive cocci, Gram positive bacilli, Streptococcus
Treatment F	Gram positive bacilli, Streptococcus Gram-positive, Gram negative bacilli, Diplococcus
Treatment G	Streptococcus, Gram-negative bacilli
Treatment H	Gram negative bacilli, Gram-positive cocci
Treatment I	Gram negative bacilli, Gram-positive cocci
Treatment M	Gram negative bacilli
Treatment N	Gram negative bacilli

HPLC method for the licorice plant root

The results gained using the HPLC method showed that there were no significant differences in the percentage of *glycyrrhizin* as an essential component in the licorice root among control and selected treated samples of D, *G*, *M*, and *H* (χ^2= 4.28, P=0.11) (Table 3 and Figure 3).

Table 3 The results of the HPLC method for analysis of the *glycyrrhizin in control and treated samples with cold plasma.*

NO	Sample	Efficiency (%)	*Glycyrrhizin* (%)
1	Control	7.67	4.65
		8.12	4.47
		7.5	5.35
2	Treatment D	7.07	4.6
		7.6	4.92
		7.32	4.87
3	Treatment G	6.85	4.65
		7.8	4.9
		6.72	4.27
4	Treatment M	6.85	4
		6.9	4.57
		6.4	4.37
5	Treatment H	6.65	3.9
		7.87	4.42
		7.12	4.55

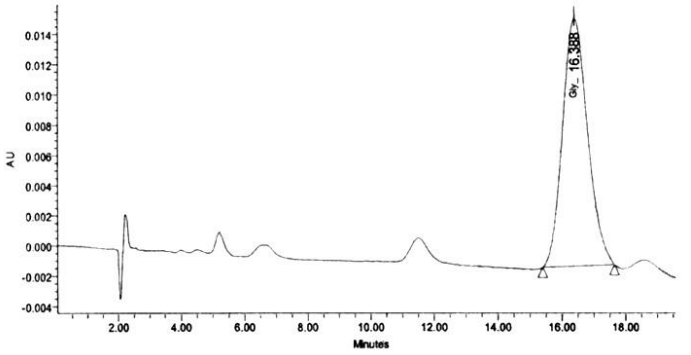

Figure 3 Peaks present on a HPLC chromatogram of glycyrrhizic acid in samples treated with cold plasma.

DISCUSSION

Both the intrinsic and extrinsic (environment, collection, and transport methods, cultivation, harvest, and storage facilities) factors are involved in the quality and safety of medicinal and pharmaceutical plant products for human consumers, so the plant products may be failed to resist contamination **(Idu *et al.*, 2010)**. Also, literature reviews that herbs and plant materials have high bacterial counts **(Pogorzelska-Nowicka *et al.*, 2021)**. Microbial contaminants in plant and herbal products may be affecting the health status of consumers, so they are considered a common health problem worldwide. Thus, selecting the appropriate and non-thermal method is important for decontaminating plants or obtained extracts based on assuring the quality and safety of the produce **(Gil, Selma, López-Gálvez, & Allende, 2009)**.

Different studies have touted the decontamination methods in plant materials with several physical, chemical, and biological processes such as methyl bromide, ethylene oxide, thermal (steam sterilization) treatment, and gamma rays **(Pewlong, Sajjabut, Eamsiri, Chookaew, & Boonsirichai, 2016; Yeh, Line, Hinton, Gao, & Zhuang, 2019)**. Gamma irradiation was used for the decontamination of different tissues, extracts, and plant byproducts **(Aouidi, Ayari, Ferhi, Roussos, & Hamdi, 2011; Harrison & Were, 2007; Koseki *et al.*, 2002)**. For example, **Pewlong *et al.*, 2016** evaluated the effect of gamma radiation on the microbial population of *Glycyrrhiza glabra*; they included that the microbial quality of licorice powder can be improved by treating with gamma radiation **(Pewlong *et al.*, 2016)**. These decontamination methods have some disadvantages, too. For example, ethylene oxide and methyl bromide are toxic compounds and have carcinogenic effects on the environment and humans. Thermal treatment can modify the color and effective and active substances of treated samples and gamma irradiation is poorly accepted by consumers **(Ebadi, Abbasi, Harouni, & Sefidkon, 2019)**. So, scientists have focused more on an alternative and appropriate decontamination method that does not have these disadvantages and limitations mentioned above **(Ebadi *et al.*, 2019)**.

Cold atmospheric plasma (CAP) is non-thermal, fast, and safe for the environment and has been effectively recruited to inactivate a wide range of microorganisms; therefore, the use of this technology has been widely suggested **(Hosseini, Rostami, Hosseinzadeh Samani, & Lorigooini, 2020; Rahmati, Khoshtaghaza, Banakar, & Ebadi, 2022)**. Our research studied the effect of cold plasma treatment on the microbial population of licorice. Samples of licorice root were treated with cold plasma in different conditions. The results indicated that cold plasma technology reduced the microbial population in the treated samples. In our study, all treatments significantly reduced the microbial growth and population compared to the control treatments at the probability level of 1%. However, the highest inhibition rate of microbial growth was related to the treatments N and M, with 97.38% and 97.08%, respectively. The highest microbial population reduction was related to these treatments, with 69.6% and 67.47%, respectively. These treatments (*N* and *M*) decontaminating licorice by cold plasma at pressure with 10^{-2} TORR, injected gas with oxygen and argon, radio frequency energy (RF) with a frequency of 13.56 MHz, exposure time of 3 minutes, and power of 100 watts.

Based on literature reviews, depending on the exposure time, input power, exposure frequency, gas type, and cold plasma sources, the effect of cold plasma treatment on bacterial inactivation is different **(Ulbin-Figlewicz *et al.*, 2015)**. Our study observed a higher antimicrobial effect for nitrogen plasma treatment injected gas with a mix of oxygen and argon. **Ulbin-Figlewicz *et al*., 2015)** found that using argon and helium can provide more antimicrobial activity. **Song *et al.*, 2009,** considered exposure time (60, 90, and 120 s) of atmospheric pressure plasma to improve the safety of sliced cheese and ham inoculated by a 3-strain cocktail of *Listeria monocytogenes*; their results showed that microbial log reduction increased with increases of input power and plasma exposure time **(Song *et al.*, 2009)**. In our study, in the best treatment (*M* and *N*), the microbial population was reduced by increasing power to 100 W. The rate of microbial cell reduction increases with higher power levels. The amount of reactive species produced varies depending on the power and voltage used. The increase in power creates a stronger electric field, which results in the production of more energetic ions, allowing reactive species to penetrate and destroy microbial cells **(Lu *et al.*, 2016)**.

Reported data indicated that cold plasma could improve crop yields and germination rates and decrease microbial population **(Abarghuei, Etemadi, Ramezanian, Esehaghbeygi, & Alizargar, 2021)**. **Liu *et al*., 2021**, concluded that the treatment with cold Atmospheric plasma was a promising tool for maintaining the quality of minimally processed kiwifruit **(Liu *et al.*, 2021)**. **Idu *et al*., 2010**, concluded that herbal remedies were not sterile and that constant quality assessment of therapeutic products is needed to ensure human consumption **(Idu *et al.*, 2010)**. **Pogorzelska-Nowicka *et al*., 2021**, studied the effect of nitrogen cold plasma pretreatment on the content of antioxidants, antioxidant activity, the profile of volatile compounds, and microbial count in herb extracts. Their results confirmed that cold plasma treatment can decrease total aerobic bacteria **(Pogorzelska-Nowicka *et al.*, 2021)**. **Abarghuei *et al*., 2021**, analyzed the effects of atmospheric plasma on the performance of basil (*Ocimum basilicum* L. cv. Genovese Gigante). The results indicated that cold atmospheric plasma can significantly enhance the physiological and biochemical traits of basil **(Abarghuei *et al.*, 2021)**. In accordance with our study, **Lee *et al*., 2022,** applied three gases (argon, air, and nitrogen) to reduce *E. coli* in wheat. In their study, the optimized conditions to reduce *E. coli* in wheat flour using atmospheric cold plasma were 15 min of treatment time and 0.20 L/min of nitrogen gas flow rate **(Lee, Park, Korber, & Baik, 2022)**. **Hosseini *et al*., 2020**, evaluated the effect of cold plasma on the reduction of *E. coli* bacteria in sour cherry juice and its qualitative properties **(Hosseini *et al.*, 2020)**. They indicated that decontamination of sour cherry juice by atmospheric pressure cold plasma has little effect on the juice's qualitative properties. **Jerushalmi *et al*., 2020**, examined three different sterilization methods of medical cannabis inflorescences; including gamma irradiation, beta irradiation, and cold plasma to determine their efficacy in the reduction of fungal colony-forming units. Their results included that both beta irradiation and cold plasma treatments are cheaper and simpler to apply and have greater potential and effectiveness for sterilization **(Jerushalmi, Maymon, Dombrovsky, & Freeman, 2020)**.

Even though there are several proposed applications for non-thermal plasma in microbial decontamination, this technology is still in its infancy, and its limitations need to be addressed before it can be commercialized. Key limitations for cold plasma are the relatively early state of technological development, the variety and complexity of the required equipment, and the largely unexplored impacts of cold plasma on the quality of products. The commercialization of this technique for microbial decontamination can be facilitated by further comprehensive evaluations. Our results emphasize the importance of adjusting treatment parameters to achieve optimal microbial population reduction while also highlighting the promising role of cold plasma in enhancing food safety. Additional research is suggested, involving different voltage levels, varying time periods, and the inclusion of other medicinal plants.

CONCLUSION

In conclusion, in our study, we aimed to optimize the effect of cold plasma treatment on the microbial population of licorice. Our results indicated that conditions with cold-vacuumed plasma systems, pressure at 10^{-2} TORR, injected gas with oxygen and argon (60 L/min), radio frequency energy (RF) with a frequency of 13.56 MHz, exposure time of 3 min, the temperature at 30ºC, and power of 100 w were the most effective condition for microbial inactivation. Therefore, the cold plasma method can be used as a safe method to decrease the microbial population in the plant materials, and this method has no negative

influence on the qualitative properties of treated samples. Lastly, our results derived at the laboratory scale provide an optimized set of conditions and basic data for further studies involving the industrial sterilization of medicinal plants.

AUTHOR CONTRIBUTIONS

Peyman Yari: Data curation, methodology, writing-original draft; **Kheirollah Yari and Masoumeh Khanahmadi**: Funding, methodology, administration of project, & manuscript editing. **Ahmad Tajehmiri**: Data curation, methodology; **Hosseininezhadian koushki**: Writing-review; **Saman Hosseini**: Formal analysis, Data curation, Writing-review.

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