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Review

Perspectives and Applications of Natural Products as Civilian Defense Devices

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Civilian defense devices are physical and chemical artefacts used to incapacitate an aggressor without causing death or injury. As city violence increases, safety feelings became worst and a growing commercial demand for these materials supply faces technologically challenges not only to riot control but also to self-protection. Controversies on the use of these devices have been discussed, especially on their toxicity when synthetic chemical agents are used. Natural metabolites are a less explored alternative to meet this demand. They have the capacity to generate a temporary disabling effect due to their pungency. Those metabolites, which include capsaicinoids, gingerols and piperine, can be used as deterrent agents in civilian defense devices due to their low toxicity to humans. Although they are a viable alternative, there are few studies focused on their quality control, efficiency and toxicity. This review aimed to present relevant aspects about the development and use of natural products as deterrent agents in self-defense devices against aggressors and animals, and as non-lethal weapons for riot control in order to help the scientific development of this type of bioproduct.

Keywords: disabling agents, *Capsicum*, *Piper*, *Zingiber*, self-defense devices

1. Introduction

The use of natural products as an instrument of defense or hunting date back to Paleolithic Era, where plant extracts were applied to poison water and arrows for fishing and fighting.[1] Examples of botanic genus which were used in that time include *Derris*, *Euphorbia* and *Aconitum*.[1,2] Moreover, from the 4th century BC to 16th century AD, several records of communities in China and Brazil reported the use of pepper powder and the smoke from the burn of its fruits as weapons to blind, disorient or chase away single or multiple aggressors.[1,3,4]

Those ancestral usages inspired not only the research of natural products for medicinal purposes but also the modern military applications, mainly during the period of the World Wars I and II.[1,5] Among the developed technologies regarding natural products as weapons, the application of extracts and essential oils of *Capsicum* species has become popular as a stunning agent.[6,7] The pepper spray, as it is currently commercialized, was developed in the end of the 70th, to be used against wild animals (such as dogs or bears). In 1987, a study of pepper spray made by the FBI agent Thomas Ward endorsed this device as a safe agent to control offenders. After this study, more than 3,000 police stations in the United States adopted pepper spray as a security device, spreading the practice around the world.[5,8] Years later, in 1996, Ward was arrested for receiving kickback from a pepper spray-producing company to produce the study that led the weapon to be widespread.[8]

Besides its popularity, the use of pepper spray and its bioactive component, capsaicin, were banned by the International Convention on Chemical Weapons in 1993, fearing the onset of a chemical war.[1,7] Although, the low toxicity of pepper spray contributes to its continuous use in crowd control as an effective non-lethal weapon by public and private security forces.[7,9,10] Commercialization of non-lethal weapons became a trending topic in the last decade due to the increasing in violence around the world. Government departments and the United Nations Office of Drugs and Crime (UNODC)[11-14] had reported an increase from 5 to 41% in crimes, in different countries between

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the years of 2010 and 2016, in which more than 50% of the homicides and assaults were committed by the use of guns or knives.[14] These data also indicate that there are some occasions where the victim could defense herself if she had instruments to help her. The question raised by those data is: how to provide the civilian their right of self-defense without deprive the right of life? One of the possible answers is to provide the population an effective and safe defensive device.[15-18]

A defensive device is defined as a non-lethal weapon (NLW) and consists of a dispositive capable of repelling or incapacitating aggressors (animals or humans) with a low probability of causing death, permanent injury or property damage. Its main objective is to end a conflict quickly, with safety, respecting the right of life and with the minimum use of force, acting as an alternative to firearms.[19] The main substances used worldwide in the formulation of these devices are chloroacetophenone, chlorobenzylidene malononitrile (tear gas) and dibenzoxazepine.[7,20] Most of these substances are highly toxic, carcinogenic, imperishable, present long effect time and require specific antidotes.[19,21,22] Because of its recent popularization among civilians, many countries still do not have clear laws or specific regulations about the commercialization and use of personal defensive devices. Due to those circumstances, the use of natural products in the development of those devices became crucial since they have low toxicity and can be easily manipulated by people without specific training.

In this context, this article aims to review chemical aspects about the application of natural products in NLW formulations, to generate subsidy for the development and quality control of these products, following the main criteria necessary for their commercialization.

2. Legalization of NLW Formulations Based on Natural Products

Among the natural products that can be used as NLW, only those based on capsaicin have some type of regulation to production, usage and marketing. The use of the devices that employ this substance is still controversial, mainly due to the toxicity of pure capsaicin.[23]

Nowadays, there is no universal regulation regarding the use, purchase and sale of pepper spray. The main motivating factors to the absence of a consensus on its legalization at global level includes: the toxicity to humans, the low amount of studies related to its clinical effects and reaction mechanisms in different organs, the absence of quality control of the production process and the low technological development.[19,23-25]

In the United States, the use of pepper spray as a personal defense weapon is legalized in all states. However, some of them have restrictions (Figure 1) regarding the buyer's minimum age, criminal record, use against police officers, maximum volume of the device, maximum concentration of active principle and their usage inside educational institutions. Other locations that allow its use by civilians include Russia, Romania and India.[26,27]

In the United Kingdom, Canada, China and Germany, the use of pepper spray is illegal since it is considered a

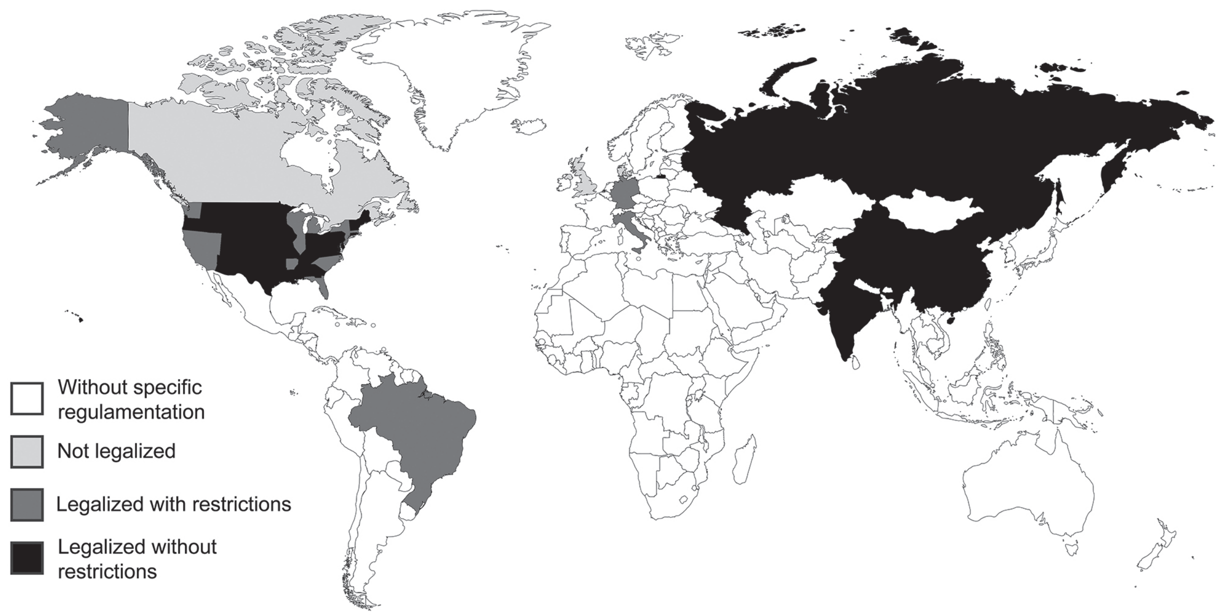


Figure 1. Legalization of the use of pepper spray in the world.

lethal and torture weapon. In these localities, only small size devices are allowed when it is proven the need of protection against wild animals. Countries such as Denmark and Brazil authorize the use of pepper sprays only by members of the armed forces, public and private security agents. In such cases, the law requires that the use of NLW have to be prioritized whenever the aggressor does not represent an immediate risk to the physical or psychic integrity of the security agent.[26]

The heterogeneity in global regulation is a consequence of the controversial view of this question, which includes the availability of weapons to civilians. The lack of consensus avoids the technological advancement in new formulations with less toxicity and short-term disabling effects. Among the strategies to support the development and legalization of NLW based on natural products it can be highlighted the dissemination of qualified information, which can demystify the safety use.

Additionally, the use of NLW is forbidden in warfare. Such prohibition was set out in the Chemical Weapons Convention (CWC), established by the Organization for the Prohibition of Chemical Weapons (OPCW) in 1997. OPCW is an autonomous international organization, which represents approximately 98% of the world's population and industry, with the mission to implement the statements of the CWC.[28]

In its 25 articles and annexes, CWC establishes conditions to promote international cooperation to strength its implementation and the peaceful use of chemistry. Thus, its main items within the context of this article can be summarized as: each State Party undertakes not to use riot control agents as a method of warfare; toxic chemicals are any substance capable of causing death, temporary incapacity or permanent damage to humans or animals, regardless of their origin or method of production; riot control agent is any chemical, not listed in the schedules of chemicals of the CWC, with the potential to rapidly cause sensory irritation or disabling physical effects in humans for a short time; non-prohibited purposes are those intended for industrial, agricultural, research, pharmaceutical, other peaceful purposes; protection against toxic chemicals or chemical weapons should be provided; and military purposes are related to the use of chemical weapons in war.[28] According to CWC, the use of domestic riot control agent is not forbidden since its composition is submitted to OPCW containing specific chemical name, structural formula and Chemical Abstracts Service (CAS) registration number. Finally, the OPCW Member States collectively share the objective of strengthening international security, avoiding the use of chemistry for war again.[28]

3. Natural Products Application in Defense Devices

Non-lethal weapons must follow a series of criteria that involves the principles of reasonableness, reversibility and their biodegradability. The principle of reasonableness is the main incentive in developing personal defensive devices. This principle discusses the restrained use of force to stop an aggressor, preserving their life and physical integrity during the immobilization process. For example, security forces should not use firearms against individuals who are on the run or those that cannot be considered a life-risk.[23,29]

To use the force necessary to contain an individual, it is also not reasonable to cause permanent physical injuries, which would categorize it as a physical punishment to the offender or even torture. In this context, there is the principle of reversibility, which says that a personal defense device should not cause permanent damage, has an easily accessible antidote and does not present extended effects.[9,30-32] Ideally, the active principle of these weapons must be biodegradable in order to not act as an environmental pollutant (when operated in open places) and to reduce the possibility of recontamination or contamination of the individuals who are immobilizing the aggressor.[6,9,32]

Incapacitating agents, such as chloroacetophenone, chlorobenzylidene malononitrile and pure synthetic capsaicin do not follow the principles of reasonableness and reversibility previously mentioned.[19,21,22] Because of their physiological characteristics, the use of these substances in personal defense weapons was discouraged and restricted to military forces. Thus, it was proposed the use of natural source substances as active principle on personal NLW formulations.[7,19] Metabolites produced by species of the genera *Capsicum*, *Piper* and *Zingiber* (Figure 2), for example, have been shown to act as efficient deterrent agents, presenting lower toxicity and being readily biodegradable.[19,21,22]

Those metabolites can be efficiently used due to their pungency, which can be defined as the ability to cause an irritation in the terminal nerves, producing pain and heat sensation.[33] This characteristic is caused by their interaction with the transient receptor potential vanilloid-1 (VR1), a neural receptor which, in summary, responses to temperature changes creating a sensation of heat or pain.[33,34] As the VR1 is a nonselective cation channel, it can be readily activated by proton sites present in different molecules, which are usually classified as vanilloids or non-vanilloids substances.[34,35]

Metabolites that contain the vanillyl moiety, amide functions, triprenyl phenols and unsaturated dialdehyde

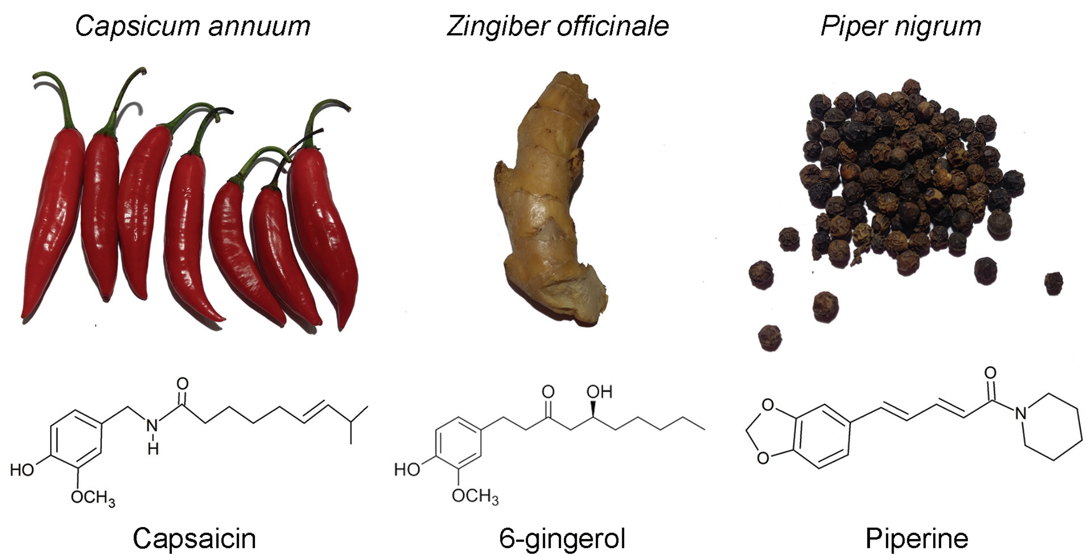


Figure 2. Major pungent metabolites of the genera *Capsicum*, *Zingiber* and *Piper*.

sesquiterpenes had demonstrated to act as agonists of the VR1, being capable to produce a certain degree of pungency.[34-36] This behavior had been correlated to molecules that present hydrophilic, dipolar and lipophilic regions across their structures. These regions provide them an amphiphilic behavior, which allows their attachment to the VR1, without suffering cleavage, enabling its down regulation or desensitization.[33,34]

The three botanical genera previously cited include the most famous pungent food additives, however, it must become clear that they are not the only options among the worldwide flora that could be used in the formulation of NLW. There are other genera and species that could provide an efficient and less toxic formulation, though the controversy of this topic avoids the technological improvement and discovery of natural product as chemical weapons.[2]

4. Main Natural Sources of Disabling Agents

4.1. The genus *Capsicum*

One of the most economically important Solanaceae genus is *Capsicum*, which is composed by approximately 30 species. Their popularity is due to their ability to generate pungency, making them one of the most widely used seasonings.[37] The economic importance of peppers is mainly related to the chemical profile of the fruits, providing flavors, odors and biological properties largely explored in the manufacture of cosmetics, nutritional supplements, food additives and as an ingredient in different meals recipes.[38,39]

The pungency of *Capsicum* is attributed to the presence of a chemical class produced exclusively by the species of this botanical genus, the capsaicinoids (CAPS).[40] This chemical class comprises a set of alkaloids with molecular structure consisting of a vanillic group, an amide and a carbon chain, which is responsible for the formation of different types of capsaicinoids (Figure 3). Their structure confers an amphiphilic character, making them capable of interacting with cell membranes and the vanilloid receptors of the nervous system.[40-42]

Approximately 25 different natural capsaicinoids have been recorded.[43] Among them, capsaicin and dihydrocapsaicin are considered the major ones, comprising from 89 to 98% of the total CAPS concentration in *Capsicum* fruits.[33] It is worth mentioning that the total CAPS concentration may vary from 0.003 to 1% of the total fruit weight, depending on the species, life cycle, environmental conditions of cultivation and geographical distribution.[33,44-47] The concentration of capsaicinoids in each species is closely linked to the process of evolution and adaptation of these to the ecosystem, since the alkaloids of this class act in the defense mechanisms of the plant against arthropods.[48-50] In this way, there is a great variability in the contents of capsaicinoids in *Capsicum* species (Table 1), including the non-pungent ones.[33,54]

The varieties belonging to the species *Capsicum chinense* are known by their high pungency. In fact, one of the varieties of this species had being classified as the most pungent pepper in the world.[55] Meanwhile, *C. annuum* species, whose capsaicinoid contents make a product with lower toxicity, are preferable to NLW formulations.[19,22,38]

The first use of *Capsicum* extracts in NLW formulation was registered in 1982, and since then the technology involving its use as an active ingredient has been practically unchanged. The popularity of capsaicinoid products is due

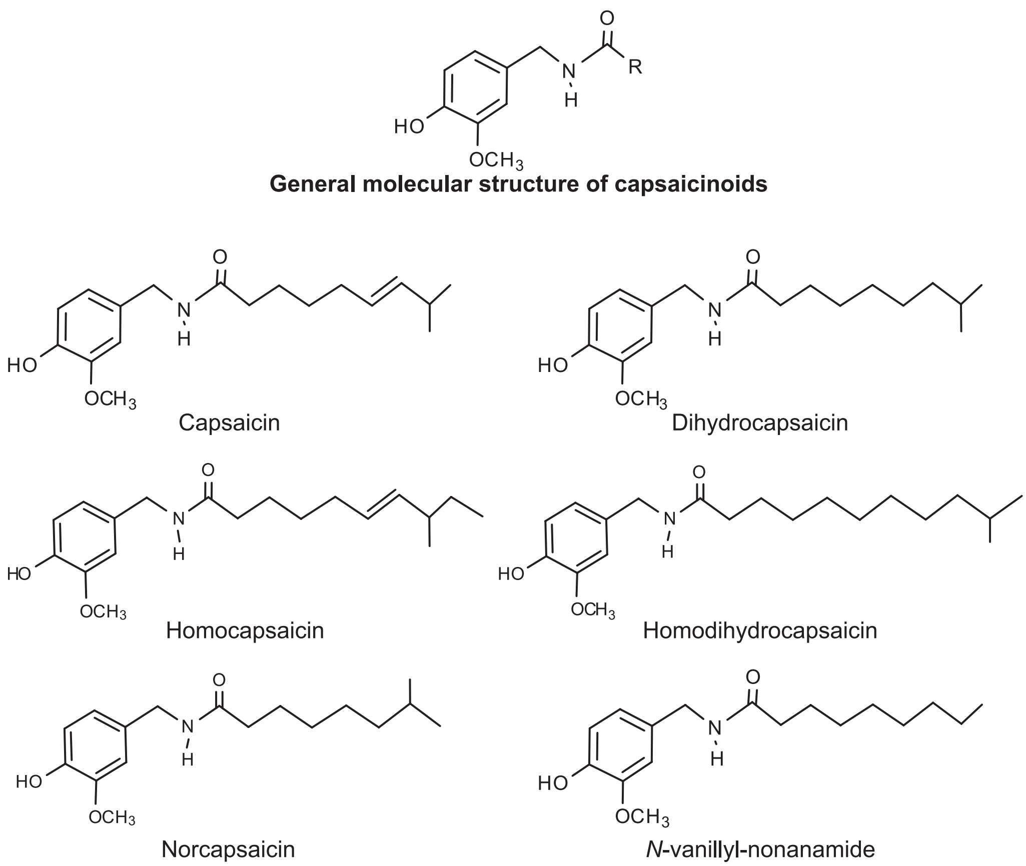


Figure 3. Molecular structure of the main capsaicinoids.

Table 1. Major capsaicinoid contents in different *Capsicum* species

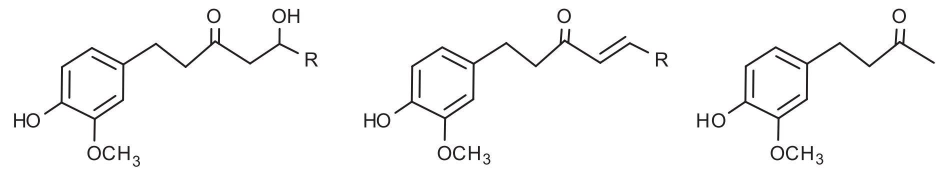
Species	Variety	Capsaicin / ($\mu g\ g^{-1}$)	Dihydrocapsaicin / ($\mu g\ g^{-1}$)	Reference
C. annuum	cayenne	211.07	114	51
C. annuum	black pepper	277	1.74	52
C. baccatum	“dedo-de-moça”	280	18	52
C. frutescens	malagueta	984	104	52
C. chinense	habanero	38,871	14,132	53
C. chinense	“murupi”	1,753	237	52

to their efficacy against individuals under the influence of depressants and central nervous system stimulants.[56]

Among the active compounds most used in defensive devices, *Capsicum* oleoresin (OC) is the only one of natural origin.[22] Current formulations of capsaicinoid as NLW generally use *Capsicum* oleoresin (food grade),[22,30] capsaicin extracted from OC[56] or synthetically produced.[57]

4.2. The genus *Zingiber*

The genus *Zingiber* (Zingiberaceae) comprises approximately 85 species of tropical occurrence, among which the *Zingiber officinale* (popular name: ginger) is widely known due to its applications in food and pharmaceutical industries, in the form of essential oil, oleoresin or extract.[58-60] The rhizome of ginger corresponds to the portion usually used in the preparation of these natural products, presenting from 1 to 7.5% of its weight in volatile compounds and pungent substances.[61] The pungent compounds present in ginger are the gingerols and correspond to approximately 33% of the weight of the *Zingiber* oleoresin. The major pungent compound present in these rhizomes is the 6-gingerol, which is considered an economically important substance, not only because of its pungency but also because it was reported to present anticarcinogenic and antitumor activities, among



R	Substance	Substance
$(CH_2)_4CH_3$	6-gingerol	6-shogaol
$(CH_2)_6CH_3$	8-gingerol	8-shogaol
$(CH_2)_8CH_3$	10-gingerol	10-shogaol

Gingerone

Figure 4. Molecular structure of gingerols and shogaols.

others.[59,62,63] It must be highlighted that these compounds are extremely heat-sensitive and can be readily converted into shogaols, their dehydrates, or gingerone (Figure 4).[60,63]

Ginger has an economic importance like the *Capsicum* fruits, being cultivated in several countries, among which Brazil is considered the third largest exporter of the dried rhizome. It is estimated that the annual worldwide production of each by-product derived from ginger (essential oil, oleoresin, fresh or dried rhizome) varies between 100 and 200 tons.[60]

Worldwide cultivation of this botanical species has led to the development of varieties that better adapt to each ecosystem, generating the formation of an intraspecific variability within the species of *Zingiber* similar to those from *Capsicum*, where within the same species can be found cultivars with different chemical profiles.[61,64,65]

The maturation stage is also one of the factors capable of changing the contents of the pungent compounds in ginger. The levels of 6-gingerol decrease as the specimen reaches maturity, with concentrations varying between 0.95 and 30.0 g *per* 100 g of fruit.[61,66] These levels are lower than those found in capsaicin in *Capsicum annuum* cayenne (main variety used in NLW manufacturing) (Table 1), evidencing that in economic terms, it would still be necessary to evaluate varieties with high levels of the major pungent compound.

4.3. The *Piper* genus

The genus *Piper* belongs to the Piperaceae family and consists of approximately 700 species, of which 170 are Brazilian. Like *Capsicum* and *Zingiber*, species of this genus are also popularly used by the pharmaceutical and food industries.[67-70] Species such as *Piper gaudichaudianum* and *Piper aduncum* are known by their antitumoral, antimicrobial, antifungal, insecticidal and anti-inflammatory activities.[71]

The main metabolite responsible for *Piper* species pungency is piperine (Figure 5).[35,72] This substance is the

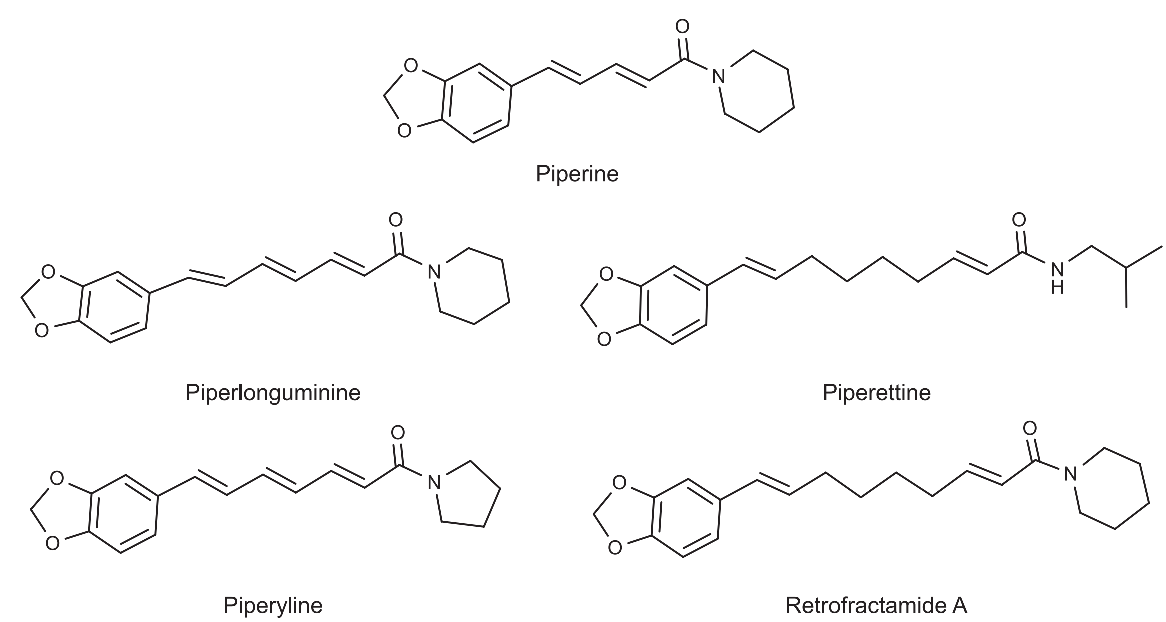


Figure 5. Molecular structure of pungent compounds in the genus *Piper*.

main alkaloid found in *Piper nigrum*, which is considered the species capable of producing highest levels of piperine, with concentrations ranging from 2 to 10% of the fruit weight.[21,73,74] Due to the high concentrations of piperine in *Piper nigrum*, this species is also considered the most economically important within the genus.[73,75-78] Piperine can be found in both oleoresin and essential oil of *Piper* species and can be used as a medicinal additive because it has antioxidant, antimicrobial, antifungal, hepatoprotective and antimalarial activities.[75,77] In addition to piperine, other less pungent alkaloids such as piperlonguminine, piperyline and piperettine can be found in *Piper* genus.[36,79]

Regarding the pungency, *Piper nigrum* has a small concentration of its major pungent compound when compared to *Zingiber* and *Capsicum*. It can be faced as an advantage, in a toxicological perspective. Besides, it can also difficult the production of an oleoresin with the indicated pungency to be effective. Among the species of interest for the manufacture of defensive devices can be highlighted *Piper nigrum*, *Piper longum*, *Piper officinarum*, *Piper methysticum*, *Piper parthenium* and *Piper angustifolium.*[21,80]

5. Methods of Extraction of Oleoresins

NLW based on natural products are usually manufactured with food-grade oleoresins whose use are authorized for human ingestion because they do not present lethality in individuals who do not have allergy to the active principle.[19,56] The oleoresins are defined as organic resinous oil extracts which chemically differ from essential oils because they are constituted mainly by non-volatile compounds. This natural product is used in the food industry as a seasoning, imparting flavors and aromas to beverages and meats.[81,82]

The extraction of the oleoresin can be achieved by extracting the whole fruit, specific portions of the fruit (such as seeds and pericarp) or roots. It depends on the botanical species and the portion of the specimen with the highest accumulation of the substance of interest.[83-85] The most commonly used extraction techniques include organic solvent maceration, Soxhlet extraction, microwave assisted extraction (MAE), ultrasonic assisted extraction (UAE) and supercritical fluid extraction.[24,82,86] Among these, the most used by the food industry is organic solvent maceration due to its low costs related to instrumentation and operation.[24,87] More expensive and accurate methods, such as supercritical fluid extraction, are usually performed during scientific research. To produce analytical reliable data, high standard levels of reproducibility, recovery, precision and accuracy are necessary. It allows the fruit extract to have a complete chemical and pharmacological characterization to evaluate the quality control of the processes.[40,88,89]

The exhaustive extraction is the older technique, with optimization studies that refer to its use registered in the 1920's.[90] In general, this method is performed through one or more cycles of mechanical agitation of a mixture containing the plant material and the extractive solvent, for periods of time ranging from 12 to 72 h. After percolation, the mixture is filtered and the extractive solvent is removed by the use of centrifugation or distillation techniques to obtain the oleoresin.[86,91] This method, although simple, may be influenced by different factors such as the type of solvent extraction, solvent removal method, extraction temperature and the pre-processing of the fruit.[86,90]

During exhaustive extraction, and in the other methods that are going to be discussed, each pungent mixture is obtained in different optimal conditions, that include not only the chosen method but also the inherent characteristics of the extraction solvent and its compatibility with the sample (Table 2).[92-94] The efficacy of a specific solvent to extract a specific pungent compound is mainly related to their viscosity and polarity.[93] In general terms, a lower viscosity can enhance the extraction yield since it provides a better diffusion of the substances in the matrix-solvent interface.[93,94,96,100] The main pungent compounds applied in NLW (capsaicinoids, piperinoids and gingerols) present a structure with polar and non-polar regions, which allow them to have a better interaction with solvents that have a medium-to-low polarity index (Table 2).

It also must be clear that in exhaustive extraction the viscosity of the solvent does not play a crucial role in the solvent determination as the extraction mechanism is mainly related to diffusion process. Therefore, the usage of acetone or ethanol usually provides statistically similar results (Table 2).

The extraction of pungent compounds by exhaustive maceration can also be enhanced using mild temperature gradients[96,100] and longer cycles of extractions. For example, in the extraction of piperine an increase of 5.32% on yield was obtained when applying an extraction time of 3 days,[97] instead of only 8 h.[96] There are some questions that must be carefully evaluated to establish if an increase in process time is beneficial, such as the species content of pungent compounds and the time required to saturate the extractor solvent.[96-98]

The Soxhlet extraction method has as main advantage the reduced extraction time when compared to exhaustive maceration to obtain a similar or better result, although it is more favorable to the degradation of the pungent compounds due to the use of milder heating. While the extraction time by exhaustive maceration varies between

Table 2. Extraction yields of pungent compounds by exhaustive maceration

Sample	Extracting solvent	Yield of oleoresin / %	Yield of the pungent compound in the oleoresin[a] / %	T / °C	Reference
Pericarp of commercial *C. annuum* var. *accuminatum* dried fruit	ethanol	17.5	0.52	25	90
	acetone	15.6	0.52	25	90
	chloroform	16.4	0.58	25	90
	ethyl ether	16.1	0.70	25	90
	hexane	15.0	0.60	25	90
Dried *C. annuum* fruit	acetone	12.6	1.24	37	95
Dried *C. annuum* fruit	ethyl acetate:hexane (60:40 v/v)	12.7	1.54	37	95
Dried *Piper longum* fruit	acetone	n.i	0.101	40	96
Dried *Piper longum* fruit	ethanol	n.i	0.098	40	96
Dried *Piper longum* fruit	hexane	n.i	0.084	40	96
Dried *Piper nigrum* fruit	ethanol	10.30	5.36	25	97
Dried *Piper nigrum* fruit	ethanol	n.i	2.7	25	98
Dried ginger rhizome	ethanol	n.i	3.81	25	99
Dried ginger rhizome	isopropanol	n.i	1.29	25	99

[a]Capsaicin for *Capscium* fruits, piperine for *Piper* fruits and 6-gingerol for *Zingiber* rhizome. n.i: not informed; T: extraction temperature.

3 and 15 days, Soxhlet extraction lasts from 2 to 12 h, representing a significant increase in the production efficacy.[78,87,101]

The solvent applied during Soxhlet extraction must also be carefully planned, since the major pungent compounds are thermosensitive, and the boiling point of the solvent will determinate the temperature that the sample will be exposed (Table 3). The use of Soxhlet provides lower yield of 6-gingerol when comparing to the results obtained by exhaustive maceration (Tables 2 and 3) due to its thermal degradation. When the pungent compound is less sensible, it is possible to obtain higher concentrations, which is the case of piperine and capsaicin (Tables 2 and 3). This behavior can be attributed to an increase in the solubility of piperine and capsaicin when a higher temperature is applied and by the continuous cycles of extraction performed during the Soxhlet method, which act as new extractions of the same sample, avoiding the saturation of the solvent and enhancing the diffusion of the analytes.[69,93,94,100]

The effect of the solvent boiling point in the efficacy of the extraction of piperine by Soxhlet extraction was demonstrated comparing the use of hexane, ethyl acetate and ethanol.[69] It was observed that ethyl acetate produced an yield in the extraction of piperine approximately 10% greater than the obtained by ethanol and hexane. Ethanol can be considered an efficient solvent to extract piperine by percolation (Tables 2 and 3), though its slightly higher boiling point could cause a certain degree of thermal degradation.

One way to increase the yield of capsaicinoids and gingerols in the Soxhlet and maceration methods is

Table 3. Extraction yields of pungent compounds by Soxhlet extraction

Sample	Extracting solvent	Yield of the pungent compound in the extract[a] / %	Temperature of extraction / °C	Reference
Fresh rhizome of *Zingiber officinale*	methanol	4.2	65	102
Dried rhizome of *Zingiber officinale*	ethanol	0.97	85	103
Fresh *Piper nigrum* fruit	ethanol	3.91	70	74
Dried *Piper nigrum* fruit	methanol	3.8	70-80	104
Dried *C. chinense* fruit	ethanol	2.2	90	100
Dried *C. chinense* fruit	acetone	2.4	65	105

[a]Capsaicin for *Capscium* fruits, piperine for *Piper* fruits and 6-gingerol for *Zingiber* rhizome.

through the use of an enzymatic pretreatment.[86,106,107] Baby and Ranganathan[40] reported that the use of Viscozyme L enzyme provided an increase of up to 22% in the extraction of capsaicinoids using hexane:acetone (1:1 v/v) as the extractive solvent during the maceration technique.[40] In the case of 6-gingerol, the use of Accelerase® could increase the extraction efficiency by 30 to 70% when compared to conventional Soxhlet methods and maceration using methanol as the extracting solvent.[102]

The UAE and MAE techniques are preferable in laboratory scale because they require a lower extraction time and solvent, which makes them more ecologically viable (Table 4).[60,86,109] Besides these advantages, both methods are not widespread on an industrial scale, because of the high instrumental cost necessary to use them in large scale when comparing to exhaustive extraction or even Soxhlet. A reduction of 83% in the extraction time was achieved when the UAE technique was used instead of the Soxhlet method.[110] Also, the use of MAE is even faster, reducing the time by up to 99.8% (Table 4).

In general, the most used solvents in the extraction of oleoresins of *Capsicum*, *Zingiber* and *Piper* are ethyl acetate, acetone, ethanol, methanol, hexane, propanol, trichloroethane and dichloroethane, though in the manufacture of food grade products it is recommended the selection of the least toxic solvent, which usually is ethanol.[60,86,109]

An alternative method that has gained space in the food industry is the supercritical fluid extraction (SFE). Its main advantage is the decrease of solvent residues in the product, since the fluid used is promptly removed by the pressure change of the system.[86,101] This extraction is usually developed with carbon dioxide, which is economically profitable, costing US$ 125.41 kg^{-1} of oleoresin using: two extractor units of 0.5 m^3; supercritical dioxide fluid carbon; working pressure of 15 MPa; extraction temperature of 40 °C; and extraction time of 240 min.[111] Under these conditions (large scale), the extraction yield of capsaicinoids is 0.4%, substantially lower than the obtained on a laboratory scale by the use of the same technique (1.9%).[101]

Depending on the pressure conditions, temperature and supercritical solvent used (acetone or carbon dioxide, for example), the yield of gingerol extracted may increase by approximately 50%, when compared to the production of oleoresin by maceration or Soxhlet at room temperature.[58,102,112] The quality of oleoresin as a function of the 6-gingerol content using SFE was higher,[112] producing an extract in which 22.30% of the total weight was 6-gingerol, whereas using Soxhlet and maceration it was 4.59 and 6.26%, respectively.[99]

Piper oleoresin is more difficult to obtain by supercritical fluid extraction than *Capsicum* and *Zingiber*. Piperine oleoresin extraction demands high pressures and working temperatures to make it soluble in carbon dioxide, which increase the operating costs of the method.[76,101,113] Piperine extraction by supercritical fluid using carbon dioxide can be achieved using working pressure of 250 bar, at 45 °C during 120 min.[113] Thus, it is possible to double the extraction efficiency when comparing to conventional methods, obtaining 48% of the total weight of oleoresin in piperine. In addition, similar yields (38.73%) were obtained by reducing extraction time to 60 min and raising the temperature to 50 °C.[76] The change from laboratory to industrial scale also affects the performance of the other types of oleoresin. In the case of 6-gingerol extraction, the scaling up of supercritical fluid extraction using 15 MPa, 35 °C and 15 g min^{-1} CO_2 flow has a reduction of 13% in

Table 4. Comparison of extraction techniques in Soxhlet, ultrasound assisted extraction and microwave assisted extraction

Pungent compound[a]	Method	Extractor solvent	Volume of the extractor solvent / mL	Yield of the pungent compound / %	Extraction time / min	Reference
Capsaicin	UAE	acetone	10	2.4	60	105
	MAE	acetone	10	2.4	60	105
	Soxhlet	acetone	100	2.4	360	105
6-Gingerol	UAE	$[C_4mim]BF_4$	20	1.09	10	103
	MAE	ethanol	26	1.5	0.51	108
	Soxhlet	ethanol	15	0.97	480	103
Piperine	UAE	ethanol	10	3.7	30	74
	MAE	ethanol	10	3.8	1	74
	Soxhlet	ethanol	300	3.9	720	74

[a]Capsaicin for *Capscium* fruits, piperine for *Piper* fruits and 6-gingerol for *Zingiber* rhizome. UAE: ultrasound assisted extraction; MAE: microwave assisted extraction.

yield compared to laboratory scale.[112] Thus, its industrial application still requires more studies on optimization and scale up to become economically viable.

It must be highlighted that, in the concern of method development of oleoresin extraction for use in NLWs, only a restricted amount of studies is available, and most of the existent information is patent protected.[19,114] The scientific investigation designed to optimize the extraction method of pungent substances should be design aiming the production of NLW, with a chemical profile that could provide an effective and safe final formulation.[81,115,116]

Considering that natural defensive devices still have a lower commercial demand than products designated to culinary, it is recommended that the extraction method should have a low cost and a high recovery. In this context, the exhaustive maceration method can provide better results in industrial scale.[112] Regarding the safety of the formulation, it is also recommended to use low toxicity solvents, such as ethanol. Technological development must be addressed to develop a method that can combine the low cost of maceration method with the safety of the supercritical fluid extraction technique, which generates a product with the lowest concentration of solvent residues.

An issue always present in studies focused on the production and evaluation of food grade oleoresin is the lack of representativeness in the samples.[66,74,90,96,97,105] The sampling is subjected to variances in the manufacturing conditions regarding different producers. The use of single or composed raw material reduces the significance of the result on pungency of commercial products because the fruit origin and the presence of a single specie cannot be guaranteed.

An area that also requires greater development regarding the commercialization of defensive devices based on natural products is the method designed to accomplish the quality control of the NLW.[32] As previously discussed, *Capsicum*, *Zingiber* and *Piper* genera have a great intraspecific variability, which can cause a variation in the chemical profile of oleoresin produced by the same manufacture. The variability in the composition of the oleoresin may cause unpredictable effects in the toxicity of the NLW. Thus, extraction methods and chemical profiles must be standardized.

6. Formulations and Toxicity of Defensive Devices

The formulation of natural non-lethal devices is composed by a propellant, a dilution solvent and a deterrent substance. The efficacy and safety of these devices will depend on the characteristics of these three components, although there is no specific regimentation regarding the concentration of propellant and dilution solvent that should be applied.[22,38,56,117]

The propellants and diluents used must have low flammability, toxicity and vapor pressure. These features ensure product safety, avoid cross-contamination and injuries during use.[30] The first propellants used were chlorofluorocarbons, which the environmental risks and toxicity made them being quickly replaced by alternatives such as ethanol, carbon dioxide, nitrogen oxide and mixtures of propylene glycol dicaprylate/dicaprate and tris-glycerol-2-(ethyl hexanoate).[19,30] Despite the flammability of ethanol, this is the main propellant and diluent used due to the pungent compounds low solubility in water.[19,30,114,118]

Regarding the deterrent substance, it is important to emphasize that only law enforcement agencies, police and military forces can use devices produced with high purity capsaicin or pungent substances produced synthetically.[1,23,119] For other purposes, as the use against civilians and wild animals, the formulation of NLWs is based on the usage of oleoresin from natural products (Table 5).

As previously discussed, the manufacture of oleoresin is mainly accomplished using organic solvents, which should be eliminated to enable the safety of the oleoresin for mammalians. The presence of organic solvents residues in the oleoresin used in NLWs must follow the standard regulated by the Food and Agriculture Organization of the United Nations (FAO),[109] since the formulation will have

Table 5. Concentrations of natural pungent compound in non-lethal weapons

Pungent compound	Concentration in NLWs / %	Application	Reference	50% of lethal dose (mice) / (mg kg^{-1})	Reference
Capsaicin powder	0.1-1.0	military	56, 120	161	121
Capsicum oleoresin	12-60	civilians and military	7, 56		
Zingiber oleoresin	5-30	civilians	19, 114	155	122
Piper oleoresin	0.1-10	civilians	21, 80, 123	43	124
	< 30	animal repellent			

NLW: non-lethal weapon.

direct contact with the victim's eyes, respiratory system and occasionally with the mouth.[31,125] It is estimated that part of the high severity lesions caused by NLWs are due to residual solvents present together in the formulation.[29,126] FAO regulation states that solvent residues should not exceed 30 mg kg^{-1} of dichloromethane and/or acetone; 50 mg kg^{-1} of 2-propanol, ethanol and/or methanol.

The concentration of the pungent compound in NLW should be enough to have a reproducible clinical effect. In the case of capsaicin, concentrations lower than 0.5% of the net weight are not recommended, because they often do not cause the incapacitating effect. Contents of capsaicin between 0.90 and 1.00% generate homogeneous clinical effects and present 90% efficiency in reducing the aggressiveness of an offender. Contents between 1.45 and 1.60% do not present increase in toxicity in relation to the prior concentration level, however, its efficiency in aggressiveness reduction is 100%. In other words, any human exposed to this concentration will be temporarily incapacitated. In addition, concentrations of more than 1.60% of capsaicin are not recommended, since they cause symptoms that last for at least 1 h.[22,30] It is noteworthy that up until our knowledge extends there are no studies focused on the effectiveness and reproducibility of clinical effects related to the usage of ginger and black pepper oleoresin in NLWs.

Gingerols, piperine and capsaicinoids act in the body through their interactions with the vanilloid neuroreceptors, which are responsible for the sensation of pain and burning.[34,127] Regarding 6-gingerol and piperine, there are few records of its clinical effects when used as defense aerosols, with only nausea, temporary blindness and small respiratory disorders being reported.[19,35,72,118,127]

The lower toxicity of 6-gingerol and piperine in comparison to capsaicin is due to the lower interaction of these active principles with the neuroreceptors, remaining bound to the cell membranes of these for less time than capsaicin.[35,128] Because of this, Fedida[21] proposed a formulation that uses capsaicin as synergist agent to increase the effectiveness of a piperine-based device, which would cause blindness only for a period of 30 min.

Other formulations in the use of *Capsicum* oleoresin defense devices suggest their association with other agonist and antagonistic agents. In the patent of Bahary,[7] for example, a formulation with military antiterrorist application was proposed, where the OC was used together with *O*-aminoacetophenone and α-chloroacetophenone.[7] Moreover, Blumberg and Pearce patent[22] proposes to control the incapacitating action time by the use of antagonists of the active principle with the formulation. In the proposed formulation,[22] a number of synthetic antagonists (including BCTC, IodoRTX, JYL-827, AMG9810 and capsazepine) were used together with capsaicin to produce an NLW that lost 80% of its disabling effects in a 20 min period, considering that the concentration of the antagonist varies from 0.1 to 10%, and that of the active principle ranges from 0.5 to 1.6%.

The application of agonist and antagonistic agents in NLW formulations is promising regarding the effectiveness and safety of these devices, however, there are few studies addressing such modifications in the formula and any of the existent data had tested its toxicity clinically. Such advances in formulations could facilitate legalization and commercialization of those devices to civilians.

Among the symptoms experienced by individuals exposed to defensive sprays based on *Capsicum* oleoresin are the sensation of burning and erythema in the skin and eyes, dermatitis, lacrimation, blepharospasm, conjunctival injection, conjunctival proliferation, abrasion/erosion/corneal ulcer, cough, coryza, apnea, shortness of breath, inflammation of the throat.[9,38,129] When the pepper spray (*Capsicum*) is properly used, the clinical symptoms are extinguished between 30 and 60 min.[38,81]

Exposure of the eyes to capsaicinoid devices produces the longest lasting clinical effects. Exposure to 0.1 mL of 5% of *Capsicum* oleoresin can generate erythema with an average duration of 72 h,[81] and a reduction in corneal sensitivity for 1 week.[125] In addition, severe corneal abrasion symptoms reported in cases of intoxication may last from 3 h to 5 days, which may be aggravated by the use of propellants and toxic solvents in the formulation (such as trichloroethylene and dichloromethane residues).[6,126,130] Changes in the respiratory functions caused by the solvents used in the preparation of OC-based device are the same as those caused by the formulation of the device with the OC, emphasizing the need for technological development of the propellants and diluents used in the formulations.[22,116,126]

Despite the low lethality, there are cases of death associated with the use of NLW based on *Capsicum* oleoresin. Ideally, the spray should be used only one time, with its jet being pointed towards the face of the aggressor. A citizen who came into conflict with an out-of-service police officer with a pepper spray died even after receiving medical care 18 min after the spray exposure began. During the conflict, the policeman used the device 12 times against the face of the victim, who had a history of asthma, and had the cause of death determined as suffocation due to bronchospasm precipitated by the *Capsicum* oleoresin.[31] Among the risk factors recorded as aggravating factors for OC toxicity are: history of asthma, coronary diseases, obesity and use of narcotics.[29,31,38,131]

Given the different toxicity, control of the clinical effects that the NLW will cause and the profitability of the raw material used in the formulation, it becomes evident that, the choice of the species, stage of maturation and manner of cultivation of the fruits are fundamental to guarantee the quality control of the marketed product. However, these steps are generally neglected when using food grade oleoresins, which do not require the same degree of care with the concentration range obtained from the pungent compound.

The quality control of NLW formulation misses to truly ensure the safety of the device since there are few data available regarding the toxic effects of different types and concentration of propellants;[126] clinical effect of different oleoresin considering their composition of major and minor pungent and non-pungent substances; the absence of validated method for the quality control of natural NLWs; and evaluation of clinical effects caused after prolonged expositions of individuals with historic of chronic diseases. Additionally, most of researches regarding clinical effects of natural NLWs are related to pepper spray and do not present a consistent evaluation of the data.[29]

7. Conclusions

Commercialization of non-lethal defensive devices in regions with increasing violence, such as Latin America, corresponds to an area that needs advances to guarantee the safety of both user and aggressor. The preservation of physical integrity must always be in first place during the development of this type of device.

The currently employed NLW formulations use pungent metabolites from natural bioproducts as active principle. Despite the low lethality of these type of natural products, standardized studies are still lacking to ensure product reliability, efficiency and toxicity.

Although most studies are focused in *Capsicum* based NLWs, there are several gaps in scientific knowledge that must be addressed to ensure their safety to civilians. The usage of food grade oleoresin creates a false sensation of safety where the quality control of the defensive device is only assessed by their pungency, ignoring the effects of other chemical constituents present in the oleoresin and on the final formulation. Since the spray will enter in contact with the eyes and respiratory system, the toxicity evaluation of the composition should be more careful than the food grade products. The chemical composition of oleoresin requires standardization to allow the quality control of this type of device. The variability of the pungent compounds in the cultivars/varieties present in the pungent botanic species covered in this review should be considered in the development of new formulations.

To improve quality control of those devices it also must be developed a validated method based on the use of standardized oleoresin and specific possible propellant and diluents. Considering the usage by civilians, the safety of storage, thermal stability and expiration date are technical points that still require further evaluation and are not reported in the literature so far.

Although the use of *Piper* and *Zingiber* oleoresins is legalized, the standardization and clinical studies of these formulations should be provided, aiming at the consumer rights and International Amnesty applications on the use of these devices as an instrument of torture. The species of both genera can be reliable sources of raw material for NLW with lower toxicity than OC, after a careful clinical evaluation of proposed formulations.

Although natural products present a reliable alternative to personal defensive devices, their proper usage still requires several technological development and clinical evaluation, which would also assist in their regulation worldwide.

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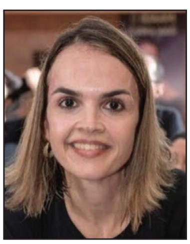

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