Phytochemical composition and biological activities of *Geranium robertianum* L.: a review

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Abstract

After a period of a certain indifference, in which synthetic compounds were favored, the interest in the study of the biological properties of plants and the active principles responsible for their therapeutic properties has been growing remarkably. *Geranium robertianum* L., commonly known as Herb Robert or Red Robin, is a spontaneous, herbaceous plant that has been used for a long time in folk medicine of several countries and in herbalism's practice for the treatment of a variety of ailments. Herein, we present a comprehensive review on the phytochemical characterization and the biological activities of this species, which, accompanying the remarkable increase of its use in herbal medicine, has been disclosed in the literature mainly in the last decade. The phytochemical characterization of *G. robertianum* has been focused mostly on the investigation of solid-liquid extracts of the plant, with special emphasis on phenolic compounds, particularly flavonoids. Studies concerning the essential oils of this species are still scarce but the number of identified compounds is high. The chemistry of *G. robertianum* is clearly dominated by phenolic constituents, the most studied classes of compounds being tannins, flavonoids and phenolic acids. The confirmation of the antioxidant, antimicrobial, anti-inflammatory, anti-hyperglycaemic and

cytotoxic activities of *G. robertianum*, closely related to the high content of phenolic compounds, has come to corroborate in some extent the recognized beneficial proprieties of this medicinal plant.

Keywords

Geranium robertianum L; medicinal plants; phytochemistry; biological properties; geraniin

1. Introduction

The knowledge related to the popular uses of plants is based on thousands of years of experience. In the constant search for their wellbeing, humans learned, by trial and error, to recognize useful plants, including those playing a magical-religious function (Camejo et al., 2003). As a result of the accumulated empirical information, the most effective plants in controlling or curing diseases have been selected (Cunha et al., 2010).

The knowledge concerning the use of plants for medicinal purposes was highly valued in ancient civilizations. Until the mid-nineteenth century, plants were the main therapeutic agents used by humans, and still have an important role in medicine (Camejo et al., 2003). The difficulty or inability to find adequate and affordable solutions for the treatment of various diseases, including cancer, HIV/AIDS, diabetes, hepatitis, allergies, and mental disorders, has driven conventional and traditional medicines in the search for new phytochemicals and other natural products for their prevention and/or treatment, as well as for health promotion in general (Slikkerveer, 2006).

The discovery of new active molecules is based on the knowledge inventoried by the methodologies of ethnobotany and etnopharmacology (Gomes, 2005; Maciel et al., 2002), disciplines which are essential to secure vegetable raw material for the production of phytotherapeutic products and for the isolation of new drugs and/or lead compounds (Foglio et al., 2006; Gomes, 2005). In the search for new bioactive natural molecules, preference is naturally given to the study of plants that, for their use in folk medicine, have already shown to display pharmacological activity (Montanher et al., 2002). Part of this activity comes from the high capability of these species to scavenge free radicals responsible for cell damage (Mantle et al., 2000).

In many developing countries, the primary health care of a large part of the population relies greatly on traditional practitioners and medicinal plants and, although modern medicine may be available in those countries, herbal medicines have often maintained popularity for historical and cultural reasons (WHO, 1999). In developed countries, after a period of indifference in which synthetic compounds were favored, the interest for herbalism resurged in the beginning of the last quarter of the 20th century, mainly in Germany, France and the United Kingdom, later passing to other European countries and to North America (Cunha, 2005; WHO, 2002, 2010), and keeps growing.

Geranium robertianum L., commonly known as Herb Robert or Red Robin, has long been used in the folk medicine of several countries and in herbalism's practice for a number of different therapeutic purposes. This medicinal plant owes its popularity to the use as remedy for a variety of digestive system disorders, and also to a series of properties such as anti-inflammatory, haemostatic, antidiabetic, antibacterial, antiallergic, anti-cancer and diuretic often ascribed to it.

2. Botanical description

2.1. The Geraniaceae family

The Geraniaceae family is a small botanical family that contains about 750 species, grouped into to 5 to 11 genera (Ávila et al., 2013). Generally, it includes herbaceous plants, covered by glandular simple leaves usually deeply lobed, flowers with five petals, five sepals, five to fifteen stamens and five carpels yielding five fruits. The most distinctive feature of this family is a beak-shaped fruit that is made up of five mericarps arranged around an inner central column. During the maturation this column expands, acquiring the shape of a heron's or stork's beak (Abraham and Elbaum, 2013; De Jussie, 2010). Most species of this family are distributed worldwide, mainly in temperate areas, with the Mediterranean region and Southern Africa being centers of greater diversity (Fiz et al., 2008). The largest genera of this family are *Geranium* (300 species), *Pelargonium* (250 species), *Erodium* (80 species) and *Monsonia* (25 species) (De Jussie, 2010).

The economic importance of this family includes the cultivation and marketing of species for ornamental use, especially of *Geranium* and *Pelargonium* genera, and extraction of essential oils used in perfumery, cosmetics and aromatherapy. *Erodium* genus, which is hygroscopic, is often used to indicate changes in humidity (Lis-Balchin, 2004; De Jussie, 2010).

2.2. *Geranium robertianum* L.

G. robertianum, commonly known as Herb Robert or Red Robin, is a species from the botanical family of Geraniaceae. This plant can be found widely in Europe, with the exception of the far north, in temperate parts of Asia, North Africa, Atlantic area of North America, and temperate parts of South America (Allen and Hatfield, 2004; Gruenwald et al, 2000). *G. robertianum* is an annual or biennial herbaceous plant, which grows spontaneously, especially in fresh and moist places. It is found most commonly in shaded or partly shaded habitats, such as woodlands, waste lands, woods or old walls located above 1500 m up to 1800 m and can be gathered between May and October (Cunha et al., 2012; Gruenwald et al, 2000; Ribeiro et al., 2000).

G. robertianum is a plant with a pungent smell. Its height may vary from 10 to 60 cm, has a fibrous rooting system and usually stems branched in many directions from the base. It has long stems and petioles. These stems can present green color but are usually reddish, long, thin and fragile. Leaves are green light and can later acquire a reddish board. It is triangular in shape, with over 10 cm across, and generally divided into three deeply pinnately divided segments. This plant bears two to four pink or violet open flowers at a time with a 13–15 mm corolla consisting of five

rounded petals arranged radially around the superior ovary. Flowers contain 10 anthers and 10 ovules, and individual plants typically produce fewer than the full 10 seeds. Fruits resemble a crane's beak (Frey and Bukoski, 2014; Gruenwald et al, 2000; Miller, 2004; Pedro et al., 1990; Tofts, 2004).

2.3. Medicinal uses of *Geranium robertianum* L.

G. robertianum has been used for a long time in the folk medicine of several countries in different preparations, for a multitude of therapeutic purposes (Table 1). Its anti-inflammatory, haemostatic, antidiabetic, antibacterial, antidiarrhoeic, antiallergic, anti-cancer, antihepatotoxic, diuretic and tonic properties, as well as its suitability for the treatment of digestive system ailments has made this species very appreciated in herbal medicine.

3. Phytochemical characterization

The chemistry of *Geranium* genus is reasonably well-known and clearly dominated by phenolic constituents (Harborne and Williams, 2002), the most studied classes of compounds being tannins, flavonoids and phenolic acids. The phytochemical caracterization of *Geranium robertianum* L. has been focused mostly on the investigation of solid-liquid extracts of the plant, with special emphasis on phenolic compounds, particularly flavonoids. Studies concerning the essential oils of this species are still scarce.

3.1. Solid-liquid extracts

3.1.1. Tannins

According to Hegnauer's dictionary of plant chemistry (Hegnauer, 1966) tannins were the first phytochemicals to be reported in *G. robertianum*. The Geraniaceae family is known to be rich in tannins (Bate-Smith, 1973a) and *G. robertianum* is not an exception (Igwenyi and Elekwa, 2014; Paun et al., 2014; Piwowarski et al., 2011).

Although the structures of the tannins in most *Geranium* species are largely unknown (Okuda et al., 1980), the presence of ellagitannins, a class of hydrolysable tannins containing hexahydroxydiphenic (HHDP) acid (**1**) units esterified to a core polyol, has been reported based on the detection and quantification of ellagic acid (**2**) (Ascacio-Valdés et al., 2011). In leaves and other tissues there seems to be little if any free ellagic acid (Bate-Smith, 1973a) but this is readily produced by the spontaneous lactonization of the HHDP esters upon acid hydrolysis in aqueous solution and, therefore, it is considered mostly as product of the hydrolysis of ellagitannins in plants

(Scheme 1). The ellagitannin content of an aqueous-methanolic extract of *G. robertianum* from Cambridge (England), expressed in terms of hexahydroxydiphenylglucose (HHDPG), was found to be 5% (HHDPG/dry weight) (Bate-Smith, 1972). Ellagitannins are endowed with several beneficial biological activities (Landete, 2011; Lipińska et al., 2014).

The main hydrolysable tannin in *Geranium* genus is geraniin (**3**) (Harborne and Williams, 2002; Okuda et al., 1980), formed by one hexahydroxydiphenic acid unit (HHDP), one unit of dehydrohexahydroxydiphenic acid (DHHDP) and one gallic acid unit linked to a glucose molecule (Figure 1). Geraniin was first isolated from the tannin-rich plant *Geranium thunbergii* Sieb. et Zucc., a medicinal plant with long tradition as remedy for intestinal disorders in Japan popular, by Okuda et al. (1976), as a crystalline solid devoid of astringency. Since its discovery, geraniin has been identified as a constituent in extracts of a number of plants of various families, particularly medicinal plants, and it has been showed to be endowed with a range of beneficial bioactive properties (Pereraa et al., 2015). Geraniin has been identified in acetone/water extracts from the leaves of *Geranium robertianum* native from Japan to as much as 9.8% (Okuda et al., 1980). Geraniin was also identified in aqueous extracts of specimens harvested in Poland (Piwowarski et al., 2014).

Plants of the *Geranium* genus contain both hydrolysable and condensed tannins (proanthocyanidins). Their distribution in the various organs differs substantially, condensed tannins occurring mainly in the rootstocks (Bate-Smith, 1973b), while in the leaves ellagitannins seem to predominate (Harborne and Williams, 2002). Although no detailed phytochemical studies have yet been carried out concerning condensed tannins of Geranium species, it is likely that procyanidins are a common type (Harborne and Williams, 2002). To date the only data related to condensed tannins in *G. robertianum* seems to have been reported by Ben Jemia et al. (2013) who determined the proanthocyanidins content in a methanol extract of plants native from Tunisia to be 0.86 mg catechin equivalents/g dry weight. This very low amount of tannins was attributed to the low extractability of methanol.

3.1.2. Phenolic acids

Ellagic acid found in plants is believed to result mostly from the hydrolysis of endogenous ellagitannins (Bate-Smith, 1973a) and is commonly used to indirectly ascertain the existence and quantification of the later compounds (Ascacio-Valdés et al., 2011). Ellagic acid has been detected frequently, sometimes in considerable amounts, in different extracts of *G. robertianum* (Bate-Smith, 1962; Kobakhidze and Alaniya, 2004; Neagu et al., 2013; Paun et al., 2014; Santos et al., 2013). Fodorea et al. (2005) observed an increase in the amount of ellagic acid in an aqueous ethanolic

extract of specimens from Romania upon hydrolysis demonstrating indirectly the presence of ellagitannins in the plant.

Gallic acid (**4**), which is a presumed precursor of ellagic acid and a key unit of gallotannins, the simplest hydrolizable tannins, has been frequently detected in different extracts of the plant in relatively high amounts (Kobakhidze and Alaniya, 2004; Neagu et al., 2013; Paun et al., 2014) (Figure 2).

Together with ellagic acid, these are the two main phenolic acids of *G. robertianum* and commonly found in Geraniaceae species (Ávila et al., 2013; Bate-Smith, 1962; David and Giannasi, 1988). Gallic and ellagic acids are two endogenous plant phenolic compounds whose inhibitory effect on carcinogenesis is documented (Verma et al., 2013; Zhang et al., 2014). The 3,5-dimethyl ether of gallic acid (syringic acid) (**5**) was also detected in a commercial aqueous ethanolic extract of this species (Amaral et al., 2009).

G. robertianum contains other phenolic acids also resulting from the shikimate biosynthetic pathway such as ferulic acid (**6a**) and its precursor, the caffeic acid (**7**) (Bate-Smith, 1962; Fodorea et al., 2005; Kobakhidze and Alaniya, 2004; Paun et al., 2011; Paun et al., 2012). Ferulic acid was additionally detected as methyl (**6b**) and ethyl (**6c**) esters (Amaral et al., 2009). Chlorogenic acid (**8**), which is the ester of caffeic and (-)-quinic acids, was also detected in the decoction of *G. robertianum* leaves (Santos et al., 2013). Caftaric acid (**9**), formed when caffeic acid and tartaric acid undergo esterification, was detected in an aqueous ethanolic extract of a sample from Romania (Fodorea et al., 2005). Caffeic acid, which was not detected in the later extract, was found in an acid hydrolysed methanolic extract of the same plant sample, thus indicating the existence of bi- or polycaffeoil derivatives. The inexistence of caffeic acid in some extracts is not unusual (Neagu et al., 2013; Paun et al., 2014).

3.1.3. Flavonoids

Geranium genus exhibits a wide range of flavonoid pattern, quercetin (**10a**) and kaempferol (**11a**) being present in almost every species (Bate-Smith, 1973b; Ivancheva and Petrova, 2000) (Figure 3). Variation in the relative amounts of these two flavonols is to some extent correlated with the geography of the genus (Bate-Smith, 1973b).

Flavonoids constitute the main compounds found in *G. robertianum* (Table 2) and can be found in relatively high amounts (Igwenyi and Elekwa, 2014; Ben Jemia et al. 2013; Neagu et al., 2010a; Paun et al., 2011; Paun et al., 2012; Paun et al., 2014). Within this class of secondary metabolites, flavonols, namely, quercetin and kaempferol, are predominant. The first description of flavonoids from *G. robertianum* appears to be an early study by Bate-Smith (1962) who identified by paper chromatography those two compounds in the leaves hydrolysate of the plant.

Quercetin and kaempferol occur in *G. robertianum* either as aglycones or in glycosidic combination. Kartnig and Bucar-Stachel (1991) first isolated and identified a number of flavonoid glycosides present in the methanol extract of the aerial parts of the plant: six monoglycosides - quercetin-3-*O*-glucoside (isoquercitrin) (**10b**), quercetin-3-*O*-rhamnoside (quercitrin) (**10c**), quercetin-4′-*O*-glucoside (spiraeoside) (**10d**), quercetin-7-*O*-glucoside (quercimeritrin) (**10e**), quercetin-3-*O*-galactoside (hyperoside) (**10f**) and kaempferol-3-*O*-glucoside (astragalin) (**11b**) - and four diglycosides - kaempferol-3-*O*-rhamnogalactoside (**11c**), kaempferol-3-*O*-rutinoside (nicotiflorin) (**11d**), quercetin-3-*O*-rutinoside (rutin) (**10g**) and quercetin-3-*O*-rhamnogalactoside (**10h**).

The flavonol glycosides rutin, quercitrin and kaempferol-3-*O*-rhamnoside (**11e**) were also isolated from a methanol extract of *G. robertianum* native from Bulgaria (Ivancheva and Petrova, 2000). This extract also yielded several methoxy derivatives of kaempferol and quercetin: kaempferol 3-methyl ether (isokaempferide) (**11f**), kaempferol 4'-methyl ether (kaempferide) (**11g**), kaempferol 3,7,4'-trimethyl ether (**11h**), quercetin 3,7-dimethyl ether (**10i**), quercetin 3,3'-dimethyl ether (**10j**), and quercetin 3,7,3'-trimethyl ether (pachypodol) (**10k**).

Quercetin-3-*O*-galactoside (hyperoside) was also found in the ethyl acetate extract of the aerial parts of *G. robertianum* from Georgia (Kobakhidze and Alaniya, 2004) and in ethanol/water (50/50) extracts of specimens native from Romenia, together with isoquercitrin and rutin (Fodorea et al., 2005). In this later study the existence of quercetin and kaempferol in glycosidic combination was additionally ascertained indirectly by the increase of their amounts after hydrolysis.

3.1.4. Other constituents

Other non-phenolic compounds have also been found in *G. robertianum* extracts and occasionally quantified.

Rybak and Rudik (2013) found small amounts of lectins, a class of carbohydrate-binding proteins displaying numerous important biological activities, including anticancer properties (Teixeira et al., 2012), in the rhizomes of *G. robertianum* native from Ukraine. The lectin content, determined by the reaction of hemagglutination of human erythrocytes, was observed to increase during seasonal growth.

Individual alkaloids from *G. robertianum* have not hitherto been reported. However, Hultin and Torssell (1965) detected the presence of alkaloids in dried Swedish plants by a semi-quantitative method. Recently, using the Harborne procedure (Harborne, 1973) for quantification of alkaloids, Igwenyi and Elekwa (2014) determined the total amount of alkaloids in an aqueous extract of fresh leaves of plants native from Nigeria to be 1.20 ± 0.10 mg/100 g.

The later authors also determined the content of other secondary metabolites such as glycosides (0.20 ± 0.06 mg/100 g) and saponins (1.43 ± 0.06 mg/100 g) in the same extract. Saponins were also detected in aqueous extracts of the plant (Paun et al., 2011).

Malic and citric acids were found in *G. robertianum* but tartaric acid was not detected (Hegnauer, 1966), although it occurs regularly in members of the Geraniaceae family (Stafford, 1961).

The nutritional characterization of *G. robertianum* has been rarely addressed. Neagu et al. (2010a) determined the content of proteins (1.117-2.242 μg/mL) and reducing sugars (257.2-479.5 μg/mL) of several aqueous ethanolic extracts (50%, 70% and 96% ethanol) from the leaves of plants harvested in Romania (herbal mass concentration in the solvent 8%, 10% and 15%). The amount of both nutrients was found to be correlated, although not always consistently, with the herbal mass concentration and composition of the solvent mixture. Reducing sugars, polysaccharides, proteins and amino acids were also found in aqueous extracts of the plant (Paun et al., 2011).

Leaves of *G. robertianum* native from Cardiff (United Kingdom) were found to contain a relatively high amount of ascorbic acid (vitamin C) (Jones and Hughes, 1983). The determined content (156.8 mg/l00 g) was close to the foliar mean ascorbic acid concentration of 213 angiosperm species. In an aqueous extract of fresh leaves of plants from Nigeria, besides vitamin C (14.76 ± 5.1 mg/100 g) other vitamins were recorded: vitamin A (1.44 ± 0.02 mg/100 g), vitamin B_1 (288.17 ± 0.12 mg/100 g), vitamin B_2 (818.21 ± 0.07 mg/100 g), vitamin B_3 (319.13 ± 0.12 mg/100 g) and vitamin E (0.016 ± 0.02 mg/100 g) (Igwenyi and Elekwa, 2014).

Lutein, a carotenoid, was detected in infusions of the aerial parts of *G. robertianum* and quantified to as much as 92.3 mg/g dry weight (Loranty *et al.*, 2010).

G. robertianum has a good capacity to exploit the mineral elements of the soil, which results in significant amounts of mineral elements accumulated in plants (Stratu et al., 2011). The foliage of specimens from England was referred to contain high concentrations of Ca, Na and Fe (Grime et al., 1988). Aqueous and hydro-alcoholic extracts of plants native from Romania were also screened for dietary elements and Ca, Mg, Mg, Zn and Fe were found to be present in reasonable amounts (Table 3) (Paun et al., 2012).

Plants can contain heavy metals which they accumulate mainly as result of the pollution spread by anthropogenic activities and therefore herbal medicines can present important health risks (Locatelli et al, 2014). The same aqueous and hydro-alcoholic extracts of plants native from Romania were additionally inspected for the toxic metals Pb and Ni, but none exceeded the limits recommended for medicinal plants (Paun et al., 2012).

3.2. Essential oils

Compared to the extracts resulting from solid-liquid extraction, the essential oils from *G. robertianum* have received much less attention. Conversely, due to the volatile nature of the constituents, their compositional analysis is far more extensive.

The first study describing the composition of the essential oil of *G. robertianum* was carried out by Pedro et al. (1992). The oil was obtained from the aerial parts of plants native from the Netherlands, by hydrodistillation (Clevenger type), and it was verified by GC and GC-MS to be a complex mixture of more than 100 compounds, 72 of which could be identified (Table 4), representing 86% of the oil. The oil consisted mainly of monoterpenes, linalool (**12**) being the most abundant compound (22.9%). The other major components were γ-terpinene (**13**), germacrene-D (**14**), limonene (**15**), geraniol (**16**), α-terpineol (**17**) and phytol (**18**) (Figure 4).

More recently, the detailed compositional analysis of the volatiles isolated from extracts of *G. robertianum* (aerial and underground parts) native from Serbia, obtained by hydrodistillation (Clevenger type), was described (Radulović et al., 2012). It was possible to identify by GC and GC-MS 152 compounds from the aerial parts (95.8% of the oil), and 53 compounds from the underground parts of the plant (98.0% of the oil) (Table 4). Fatty acids and fatty acid-derived molecules predominated, constituting 49.2% of the aerial parts oil and 93.4% of the underground parts oil. The most abundant components in the *G. robertianum* oils were hexadecanoic acid (palmitic acid), pentacosane, hexahydrofarnesyl acetone (**19**) and caryophyllene oxide (**20**). Significant differences were observed in relation to the composition of the *G. robertianum* essential oil previously reported by Pedro et al. (1992). The most abundant components of the later oil were not detected in the oil of aerial parts from Serbian plants (e.g. γ-terpinene, germacrene-D, limonene) except for linalool and geraniol, which were present in a much less extent (1.4% and trace amounts, respectively). It was suggested that the above-mentioned differences in composition are possibly due to the influence of environmental factors and/or genetic variability of the investigated populations.

Fatty acids were also found to be the dominant class of compounds in an essential oil obtained by hydrodistillation (Clevenger type) from the aerial parts of *G. robertianum* collected in the Midi-Pyrénées, France (Zhao, 2014). Amongst the 32 compounds identified by GC-MS and GC-FID (80.6% of total volatile extracts), hexadecanoic acid (palmitic acid) (33.4%) was the major component (Table 4). Dodecanoic acid (lauric acid) (10.3%), tetradecanoic acid (myristic acid) (7.0%) and (9*Z*,12*Z*)-octadecadienoic acid (linoleic acid) (3.7%) were the other main constituents of the oil.

These studies pointed to *G. robertianum* as an essential oil "poor" species (oil yields < 0.1%). Although a diversity of factors might influence the yield of an essential oil, the particularly

low yields obtained may be directly related to a very deficient production of volatile secondary metabolites, which is consistent with the observed dominance of the oil composition by fatty acids, fatty acid-derived and/or carotenoid-derived compounds (Radulović et al., 2009; 2012).

4. Biological activity

Geranium robertianum L. is commonly used in the folk medicine of several countries and in herbalism's practice for the treatment of a variety of ailments. Although its beneficial properties have long been recognized, the scientific evaluation of such properties has been only barely addressed. In the last decade, following the resurgence of the interest to herbal medicines, increasing attention has been given to the biological properties of this plant and several studies have been conducted aiming at assessing its various biological activities.

4.1. Antioxidant activity

The antioxidant ability of *Geraniaceae* species is reasonably well-known (Ávila et al., 2013; Camacho-Luis et al., 2008; Ismail et al., 2009; Nikolova et al., 2010; Şöhretoğlu et al., 2008; Şöhretoğlu et al., 2011; Sokmen et al., 2005). It has been the most extensively studied biological activity of *G. robertianum* and the literature encompasses a number of reports on the antioxidant properties of this plant contemplating a diversity of geographic origins, parts of the plant used and evaluation methods (Table 5). The antioxidant activity is of particular importance given the proven beneficial role of antioxidants in human health (Sen and Chakraborty, 2011).

The majority of the assessments have been performed using the popular DPPH (2,2-diphenyl-1-picrylhydrazyl) antioxidant assay based on the scavenging of the DPPH radical, and the antioxidant capacity quantified in different ways (EC_{50} – effective concentration to scavenge 50% of the radicals, IC_{50} – inhibition concentration of 50% of the radicals, TEAC - trolox equivalent antioxidant capacity, % of inhibition, etc.). Other methods for assessing the antioxidant capacity such as ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)), β-carotene bleaching inhibition, reducing power, and metal chelating activity assays have also been used, although in a much lower extent. Althoughresults regarding the antioxidant activity of *G. robertianum* from the various research groups were not comparable, since each antioxidant assay has a different mechanism, redox potential, reaction media, etc. (Apak et al., 2013), the diverse authors are unanimous in referring the powerful antioxidant capacity of extracts.

Some comparisons with conventional antioxidants have been made that consubstantiate the above. For example, DPPH scavenging activity promoted by Trolox, a common standard for the antioxidant capacity of a substance, was observed to be only *ca.* 20%, against around 90% by concentrated aqueous and hydroethanolic *G. robertianum* extracts obtained by membrane processes

(Paun et al., 2011; Paun et al., 2012). In a β-carotene/linoleic acid bleaching assay, the inhibition capacity of a methanolic extract of *G. robertianum* (IC_{50} = 6.8 ± 1.32 μg/mL) was found to be stronger than that of BHT (butylated hydroxytoluene) (IC_{50} = 85 ± 0.11 μg/mL), an antioxidant food additive (Ben Jemia et al., 2013). The same methanolic extract displayed a reducing power (EC_{50} = 20 ± 4.53 μg/mL) higher than that of ascorbic acid (EC_{50} = 40 ± 1.31 μg/L).

The antioxidant properties of plants are closely related to the presence of phenolic compounds, a large family broadly distributed in the plant kingdom and the most abundant secondary metabolites of plants (Dai and Mumper, 2010). A direct relation between the phenolic content and the antioxidant ability of *G. robertianum* was also observed in several studies (Table 5), i.e., the higher the extract‘s polyphenolic content, the higher the antioxidant activity. Moreover, as ethanol and methanol are typically better solvents for polyphenols’ extraction than water (Azmir et al., 2013), the hydroalcoholic extracts of the plant invariably display higher antioxidant activity than the aqueous ones.

The polyphenolic content of the *G. robertianum* extracts and, consequently, their antioxidant capacity, could be improved, sometimes significantly, by the purification and concentration of different extracts by membrane-based procedures, namely microfiltration, ultrafiltration and nanofiltration (Neagu et al., 2010b; Neagu et al., 2013; Paun et al., 2011; Paun et al., 2012, Paun et al., 2014). Although membrane based filtration techniques have become an important industrial separation technique and has been applied extensively to various fields, single membrane techniques, such as micro-, ultra- and nanofiltration only recently started to be regarded as interesting techniques for the purification of natural products from plant sources due to their inherent advantages (Li and Chase, 2010).

4.2. Antimicrobial activity

Investigation on the antimicrobial activity of *G. robertianum* was referred for the first time a decade ago (Hersch-Martínez et al., 2005). A commercial essential oil, obtained by steam distillation, was tested against a number of locally prevalent pathogenic bacteria strains isolated from pediatric patients in Mexico, by the Kirby-Bauer agar diffusion method, but no significant activity was observed.

Another *G. robertianum* essential oil, also of commercial origin, was investigated by Hungarian researchers and found to be relatively effective against Gram-positive *Staphylococcus epidermidis* and against two strains of *Saccharomyces cerevisiae* (Schelz et al., 2006) (Table 6). In both studies (Hersch-Martínez et al., 2005; Schelz et al., 2006) the parts of the plant used for the isolation of the essential oil was not mentioned.

In a more complete survey, the antimicrobial activity of essential oils of *G. robertianum* (aerial and underground parts) native from Serbia, obtained by hydrodistillation (Clevenger type), was evaluated against a panel of microorganisms including several Gram-positive and Gram-negative bacterial strains, and fungal strains (Table 6), which are very common molds in human habitats and also responsible for human respiratory allergic diseases (Radulović et al., 2012). The oil from the underground parts revealed higher antibacterial and antifungal activity than the one from the aerial parts, the former having found only one resistant strain (*Salmonella enteritidis* ATCC 13076) at the tested concentrations. The *G. robertianum* oils studied exhibited the strongest activity against *Escherichia coli* and *Aspergillus fumigatus*. The resulting microbicidal activities were attributed to the presence of the main oils constituents that have established antimicrobial properties such as caryophyllene oxide, phytol and hexadecanoic acid.

An aqueous extract of commercially available *G. robertianum* (whole plant) was observed to display bactericidal action against two closely related species of *Streptococcus mutans* and *Streptococcus sobrinus*, associated to human cariogenesis (Lima, 2009). The determined MICs (minimum inhibitory concentrations) are at least of the same order as those of other medicinal plants commonly used in the treatment and prevention of oral diseases such as caries and dental plaque (Alviano et al., 2008; Song et al., 2006, Tsai et al., 2007).

The antimicrobial activity of *G. robertianum* was also tested against *Mycobacterium tuberculosis* H_{37}Rv, the best-characterized strain of this pathogen. However, in a survey of the antimycobacterial activity of 107 plants from Turkey, a 70% aqueous ethanol extract from the aerial parts of plant did not display any relevant activity amongst the studied specimens (Tosun et al., 2005).

Recently, the antimicrobial efficacy of herbal drops composed of essential oils of *G. robertianum*, *Syzygium aromaticum* L. and *Lavandula angustifolia* Mill. in patients with acute external otitis, a condition caused primarily by bacterial infection, with *Pseudomonas aeruginosa* and *Staphylococcus aureus* being the most common pathogens (Schaefer and Baugh, 2012), was investigated and compared to that of ciprofloxacin, a widely used antibiotic for its treatment (Panahi et al., 2014). The herbal composition was found to be as effective as ciprofloxacin 0.3% in terms of antibiotic effects, as well as alleviating pain and symptoms associated to acute external otitis.

4.3. Anti-inflammatory activity

The anti-inflammatory activity of a commercially available 50% aqueous ethanolic extract of *G. robertianum* was investigated for its ability to scavenge hypochlorous acid (HOCl), the major strong oxidant produced by neutrophils, which plays an important role in inflammation processes (Amaral et al., 2009). The extract was found to be moderately protective against HOCl with an IC_{50}

of 111.94 ± 1.79 μM (concentration able to inhibit 50% of HOCl-mediated 5-thio-2-nitrobenzoic acid oxidation) when compared to quercetin, a flavonol considered to be a very effective antioxidant (Silva et al., 2002), which was used as positive control presenting an IC_{50} of 34.22 ± 0.72 μM.

Piwowarski et al. (2011) determined the anti-hyaluronidase and anti-elastase activity of aqueous extracts of the aerial parts of *G. robertianum* native from Poland. Hyaluronidase and elastase are two enzymes that participate in the degradation of the extracellular matrix, a process that plays an essential role in the development of many diseases with inflammatory background (Adair-Kirk and Senior, 2008). The assays showed 7.2% hyaluronidase inhibition and 34.7 % elastase inhibition, at a concentration of 10 μg/mL.

The anti-inflammatory action of aqueous extracts of *G. robertianum* from Poland on human THP-1 cell line-derived macrophages was recently demonstrated (Piwowarski et al., 2014). It was shown that the plant is a source of bioavailable gut microbiota metabolites, i.e. urolithins, which have a detrimental action on the pro-inflammatory functions of the macrophages, clearly indicating that in the case of the perioral use of the plant the bioactivity of gut microbiota metabolites has to be taken into consideration. Urolithins are well established as gut microbiota catabolites of dietary ellagitannins in various animals and are most likely the ultimate molecular species responsible for the health benefits usually atributed to ellagitannins and ellagic acid (Espín et al., 2013). Geraniin, the main ellagitannin in the *Geranium* genus (Harborne and Williams, 2004; Okuda et al., 1980), was observed to be a metabolic source of several urolithins exhibiting potent antioxidant activities, higher than that of the intact geraniin (Ito, 2011).

4.4. Anti-hyperglycaemic activity

Ferreira et al. (2010) evaluated the anti-hyperglycaemic effect of decoctions of *G. robertianum* leaves from Portugal in Goto–Kakizaki rats, a non-obese spontaneous animal model of type 2 diabetes mellitus. The results showed that the oral administration of the decoctions over a period of four weeks lowered the plasma glucose levels in the diabetic rats. Additionally, it was demonstrated that the *G. robertianum* decoctions improved liver mitochondrial respiratory parameters and increased oxidative phosphorylation efficiency, which is particularly relevant since mitochondrial impairment is a common feature of several metabolic alterations, including diabetes mellitus (Sivitz and Yorek, 2010).

4.5. Enzyme's inhibitory activity

The inhibitory activity of *G. robertianum* against the enzymes urease and α-chymotrypsin was recently demonstrated for aqueous extracts (Paun et al., 2014). Urease is central to the

virulence of *Helicobacter pylori* and hence plays an important role in the pathogenesis of peptic ulcers and gastric cancer (Follmer, 2010). α-Chymotrypsin, a serine protease enzyme, is an important target for protease inhibitors, a well-established class of cancer chemopreventive agents (Kennedy, 1998). Polyphenol-rich extracts, obtained by purification and concentration through membrane micro- and ultrafiltration, showed significant urease inhibition activity (91.96%) and moderate inhibition of α-chymotrypsin (*ca.* 50%). The authors suggested that the enzyme inhibitory effect is due to the synergistic actuation of the polyphenolic compounds present in the extracts. Different polyphenols (present in extracts or as pure compounds) from natural origin have been reported to display inhibitory activity against urease (Modolo et al., 2015) and serine proteases (Ismail et al., 2012; Rachel and Sirisha, 2014).

An aqueous extract obtained from decoction of commercially available *G. robertianum* was also tested for the inhibition of acetylcholinesterase (Lima, 2009). This enzyme has been an important target of the strategy for the treatment of Alzheimer's disease, a neurodegenerative condition characterized by a cholinergic deficit (decrease of acetylcholine levels). The observed anticholinesterase activity (IC_{50} = 765.9 ± 15.4 μg/mL) is not noteworthy when compared to that of other specimens (Adewusi, 2010; Mukherjeea, 2007), although the majority of the studies regarding plants acetylcholinesterase inhibitory activity have been performed using methanol and ethanol extracts of the plants rather than water extracts.

4.6. Cytotoxic activity

Despite being used in the folk medicine of several countries for the treatment of cancer, the evaluation of the toxicity of *G. robertianum* against cancer cells has rarely been addressed.

Recently, Paun et al. (2012) assessed the cytotoxity of aqueous and 50% aqueous ethanolic extracts of *G. robertianum* plants from Romania purified and concentrated by membrane processes (micro- and ultrafiltration), against human epidermoid laryngeal carcinoma cells (Hep-2p) and normal monkey kidney cells (extract dose of 1.5 mg/L). The concentrated extracts displayed very low cytotoxicity against healthy cells (4.7 - 22.3% in the 50% aqueous ethanolic extracts; 1.5 - 9.2% in the aqueous extracts), but a significant cytotoxic effect on Hep-2p cancer cells (6.1 - 25.9% in the 50% aqueous ethanolic extracts; 0.9 - 32.5% in the aqueous extracts). The highest selective cytotoxicity was showed by one of the aqueous extracts. The effect of the concentrated aqueous extracts on the viability of the HEp-2p cells was demonstrated to be dependent on the exposure time and concentration, the later playing a much more important role (Neagu et al., 2013). A direct correlation between the cytotoxic effect and the polyphenols content of extracts could also be established. The ability of this important group of phytochemicals to inhibit cancer cell growth is now well recognized (Kampa et al., 2007).

5. Conclusions

Following an intrinsic characteristic of its taxonomic genera, the phytochemistry of *Geranium robertianum* L. is clearly governed by phenolic constituents. To date, studies concerning the chemical characterization of *G. robertianum* have been directed predominantly towards solid-liquid extracts, targeting with special emphasis phenolic compounds, particularly flavonoids. The compositional analysis of the essential oils has received lesser attention, but conversely, the number of identified compounds is considerably higher.

The scientific evaluation of the beneficial properties of *G. robertianum* for human health, which have long been recognized in folk medicine and herbalism, has being only barely addressed, mostly in the last decade, probably as a consequence of the growing interest on herbal medicines. So far the established bioactive properties of *G. robertianum* such as antioxidant, antimicrobial, anti-inflammatory, anti-hyperglycaemic and cytotoxic activities, appear to be associated with the type and quantity of particular phenolic compounds present and seem to corroborate the beneficial properties of the plant ascribed by the traditional medicine.

Despite the number of compounds hitherto identified in *G. robertianum*, its full phytochemical complexity still remains to be explored. In parallel, much additional investigation is necessary to adequately understand the relation between the chemical composition and the biological properties, and to establish the molecular entities eventually responsible for the therapeutic properties attributed to this plant.

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Figure Captions:

Figure 1. Chemical structure of Geraniin (**3**).

Figure 2. Phenolic acids found in *Geranium robertianum* L. extracts.

Figure 3. Some flanovoids and flavonoid glycosides found in extracts of *Geranium robertianum* L..

Figure 4. Some of the major compounds found in the essential oils of *Geranium robertianum* L..

Scheme Captions:

Scheme 1: Ellagic acid from the hydrolysis of ellagitannins

Table 1
Medicinal uses of *Geranium robertianum* L. and common modes of administration.

Part used	Mode of administration (preparation)	Popular uses/therapeutic properties	References
Flowers	Oral (infusion)	Gripes, headaches, stomach problems, liver problems	Ribeiro et al. (2000)
	Gargle (maceration)	Tonsillitis	Ribeiro et al. (2000)
Flowers and leaves	Oral (infusion)	Diarrhea	Ribeiro et al. (2000)
	Rubbing (decoction[a])	Rheumatic	Marc et al. (2008)
Leaves	Oral (infusion/decoction)	Prevention of cancer, diabetes, astringent	Ferreira et al. (2010), Loi et al. (2004), Ribeiro et al. (2000)
	Mouthwashes/gargles (infusion/decoction)	Oropharyngeal inflammation	Gruenwald et al. (2000)
	Topical (infusion/decoction)	Poorly healing wounds, mosquito bites	Gruenwald et al. (2000), Loi et al. (2004)
Aerial parts	Oral (decoction/infusion)	Diabetes, diarrhea, gastritis, gout, gallbladder inflammation, kidney inflammation, liver problems, bladder inflammation, calculosis, sinuses diseases, nose bleeding, sciatica, tonic, hypertension, diuretic, cancer, antispasmodic	Bnouham et al. (2002), Cunha et al. (2012), Gruenwald et al. (2000), Menkovic et al. (2011), Neto and Simões (2007), Ribeiro et al. (2000)
	Poultice	Antipneumonic	Rigat et al. (2013)
	Gargle	Sore throat	Rigat et al. (2013)
	Topical (infusion/decoction)	Poorly healing wounds, mild rashes, osteoarticular diseases, parasitosis of the scalp, oropharyngeal inflammation	Cunha et al. (2012), Guarrera (1999), Menkovic et al. (2011), Neto and Simões (2007)
	Topical (liniment)	Labial herpes	Rigat et al. (2007)
	Compress (decoction)	Sciatica	Marc et al. (2008)
Leaves and roots	Oral (decoction)	Cholesterol	Said et al. (2002)
Whole plant	Topical (decoction)	Ovine, cattle and horses scab	Menale and Muoio (2014)

[a]In association with *Ranunculus* sp. (aerial parts), *Juglans regia* L. (leaves), *Origanum syriacum* L., *Pinus pinea* L. (Oleoresin) and olive oil.

Table 2
Composition of extracts of *Geranium robertianum* L.

Part used	Extract	Component	Quantity	Method of analysis	Reference
Leaves[a]	H_2O	Ellagic acid	744.15[h]	HPLC-PDA	Neagu et al. (2013)
		Quercetin-3-*O*-rutinoside	1.73[h]		
		Luteolin	3.56[h]		
		Kaempferol	1.86[h]		
		Gallic acid	978.86[h]		
Leaves[a]	H_2O	Chlorogenic acid		HPLC-PDA-ESI-MS	Santos et al. (2013)
		Ellagic acid			
		Quercetin			
		Homoeriodictyol			
		Kaempferol			
Leaves[b]	Me_2CO/H_2O (50/50)	Geraniin	9.8[i]	HPLC-UV	Okuda et al. (1980)
Leaves[a]	2 N aq. HCl	Kaempferol		Paper chromatography	Bate-Smith (1962)
		Quercetin			
		Ellagic acid			
		Caffeic acid			
		Ferulic acid			
Aerial parts[a]	H_2O	Lutein	92.3[h]	HPLC-PDA	Loranty et al. (2010)
Aerial parts[a]	MeOH	Quercetin-3-*O*-glucoside		UV-Vis, ^{1}H-NMR, DC	Kartnig and Bucar-Stachel (1991)
		Quercetin-3-*O*-rhamnogalactoside			
		Quercetin-3-*O*-rhamnoside			
		Quercetin-3-*O*-rutinoside			
		Quercetin-4′-*O*-glucoside			
		Quercetin-7-*O*-glucoside			
		Quercetin-3-*O*-galactoside			
		Kaempferol-3-*O*-glucoside			
		Kaempferol-3-*O*-rhamnogalactoside			
		Kaempferol-3-*O*-rutinoside			
Aerial parts[c]	AcOEt	Quercetin-3-*O*-galactoside		UV, IR, m.p.	Kobakhidze and Alaniya (2004)
		Quercetin			
		Kaempferol			
		Ellagic acid			
		Caffeic acid			
		Gallic acid			
Whole plant[a]	H_2O	Quercetin-3-*O*-rutinoside		HPLC-PDA	Paun et al. (2011)
		Quercetin			
		Kaempferol			
		p-Coumaric acid			
		Caffeic acid			
		Ferulic acid			
Whole plant[a]	H_2O	Quercetin-3-*O*-rutinoside	2.67[h]	RP-HPLC-PDA	Paun et al. (2012)
		Quercetin	4.83[h]		
		p-coumaric acid	0.77[h]		
		Caffeic acid	8.18[h]		
		Ferulic acid	1.18[h]		

Table 2 (*Continued*)

Part used	Extract	Content/Component	Quantity	Method of analysis	Reference
Whole plant[a]	H_2O	Ellagic acid	900.13[h]	HPLC-PDA-MS	Paun et al. (2014)
		Gallic acid	1070.78[h]		
		Quercetin-3-*O*-rutinoside	23.83[h]		
		Luteolin	4.03[h]		
		Kaempferol	1.96[h]		
Whole plant[d]	H_2O	Geraniin		UHPLC-PDA-MS/MS	Piwowarski et al. (2014)
Whole plant[e]	EtOH/H_2O (50/50)	Quercetin-3-*O*-rutinoside	38.95[h]	RP-HPLC-PDA	Paun et al. (2012)
		Quercetin	54.60[h]		
		Kaempferol	284.57[h]		
		p-coumaric acid	9.22[h]		
		Caffeic acid	20.18[h]		
		Ferulic acid	11.00[h]		
Whole plant[f]	EtOH/H_2O (50/50)	Quercetin-3-*O*-galactoside	36.4[h]	HPLC-UV	Fodorea et al. (2005)
		Ellagic acid	75997.6[h]		
		Quercetin-3-*O*-glucoside	494.9[h]		
		Quercetin-3-*O*-rutinoside	722.3[h]		
		Quercetin	839.2[h]		
		Kaempferol	1434.3[h]		
		Caftaric acid	1669.2[h]		
Whole Plant[g]	MeOH	Kaempferol		Chromatography[h]	Ivancheva and Petrova (2000)
		Kaempferol 3-methyl ether			
		Kaempferol 4'-methyl ether			
		Kaempferol 3,7,4'-trimethyl ether			
		Quercetin 3,7-dimethyl ether			
		Quercetin 3,3'-dimethyl ether			
		Quercetin 3,7,3'-trimethyl ether			
		Kaempferol-3-*O*-rhamnoside			
		Quercetin-3-*O*-rhamnoside			
		Quercetin-3-*O*-rutinoside			
Whole Plant[f]	MeOH/ 2 M aq. HCl	Caffeic acid	66.2[h]	HPLC-UV	Fodorea et al. (2005)
		Ellagic acid	105506.5[h]		
		Quercetin	2034.5[h]		
		Kaempferol	2318.0[h]		
		Caftaric acid	474.1[h]		
Commercial extract[a]	EtOH/H_2O (50/50)	Acetovanillone		ESI-MS/MS, SPE/LC-PDA	Amaral et al. (2009)
		Gallic acid 3,5-dimethyl ether			
		3',4'-Dimethoxyflavone			
		Homoeriodictyol			
		Ferulic acid methyl ester			
		Ferulic acid ethyl ester			
		Kaempferol			

[a] Unspecified origin. [b] Plant native from Japan. [c] Plant native from Georgia. [d] Plant native from Poland. [e] Plant native from Romania. [f] Presumably whole plant, native from Romania. [g] Presumably whole plant, native from Bulgaria. [h] µg/g dw. [i] % dry leaves. [j] Technique not specified. CD - Circular dichroism. Dw - dry weight. ESI-MS/MS - Electrospray ionization tandem mass spectrometry. ^{1}H-NMR - Proton Nuclear Magnetic Resonance. HPLC-PDA - High-performance liquid chromatography -photodiode array detection. HPLC-PDA-ESI-MS - HPLC-PDA-electrospray ionization-mass spectrometry. HPLC-PDA-MS - HPLC-PDA-mass spectrometry. HPLC-UV - HPLC-utraviolet

detection. IR - Infrared. . m.p. - Melting point. RP-HPLC-PDA - Reversed phase-HPLC-PDA. SPE/LC-PDA - Solid phase extraction/liquid chromatography-PDA. UHPLC-PDA-MS/MS - Ultra-HPLC-PDA-tandem mass spectrometry. UV - Ultraviolet. UV-Vis - Ultraviolet-Visible.

Table 3
Content of metal elements in *Geranium robertianum* L. extracts.

Extract	Content of element, mg L^{-1}						
	Ca	Mg	Mn	Zn	Fe	Ni	Pb
H_2O	0.935 ± 0.08	10.4 ± 0.3	0.893 ± 0.07	0.071 ± 0.006	3.2 ± 0.1	0.051 ± 0.003	< DL
EtOH/H_2O (50/50)	0.927 ± 0.08	9.78 ± 0.7	0.819 ± 0.07	0.069 ± 0.006	1.8 ± 0.1	0.047 ± 0.003	< DL

DL - detection limit.

Table 4
Percentage composition of essential oils of *Geranium robertianum* L.

Component	Percentage				Component	Percentage			
	A	B	C	D		A	B	C	D
Fatty acids and fatty acid derived compounds					Octadecanoic acid	-	-	0.9	0.8
2-Butylfuran	-	-	0.1	-	1-Octadecanol	-	-	-	0.3
(2*E*,4*Z*)-Decadienal	-	0.1	-	-	(Z)-9-Octadecenoic acid	-	-	0.6	0.6
Decanal	0.3	0.1	0.1	-	Octanal	-	-	0.1	-
Decane	-	-	tr	-	Octanoic acid	-	-	0.1	tr
Decanoic acid	-	0.1	0.6	tr	1-Octanol	-	-	0.8	-
3,4-Dimethyl-5-pentyl-2(	-	-	-	tr	*(E)*-2-Octenal	-	-	tr	-
5H)-furanone[a]					Octyl formate	-	-	0.1	-
Docosanal	-	-	0.1	-	γ-Palmitolactone	-	-	0.4	0.4
Docosane	-	-	0.3	tr	Pentacosane	-	-	1.3	28.5
1-Docosanol	-	-	tr	-	Pentadecanal	-	-	0.8	tr
Dodecanal	-	0.1	0.1	-	Pentadecane	0.1	-	0.7	tr
Dodecanoic acid	-	10.3	1.0	0.5	Pentadecanoic acid	-	-	1.8	2.3
1-Dodecanol	0.1	1.5	0.1	tr	1-Pentadecanol	-	-	0.8	tr
Eicosanal	-	-	0.1	-	(*E*)-2-Pentenal	-	-	tr	-
Eicosane	-	-	0.7	tr	(*Z*)-2-Pentenol	-	-	tr	-
1-Eicosanol	-	-	tr	tr	2-Pentylfuran	tr	-	tr	tr
Ethyl hexadecanoate	-	-	0.4	-	Tetracosane	-	-	0.9	8.9
Heneicosanal	-	-	tr	-	γ-Tetradecalactone	-	-	-	tr
Heneicosane	-	-	3.9	0.5	Tetradecanal	-	-	0.3	tr
Heptadecane	0.3	-	0.3	tr	Tetradecane	tr	-	0.4	-
Heptadecanoic acid	-	-	0.3	0.6	Tetradecanoic acid	-	7.0	3.4	2.4
Heptanal	-	-	tr	tr	1-Tetradecanol	0.6	-	0.9	0.6
Heptanoic acid	-	-	tr	tr	(*E*)-2-Tetradecen-1-ol	-	-	0.2	-
Hexadecanal	-	-	-	tr	Tricosane	-	-	4.8	0.7
Hexadecane	0.2	-	0.3	tr	Tridecanal	-	-	tr	tr
Hexadecanoic acid	-	33.4	16.6	45.3	Tridecane	-	-	0.1	-
1-Hexadecanol	0.9	-	tr	tr	1-Tridecanol	0.3	-	0.3	tr
Hexanal	-	-	0.1	tr	2-Tridecanone	-	-	0.1	tr
Hexanoic acid	-	-	0.3	tr	Undecanal	-	-	0.3	-
1-Hexanol	-	-	0.1	-	Undecanoic acid	-	-	0.2	-
(*E*)-2-Hexen-1-ol	-	-	tr	-	1-Undecanol	-	-	tr	-
(*Z*)-3-Hexen-1-ol	-	-	0.1	-					
(*E*)-2-Hexenoic acid	-	-	0.3	-	*Hemiterpenoids*				
(*Z*)-3-Hexenyl formate	-	-	tr	-	3-Methyl-2-butenal	-	-	0.1	-
Methyl hexadecanoate	-	-	0.4	0.6	(*E*)-2-Methyl-2-butenoic acid	-	-	tr	-
Methyl tetradecanoate	-	-	-	0.4					
3-Methyl-2-hexanone	-	-	0.2	tr	*Monoterpenoids*				
Nonadecane	-	-	0.7	tr	Borneol	0.1	-	1.0	-
Nonanal	-	-	0.4	-	Borneol isomer	0.4	-	-	-
Nonanoic acid	-	0.1	0.8	tr	Bornyl acetate	-	-	0.1	-
1-Nonanol	-	0.1	-	-	Bornyl formate	-	-	0.2	-
(*Z*)-2-Nonenal	-	-	tr	-	Camphene	0.2	-	-	-
(*Z*,*Z*)-9,12-Octadecadienoic	-	3.7	-	-	6-Camphenone	0.1	-	-	-
acid					Camphor	-	-	0.1	-
Octadecanal	-	-	tr	-	Δ^3-Carene	0.2	-	-	-
Octadecane	-	-	0.4	tr	Carvacrol	-	-	tr	-

Table 4 (*Continued*)

Component	Percentage				Component	Percentage			
	A	B	C	D		A	B	C	D
Carvacrol methyl ether	-	-	0.2	-	α-Terpineol	3.8	0.1	0.5	-
trans-Carveol	0.1	-	0.5	-	Terpinolene	0.7	-	-	-
Carvone	-	-	tr	-	α-Thujene	1.0	-	-	-
Carvone hydrate	-	-	0.4	-	Thymol methyl ether	-	-	0.1	-
Cuminal	-	-	0.1	-	Tricyclene	2.2	-	-	-
p-Cymen-7-ol	-	-	0.1	-					
p-Cymen-8-ol	-	-	0.6	-	*Sesquiterpenoids*				
p-Cymene	0.4	-	0,1	-	α-Bisabolol	-	0.9	-	-
p-Cymenene	-	-	tr	-	β-Bourbonene	0.2	-	0.3	-
2,6-Dimethyl-1,7-octadien-	-	-	0.7	-	Cadalene	-	-	tr	-
-3,6-diol[a]					γ-Cadinene	0.1	1.1	tr	-
2,6-Dimethyl-3,7-octadiene-	-	-	0.8	-	δ-Cadinene	0.4	4.0	-	-
-2,6-diol					α-Cadinol	0.5	3.5	0.9	-
α,*p*-Dimethylstyrene	0.2	-	-	-	δ-Cadinol	0.1	-	-	-
(E)-Furan linalool oxide	-	-	0.6	-	τ-Cadinol	0.2	-	-	-
(Z)-Furan linalool oxide	0.2	-	0.6	-	α-Calacorene	-	-	tr	-
Geraniol	4.4	0.1	tr	-	β-Calacorene	-	-	0.2	-
Geranylacetone	tr	0.2	-	-	*trans*-Calamenene	-	-	tr	-
Hotrienol	-	-	tr	-	Caryophylla-4(12),8(13)-	-	-	0.5	-
exo-2-Hydroxycineole	-	-	0.4	-	dien-5-β-ol				
p-Isopropylbenzoic acid	-	-	tr	tr	β-Caryophyllene	0.2	0.4	-	-
cis-Jasmone	0.1	-	-	-	Caryophyllene oxide	0.3	0.7	5.4	-
Limonen-4-ol	-	-	tr	-	Clovane-2,9-diol[a]	-	-	1.2	-
Limonene	5.3	-	-	-	α-Copaene	0.1	0.1	-	-
Linalool	22.9	1.1	1.4	-	Cubeban-11-ol	-	0.4	-	-
Trans-p-Menth-2-en-1-ol	tr	-	-	-	Cubebol	-	1.5	-	-
trans-p-Menth-6-en-2,8-diol	-	-	0.2	-	β-Elemene	0.4	0.1	0.2	-
Menthol	0.1	-	-	-	β-Eudesmol	-	-	0.8	-
O-Methylthymol	0.2	-	-	-	Farnesane[a]	-	-	0.2	-
β-Myrcene	3.3	-	-	-	α-Farnesene	tr	-	-	-
Nerol	1.5	-	-	-	*trans*-β-Farnesene	0.9	-	-	-
allo-Ocimene	0.1	-	-	-	Germacrene-D	7.8	0.9	-	-
cis-β-Ocimene	0.4	-	-	-	Globulol	-	-	0.2	-
trans-β-Ocimene	0.5	-	-	-	Homofarnesane[a]	-	-	0.4	-
α-Phellandrene	0.1	-	tr	-	α-Humulene	0.1	-	-	-
β-Phellandrene	0.2	-	-	-	Humulenepoxide II	-	-	0.8	-
α-Pinene	0.4	-	-	-	α-Isocomene	-	-	0.1	-
β-Pinene	0.1	-	-	-	β-Isocomene	-	-	0.1	-
trans-Pinocarveol	0.1	-	-	-	Longifolene	-	0.1	-	-
Pinocarvone	0.1	-	-	-	Modhephene	-	-	0.2	-
Piperitone	-	-	tr	-	α-Muurolene	0.2	-	-	-
(*E*)-Pyran linalool oxide	-	-	0.8	-	γ-Muurolene	0.8	-	-	-
(*Z*)- Pyran linalool oxide	-	-	0.7	-	*epi*-α-Muurolol	-	1.8	0.3	-
Sabinene	0.6	-	-	-	τ-Muurolol	0.1	-	-	-
trans-Sabinene hydrate	tr	-	-	-	(*E*)-Nerolidol	0.4	-	-	-
α-Terpinene	0.4	-	-	-	β-Selinene	-	-	0.4	-
γ-Terpinene	13.9	-	-	-	α-Ylangene	0.1	-	-	-
Terpinen-4-ol	0.9	0.1	0.1	-					

Table 4 (*Continued*)

Component	Percentage				Component	Percentage			
	A	B	C	D		A	B	C	D
Diterpenoids					Benzyl alcohol	-	-	0.4	-
Isophytol	-	-	0.9	-	Benzyl benzoate	0.3	-	0.4	-
13-*epi*-Manoyl oxide	-	-	1.1	1.5	Benzyl salicylate	-	-	tr	-
Neophytadiene (isomer I)	-	-	0.3	-	5,5-Dimethyl-2(5*H*)-furanone	-	-	0.1	-
Phytol	3.8	4.8	1.9	-	5,5-Dimethyl-4-(3-oxobutyl)-	-	-	0.2	-
8(14),15-Pimaradiene	-	-	0.5	-	dihydro-2(3*H*)-furanone				
					Furfural	-	-	tr	-
Carotenoid derived compounds					*cis*-Hex-2-enal	0.3	-	-	-
Dihydroactinidolide	-	-	0.2	tr	(*Z*)-3-Hexenyl benzoate	-	-	tr	-
2,6-Dimethylundecane	-	-	tr	-	5-Methyl-5-(4,8,12-tri-	-	-	tr	-
(5*E*, 9*E*)-Farnesyl acetone	-	1.3	-	-	methyltridecyl)dihydro-				
Hexahydrofarnesyl acetone	-	-	6.5	1.9	2(3*H*)-furanone				
β-Ionone	0.4	0.9	-	-	Phenanthrene	-	-	tr	tr
(*E*)-β-Ionone 5,6-epoxide	-	-	tr		Phenylacetaldehyde	tr	-	1.7	-
Ishwarane	-	-	0.2	-	Phenylacetic acid	-	-	0.9	-
6-Methyl-5-hepten-2-one	-	-	tr	-	Pyridine	-	-	0.1	-
Tetrahydrogeranyl acetone	-	-	0.2	-	Toluene	-	-	tr	-
					1,1,6-trimethyl-1,2-dihydro-	0.1	-	-	-
Others[b]					naphthalene				
Benzaldehyde	0.1	-	0.4	-	1,1,6-trimethyl-1,2,3,4-tetra-	0.2	-	-	-
Benzoic acid	-	-	0.3	tr	hydronaphthalene				

[a] Correct isomer not defined. [b] Unclassified constituents; compounds of possible anthropogenic origin. A - Aerial parts of *Geranium robertianum* native from Leiden, Netherlands (Pedro et al., 1992); B - Aerial parts of *G. robertianum* native from Midi-Pyrénées, France (Zhao, 2014). C - Aerial parts of *G. robertianum* native from Suva Planina Mountain, Serbia (Radulović et al., 2012). D - Underground parts native from Suva Planina Mountain, Serbia (Radulović et al., 2012). tr - Trace amounts (<0.05%).

Table 5
Antioxidant activity and total phenolics content of *Geranium robertianum* L. extracts.

Part used	Extract	Method	Antioxidant activity	Total Phenolics Content	References
Leaves[a]	H_2O	DPPH (EC_{50},[d] μg/mL)	4.53 ± 0.26	~ 60 %	Santos et al. (2013)
Whole plant[a]	H_2O	DPPH (EC_{50},[d] μg/mL)	6.53 ± 0.58	106.96 ± 2.37 μg PE/mg	Lima (2009)
Whole plant[a]	H_2O	DPPH (% inhibition)	88.0[f]	1449 ± 8.6 mg GAE/L	Paun et al. (2011)
			92.9[g]	1910.7 ± 11.2 mg GAE/L	
Leaves[a]	H_2O	DPPH (% inhibition)	69.07[h]	-	Neagu et al. (2013)
			73.30[i]	745.61 ± 9.1 μg/mL	
			81.02[j]	763.89 ±9.3 μg/mL	
		DPPH (μmol TE/g)	169.02 ± 6.86[h]	-	
			180.91 ± 6.04[i]	-	
			216.39 ± 6.64[j]	-	
		ABTS (μmol TE/g)	539,56 ± 11,21[h]	-	
			573.20 ± 9.29[i]	-	
			1286.96 ± 3.89[j]	-	
Leaves[a]	H_2O	DPPH (% inhibition)	80.0[h]	3.15 mg CAE/mL	Neagu et al. (2010b)
			92.6[i]	4.22 mg CAE/mL	
			95.3[k]	4.68 mg CAE/mL	
		DPPH (μmol TE/g)	223.99	-	
			220.20[h]	-	
			418.02[i]	-	
			464.40[k]	-	
		ABTS (μmol TE/g)	768.22	-	
			772.10[h]	-	
			1239.93[i]	-	
			2609.00[k]	-	
Leaves[a]	$EtOH/H_2O$ (50/50)	DPPH (% inhibition)	86	4.21 mg CAE/mL	
		DPPH (μmol TE/g)	217.06	-	
		ABTS (μmol TE/g)	1509.27	-	
	$EtOH/H_2O$ (70/30)	DPPH (% inhibition)	90.4	3.38 mg CAE/mL	
		DPPH (μmol TE/g)	242.75	-	
		ABTS (μmol TE/g)	782.30	-	
Aerial parts[b]	$MeOH/H_2O$ (80/20)	DPPH (IC_{50},[e] μg/mL)	14.93	-	Nikolova et al. (2010)
Leaves[c]	MeOH	DPPH (IC_{50},[e] μg/mL)	19.98 ± 0.05	32.24 ± 0.02 mg GAE/g	Ben Jemia at al. (2013)
		β-Carotene/linoleic acid (IC_{50},[e] μg/mL)	6.8 ± 1.32	-	
		Reducing power (EC_{50},[d] μg/mL)	20 ± 4.53	-	
		Chelating power	20[l]	-	

[a] Unspecified origin. [b] Plant native from Bulgaria. [c] Plant native from Tunisia. [d] EC_{50} - concentration at which antioxidant activity is 50%. [e] IC_{50} - concentration at which inhibition is 50%. [f] Concentrated successively by MF, UF1 and NF1. [g] Concentrated successively by MF,UF1 and NF2. [h] Purified by MF. [i] Concentrated successively by MF and UF. [j] Concentrated successively by MF, UF1 and UF2. [k] Concentrated successively by MF and UF3. [l] PI of chelating metal (as %). AAE - $_L$-Ascorbic acid equivalent. ABTS - 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid). CAE - Caffeic acid equivalent. CE - Catechin equivalents. DPPH - 2,2-Diphenyl-1-picrylhydrazyl. GAE - Gallic acid equivalent. MF - microfiltration using 0.45 μm pore size Millipore membrane. NF1 - nanofiltration using Koch SelRO MPF-36 membrane. NF2 - nanofiltration using polysulphone/SBA-15-NH_2 membrane. PE - Pyrogallol equivalente. TE - Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalent. UF1 - ultrafiltration using Millipore membrane with 10000 Da cut-off. UF2 - ultrafiltration using Millipore membrane with 1000 Da cut-off. UF3 - ultrafiltration using polysulphone membrane with 10000 Da cut-off.

Table 6
Minimum inhibitory concentrations (MIC) of *Geranium robertianum* L. extracts.

Microorganism	MIC (mg/mL)				References
	A	B	C	D	
Gram-positive bacteria					
Staphylococcus epidermidis (clinical isolate)	5.6	-	-	-	Schelz et al. (2006)
Staphylococcus aureus ATCC 25923	-	10.0	10.0	-	Radulović et al. (2012)
Staphylococcus aureus (clinical isolate)	-	5.00	5.00	-	
Clostridium sporogenes ATCC 19404	-	5.00	5.00	-	
Sarcina lutea ATCC 9341	-	10.0	10.0	-	
Micrococcus flavus ATCC 10240	-	2.50	5.00	-	
Bacillus subtilis ATCC 6633	-	5.00	2.50	-	
Streptococcus sobrinus CETC 4010	-	-	-	19.70	Lima (2009)
Streptococcus mutans CETC 479	-	-	-	12.99	
Gram-negative bacteria					
Escherichia coli (clinical isolate)	-	0.312	0.156	-	Radulović et al. (2012)
Escherichia coli ATCC 25922	-	10.0	5.00	-	
Escherichia coli ATCC 8379	-	5.00	2.50	-	
Escherichia coli Torlak 95	-	>10.0[a]	5.00	-	
Klebsiella pneumoniae ATCC 10031	-	>10.0[a]	0.156	-	
Klebsiella pneumoniae (clinical isolate)	-	5.00	5.00	-	
Proteus vulgaris ATCC 8247	-	2.50	10.0	-	
Salmonella enteritidis ATCC 13076	-	>10.0[a]	>10.0[a]	-	
Fungal strains					
Saccharomyces cerevisiae 0425 δ/1	1.4	-	-	-	Schelz et al. (2006)
Saccharomyces cerevisiae 0425 52C	1.4	-	-	-	
Penicillium chrysogenum (mattress dust isolate)	-	>10.0[a]	10.0	-	Radulović et al. (2012)
Aspergillus restrictus (mattress dust isolate)	-	>10.0[a]	10.0	-	
Aspergillus fumigatus (mattress dust isolate)	-	0.312	0.312	-	
Candida albicans ATCC 10231	-	10.0	5.00	-	
Saccharomyces cerevisiae ATCC 9763	-	>10.0[a]	2.50	-	

[a] Not active in the given concentration. A - Essential oil from whole plant. B - Essential oil from the aerial parts. C - Essential oil from the underground parts. D - Aqueous extract of whole plant.