Journal of King Saud University – Science 33 (2021) 101513

Contents lists available at ScienceDirect

Journal of King Saud University – Science

journal homepage: www.sciencedirect.com

Review

A review on antibacterial and therapeutic plasma-enhanced activities of natural extracts

F.J. Alonso-Montemayor [a], R. Reyna-Martínez [a], M.G. Neira-Velázquez [b], A. Sáenz-Galindo [a], C.N. Aguilar [c], R.I. Narro-Céspedes [a,*]

[a] School of Chemistry, Autonomous University of Coahuila, Saltillo, Mexico
[b] Department of Advanced Materials, Applied Chemistry Research Centre, Saltillo, Mexico
[c] Bioprocesses & Bioproducts Research Group, Autonomous University of Coahuila, Saltillo, Mexico

ARTICLE INFO

Article history:
Received 30 September 2020
Revised 13 February 2021
Accepted 7 June 2021
Available online 10 June 2021

Keywords:
Cold plasma
Natural extracts
Antibacterial
Therapeutic

ABSTRACT

Emerging traditional drug-multiresistant bacteria has become a critical health problem worldwide, which has motivated the development of alternative therapies and technologies to combat infections associated with such bacteria. The application of antibacterial and non-cytotoxic natural extracts combined with the therapeutic use of cold plasma offers alternatives to antibiotics and conventional therapies. Thus, the present review aims to show that research of the synergistic effect of plasma treatment on the antibacterial and therapeutic properties of natural extracts (e.g., *Rosmarinus officinalis, Citrus sinensis, Azadirachta indica, Rhizome Atractylodes macrocephala*) is a relatively new field with few reports, but with promising published results. The cited publications were recovered from scientific databases such as Google Scholar, SpringerLink, Wiley, and Elsevier – ScienceDirect through an extensive search. In this concern, it is reported that a more significant reduction of the bacterial population in wet samples (e.g., food material, cell cultures, broths, tissues) and polymer fabrics (e.g., polyethene terephthalate, cellulose) could be achieved by using cold plasma treatments combined with natural extracts rather than use them separately. Simultaneously, it is reported that the use of cold plasma and natural extracts enhances cell growth and attachment under *in vitro* and *in vivo* conditions. It was found that atmospheric-pressure plasma jet devices, instead of the dielectric barrier discharge ones, have primarily been used to improve the antibacterial activity of polymer fabrics and in wound healing therapies. Thus, some promising results on the antibacterial and non-cytotoxic properties of plasma-enhanced natural extracts have been reported, but more research (especially comparative studies) is needed to determine their therapeutically safe use.

Contents

Abbreviations: e^-, Electron; γ_{UV}, Ultraviolet radiation/photon; μ, Comparative inactivation yield; C_1, Bacteria amount on the plasma-treated sample; C_2, Bacteria amount on the untreated sample; APPJ, Atmospheric-pressure plasma jet; DBD, Dielectric barrier discharge; DNA, Deoxyribonucleic acid; FCDF, Fresh-cut dragon fruit; PET, Polyethene terephthalate; RONS, Reactive oxygen and nitrogen species.

* Corresponding author at: Departamento de Polímeros, Facultad de Ciencias Químicas, Universidad Autónoma de Coahuila, Blvd. V. Carranza s/n esq. con Ing. J. Cárdenas Valdés, República, 25280 Saltillo, Coahuila, Mexico.
E-mail address: rinarro@uadec.edu.mx (R.I. Narro-Céspedes).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jksus.2021.101513

1. Introduction

Antibiotic-resistant bacteria have become a worldwide health problem mainly attributed to the rapid evolution of the treated bacteria and self-medication habits. In this concern, the microbial antibiotic resistance could be responsible for more than 10×10^6 human deaths per year worldwide by 2050, overcoming pathologies such as cancer and heart diseases. The development of new therapeutic options to treat multi-resistant bacteria is crucial (Pech-Puch et al., 2020). It is in this sense that medicinal plants have emerged as an alternative source of new drugs (Hossain et al., 2019) used to treat various diseases a such as cancer, and their medical properties came from any part of the plants (leaves, stems, roots, flowers, or seeds) which produce active principles (Grzegorczyk-Karolak et al., 2015; Peran et al., 2020). Thousands of plant extracts and pure compounds have been tested *in vitro* against many Gram-positive and Gram-negative bacteria. However, only a few antibacterial extracts and isolated compounds have been tested in pre-clinical and clinical conditions (Sharma et al., 2017; Andleeb et al., 2020).

Researchers entrusted to develop extract-based drugs have also been committed to foster auxiliary technologies to overcome the deficiencies of conventional drugs (e.g., vancomycin, carbapenems). It is in this sense that cold plasma has emerged as an auxiliary pharmaceutical technology. Cold plasma is a partially ionized gas containing neutral and energized molecules, radicals, ions, electrons (e^-), ultraviolet photons (γ_{UV}), among other particles. Also, since low-temperature conditions are required for the treatment of human tissues and other soft materials, which can be damaged by even weak thermal stimulation, plasma has been considered a suitable pharmaceutical tool (Nam et al., 2018; Faria et al., 2020; Mazhir et al., 2020). In general, it has been established that plasma has three main pharmaceutical applications: *i*) structural and functional modification of drugs; *ii*) improvement of drug delivery systems; *iii*) synergistic effect-based treatment of diseases. For instance, innovative pharmaceutical applications of plasma are the production of plasma-activated solutions with antibacterial and anticancer effects, as well as the development of potential inactivated vaccines (Gao et al., 2020).

Although cold plasma applied in pharmacy is a novel field, it has recently exhibited an outstanding development. However, any published review analyzing cold plasma-supported utilization of natural extracts was not found (Gao et al., 2020; Reyna-Martínez et al., 2018; Belete, 2019; Brown et al., 2014; Martelli and Giacomini, 2018; Dai et al., 2020). As this literature analysis shows, there have been reported promising results on the synergistic effect of cold plasma treatment on antibacterial and therapeutic activities of natural extracts that must be analyzed. The conclusions reported here could encourage researches on prospective extract-based therapies to treat diseases provoked by antibiotic-resistant bacteria.

2. Cold plasma generation devices in pharmaceutics

First, it is necessary to explain that cold plasma, which is also called physical plasma, is a partially ionized gas, without thermal equilibrium, composed of ions, electrons, electromagnetic radiation, radicals, among other highly reactive species. In controlled environments, cold plasma is mainly generated by supplying electrical energy to not directly effective gases (e.g., Ar, He, O_2, N_2, air) to ionize them and subsequently produce reactions in the plasma state (von Woedtke et al., 2013). Physical plasma is a relatively new and environmentally friendly technology that has broken into many science areas, such as medicine. For physical plasma in pharmaceutical applications, two devices have been mainly used (Fig. 1): *i*) in dielectric barrier discharge (DBD) devices, plasma is generated by using a single or two different electrodes covered by a dielectric layer, which prevents sudden discharge current and streamer formation. However, it has been reported that DBD can damage living targets, but these drawbacks can be minimized under proper application conditions (Kim et al., 2014); *ii*) atmospheric-pressure plasma jet (APPJ) devices which consist of a powered electrode inside a dielectric tube with an external grounding electrode. The working gas flows over the power electrode and is ionized. A streamer discharge is then formed and propagated through the tube and into the ambient until the energy dissipates. The jet plasma becomes non-arcing; meanwhile, the surrounding environment remains low-temperature by adding a dielectric barrier around the powered electrode. Besides, compared to DBD devices, APPJ ones allow *in situ* experiments, meaning that biological and heat-sensitive materials can be treated with plasma in outdoor conditions. (Gott and Xu, 2019). In general, cold plasma devices have been applied in specific pharmaceutical fields as the generation of biologically active solutions, the support of drug transport across biological barriers, the stimulation of biological processes, among others (von Woedtke et al., 2013).

According to the present review, DBD devices have been the most used plasma generation system in investigations on enhancing the antibacterial activity of extract-soaked samples. In general, such samples have comprised in-packed food materials coated with extracts (Gao et al., 2019; Yeh et al., 2019; Shiekh and Benjakul, 2020) and extract-coated fabrics (Vajpayee et al., 2020; Gorjanc et al., 2016, 2010; Vaideki et al., 2011), rather than biomaterials. The atmosphere contained in packages that usually implies a specific composition (e.g., O_2/CO_2, $O_2/CO_2/Ar$, O_2/Ar, O_2/Air), is ionized, and it modifies the surface of the food material exposed to plasma. Thus, DBD plasma is especially suitable for treating in-package food materials since they are not easily contaminated because they remain sealed from the treatment to the storage (Olatunde et al., 2020). However, DBD plasma pre-treatment of extract-coated fabrics usually requires specific atmospheres (dry or specifically humidified air, Ar, H_2O, O_2) at low-pressure. On the other hand, APPJ devices have not been frequently used to enhance the antibacterial activity of natural extract soaked on food materials. Only one research on enhancing the antibacterial activity extract-coated fresh-cut dragon fruit, after Ar plasma treatment, was found (Matan et al., 2015). It has been reported that APPJ-treated extract extended the shelf-life of dragon fruit for about 15 days while the fruit dipped with untreated extract exhibited a shorter shelf-life of fewer than 5 days. In this sense, APPJ-treated extract provoked a more significant reduction of bacteria present in dragon fruit than untreated extract, which could explain the synergistic effect of plasma treatment and extract on the fruit shelf-life.

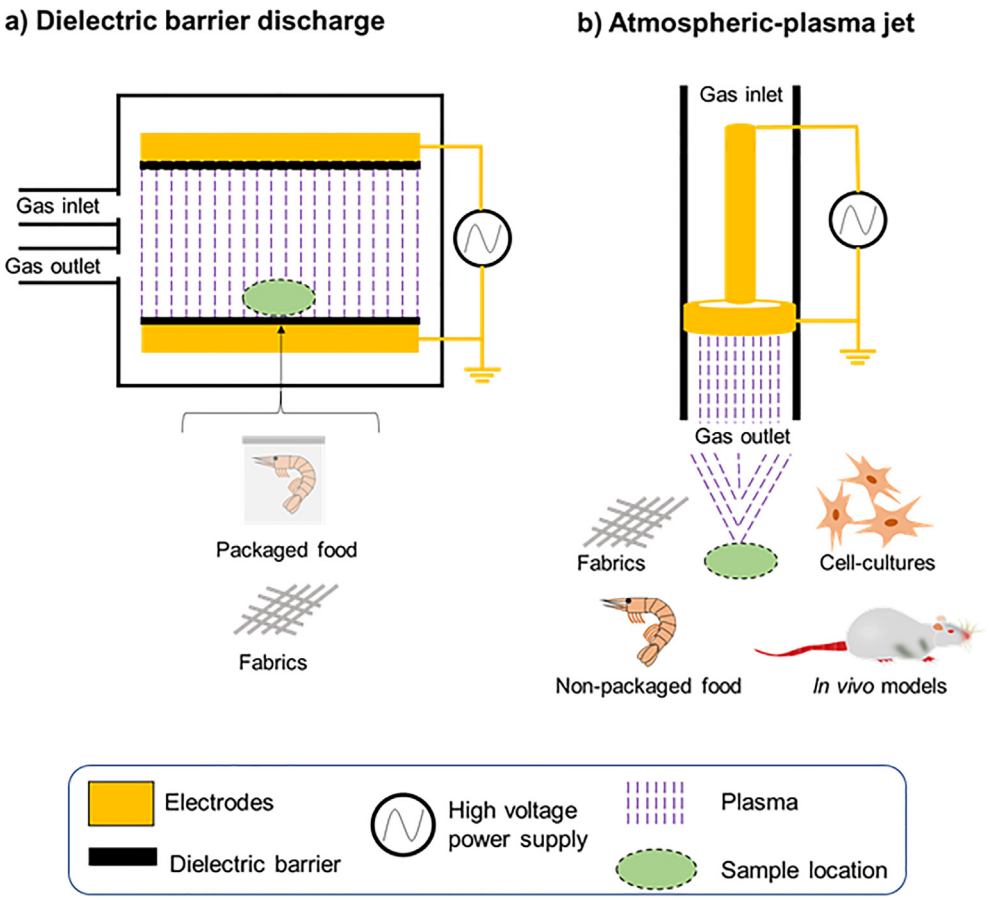

Fig. 1. Schematics of a) dielectric barrier discharge and b) atmospheric pressure plasma jet devices. Some kinds of treated samples are also depicted.

On the contrary, for investigations on the enhancement of the therapeutic activity of natural extracts soaked on samples, the utilization of APPJ devices is standard. Such samples have involved polyethene terephthalate fabrics, cultures of mice fibroblasts, and *in vivo* mice skin (Shu et al., 2017; Nam et al., 2018; Rahayu et al., 2019). In these reports, it is demonstrated that APPJ technology can increase the workability and efficacy of antibacterial dressing containing natural extracts. Additionally, it is reported that APPJ-treated extracts do not exhibit cytotoxic activity, encouraging cells to grow and attach. Although DBD devices have been preferred over APPJ ones, it has been reported that APPJ devices have benefits for industries and businesses due they can be operated at temperatures lower than 50 °C by using the afterglow zone of the plasma plume, and vacuum conditions are not required, reducing operational cost. Thus, APPJ is particularly suitable for treating non-packaged food (Matan et al., 2015) and living matter.

3. Plasma-enhanced antibacterial activity natural extracts

The antibacterial properties of plant extract have been attributed to their capacity to disrupt bacterial membranes through envelope rigidification and inhibition of succinate dehydrogenase (Rempe et al., 2017). Phenolic and polyphenolic compounds found in plants compile the main active principles used to combat multidrug-resistant bacteria. For instance, it has been reported that thymol, carvacrol, eugenol, guaiacol, amino guaiacol, curcumin, some curcuminoid derivatives, cinnamic acids, and derivatives, among others, exhibit antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* strains, which are common bacteria in clinical studies (Martelli and Giacomini, 2018). It has been reported that mechanisms of action of phenolic compounds against bacteria include damage of cytoplasmatic membrane and inhibition of deoxyribonucleic acid (DNA)- and adenosine triphosphate-related enzymes. Besides, many synergistic antibacterial effects have been attributed to the presence of phenolics, which allow intracellular toxins access to their targets by both disrupting bacterial membrane and blocking toxins-removal efflux pumps of specialized strains. Other kinds of synergistic effects, such as hindering carbon sources to bacterial communities by promoting the formation or delivery of non-metabolizable compounds (for bacteria) from plasma-treated extracts (Yeh et al., 2019), could improve the functionality of the antibacterial compounds of extracts, especially against drug resistance mechanisms (Rempe et al., 2017; Faria et al., 2020).

Antibacterial agents generated in plasma can lead to synergistic effects that can enhance the antibacterial activity of extracts. These agents are known to involve reactive oxygen and nitrogen species (RONS), such O_3, O_2^-, H_2O_2, HO^- (Kim et al., 2014), and NO_x. Besides RONS, e^-, and γ_{UV}, from plasma, exhibit well-knowledge antibacterial activity, it has been reported that they increase the phenolic content of antibacterial plasma-treated extracts (Kashfi et al., 2020), as Table 1 shows. Such increment has been attributed to the release of phenolic compounds from glycosidic components, and the degradation of phenolic compounds into smaller ones after exposing the extract to RONS from plasma (Kim et al., 2014) improve the antibacterial activity of the extract. However, a recent investigation reports an enhanced antibacterial activity of plasma-treated extracts, even when phenolic content remains constant. Thus, it has been proposed that RONS diffused in the plasma-treated extract (exceptionally long-lived species such as NO_x and

Table 1
Total phenolic content of some plant extracts before and after plasma treatment.

Reference	Extract source	Total phenolic content		
		Unit	Before treatment	After treatment
Faria et al., 2020	*Salicornia neei*	mg/g	20	18
Jung et al., 2017	*Perilla frutescens*	g/L	1800*	1900*
Kashfi et al., 2020	*Mentha piperita*	mg/g	263	293
Kim et al., 2014	Naringin	ppm	173	226
Matan et al., 2015	*Citrus sinensis***	mg/100 g	2.8	3.4

* The total phenolic content was estimated from graphics.
** Treatment of dragon fruit with 5% of *Citrus sinensis* extract.

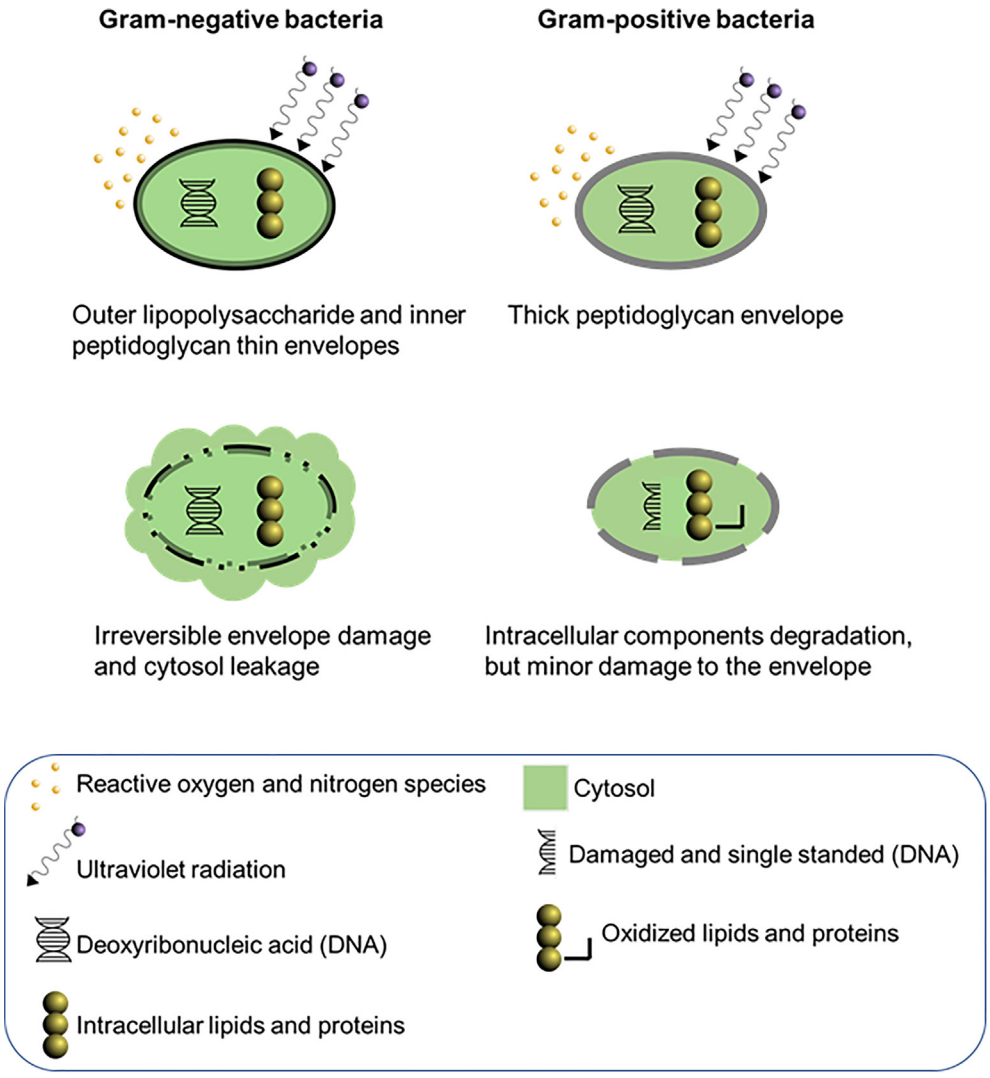

Fig. 2. Hypothetical main mechanisms of action of plasma reactive species against Gram-negative and Gram-positive bacteria.

$ONOO^-$), increase the antibacterial activity of the extract, in addition to that provided by phenolic compounds and other active principles. NO_x species are generated in the acidic conditions produced by O_2 and then diffused in the treated natural extract. Following the reaction expressed by Eq. (1), unstable NO_2^- decomposes to form HNO_2 and, subsequently, a variety of NO_x, accordingly to the reactions described by Eqs. (2)-(4). Moreover, the generation of $ONOO^-$ in plasma is possible via reactions represented by Eqs. (5)-(6) (Jung et al., 2017). Then, as Fig. 2 depicts, once RONS dissolved in the extract interact with bacterial cells, two main specific inactivation mechanisms occur for Gram-negative and Gram-positive bacteria, respectively. For Gram-negative bacteria, which have a thin cytoplasmic membrane and cellular wall, once the damaged envelope has lost its osmotic capacity, intracellular content leaches, which provokes irreversible damages and cell death. On the other hand, for Gram-positive bacteria, which have a thick envelope, RONS provoke oxidation of intracellular components (e.g., DNA, lipids, proteins) rather than envelope disruption, and, in consequence, causes the bacteria death (Olatunde et al., 2019; Han et al., 2016).

$$NO_2^- + H^+ \rightarrow HNO_2 \quad (1)$$

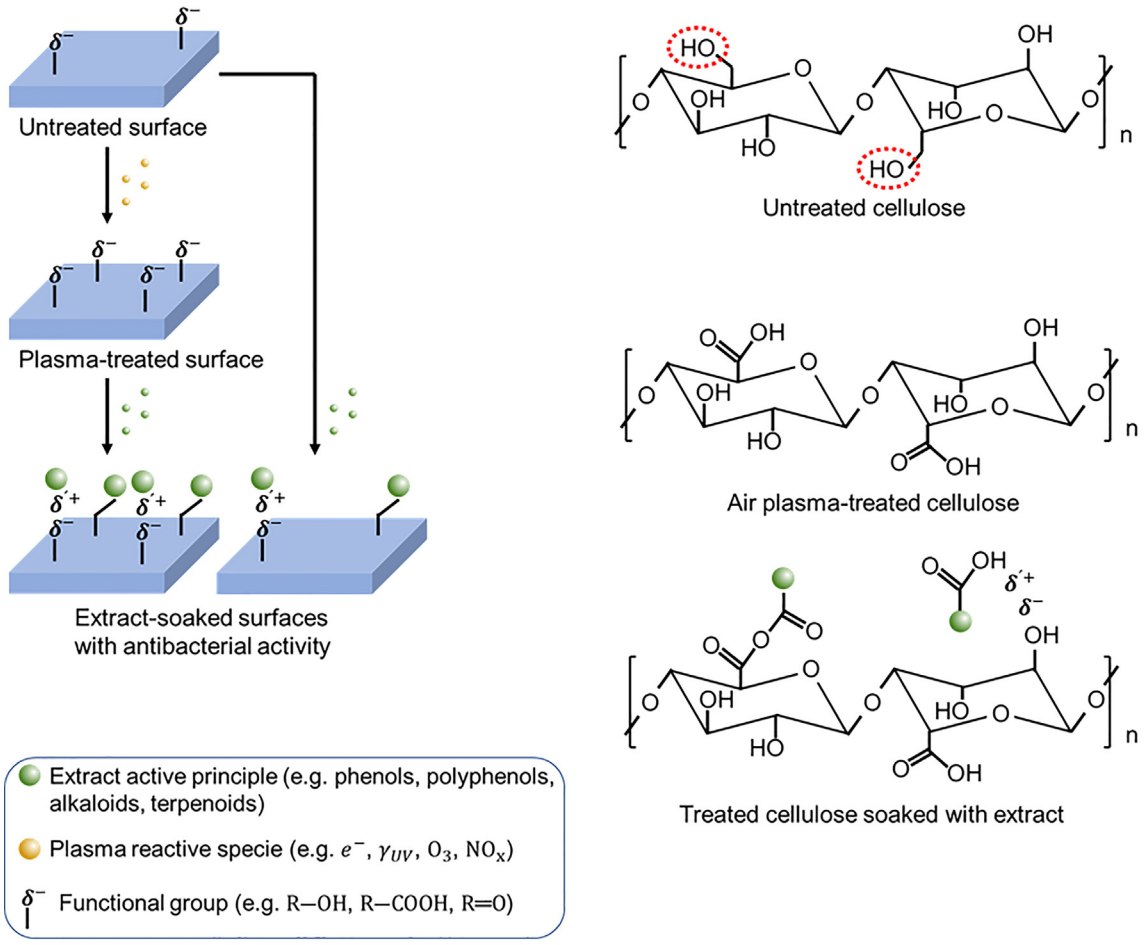

Fig. 3. Hypothetical mechanisms of plasma pre-treatment synergistic effect on the antibacterial activity of natural extracts soaked on materials. A red dotted perimeter frames the primary alcohols of cellulose.

Table 2
Investigations about enhancing the antibacterial activity of natural extracts by applying plasma treatment to samples pre-soaked with them.

Reference	Extract source	Sample	Inactivated bacteria	$\mu \cdot 100$
Gao et al., 2019[a]	*Rosmarinus officinalis*	Chicken breast	Undefined microbiota	9
Yeh et al., 2019[b]	*Rosmarinus officinalis*	Chicken breast	Undefined microbiota	39
Matan et al., 2015[c*]	*Citrus sinensis*	Inoculated FCDF	*Salmonella typhimurium*	61
			Escherichia coli	47
			Listeria monocytogenes	75
Olatunde et al., 2020[d,*]	*Cocos nucifera*	Asian seabass slice	Mesophilic bacteria	30
Shiekh and Benjakul, 2020	*Garcinia cowa* Roxb	Pacific white shrimp	Mesophilic bacteria	22[e]
				16[f]

$\mu \cdot 100$: Percentual enhancement of bacterial inactivation after plasma treatment.
FCDF: Fresh-cut dragon fruit.
[*] The bacteria amounts were estimated from graphics.
[a] Total viable counts of O_2/CO_2 plasma-treated (at 70 kV) and untreated 5 days-stored samples, after being soaked with 1% extract, were 3.62 and 3.96 log CFU g^{-1}, respectively.
[b] Maximum population size of O_2/CO_2 plasma-treated and untreated extract-soaked samples, stored for 5 days, were 71.21 and 117.5 (in terms of optical density readings at the wavelength of 590 nm).
[c] Estimated total viable counts of Ar plasma-treated (at 40 W) and untreated 2.5% extract-treated samples were 5.6 and 2.2 log CFU g^{-1}, for the *Salmonella typhimurium* strain, 5.5 and 2.9 log CFU g^{-1}, for the *Escherichia coli* strain, and 5.9 and 1.5 log CFU g^{-1}, for the *Listeria monocytogenes* strain, respectively.
[d] Estimated total viable counts of air plasma-treated and untreated extract-soaked samples, stored for 6 days, were 4.4 and 3.1 log CFU g^{-1}, respectively.
[e] Total viable counts of 1% Ar/Air plasma-treated and untreated extract-soaked samples, stored for 15 days, were 5.00 and 6.42 log CFU g^{-1}, respectively.
[f] Total viable counts of 1% Ar/O_2 plasma-treated and untreated extract-soaked samples, stored for 15 days, after being soaked with extract, were 5.50 and 6.57 log CFU g^{-1}, respectively.

$$HNO_2 + H^+ \rightarrow H_2NO_2^+ \rightarrow NO^+ + H_2O \quad (2)$$

$$2HNO_2 \rightarrow NO + NO_2 + H_2O \quad (3)$$

$$3HNO_2H^+ \rightarrow NO_3^- + 2NO + H_2O \quad (4)$$

$$O_2^- + NO \rightarrow ONOO^- \quad (5)$$

Table 3
Investigations about enhancing the antibacterial activity of natural extracts by applying plasma treatment to samples post-soaked with them.

Reference	Extract source	Sample	Inactivated bacteria	$\mu \cdot 100$
Shu et al., 2017[a]	*Rhizome Atractylodes macrocephala*	PET fabric	*Staphylococcus aureus*	29
			Escherichia coli	40
Vajpayee et al., 2020[b]	*Ocimum sanctum*	Banana fabric	*Escherichia coli*	3
			Staphylococcus aureus	15
	Citrus sinensis		*Escherichia coli*	3
			Staphylococcus aureus	7
Gorjanc et al., 2016[c]	*Fallopia japonica*	Bamboo rayon fabric	*Staphylococcus aureus*	10
		Cotton rayon fabric		47
Vaideki et al., 2007[d]	*Azadirachta indica*	Cotton fabric	*Staphylococcus aureus*	14
			Escherichia coli	14
Vaideki et al., 2009*	*Azadirachta indica*	Cotton fabric	*Staphylococcus aureus*	18[e]
			Escherichia coli	20[e]
			Staphylococcus aureus	14[f]
			Escherichia coli	16[f]
Vaideki et al., 2011	*Azadirachta indica*	Cotton fabric	*Staphylococcus aureus*	25[g]
			Escherichia coli	27[g]
			Staphylococcus aureus	22[h]
			Escherichia coli	22[h]
			Staphyloccus aureus	10[i]
			Escherichia coli	14[i]

$\mu \cdot 100$: Percentual enhancement of bacterial inactivation after plasma treatment.
PET: Polyethylene terephthalate.
* The bacteria amounts were estimated from graphics.
[a] Inhibition zones of Ar plasma-treated and untreated samples, after being soaked with extract, were 9.85 and 7.03 mm, for the *Staphylococcus aureus* strain and 12.28 and 7.34 mm, for the *Escherichia coli* strain, respectively.
[b] Bacterial percentage reductions of air plasma-treated (for 4 min) and untreated samples, after being soaked with 5% extract, are compiled in the cited reference (Vajpayee et al., 2020).
[c] Inhibition zones of H_2O plasma-treated and untreated samples, after being soaked with extract, were 0.72 and 0.65 mm, for bamboo rayon fabric and 0.60 and 0.32 mm, for cotton fabric rayon, respectively.
[d] Bacterial percentage reductions of O_2 plasma-treated and untreated samples, after being soaked with extract, were 100 and 86, for both *Staphylococcus aureus* and *Escherichia coli* strains, respectively.
[e] Inhibition zones of O_2 plasma-treated and untreated samples, after being soaked with extract, were 6.6 (estimated) and 5.4 mm, for the *Staphylococcus aureus* strain, and 6.4 (estimated) and 5.1 mm, for the *Escherichia coli* strain, respectively.
[f] Inhibition zones of air plasma-treated and untreated samples, after being soaked with extract, were 6.3 (estimated) and 5.4 mm, for the *Staphylococcus aureus* strain, and 6.1 (estimated) and 5.1 mm, for the *Escherichia coli* strain, respectively.
[g,h,i] Inhibition zones of O_2, air, and Ar plasma treated samples, respectively, and untreated one, after being soaked with extract, are compiled in the cited reference (Vaideki et al., 2011).

$$OH + NO_2 \rightarrow ONOO^- + H^+ \quad (6)$$

On the other hand, it has been reported that the antibacterial properties of extract, soaked on plasma-treated materials, can also be improved. Fig. 3 depicts a hypothetical reported mechanism of plasma pre-treatment synergistic effect on the antibacterial activity of natural extract soaked on materials. Since plasma treatment increases the surface hydrophilicity of materials, the attachment of extract active principles to the modified surface is strengthened. Consequently, extract-coated fabrics, pre-treated with plasma, should display more significant antibacterial activities than untreated ones (Shu et al., 2017; Haji et al., 2016; Molakarimi et al., 2016). For instance, cellulose, which is the main component of cellulosic fabrics, suffer chemical changes after air plasma treatment. Dehydrogenation of the primary alcohol group or breaking of the ring oxygen, rather than breaking of β-glycosidic and the ether bonds, are plausible reactions. Then, the oxidation of aldehyde and hydroxyl groups leads to the formation of carboxylic acid functionalities. These functional groups increase the hydrophilic nature of the fabric, which results in more effective absorption of an antibacterial coat than the untreated one (Vajpayee et al., 2020).

Tables 2 and 3 summarize the reported results of investigations on enhancing the antibacterial activity of natural extracts by applying plasma treatment to extract soaked or post-soaked materials, respectively. It was found that most of the investigations of plasma-treated materials soaked with natural extract (e.g., from *Rosmarinus officinalis*, *Citrus sinensis*, *Cocos nucifera*, *Garcinia cowa* Roxb) have been carried out to extend the shelf life of food materials and to improve their organoleptic properties. Instead, the improvement of the antibacterial and bacteriostatic activities of fabrics coated with extract (e.g., from Rhizome *Atractylodes macrocephala*, *Ocimum sanctum*, *Citrus sinensis*, *Fallopia japonica*, *Azadirachta indica*) made of polyethene terephthalate (PET) and cellulose, against nosocomial bacteria (e.g., *Escherichia. coli*, *Staphylococcus aureus*), has been achieved through plasma pre-treatment. The comparative inactivation yield (μ) of extract soaking versus extract soaking with plasma treatment, defined by Eq. (7), was calculated for each investigation cited in Tables 2 and 3.

$$\mu = 1 - C_1/C_2 \quad (7)$$

where the ratio between the bacteria amount (e.g., expressed in total viable counts or maximum population size) on the plasma-treated sample (C_1) and the untreated one (C_2), measured under specific conditions, is subtracted from the unit. However, in the case of bacterial percentage reduction and inhibition zone measurements, C_1 and C_2 must correspond to the untreated and plasma-treated sample, respectively, to avoid indetermination and negative yields.

As Tables 2 and 3 show, since it was estimated μ ranges from 0.09 to 0.75 and 0.03 to 0.47 for reported plasma-treated food materials pre-soaked with natural extracts and plasma-treated fabrics post-soaked with natural extracts, respectively, it can be stated that that, in general, plasma treatment increases the antibacterial activity of natural extracts coatings. However, comparative experimental studies are needed to determine which methodology produces the best synergistic effects or if it does not matter which methodology is chosen. Thus, the research on the hypothetical synergistic effect of plasma treatment on the

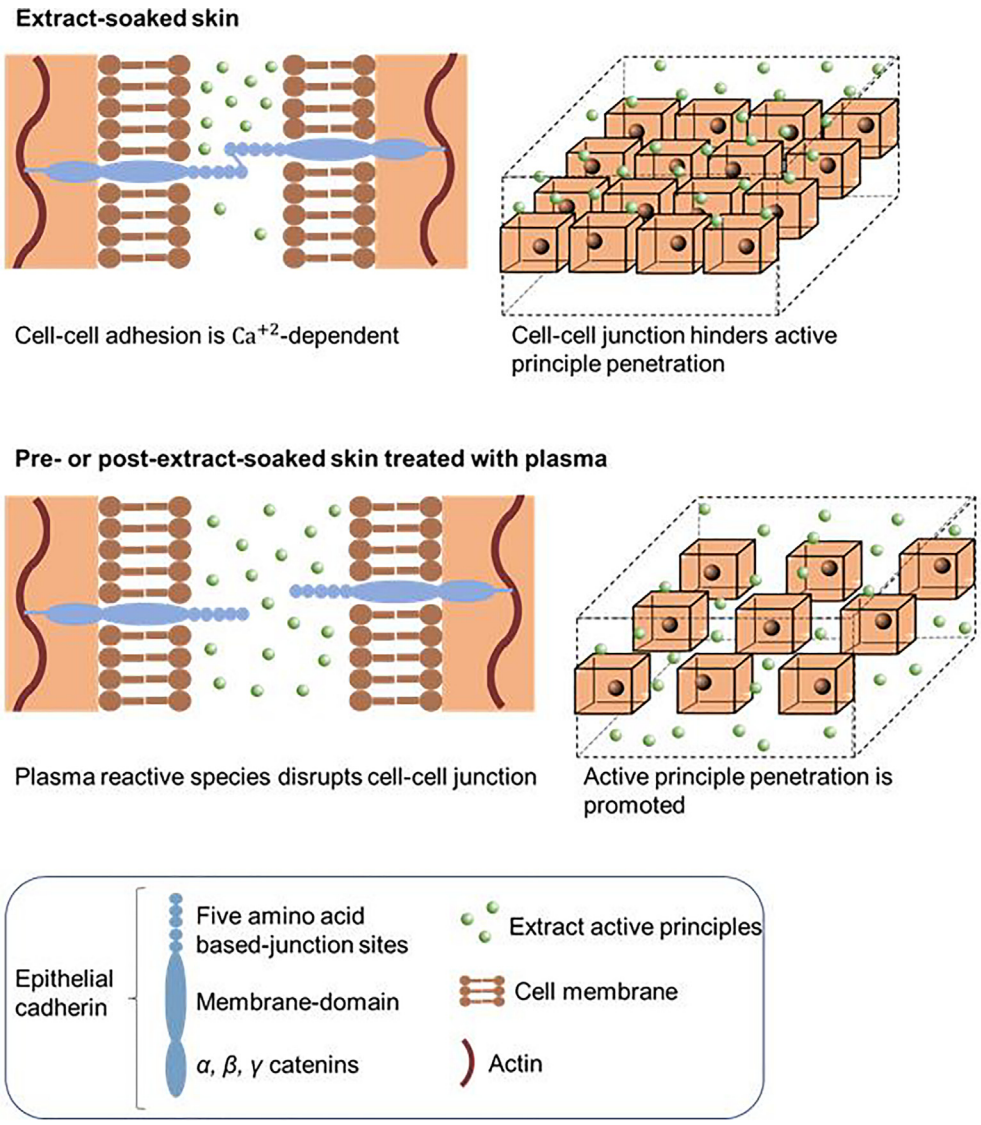

Fig. 4. Proposed mechanism of natural extract penetration in extract-soaked skin, pre- or post-treated with plasma.

antibacterial activity of natural extracts soaked on clinically relevant materials must be promoted.

4. Plasma-enhanced therapeutic properties of natural extracts

It was found that the published investigations on the enhancement of therapeutic properties of natural extracts with plasma are primarily focused on *in vivo* mice model for wound healing by using *Panax ginseng*, *Piper betle*, and *Rhizome Atractylodes macrocephala* extracts. In these researches, Type I collagen and fibronectin are extracellular matrix components that play a fundamental role in wound healing and structural preservation of the skin. It has been reported that some active principles of plant extracts promote the production of Type I collagen and fibronectin, among other structural elements of the dermis, which improve wound healing (Lee et al., 2007). For instance, it has been reported that extract coated-PET, pre-treated with plasma, did not show cytotoxic activity against mice fibroblasts. In this sense, the extract presence seemed to enhance the growth and the attachment of cells to the surface, which implies that the modified PET surface is biocompatible. (Shu et al., 2017).

Additionally, plasma treatment of skin, pre- or post-soaked with extract, accelerates wound healing and enhance the penetration efficiency of the extract active principles without provoking any histological damage (see Fig. 4). In this sense, it has been informed that during treatment of human keratinocytes with plasma, RONS temporarily open the barrier of skin through the inhibition of epithelial cadherin (Ca^{+2}-dependent cell adhesion molecule) for around three hours. Then, extract active principles present on plasma-treated skin penetrate deeper than for untreated skin (Nam et al., 2018). Moreover, it is known that the presence NO_x during plasma treatment, rather than oxygen-based ones, restore the damaged tissue (Rahayu et al., 2019). In conclusion, since the published investigations on wound healing properties of natural antibacterial extracts, enhanced with plasma treatment, have shown promising results about the safety and health benefits of potential plasma therapy and medicinal extract application, the research on wound healing and other possible therapeutic applications is encouraged.

5. Conclusions

Reports on the synergistic effect of plasma treatment on the antibacterial and therapeutic activities of natural extracts (e.g., *Rosmarinus officinalis*, *Citrus sinensis*, *Azadirachta indica*, *Rhizome Atractylodes macrocephala*) are available. Dielectric barrier

discharge (DBD) devices are the most used to enhance the antibacterial activity of extract-soaked samples treated with plasma. Such investigations have been carried out to extend the shelf life of food materials and improve their organoleptic properties, rather than address biomaterials. On the other hand, atmospheric-pressure plasma jet (APPJ) devices are frequently employed to improve the antibacterial and bacteriostatic activities of extracts soaked on plasma-treated fabrics (made of polyethene terephthalate or cellulose) against nosocomial bacteria (e.g., *Escherichia coli*, *Staphylococcus aureus*). Since the reported synergistic effect of plasma treatment on the antibacterial activity of natural extracts is considerable, investigation efforts on possible improvement on the antibacterial activity of extract-soaked biomaterials, pre- or post-treated with plasma, must be encouraged.

On the other hand, research on the therapeutic properties of natural extracts, enhanced with plasma, is mainly focused on wound healing therapies tested *in vivo* mice models and *in vitro* cultures of mice fibroblasts. APPJ devices are commonly implied in such a kind of research, rather than DBD ones. In perspective, since the available reports on the synergistic effect of plasma treatment on the therapeutic properties of natural extracts seem promising, possible therapeutic applications of natural extracts, supported applying plasma treatment, must continue to be investigated.

Disclosure of funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

Consejo Nacional de Ciencia y Tecnología of Mexico is acknowledged for the grant (no. 859503) given to Francisco Javier Alonso-Montemayor to pursuit his current doctoral study in Posgrado en Ciencia y Tecnología de Materiales at Universidad Autónoma de Coahuila.

References

Andleeb, S., Alsalme, A., Al-Zaqri, N., Warad, I., Alkahtani, J., Bukhari, S.M., 2020. *In-vitro* antibacterial and antifungal properties of the organic solvent extract of *Argemone mexicana* L. J. King Saud Univ Sci. 32, 2053–2058. https://doi.org/10.1016/j.jksus.2020.01.044.

Belete, T.M., 2019. Novel targets to develop new antibacterial agents and novel alternatives to antibacterial agents. Hum. Microbiome J. 11, 100052. https://doi.org/10.1016/j.humic.2019.01.001.

Brown, D.G., Lister, T., May-Dracka, T.L., 2014. New natural products as new leads for antibacterial drug discovery. Bioorg. Med. Chem. Lett. 24 (2), 413–418. https://doi.org/10.1016/j.bmcl.2013.12.059.

Dai, J., Han, R., Xu, Y., Li, N.a., Wang, J., Dan, W., 2020. Recent progress of antibacterial natural products: Future antibiotics candidates. Bioorg. Chem. 101, 103922. https://doi.org/10.1016/j.bioorg.2020.103922.

Faria, G.Y.Y., Souza, M.M., Oliveira, J.R.M., Costa, C.S.B., Collares, M.P., Prentice, C., 2020. Effect of ultrasound-assisted cold plasma pre-treatment to obtain sea asparagus extract and its application in Italian salami. Food Res. Int. 137, 109435. https://doi.org/10.1016/j.foodres.2020.109435.

Gao, L., Shi, X., Wu, X., 2020. Applications and challenges of low temperature plasma in pharmaceutical field. J. Pharm. Anal. https://doi.org/10.1016/j.jpha.2020.05.001.

Gao, Y., Zhuang, H., Yeh, H.-Y., Bowker, B., Zhang, J., 2019. Effect of rosemary extract on microbial growth, pH, color, and lipid oxidation in cold plasma-processed ground chicken patties. Innov. Food Sci. Emerg. Technol. 57, 102168. https://doi.org/10.1016/j.ifset.2019.05.007.

Gorjanc, M., Bukošek, V., Gorenšek, M., Vesel, A., 2010. The influence of water vapor plasma treatment on specific properties of bleached and mercerized cotton fabric. Text. Res. J. 80 (6), 557–567. https://doi.org/10.1177/0040517509348330.

Gorjanc, M., Savić, A., Topalić-Trivunović, L., Mozetič, M., Zaplotnik, R., Vesel, A., Grujić, D., 2016. Dyeing of plasma treated cotton and bamboo rayon with *Fallopia japonica* extract. Cellulose. 23 (3), 2221–2228. https://doi.org/10.1007/s10570-016-0951-9.

Gott, R.P., Xu, K.G., 2019. OH Production and jet Length of an atmospheric-pressure plasma jet for soft and biomaterial treatment. IEEE Trans. Plasma Sci. 47 (11), 4988–4999. https://doi.org/10.1109/TPS.2710.1109/TPS.2019.2942576.

Grzegorczyk-Karolak, I., Wysokińska, H., Olas, B., 2015. Studies on the antioxidant properties of extracts from the roots and shoots of two *Scutellaria* species in human blood plasma. Acta Biochim. Pol. 62, 253–258. https://doi.org/10.18388/abp.2014_944.

Haji, A., Mehrizi, M.K., Sharifzadeh, J., 2016. Dyeing of wool with aqueous extract of cotton pods improved by plasma treatment and chitosan: Optimization using response surface methodology. Fibers Polym. 17 (9), 1480–1488. https://doi.org/10.1007/s12221-016-6457-0.

Han, L., Patil, S., Boehm, D., Milosavljević, V., Cullen, P.J., Bourke, P., Dudley, E.G., 2016. Mechanisms of inactivation by high-voltage atmospheric cold plasma differ for *Escherichia coli* and *Staphylococcus aureus*. Appl. Environ. Microbiol. 82 (2), 450–458. https://doi.org/10.1128/AEM.02660-15.

Hossain, M.A., 2019. A phytopharmacological review on the Omani medicinal plant: *Ziziphus jujube*. J. King Saud Univ Sci. 31, 1352–1357. https://doi.org/10.1016/j.jksus.2018.12.003.

Jung, S., Lee, C.W., Lee, J., Yong, H.I., Yum, S.J., Jeong, H.G., Jo, C., 2017. Increase in nitrite content and functionality of ethanolic extracts of *Perilla frutescens* following treatment with atmospheric pressure plasma. Food Chem. 237, 191–197. https://doi.org/10.1016/j.foodchem.2017.05.095.

Kashfi, A.S., Ramezan, Y., Khani, M.R., 2020. Simultaneous study of the antioxidant activity, microbial decontamination, and color of dried peppermint (*Mentha piperita L.*) using low pressure cold plasma. LWT-Food Sci. Technol. 123, 109121. https://doi.org/10.1016/j.lwt.2020.109121.

Kim, H.-J., Yong, H.I., Park, S., Kim, K., Kim, T.H., Choe, W., Jo, C., 2014. Effect of atmospheric pressure dielectric barrier discharge plasma on the biological activity of naringin. Food Chem. 160, 241–245. https://doi.org/10.1016/j.foodchem.2014.03.101.

Lee, J., Jung, E., Lee, J., Huh, S., Kim, J., Park, M., So, J., Ham, Y., Jung, K., Hyun, C.-G., Kim, Y.S., Park, D., 2007. *Panax ginseng* induces human Type I collagen synthesis through activation of Smad signaling. J. Ethnopharmacol. 109 (1), 29–34. https://doi.org/10.1016/j.jep.2006.06.008.

Martelli, G., Giacomini, D., 2018. Antibacterial and antioxidant activities for natural and synthetic dual-active compounds. Eur. J. Med. Chem. 158, 91–105. https://doi.org/10.1016/j.ejmech.2018.09.009.

Matan, N., Puangjinda, K., Phothisuwan, S., Nisoa, M., 2015. Combined antibacterial activity of green tea extract with atmospheric radio-frequency plasma against pathogens on fresh-cut dragon fruit. Food Control. 50, 291–296. https://doi.org/10.1016/j.foodcont.2014.09.005.

Mazhir, S.N., Ali, I.A.M., Al-Ahmed, H.I., Bououdina, M., Ali, F.A.M., 2020. Dependence of *O. Vulgare* extract and cold plasma on the formation of silver nanoparticles: Anticancer activity against L20B cells. AIP Conf. Proc. 2213,. https://doi.org/10.1063/5.0000112 020149.

Molakarimi, M., Khajeh Mehrizi, M., Haji, A., 2016. Effect of plasma treatment and grafting of β-cyclodextrin on color properties of wool fabric dyed with Shrimp shell extract. J. Text I. 107 (10), 1314–1321. https://doi.org/10.1080/00405000.2015.1102459.

Nam, S.H., Choi, J.H., Song, Y.S., Lee, H.-J., Hong, J.-W., Kim, G.C., 2018. Improved penetration of wild ginseng extracts into the skin using low-temperature atmospheric pressure plasma. Plasma Sources Sci. T. 27 (4), 044001. https://doi.org/10.1088/1361-6595/aab4b4.

Olatunde, O.O., Benjakul, S., Vongkamjan, K., 2019. Dielectric barrier discharge cold atmospheric plasma: Bacterial inactivation mechanism. J. Food Saf. 39 (6). https://doi.org/10.1111/jfs.v39.610.1111/jfs.12705.

Olatunde, O.O., Benjakul, S., Vongkamjan, K., 2020. Cold plasma combined with liposomal ethanolic coconut husk extract: A potential hurdle technology for shelf-life extension of Asian sea bass slices packaged under modified atmosphere. Innov. Food Sci. Emerg. Technol. 65, 102448. https://doi.org/10.1016/j.ifset.2020.102448.

Pech-Puch, D., Pérez-Povedano, M., Gómez, P., Martínez-Guitián, M., Lasarte-Monterrubio, C., Vázquez-Ucha, J.C., Novoa-Olmedo, M.L., Guillén-Hernández, S., Vilegas-Hernández, H., Bou, G., Rodríguez, J., Beceiro, A., Jiménez, C., 2020. Marine organisms from the Yucatan peninsula (Mexico) as a potential natural source of antibacterial compounds. Mar. Drugs. 18, 369. https://doi.org/10.3390/md18070369.

Peran, J., Ercegović Ražić, S., Sutlović, A., Ivanković, T., Glogar, M.I., 2020. Oxygen plasma pre-treatment improves dyeing and antimicrobial properties of wool fabric dyed with natural extract from pomegranate peel. Color Technol. 136, 177–187. https://doi.org/10.1111/cote.12464.

Rahayu, H.S.E., Nasruddin, N., Nurani, L.H., Darmawati, S., Rohmani, A., Lutfiyati, H., Wahyuningtyas, E.S., Sikumbang, I.M., Muhlisin, Z., Sukeksi, A., Nuroini, F., Ishijima, T., Sugama, J., Nakatani, T., 2019. Ethanolic extract of the natural product of *Daun sirih (Piper betle*) leaves may impede the effectiveness of the

plasma jet contact style for acute wounds. Clin. Plasma Med. 15, 100090. https://doi.org/10.1016/j.cpme.2019.100090.

Rempe, C.S., Burris, K.P., Lenaghan, S.C., Stewart Jr., C., 2017. The potential of systems biology to discover antibacterial mechanisms of plant phenolics. Front. Microbiol. 8, 422. https://doi.org/10.3389/fmicb.2017.00422.

Reyna-Martínez, R., Narro-Céspedes, R.I., Ibarra-Alonso, M.C., Reyes-Acosta, Y.K., 2018. Use of cold plasma technology in biomaterials and their potential utilization in controlled administration of active substances. Juniper Online J. Mater. Sci. 4,. https://doi.org/10.19080/JOJMS.2018.04.555649 555649.

Sharma, A., Flores-Vallejo, R.del.C., Cardoso-Taketa, A., Villarreal, María.L., 2017. Antibacterial activities of medicinal plants used in Mexican traditional medicine. J. Ethnopharmacol. 208, 264–329. https://doi.org/10.1016/j.jep.2016.04.045.

Shiekh, K.A., Benjakul, S., 2020. Effect of high voltage cold atmospheric plasma processing on the quality and shelf-life of Pacific white shrimp treated with Chamuang leaf extract. Innov. Food Sci. Emerg. Technol. 64, 102435. https://doi.org/10.1016/j.ifset.2020.102435.

Shu, Y.-T., Kao, K.-T., Weng, C.-S., 2017. *In vitro* antibacterial and cytotoxic activities of plasma-modified polyethylene terephthalate nonwoven dressing with aqueous extract of Rhizome *Atractylodes macrocephala*. Mater. Sci. Eng, C. 77, 606–612. https://doi.org/10.1016/j.msec.2017.03.291.

Vaideki, K., Jayakumar, S., Nithya, E., Rajendran, R., 2011. Study on the effect of RF plasma pre-treatment on the antimicrobial efficacy of neem leaf extract processed cotton fabric. Sci. Technol. Microb. Pathog. Proc. Int. Conf. Antimicrob. Res. 2011, 259–267. https://doi.org/10.1142/9789814354868_0051.

Vaideki, K., Jayakumar, S., Rajendran, R., 2009. Investigation on the enhancement of antimicrobial activity of neem leaf extract treated cotton fabric using air and oxygen DC plasma. Plasma Chem. Plasma Process. 29 (6), 515–534. https://doi.org/10.1007/s11090-009-9188-9.

Vaideki, K., Jayakumar, S., Thilagavathi, G., Rajendran, R., 2007. A study on the antimicrobial efficacy of RF oxygen plasma and neem extract treated cotton fabrics. Appl. Surf. Sci. 253 (17), 7323–7329. https://doi.org/10.1016/j.apsusc.2007.03.014.

Vajpayee, M., Singh, M., Ledwani, L., Prakash, R., Nema, S.K., 2020. Investigation of antimicrobial activity of DBD air plasma-treated banana fabric coated with natural leaf extracts. ACS Omega. 5 (30), 19034–19049. https://doi.org/10.1021/acsomega.0c02380.

von Woedtke, T., Haertel, B., Weltmann, K.D., Lindequist, U., 2013. Plasma pharmacy–physical plasma in pharmaceutical applications. Pharmazie. 68, 492–498. https://doi.org/10.1691/ph.2013.6521.

Yeh, H.-Y., Line, J.E., Hinton, A., Gao, Y., Zhuang, H., 2019. The effect of rosemary extract and cold plasma treatments on bacterial community diversity in poultry ground meats. Heliyon. 5 (10), e02719. https://doi.org/10.1016/j.heliyon.2019.e02719.