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Review

Plants of the genus *Allium* as antibacterial agents: From tradition to pharmacy

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Abstract: Plants belonging to the genus *Allium* are widely cultivated and used all over the world as food and medicinal plants. Since ancient times, these plants, particularly garlic (*Allium sativum* L.) and onion (*Allium cepa* L.), have represented important components of typical recipes and traditional healing systems. Not the least of which, their use as food biopreservatives is well documented, due to the relevant antibacterial activity of their extracts and essential oils. In addition to garlic and onion, this review article deals with the main members of the genus *Allium*, including *A. ampeloprasum* (Leek), *A. schoenoprasum* (Chive) and *A. ascalonicum* (Shallot), focusing both on their ethnonutritional uses and potential as promising food biopreservative agents. Noteworthy, recent research has demonstrated *Allium* derivatives to be novel components in active edible coatings as well as nanoformulates.

Key words: Alliaceae, *Allium sativum* L., ethnobotany, ethnonutrition**,** antimicrobial activity**,** food biopreservatives.

Introduction

The genus *Allium* belongs to the monocot family Alliaceae, formerly considered part of Liliaceae and Amaryllidaceae. It is a large and heterogeneous taxon including approximately 850 species that grow wild throughout the temperate, semi-arid and arid regions of the northern hemisphere. These plants are hardy perennials with underground storage organs consisting of true bulbs, or less-developed vestigial bulbs attached to rhizomes, the latter known as rhizomatous alliums. The main centre of evolution of the genus extends along the Irano-Turanian biogeographical region. Secondary centres of diversity are found in the Mediterranean basin and western North America (The California Floristic Province). From these centres, *Allium* plants have widely spread all over the northern hemisphere (1).

Many plants of this genus are of high economic significance as vegetables [*A. cepa* (onion, shallot and scallion); *A. sativum* (garlic); *A. ampeloprasum* (leek); *A. schoenoprasum* (chive); *A ascalonicum* (shallot)], ornamentals and medicinal plants (particularly garlic and onion). For instance, in case of garlic, Asian countries are the most important producers of this species (Figure 1A), with China at the top of the ranking (Figure 1B). In addition, garlic is a significant source of micronutrients (minerals and vitamins) (Table 1) and its pharmacological activities are currently investigated in a number of clinical trials (Table 2).

This review focuses on plants belonging to genus *Allium*, with emphasis on their antimicrobial properties, active constituents, ethnopharmacological uses and horticultural traits.

Cultivation of *Allium* spp. plants

As previously introduced, the genus *Allium* is originated from south west Asia in dry, arid and moderate humid climatic regions. Therefore, its main centre of diversity extends from Mediterranean basin to central Asia and Pakistan. Second centre of diversity, comparatively less pronounced, occurs in western North America (2). Improper management and intense gathering caused severe decline of wild sources. Findings from ancient Egyptian burials, suggest that the history of onion cultivation may date back to at least 3200-2800 BC. No data are available on evolutionary history of cultivated *Allium* (3). Now, *Allium* species are cultivated as annuals and appropriate sowing season is from July to November. Seed germination temperature is around 13

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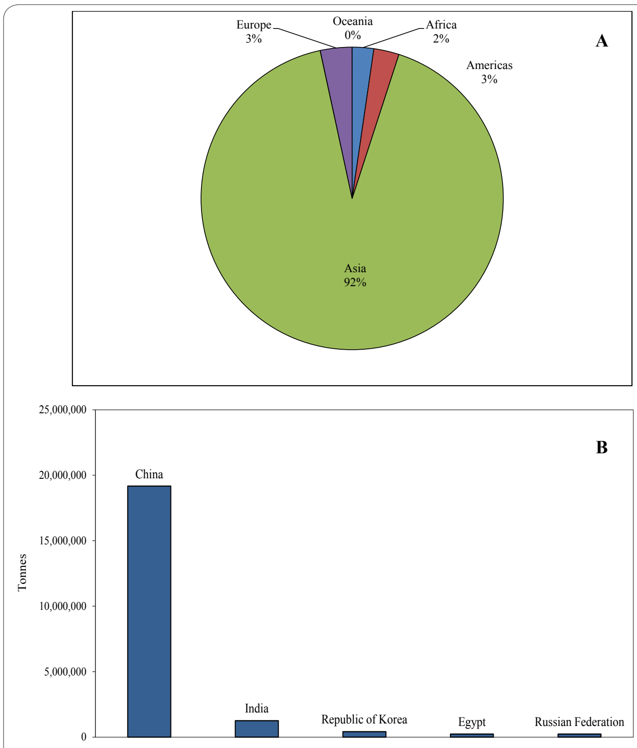

Figure 1. Garlic (*Allium sativum* L.) production in the world: A) ranking of garlic production by continents in 2013; B) top 5 garlic producer countries in 2013 (http://faostat.fao.org).

°C and the optimum leaf growth occurs at 20-25 °C (4). The seed germination conditions vary within the taxa. Flowering initiates after juvenile phase, *i.e.*, with 4-14 leaves. The optimum temperature for flowering ranges from 5 °C to 13 °C for 90 to 120 days (5). Flowering is reduced or even suppressed with increase (from 15.5 °C to 30 °C) or decrease (from -3 °C to 0 °C) in temperatures. The emergence of inflorescence is faster at high temperatures (20-30 °C) and long days (14-16 hours). Suitable pH for *Allium* is 6.0-6.8 (6). The components of crop yield such as number of seed stalks per plant, flowers per umbel, umbel diameter and seed yield depend on climatic and agronomic factors such as time of planting, irrigation, fertilization, spacing, plant protection and other measures (7). Luxuriant *Allium* growth is observed in muck soil (with at least 20% organic matter). Season, type of soil, method of seed production, genotype and locality directly affect average seed yield of *Allium* species (8).

Plant spacing, an important factor in determining seed yield of onion, varies from place to place as well as from variety to variety. The more the number of tillers, the more would be the leaves and vice versa. Storage of

Table 1. Nutritional composition of garlic (*Allium sativum* L.), raw*.

Constituents	Value per 100 g
Water	58.58 g
Energy	149 kcal
Fibre	2.10 g
Nutrients	
Protein	6.36 g
Total lipids	0.50 g
Carbohydrate	33.06 g
Sugars (total)	1.00 g
Minerals	
Calcium (Ca)	181 mg
Iron (Fe)	1.70 mg
Magnesium (Mg)	25 mg
Phosphorous (P)	153 mg
Potassium (K)	401 mg
Sodium (Na)	17 mg
Zinc (Zn)	1.16 mg
Vitamins	
Vitamin C (ascorbic acid)	31.2 mg
Vitamin B1 (thiamin)	0.20 mg
Vitamin B2 (riboflavin)	0.11 mg
Vitamin B3 (niacin)	0.70 mg
Vitamin B6 (piridoxine)	1.23 mg
Folate	3.00 µg
Vitamin E (α-tocopherol)	0.08 mg
Vitamin K (phylloquinone)	1.70 µg
Lipids	
Fatty acids (total saturated)	0.089 g
Fatty acids (total monounsaturated)	0.011 g
Fatty acids (total polyunsaturated)	0.249 g

*Source: U.S. Department of Agriculture National Nutrient Database for Standard Reference (http://nal.usda.gov, retrieved on May 7th, 2016).

Table 2. Not yet recruiting and recruiting clinical trials on garlic (*Allium sativum* L.)*.

Condition(s)	Intervention(s)	Identifier number	Status§
Official title			
Topical Garlic Concentrate for Alopecia Areata in Children			
Alopecia areata	Garlic concentrate	NCT02691117	R
Garlic in Patients with Febrile Neutropenia			
Chemotherapy, neutropenia	Garlic capsule, placebo	NCT00247039	R
Supplementation with Aged Garlic Extract to Improve Endothelial Function in Patients with Metabolic Syndrome			
Metabolic syndrome	Aged garlic extract, placebo	NCT01168700	R
Dietary Intervention in Follicular Lymphoma			
Follicular lymphoma	Garlic and other dietary supplements	NCT00455416	R
Effects of Mixed Spices on Cardiometabolic Function - The PolySPice (PSP) Study			
Cardiometabolic risk	Dishes with different spices (including garlic)	NCT02599272	R
Prehypertension and Dietary Supplements - The PYRAMIDS Study			
Prehypertension	Garlic and other dietary supplements	NCT01682291	NR
Qing'E Formula Therapy on Menopausal Symptoms			
Menopause	Qing'E pills (containing garlic), placebo	NCT01805765	R
Pharmacokinetics Study of Qing'E Pill			
Menopause	Qing'E pills (containing garlic)	NCT01931436	R

bulbs in the range of temperatures from 5 °C to 15 °C stimulates subsequent inflorescence development, while higher temperatures (16 °C or above) reduce it. Significant losses in *Allium* production is observed by bacterial soft rots (*Proteobacterium carotovorum*), basal rot (*Botrytis allii* and *Fusarium roseum*), black mould (*Aspergillus niger*), blue mould (*Penicillium* spp.), botrytis leaf spot (*Botrytis* spp.), downy mildew (*Peronospora destructor*), botrytis bulb rot (*Botrytis* spp.), pink root (*Phoma terrestris*), purple blotch (*Alternaria porri*), leaf blight (*Stemphylium vesicarum*), sour skin (*Burkholderia cepacia*), white rot (*Sclerotinia cepivorum*) and rust (*Puccinia allii*).

Domestication has no effect on the ploidy status of onion, shallot, garlic and many other diploid species. The same is true for the cultivated taxa of *A. ampeloprasum*, which apparently arose from ancestors with different ploidy levels. However, diploids, triploids and tetraploids cultivars of *A. ramosum* and *A. chinense* are also in cultivation (9). Because diploid and tetraploid wild strains exist, therefore, polytopic (at different places and at several times) domestication of *A. ramosum* seems feasible. The history of domestication of *A. chinense* is still disputed. Domestication of wild species of *Allium* is in continuation. Recently, in North Korea, *A. komarovianum* was taken into cultivation as a vegetable (10).

Allium chemistry: a brief overview

As is well known, intact bulbs of garlic and onion have little odour, but, once cut or crushed, they instantly release a typical, pungent smell. The odours are associated with mixtures of reactive organosulfur compounds formed, within seconds, by enzymatic action on odourless precursors in the intact bulbs. Noteworthy, medicinal properties of representatives of the genus *Allium* have been mainly attributed to organosulfur compounds (11).

Alliin (*S*-allylcysteine sulphoxide) represents the most important *S*-alk(en)ylcysteine sulphoxide precursor occurring in intact bulbs (Figure 2). Other minor sulphur precursors include methiin, isoalliin and propiin, present at much lower concentrations (Figure 2). Upon cutting, crushing or grinding of bulbs, vacuolar enzyme aliinase (alliin lyase EC. 4.4.1.4.) is released from damaged cells. This enzyme very quickly cleaves the precursors to sulfenic acids (R-SOH), highly reactive organosulfur intermediates which self- or cross-condense giving thiosulfinates (Figure 2). In garlic, two molecules of 2-propensulfenic acid undergo condensation leading to allicin (diallyl thiosulfinate), responsible for the characteristic pungent flavour of garlic (Figure 2). In onion, 1-propensulfenic acid is transformed to sulfine 2-propanethial sulphoxide, the lachrymatory factor (LF) of onion, by LF synthase (Figure 2) (12-14).

Intact *Allium* bulbs also contain steroidal saponins, organic selenium compounds, such as *γ*-glutamyl-Se-methylselenocysteine, and flavonoids, such as quercetin-4′-*O*-*β*-glucoside (15).

Allium spp. essential oils as food preservative

In recent years, the demand for natural food additives has increased due to the negative perception of consumers on chemical preservatives. In this context, the use of essential oils as antimicrobials has been developed as safer alternative in food industries (16-20). In fact, natural compounds including essential oils show recognized

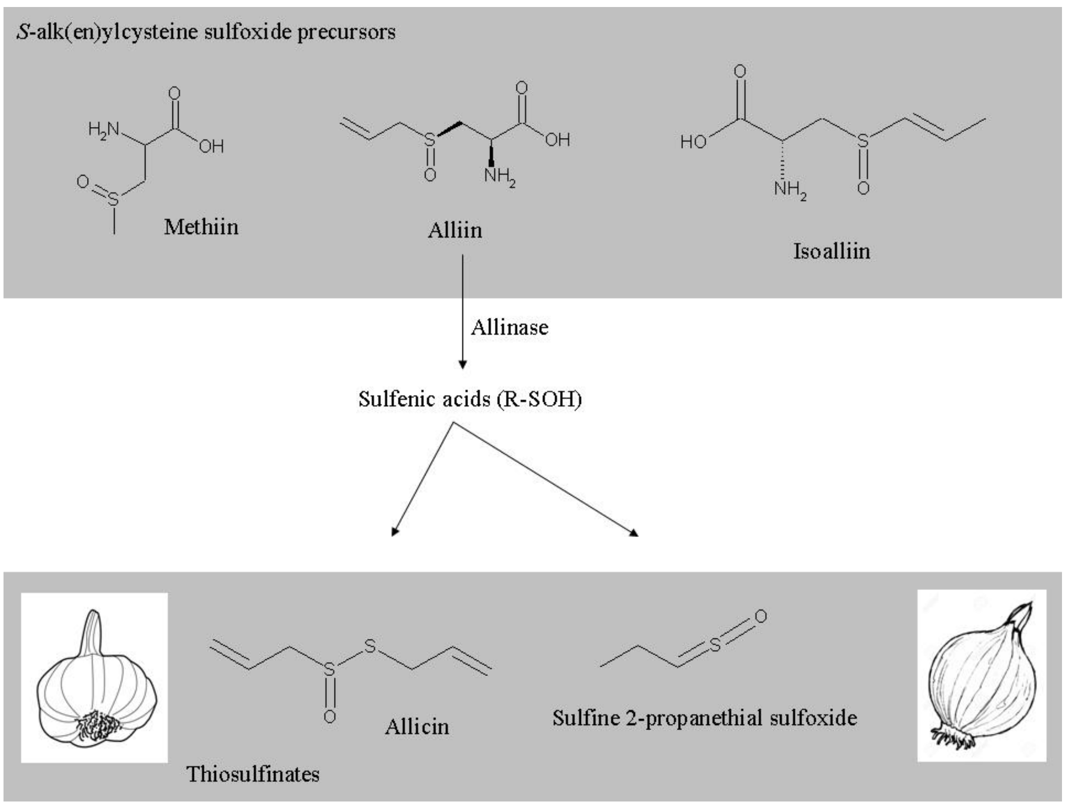

Figure 2. The main *S*-alk(en)ylcysteine sulphoxide precursors occurring in intact bulbs are alliin (*S*-allylcysteine sulphoxide), methiin and isoalliin; upon damaging of bulbs, vacuolar enzyme aliinase (alliin lyase EC. 4.4.1.4.) released from damaged cells cleaves the precursors to sulphenic acids (R-SOH), highly reactive organosulfur intermediates which self- or cross-condense giving thiosulphinates: allicin (diallyl thiosulfinate), in garlic, and sulphine 2-propanethial sulphoxide, the lachrymatory factor (LF) of onion.

antimicrobial activity and can be used to prevent the growth of pathogenic or degradative microorganisms in food (21-24).

Spices and aromatic vegetables, which are commonly employed as food ingredients and flavouring agents, can represent a good source of natural antioxidant and antimicrobial compounds (25, 26). However, chemical food structure, fat content, proteins, water activity, pH, and enzymes are factors that can potentially decrease essential oil efficacy in real food (21, 27).

Among vegetables, *Allium* spp. extracts are recognized to exert antibacterial, antifungal and antiviral properties due to sulphur-containing compounds and flavonoids (28, 29). Several researchers have reported the effects of these compounds on the growth of pathogens or food spoiling microorganisms. Although the use of these compounds is not widespread as food additives due to their relative biochemical instability and to their strong flavour, several recent studies have evaluated the potential effectiveness of genus *Allium* plants or their bioactive compounds as natural preservatives in many food systems, especially in meat and meat-related products (30, 31). In fact, since oxidation and microbial contamination are serious concerns for meat producers and consumers, the application of natural compound exerting both antioxidant and antimicrobial activities may contribute to enhance meat quality and to extend product shelf-life.

Krisch *et al*. (32) compared the antimicrobial effect of essential oils, among which garlic and onion oils, and commercial herbs and spices in minced pork. These authors observed that the best shelf-life values were obtained for pork meat with added garlic and marjoram oil. Park *et al*. (33) reported that refrigerated pork or beef meat with 5% garlic or onion powder inhibited the growth of total bacteria and Enterobacteriaceae and reduced TBARS number at 8 °C for 28 days. The antioxidant and antimicrobial effects of garlic have been tested also in ground camel meat, suggesting their potential utility in preserving these kinds of meat products in concentrations that did not negatively affect product organoleptic acceptance (34). More recently, these antioxidant and antibacterial activities of garlic peel ethanol extract have also been demonstrated in cooked beef, inoculated with selected bacteria and stored at 4°C for 9 days. The results confirmed that, when used at concentration of 10.8 mg/100 g of meat, the extract reduced the population of inoculated *Staphylococcus aureus*, *Escherichia coli* and *Proteus vulgaris* by at least one log unit compared to control at the end of storage (35). Antimicrobial activity of garlic has also been studied in meat products, *i.e*., in chicken sausages, to reduce oxidation and aerobic plate counts (36), as well as in frozen hamburger over three months to prevent *S. aureus* growth (37).

Tang *et al*. (38) focused the attention on *A. cepa* essential oil, testing its antioxidant activity in refrigerated turkey meat. This essential oil contributed to improve the sensory quality of the product and provided protection against oxidation during cooking and refrigerated storage.

In addition to meat, *Allium* plants or their bioactive compounds have been studied as natural preservatives in cheeses, fish and vegetable products.

Josipović *et al*. (39) developed a novel cottage cheese containing spices, among which garlic, having acceptable sensory properties and an extended shelf-life. Shallots (*A. ascalonicum*) together with anise (*Pimpinella anisum*) essential oils were used against *Escherichia coli* during two months of ripening of Iranian white brined cheese (40). The results showed that *A. ascalonicum* essential oil possesses higher antibacterial activity than *P. anisum* essential oil. Moreover, 2000 ppm of *A. ascalonicum* and 3000 ppm of *P. anisum* essential oils were affective in *E. coli* reduction during the cheese ripening period, although 750 ppm *A. ascalonicum* essential oil reached the highest acceptable score in the cheese organoleptic assessment.

Natural compounds, including garlic, have also been studied as preservative methods for fish products, which are highly perishable due to the activity of spoilage seafood microorganisms that produce biogenic amines and off-odours (41). Erkan (42) showed that thymol and garlic oils (1%) in hot-smoked rainbow trout, packaged under vacuum, were able to improve the product quality. Ranjan *et al*. (43) used gum acacia coating with garlic and cinnamon pastes and observed that fish microbial load was reduced during storage. The effects of a variety of spices, including garlic and green onion, were investigated to reduce biogenic amine contents in *Myeolchi-jeot*, Korean salted and fermented anchovy (44). The highest inhibitory effect on biogenic amine production was found in the culture treated by garlic extract while the other spice extracts showed minor effects.

Garlic oil has been proposed, together with other natural volatile compounds, for the biopreservation of fresh-cut tomato stored at 5 °C for 15 days (45). In addition, the same authors studied the encapsulation of garlic oil in β-cyclodextrin and tested this controlled release system during the shelf-life of fresh-cut tomato, achieving a successful control of microbial growth and good sensory quality (46). Moreover, onion bioactive compounds can also be useful as anti-browning agents, preventing vegetables and fruits from the deterioration associated to the post-harvest processing (47).

Besides variability in composition of essential oils, which influences their antimicrobial activity, the main limitation to industrial use of these products as food preservatives is their organoleptic impact on the sensory traits of foodstuffs (21, 48-50). For this reason, the choice of compatible essential oils is pivotal. The combination of different mild treatments, *i.e*., mild thermal or high pressure treatment, with low concentration of selected aroma compounds has been proposed to inhibit or delay microbial growth in many food products, reducing the problems arising from the organoleptic impact of many essential oil constituents (51-55). *Allium* spp. essential oils or extracts have been used within this strategy. In particular, Yang *et al*. (56) used onion and garlic, in combination with irradiation at 2.48-2.83 kGy, in frozen red meat, observing a positive antioxidant activity. Rico *et al*. (57) described similar results with a positive antimicrobial activity obtained in ground beef. Interesting interactive effects on *Escherichia coli* and *Listeria monocytogenes* inoculated on ground pork were reported by combining 1% garlic, leek, and onion with high pressure processing performed up to 600 MPa (58).

The active food packaging is one of the most promising frontiers in which the use of essential oils or their components can be an effective tool to inhibit undesired microflora. There are many reviews reporting packaging technologies in which these molecules are embedded in polymers, with the aim to prolong shelf-life or to assure the safety of foods (59, 60).

Films obtained from polylactic acid containing *Allium* spp. extracts were tested as antimicrobials in packaging for ready-to-eat salads, prolonging the product shelf-life at 4 °C (61). Sung *et al*. (62) proposed the use of LDPE-EVA (low-density polyethylene and ethylene vinyl acetate copolymer) films with *A. sativum* essential oil incorporated for the preservation of animal food. Interesting results were observed for ready-to-eat cooked beef, prolonging the shelf-life and reducing the concentration of *Listeria monocytogenes* (63).

***Allium* spp. plants in traditional medicine**

Plants belonging to genus *Allium* have been used since the beginning of human civilization as food and herbal remedies. Traditional knowledge of *Allium* plants is widespread in all human cultures, where garlic, onion, leeks, chives, scallions and shallot are commonly used. Documented traditional uses have shown the highest prevalence of the most famous species, *A. cepa* and *A. sativum*, which are considered to be some of the oldest cultivated plants. These two plants are mentioned, in the earliest documents (clay tablets, papyrus scrolls, pictograms and other ancient art), as cultivated and intensively used for both consumption and medicinal purposes (64). Numerous archaeological records point to extensive use of onion and garlic in Sumerian civilization, and also in Mesopotamia and ancient Egypt. Egyptian data on *Allium* plants include leek, mentioned together with onion and garlic as an important food ingredient. In Codex Elbers (1500 B.C.), 22 recipes, based on garlic, are described for the treatment of headache, body weakness and throat disorders. Indian Ayurvedic medical philosophy, which originated around 400-200 B.C., attributes numerous health beneficial effects to onion and garlic. In China, some alliums of local origin have been cultivated for more than 3000 years, such as Chinese chives (*A. tuberosum*). In ancient Greece and Rome, naturalists and physicians such as Dioscorides, Hippocrates and Pliny the Elder mentioned garlic, onion and other alliums as important medicinal plants. Garlic was recommended for a variety of illnesses: skin problems (rashes, birthmarks, leprosy, ulcers, eczema, baldness), eye diseases (cataracts), kidney disorders and for worms, lice, nits, dog and snake bites. In the Middle Ages, herbal physicians such as Paracelsus pointed to significance of garlic against intestinal warms. Later, garlic was used for the treatment of the black plaque and tuberculosis. The use of different alliums, especially garlic and onion, is still employed in folk medicine worldwide.

A. cepa is one of the most famous plants used for consumption worldwide. It possess carminative, rubefacient, cholesterol-lowering, antidiabetic, antitussive and antimicrobial effects and is also used for prevention of diseases and boosting of general health. Applications of the onion juice or bulb macerated in water are traditionally used for antiseptic, diuretic, expectorant, hypoglycaemic, antispasmodics effects and prostate relief. Topical application of fresh onion slices is used to cure acne and other skin problems (warts, boils and others), for healing of wounds, relieving toothache and for stimulation of hair growth (65). Onion juice is also a very important treatment for ear disorders (earache and ringing sound), in Russia. Roasted slices of onion can be applied topically on external haemorrhoids to decrease inflammation and reduce pain. Also, onion is a major part of many complex traditional preparations (most of them include honey), used to promote productive cough and to relieve respiratory complaints. Some infective diseases, such as cholera, are effectively treated with onion-based preparations. Bladder and some other urinary system disorders (*e.g.*, urine retention) are also traditionally treated with onion. In India, onion peeled, crushed, fried and mixed with honey is one of the most important aphrodisiac foods (66).

As already mentioned, *A. sativum* is an extremely important plant species in both ancient and modern ages. Its traditional use has spread in all world cultures, and besides its use in fresh, cooked and dehydrated forms for human consumption, there are many folk medicines based on garlic. Overall, garlic is used for the treatment of many conditions, including cardiovascular and respiratory ailments. It also is an effective treatment against diabetes and inflammatory diseases (rheumatisms). In middle ages, it was used to cure deafness, earaches, flatulence, scurvy, leprosy, black plaque, dog and snake bites and even clotting disorders in animals (horses) (65). In Ayurvedic medicine, it is an extremely important plant and is used for a variety of problems: insect bites, intestinal worms, headache and tumours, while folk medicine practioners in India use it for the management of heart disease, cancer, parasites, fungal infections and diabetes (66). In Mexico, the bulb epidermis is used to treat stomach-ache and dysentery (67). Infusion of garlic is used for the treatment of epilepsy, while ‘acetum aromaticum’ (garlic boiled with vinegar and sugar) is used as topical antiseptic. Combined with milk, it is used as a vermifuge, while mashed with honey or in a form of extract, it is effective against rheumatisms. Powdered bulb applied locally can treat alopecia (68).

Apart from garlic and onion, the most important plants, the *Allium* genus includes many other species with traditional uses. *A. ursinum* (ramson) is commonly used in Europe in fresh form as salad, and it is known as food for prevention of cardiovascular diseases and as a detoxifying agent. The entire plant is edible and effective against hypercholesterolaemia, formation of blood clots, rheumatisms and digestive complaints (69). Externally, it can be applied to promote wound healing and as an antimicrobial agent to treat acne and chronic skin disorders. It is also well known as remedy for common respiratory problems such as cold, fever, bronchitis and asthma (70).

A. cernuum, known as nodding onion or lady's leek is edible and possesses a very strong flavour of onion. It was traditionally used by Native Americans as food and also as a remedy for various disorders in a form of juice (hives, croup, colds, sore throat and kidney stones) or applied externally as a poultice in the treatment of respiratory ailments. *A. shaenoprasum* is mostly used for treatment of coughs and colds in the form of thick syrup; dried bulbs are inhaled to clean sinuses, while water infusion cures intestinal worms. The juice is used to soak moss which is then applied as a dressing for wounds. Chinese chive (*A. tuberosum*) seeds are used to treat impotence and nocturnal emissions (71). In addition, this plant is used to treat fatigue, control excessive bleeding and as antidote for ingested poisons. The leaves and bulbs are applied to insect bites, cuts and wounds, while the seeds are used to treat kidney, liver and digestive problems. Indigenous *Allium* species from Caucasus, *A. gramineum* and *A. leucanthum*, are widely used in Georgian traditional medicine as antiseptic remedies (72). *A. ampeloprasum* is native to Mediterranean region and is consumed raw as vegetable, cooked, boiled and seasoned with olive oil and vinegar. It is traditionally used as an antihelmintic, diuretic, antihypertensive and digestive agent (65), as well as for inflammatory symptoms. The fresh juice of this plant is taken orally to improve digestion, while crushed bulbs are utilized for the treatment of cough, sore throat and mucous secretion (73). Another extensively used *Allium* plant, native to North Africa, is *A. roseum*. As a food, it is used fresh in salads or as a spicy ingredient. The same plant is used in ethnomedicine as appetizer and to treat headaches, rheumatism, common cold, fever and bronchitis (74). Tribal communities in North West Himalayas use *A. wallichii* and *A. stracheyi* as both food and traditional treatments for several ailments. Ingested boiled and fried bulbs of these two species are used as a remedy for cholera and dysentery, while chewed raw bulb can cure cold, cough and altitude disease (27). The extract of *A. hirtifolium* is used in traditional treatment of rheumatism, inflammation and infectious diseases (75). *A. vineale* is applied for dermatosis and eczema, while *A. rotundum* is mentioned as an agent effective against jaundice and sinusitis (76). Another *Allium* important for cardiovascular diseases is *A. fuscum*, mentioned as antihypertensive plant in traditional medicine. Ethnopharmacological data from India recorded the use of *A. cepa*, *A. chinense*, *A. macranthum*, *A. tuberosum*, *A. prattii*, *A. rubellum*, *A. sativum* and *A. walchii* for some common health problems such as cold or skin rashes. In the same Indian province (Arunachal Pradesh), *A. chinense* is used against cough, cold and vomiting. It is also used for wound healing, while the bulb ash mixed with oil can treat skin diseases. When applied externally in a form of paste, it provides relief from body pain (77).

Antibacterial activity of genus *Allium* plants

As previously introduced, species belonging to the genus *Allium* includes among the oldest cultivated plants widely distributed all over the world. *Allium* includes several hundreds of species appreciated not only for their flavour, but also for their medicinal benefits (78). These plants are rich in thiosulphinates, which mainly contribute to their biological properties as effective antibacterial agents (79, 80). In addition, the content of these sulphur compounds may considerably vary among different species and within the same species (81).

The antibacterial activity of *Allium* spp. extracts from bulbs and leaves as well as essential oils has been extensively reported against a wide range of Gram-positive and Gram-negative bacteria (78, 82-86). The extraction methods, the solvents used and the concentration of the extracts play an important role in determining the antimicrobial activity of these plant products, mainly attributed to sulphur compounds. Other constituents such as peptides, phenols and flavonoids may also contribute to the reported antibacterial activity (87). The extracts/active compounds of the *Allium* spp., the antibacterial tests used and the bacterial strains studied are presented in Table 3.

Again, among the different *Allium* species, garlic and onion are the most studied plants. During the last decade, the antimicrobial activity of garlic and garlic-derived organosulphur compounds has been widely investigated (13, 88, 89, 90). In addition to these compounds, garlic phenolic constituents are also involved in this activity (91). However, genetic traits, agronomic factors and extraction procedures may influence the content of organosulphur compounds in plant tissues and organs (92).

Allicin and garlic extracts possess a broad-spectrum antibacterial activity against *Escherichia, Salmonella, Staphylococcus, Streptococcus, Klebsiella, Proteus, Clostridium, Mycobacterium* and *Helicobacter* species by inhibiting bacterial DNA, RNA and protein biosynthesis (91, 93). Similarly, ajoene exhibited a broad-spectrum antibacterial activity, especially on Gram-positive bacteria (94), and the antibacterial activity of allicin in garlic extracts was also reported against harmful enterobacteria (95).

A recent study showed that allicin-rich extract and ajoene-rich fraction of garlic exert good antimycobacterial activity against *Mycobacterium tuberculosis,* compared to standard drugs, whereas garlic essential oil demonstrates significant antibacterial activity against methicillin-resistant *Staphylococcus aureus* (96).

Among different fresh juices from 17 plants tested for bacteria inhibition, garlic was the most effective one while onion and leek inhibited the growth of one to three bacteria. The high activity of garlic juice was mainly attributed to allicin (97). The aqueous and ethanolic extracts of two varieties of garlic exerted an inhibitory activity against enteric pathogenic bacteria (98). Garlic crude extracts showed inhibitory activity against *Staphylococcus aureus, Escherichia coli, Streptococcus pneumoniae* and *Pseudomonas aeruginosa* at a concentration of 100 mg/mL (99). Similarly, crude and ethanolic extracts of garlic were studied against five Gram-negative and two Gram-positive bacteria; both extracts showed good antibacterial activity (91).

The antimicrobial activity of fresh garlic, garlic powder and essential oil was investigated against microbial growth in raw chicken sausage during storage at 3 °C (36). All extracts exhibited higher antimicrobial activity than synthetic antioxidant butylated hydroxyanisole (BHA). Among garlic extracts, fresh garlic exerted higher activity than garlic powder and garlic oil, probably because it provides the full range of beneficial compounds. The lower antimicrobial activity of garlic essential oil could be attributed to the loss of allicin and other volatile sulphur compounds during steam distillation. Indeed, some authors reported that garlic essential oil does not contain allicin, but derivatives of alli-

Table 3. Antibacterial activity of *Allium* genus extracts.

Plant	Extract/Active compound	Extraction method	Antibacterial test	Bacteria tested	References
Garlic *A. ophioscordon* *A. sativum*	Water, ethanol extracts allicin	Soxhlet extraction	Agar well diffusion assay	*Escherichia coli, Proteus mirabilis, Salmonella typhi, Shigella flexineri, Enterobacter aerogenes*	(98)
Garlic *A. sativum*	Water, ethanol and chloroform extracts	Solvent extraction	Minimum bactericidal concentration (MBC)	*Staphylococcus aureus, E. coli, Streptococcus pneumoniae Pseudomonas aeruginosa*	(99)
Garlic *A. sativum*	Ethanol extract	Solvent extraction	Disc diffusion test Minimum inhibitory concentration (MIC)	*E. coli*, *Enterobacter* spp., *P. aeruginosa*, *Proteus* spp., *Klebsiella* spp., *S. aureus*, *Bacillus* spp.	(91)
Garlic *A. sativum*	Essential oil (EO)		Aerobic plate count (APC) Total coliforms count Total staphylococci count	Enterobacteriaceae, coliforms, staphylococci	(104)
Garlic (*A. sativum*) Onion (*A. cepa*) Leek (*A. porrum*) Chinese chive (*A. tuberosum*) Shallot (*A. ascalonicum*) Chive (*A. schoenoprasum*)	Garlic and Chinese chive EOs (diallyl disulphide) Onion, leek, shallot and chive EOs (dipropyl disulphide, dipropyl trisulphide)	Turbo hydrodistillation	Disc diffusion method	*Salmonella typhimurium, E.coli, Campylobacter jejuni, S. aureus*, *Listeria monocytogenes*	(78)
Garlic (*A. sativum*) Chinese leek (*A. odorum*)	EOs/diallyl disulphide, trisulphide, tetrasulphide	Steam distillation	MIC	*S. aureus,* methicillin-resistant *S. aureus* (MRSA)	(102)
Garlic (*Allium sativum)* Onion (*A. cepa*) (green, yellow, red)	EOs	Steam distillation	Disc diffusion test	*S. aureus, Salmonella enteritidis*	(28)
Onion (*A. cepa*)	Ethanolic extract	Solvent extraction	Cup-plate diffusion method/MIC	*E. coli, S. typhi*	(111)
Onion (*A. cepa*)	White and red onion paste		Agar well diffusion method	*E. coli*, *Bacillus subtilis*	(109)
Onion (*A. cepa*)	Ethyl acetate fraction/ flavonoids	Solvent extraction	Disc diffusion assay/ MIC	*Bacillus cereus, S. aureus*, *L. monocytogenes*	(29)
Onion (*A. cepa*)	Extracts/ organosulfur compounds	Supercritical CO_2 extraction	Disk diffusion test/ MIC/MBC	*E. coli, B. subtilis, S. aureus*	(112)
Onion (*A. cepa*)	EO		Disc diffusion method	*Bacillus antracis*, *B. cereus, Micrococcus luteus, S. aureus, Klebsiella pneumoniae*	(113)
Shallot (*A. ascalonicum)*	Ethanol extract	Solvent extract	Total viable count	Psychrophilic bacteria, *Pseudomonas* spp.	(114)
Shallot (*A. ascalonicum)*	EO/ diallyl disulphide, trisulphide, tetrasulphide	Steam distillation	MIC	*B. cereus, C. jejuni, E. coli* O157:H7, *L. monocytgenes, Salmonella enterica, S. aureus, Vibrio cholerae*	(85)
Persian Shallot (*A. hirtifolium*)	Hydromethanolic extract 9-hexadecenoic acid 11,14-eicosadienoic acid *n*-hexadecanoic acid	Solvent extraction	Disk diffusion assay/ MIC	MRSA, methicillin sensitive *S. aureus*, *S. aureus, Staphylococcus epidermidis, Streptococcus pneumoniae, E. coli, E. coli* O157:H7, *S. typhimurium, Proteus mirabilis, K. pneumoniae*	(115)
Chive (*A. schoenoprasum*)	EO/ diallyl disulphide, trisulphide, tetrasulphide	Steam distillation	MIC	*B. cereus, C. jejuni, Clostridium botulinum, E. coli, L. monocytogenes, S. enterica, S. aureus, V. cholerae*	(105)
Chinese leek (*A. odorum*)	Water extract		MIC	*C. jejuni*, *Campylobacter coli*	(118)

cin such as diallyl sulphides. However, these derivative products have also shown a wide spectrum of antibacterial activity (100-103). Javed *et al*. (90) also showed that the antibacterial activity of garlic is related to allicin and its derivative diallyl sulphides. Another study reported that garlic oil is an effective antibacterial agent against enterobacteria, coliforms and staphylococci in refrigerated beef meat (104). The powerful antibacterial activity of essential oils from garlic, Chinese chive (78), shallot (85) and chive (105) was attributed to the presence of allyl sulphide groups in their fractions. In general, a high number of sulphur atoms results in powerful antimicrobial activity, such as in diallyl disulphide, trisulphide and tetrasulphide (101,102). Indeed, Kim *et al.* (101) demonstrated that sulphide with a single sulphur atom was not as antibacterial as sulphide with three or four sulphur atoms. Tsao & Yin reported that garlic oil, with higher concentrations of allyl groups, was more antimicrobial than Chinese leek oil on Staphylococcus aureus (102).

A recent study on six *Allium* species showed that garlic essential oil was effective on all bacteria tested. Onion and Chinese chive essential oils inhibited four bacteria; shallot essential oil inhibited three bacteria while leek and chive essential oils inhibited two out of five bacteria tested. Garlic and Chinese chive essential oils were rich in allyl sulphide, whereas onion, shallot, leek and chive essential oils were rich in dipropyl disulphide and dipropyl trisulphide (78). The high antibacterial activity of these plant products is related to presence of allyl sulphide and propyl sulphides, since the latter also showed antibacterial activity against *Staphyloccus aureus* (101). Skrinjar and Nemet also reported also that garlic essential oil was effective on a wide range of Gram-positive and Gram-negative bacteria (89). Garlic essential oil was incorporated into whey-protein-based edible coatings and showed inhibitory activity against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella entericidis*, *Listeria monocytogenes* and *Lactobacillus plantarum* (106).

The mechanism of action of essential oils has been investigated. Their hydrophobic properties allow them to interfere with the phospholipidic membrane of the bacterial cell, thus increasing its permeability, causing the loss of cell constituents and, finally, cell death (21). In addition, sulphides can damage the microbial cells by reacting with SH groups of proteins to generate mixed disulphides (87). Essential oils seem to be more active on Gram-positive than Gram-negative bacteria. This is due to cell wall composition, even if many controversies exist in the various published studies.

Onion, a versatile vegetable of the *Allium* genus, is appreciated worldwide and is the second most studied plant belonging to this genus after garlic. It is well known for its beneficial constituents such as sulphur compounds, phenols and flavonoids (107,108). Again, the levels of these compounds depend on the environment, variety, agricultural practices and the extraction processes. In general, the concentration of sulphur compounds in onion is lower than in garlic. This would explain why garlic is more antimicrobial than onion (28, 82). However, the antimicrobial activity of onion extracts is, to some extent, attributed to peptides and flavonoids (quercetin derivatives) (109,110).

Onion extracts were effective on Gram-negative *Escherichia coli* and *Salmonella thyphi*, but not on Gram-positive *Bacillus subtilis* (111). The *in-vitro* activity of onion paste showed that white and red onion bulbs were extremely active on *Bacillus subtilis* and less active on *Escherichia coli*, whereas green onion bulb and leaves did not inhibit bacterial growth (109). Only the ethyl acetate fraction of onion which contains flavonoids was active on Gram-positive bacteria *Bacillus cereus*, *Staphylococcus aureus* and *Listeria monocytogenes*, whereas the other water subfraction of methanolic extracts of three Spanish onions was not active (29). Garlic and onion extracts exerted bactericidal effects against *Streptococcus mutans*, *Streptococcus sobrinus*, *Porphyromonas gingivalis* and *Prevotella intermedia* oral bacteria (93).

Few studies reported the antibacterial activity of onion essential oil. The essential oils of three varieties of onion and garlic markedly inhibited *Staphylococcus aureus* and *Salmonella enteritidis* growth, although garlic showed the highest activity and green onion the lowest (28). Other authors (112,113) reported that onion essential oil, rich in organosulfur compounds, exhibited potent inhibitory activity against Gram-positive and Gram-negative bacteria.

Shallot (*A. ascalonicum*), leek (*A. porrum*), chive (*A. schoenoprasum*) and Chinese chive *(A. tuberosum*) have been used in many diets and in folk medicine since ancient time (13). Studies on these *Allium* plants are scarce; however, some investigations on their antibacterial activity were carried out. A recent study showed that shallot bulb extracts delayed the growth of psychrophilic bacteria and *Pseudomonas* spp. in semi-fried coated rainbow trout (*Oncorhynchus mykisss*) fillets (114). Shallot essential oil was rich in organosulphur compounds (diallyl sulphide, disulphide, trisulphide and trasulphide) and inhibited the growth of all bacteria studied (85). The hydromethanolic extract of Persian shallot (*A. hirtifolium*) was rich in 9-hexadecenoic acid, 11,14-eicosadienoic acid, and *n*-hexadecanoic acid, and was effective against 10 pathogenic bacteria (115). The inhibitory effect of elephant garlic (*A. ampeloprasum)* essential oil was studied against a major food-borne pathogen responsible for cholera, *Vibrio cholerae*. The oil was mainly rich in allyl sulphide compounds (diallyl di-, tri- and tetrasulphide) and showed *in-vitro* bactericidal activity on all tested strains of the bacteria. Moreover, elephant garlic essential oil delayed the growth of *V. cholerae* in a food model depending on its concentration (116).

The chemical composition and antibacterial activity of chive (*A. schoenoprasum*) essential oil was studied. The oil was rich in allyl derivatives (diallyl di-, tri- and tetrasulphide) and was effective on all bacteria tested with different inhibition degrees. Moreover, it was able to inhibit *E coli* O157:H7 in a food model (105). The antibacterial activity of Chinese chive extract was studied against 38 *Salmonella* strains. Results showed that chive extract had strong antibacterial activity against all strains in culture media and reduced their growth in chicken and beef broth (117). Finally, the *in-vitro* antibacterial activity of aqueous Chinese leek extracts (from leek flower stem, soft leek and green leek) was more effective than aqueous garlic extract and inhibited the growth of *Campylobacter jejuni* and *Campylobacter coli* (118).

Conclusions and future perspectives

Plants belonging to the genus *Allium* possess a very long tradition of ethnobiological uses, as foods, spices, medicinal herbs and food preservatives. As reviewed in this survey, modern science has confirmed a number of biological properties ascribed to alliums, particularly their broad-spectrum antibacterial activity. Future trends in food sciences include development of active edible films to improve food shelf-life and functionalization of nanomaterial for controlled release of active ingredients (nanobiocides), novel topics in which *Allium* bioactives may be successfully applied.

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