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Acaricidal Properties of Extracts of Some Medicinal and Culinary Plants against *Tetranychus urticae* Koch.

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Abstract

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The acaricidal activity of aqueous extracts obtained from 28 plant species was tested against *Tetranychus urticae*. The extract efficacy on the incidence of individual development stages of *T. urticae* on the treated plants was determined, compared to untreated plants. Of the 28 extracts, 24 showed an efficacy higher than 50%; of these, 16 extracts caused a reduction in the count of adults on the plants by more than 90% compared to the control. The counts of nymphs and eggs showed significant differences depending on the extract used. Only 13 extracts showed an efficacy higher than 50%, and only 2 extracts (obtained from *Ammi visnaga* and *Saponaria officinalis*) had more than 90% efficacy. Twenty extracts resulted in eggs reduction of more than 50% compared to the control; of these, 6 extracts (from *A. visnaga*, *G. glabra*, *J. palmata*, *L. carthamoides*, *O. majorana*, *S. officinalis*) exhibited an efficacy higher than 90%. Extracts with the highest efficacy were tested for their acute toxicity for *T. urticae* adults, and $LD_{50(90)}$ values were estimated. By comparing the confidence intervals (CI_{95}) for individual LD_{50} values, extracts obtained from *Saponaria officinalis* roots and *Ammi visnaga* seeds were found to provide the significantly highest efficacies, and lethal doses (LD_{50}) of 10.3 and 12.5 g/l, respectively, were estimated for them. However, wanting to compare LD_{90} values as well, we chose 5 extracts (*A. visnaga*, *C. annum*, *M.* × *piperita*, *O. majorana*, and *S. officinalis*) whose CI_{95} intervals overlapped ($P \leq 0.05$). Based on our tests, aqueous extracts from *S. officinalis* roots can be recommended for the development of products which reduce the incidence of *T. urticae* on plants.

Keywords: acaricides; base substances; bean; plant extracts; botanical pesticides

In recent decades, environmental and health risks associated with the application of synthetic pesticides have resulted in major efforts to seek safer alternatives for the protection of plants and agricultural products, which undoubtedly also include the development of products utilizing pesticidal effects of the so-called defensive substances isolated from plants (Isman & Grieneisen 2014).

Plant-based botanical pesticides (BPs) have been suggested as potential alternatives for arthropod control, largely because they constitute a potential source of bioactive chemicals that have been perceived by the general public as relatively safe and pose fewer risks to the environment, with minimal impacts on animal and human health (Dubey 2011; Miresmailli & Isman 2014; Pavela 2014a). Moreover, botanical insecticides usually contain a mixture of several active substances which exert different mechanisms of action as a rule, and thus may be able to effectively prevent the emergence of resistant pest populations (Rattan 2010; Pavela 2014b).

Until now, BPs have been developed in the EU as commercial products that find their application primarily in ecological farming; furthermore, they play a unique role as products that replace pesticides in small-scale consumer packages designed for gardeners

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and small growers. Regulations of the EU have also been adapted to this trend; according to Regulation (EC) No. 1107/2009 of the European Parliament and of the Council, BPs are considered products that are preferable to risky synthetic products (SANCO 2015).

Moreover, Regulation (EC) No 1107/2009 introduces the new category of "basic substances" (BSs), which are defined by Article 23 as active substances that do not have an immediate or delayed harmful effect on human and animal health nor an unacceptable effect on the environment, and can be legally used in the EU after having been approved as "basic" under the Regulation (EC) No. 1107/2009.

"Basic substances" are expected to be selected from a group of substances and plants characterized as foods, as defined in Article 6 of the Council Directive 98/83/EC, and without prejudice to the requirements of the Council Directives 80/778/EEC and 98/83/EC (SANCO 2012).

Three BSs (chitosan hydrochloride, *Equisetum arvense*, and talc) were approved until the end of 2014. Additional substances which could be used as BSs (Miresmailli & Isman 2014) are currently being sought. Our study is focused on this screening as well.

The purpose of this study was to find, among plants that are commonly consumed orally (in the form of teas used in folk medicine, spices or food additives), aqueous extracts that could become a suitable replacement for synthetic acaricides. The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), was used as the target organism. *T. urticae* is considered the most polyphagous species and has been reported in numerous host plants of economic value, including major food crops and ornamental plants (Ho 2000; Gotoh *et al*. 2015), causing damage to around 1200 plant species. The rapid population growth, short developmental time, high birth rate, and long adult survival lead to a high risk of outbreaks (Zhang 2003) and, coupled with male haploidy, which exposes recessive resistance genes to selection, result in a high rate of development of resistance to acaricides. The mites have evolved a resistance to more than 80 acaricides from more than 60 countries (Attia *et al*. 2013). As a result, the two-spotted spider mite imposes a great expense on greenhouse growers worldwide in terms of damage and control costs, and is therefore considered a serious pest in greenhouse production (Zhang 2003).

In our study we focused on basic acaricidal screening of aqueous extracts whose characteristics comply with Article 23 of the Regulation (EC) No. 1107/2009; as expected by this article, a "simple solvent", such as water, should be used for the preparation and application of BSs. Twenty-eight plant species, which are used in the food industry or folk medicine, and whose aqueous extracts were tested for mortality and oviposition of *T. urticae*, were selected for the extraction.

MATERIAL AND METHODS

Plant extract and extraction. Commercial plant material (Table 1) was purchased for basic screening from a company which trades in medicinal plants and spices (Byliny Mikeš, Číčenice, Czech Republic).

Dry plant material was ground using an electric grinder and was subsequently extracted using water. The highest tested dose (50 g/l) was prepared by macerating 50 g of the plant material in 1 litre of warm water at 25 ± 1°C. The extraction was done at ambient temperature (21 ± 2°C) for 10 hours. After maceration, the application solution was separated from unmacerated material using a cloth. The application solution was applied immediately. For better interpretation, doses reported herein are given as the weight of plant material macerated in 1 litre of water.

The dry mass content (% of dissolved substances in the extract) was determined for the most efficient extracts and for the highest tested dose (50 g/l) as follows: 10 g of the extract were removed before its application and all water was evaporated in a dryer at 150°C. The weight of the non-evaporated substances was used to calculate the dry mass content, expressed in % (w/w). The determination was performed 3 times.

Spider mites and host plants. Two-spotted spider mites, *Tetranychus urticae* Koch (Acari: Tetranychidae), were obtained from the cultures maintained at the Crop Research Institute (Prague, Czech Republic). The spider mites used in the experiments were reared on bean plants (*Phaseolus vulgaris* L. cv. Carmen) in a growth chamber (22–25°C; a 12 h photoperiod).

Bioassays. The extract was applied in a dose of 50 g/l in order to determine the biological efficacy of the plant extracts on the mortality of *T. urticae* adults, as well as their effect on the inhibition of oviposition in surviving adults. These were applied to bean plants (*Phaseolus vulgaris* L. cv. Aidagold), which were planted in a regular garden substrate, in pots of 10-cm diameter. At the time the experiment was being established, 2–3 leaves were found in the growth phase (the growing top of the plants was removed before application to make sure that

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Table 1. List of plants used in the tests, and their use in traditional folk medicine and the food industry.

Plant	Family	Part used	Traditional use	References
Ammi visnaga (L.) Lam.	Apiaceae	seeds	The tea made from the fruit of this species has been used as an herbal remedy for kidney stones	Bruneton (1999); Pavela (2014c)
Angelica archangelica L.	Apiaceae	seeds	Use as tea or tincture for treatment of disorders of the gastrointestinal tract, respiratory tract, nervous system, and also against fever, infections, and flu	Sarker & Naharl (2004)
Capsicum annuum L.	Solanaceae	fruits	The species is a source of popular sweet peppers and hot chilis with numerous varieties cultivated all around the world. Capsaicin is considered a safe and effective topical analgesic agent in the management of arthritis pain, herpes zoster-related pain, diabetic neuropathy, mastectomy pain, and headaches	Bruneton (1999)
Cinchona officinalis L.	Rubiaceae	bark	It is especially useful in the prevention and treatment of malaria. *Cinchona calisaya* is the tree most cultivated for quinine production	Bruneton (1999)
Cinnamomum verum J. Presl.	Lauraceae	bark	Use as flavourant and spice in foods, cinnamon-flavoured tea, also as a spice. It is principally employed in cookery as a condiment and flavouring material. Cinnamon is a popular flavoring in numerous alcoholic beverages	Bruneton (1999)
Curcuma longa L.	Zingiberaceae	roots	In Asian region, rhizomes are widely used in many dishes, in particular in the southern Thai cuisine, such as the yellow curry and turmeric soup. Traditionally used as a remedy for stomach and liver ailments, as topically to heal sores, diseases and conditions, including those of the skin, pulmonary, and gastrointestinal systems, aches, pains, wounds, sprains, and liver disorders	Bruneton (1999)
Cymbopogon citratus (DC) Stapf	Poaceae	leaves	In the Philippines and Indonesia leaves are traditionally used in cooking. In folk medicine as stimulant, sudorific, antiperiodic, and anticatarrhal	Leite *et al.* (1986)
Eucalyptus globulus Labill.	Myrtaceae	leaves	Tasmanian blue gum leaves are used as an herbal tea	WHO (2004)
Foeniculum vulgare Mill.	Apiaceae	seeds	It is an indispensable ingredient in modern French and Italian cooking. Use as an antiaging, antiallergic, anticolitic, antihirsutism, anti-inflammatory, antimicrobial and antiviral, antimutagenic, antinociceptive, antipyretic, antispasmodic, antistress, antithrombotic, anxiolytic, apoptotic, cardiovascular, chemomodulatory action, cytoprotection and antitumor, cytotoxicity, diuretic, estrogenic properties, expectorant, galactogenic, gastrointestinal effect, hepatoprotective, human liver cytochrome P450 3A4 inhibitory, hypoglycemic, hypolipidemic, memory-enhancing property, nootropic, and oculohypotensive activities	Badgujar *et al.* (2014)
Glycyrrhiza glabra L.	Fabaceae	roots	Liquorice is found in a wide variety of candies or sweets. Dried liquorice root can be chewed as a sweet. Used for antiviral, antimicrobial, anti-inflammatory, hepatoprotective, and blood pressure-increasing effects, against atopic dermatitis, hyperlipidaemia	Bruneton (1999); Pavela (2014c)
Illicium verum Hook. F.	Schisandraceae	fruits	Use as condiment for baking, in liquor production, is widely used in Chinese cuisine, and in Indian cuisine where it is a major component of garam masala, and in Malay and Indonesian cuisines. In folk medicine used in tea as a traditional remedy for rheumatism, and the seeds are sometimes chewed after meals to aid digestion	Wang *et al.* (2011)
Jateorhiza palmata (Lam.) Miers	Ranunculales	roots	Is used mainly as a Bitter Tonic especially in cases of anorexia nervosa. It contains no tannins, hence it can be safely used in iron preparations for the treatment of anaemia without the fear of precipitation resulted from *in vitro* interaction	Bruneton (1999)
Laurus nobilis L.	Lauraceae	leaves	As a spice in Mediterranean cuisines, Folk medicine as astringents, in massage therapy	Bruneton (1999)

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Lavandula angustifolia Mill.	Lamiaceae	flowers	The flowers and leaves are used as an herbal medicine, either in the form of lavender oil or as an herbal tea. The flowers are also used as a culinary herb, most often as part of the French herb blend called herbes de Provence	Bruneton (1999)
Leuzea carthamoides (Willd.) DC.	Asteraceae	roots	Use as a stimulant, and a remedy against male sex dysfunction	Solyomvary *et al.* (2014)
Lycium chinense Mill.	Solanaceae	fruits	In folk Chinese medicine for the treatment of nightsweats, pneumonia, cough, hematemesis, inflammation, and diabetes mellitus	McGuffin *et al.* 2000
Mentha arvensis L.	Lamiaceae	area part	Traditionally used to treat flatulence, digestional problems, gall bladder problems and coughs	Bruneton (1999); WHO (2004)
Mentha × *piperita* L.	Lamiaceae	area part	It is the oldest and most popular flavour of mint-flavoured confectionery and is often used in tea and for flavouring ice cream, confectionery, chewing gum, and toothpaste, also in some shampoos, soaps and skin care products. In folk med. used as antibacterial agent, antispasmodic, antifoaming, treat dyspepsia, analgetic	Bruneton (1999); WHO (2004)
Origanum majorana L.	Lamiaceae	area part	Use as condiment for seasoning soups, stews, dressings, and sauce	Pimple *et al.* (2012)
Petasites hybridus L.	Asteraceae	rhizome	Used for bronchitis, tussis and pulmonitis and as spasmolysan. Externaly allied on dermatitis, haemorrhoids, bruises, wounds and sores	Pavela (2014c)
Peumus boldus Molina	Monimiaceae	leaves	In folk med. as cholagogue, choleretic, for treatment of mild dyspepsia	Carbajal *et al.* (2014)
Saponaria officinalis L.	Caryophyllaceae	roots	Used as an emulsifier in the commercial preparation of *Tahini halva*, and in brewing to create beer with a good "head". In the Middle East, the root is often used as an additive in the process of making the popular sweet, halvah. In India, the rhizome is used as a galactagogue	Sezgin & Artik (2010)
Silybum marianum (L.) Gaertn.	Asteraceae	seeds	Used as food. The roots can be eaten raw or boiled and buttered or par-boiled and roasted. The young shoots in spring can be cut down to the root and boiled and buttered. The spiny bracts on the flower head were eaten in the past like globe artichoke, and the stems (after peeling) can be soaked overnight to remove bitterness and then stewed. *S. marianum* is used in traditional Chinese medicine to clear heat and relieve toxic material, to soothe the liver (cirrhosis, jaundice, and hepatitis) and to promote bile flow	Tamayo & Diamond (2007)
Syzygium aromaticum (L.) Merrill & Perry	Myrtaceae	flower buds	In the cuisine of Asian, African, and the Near and Middle East, lending flavour to meats, curries, and marinades, as well as fruit such as apples, pears or rhubarb. Cloves are used as as an anodyne (painkiller), carminative, to increase hydrochloric acid in the stomach and to improve peristalsis and anthelmintic	Bruneton (1999); Kamatou *et al.* (2012)
Thymus vulgaris L.	Lamiaceae	area part	Use as a common component of the condiment (e.g. bouquet garni, herbes de Provence and as an herbal medicine	Bruneton (1999); Shankar & Mohan (2014)
Tilia cordata Mill.	Tiliaceae	flowers	The flowers are also used for herbal teas and tinctures for cough and colds	Pavela (2014c)
Valeriana officinalis L.	Caprifoliaceae	roots	Used as an alternative for sedatives, such as benzodiazepines, in the treatment of certain anxiety disorders	Bruneton (1999); McGuffin *et al.* (2000)
Zingiber officinale Roscoe	Zingiberaceae	rhizome	Used as a hot, fragrant kitchen spice as an ingredient in many dishes, can be made into candy, or ginger wine. In folk med. use as a stimulant and carminative and used frequently for dyspepsia, gastroparesis, slow motility symptoms, constipation, and colic	Bruneton (1999); McGuffin *et al.* (2000)

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further plant growth did not affect the experiment). The day before application, a binocular magnifier and a fine brush were used to introduce 10 females of *T. urticae* (1–2 days old) onto each of the leaves. The extract was applied to the plants using a manual electronic atomiser in a dose approximately equivalent to the application of 600 l water/ha. Control plants were treated using only water. The experiment was repeated 5 times. The plants were placed in a growth chamber (L16 : D8, 25.0 ± 1.0°C) (Pavela 2015).

The numbers of adults, nymphs, and eggs on the plants were determined using a binocular magnifier on day 10 from the application. Extract efficacies were calculated compared to the control based on the determined counts of live adults, nymphs (without instar differentiation), and unhatched eggs. Acute toxicity for *T. urticae* adults was determined in subsequent tests for extracts showing efficacy > 80% for all development stages.

The acute toxicity, measured as mortality after 48 h of exposure, was determined by topical application to adults of *T. urticae*. One day before application of the extracts, 20 adults of *T. urticae* were infested on the bean plants. The plants, the mode of deploying the mites, and the extract applications were identical to the above-described experiment. The exact count of live adults on the plants was determined again immediately before application, and extracts were applied on the plants in the concentration series 100, 80, 50, 40, 30, 20, 10, and 5 g/l. Mortality was determined after 48 h from application, and the determined data became the foundation for estimating lethal doses. The experiment was repeated 5 times. The plants were placed in a growth chamber (L16 : D8, 25.0 ± 1.0°C).

Data analysis

Data for the toxicity of extracts against T. urticae: Percentages were transformed to arcsine square root values for the analysis of variance (ANOVA). Tukey's test ($P < 0.05$) was used to analyse for significant differences among the test extracts against numbers of adults, nymphs or eggs, or efficiency. Means (± standard errors) of untransformed data are reported.

The efficiency was determined by Henderson-Tilton's formula:

$$\text{Corrected \%} = (1 - n \text{ in Co before treatment} \times n \text{ in T after treatment} / n \text{ in Co after treatment} \times n \text{ in T before treatment}) \times 100$$

where: n – insect population; T – treated; Co – control

Data for acute toxicity. Experimental tests demonstrated that more than 20% of the controlled mortality was discharged and repeated. When the controlled mortality reached 1–20%, the observed mortality was corrected by Abbott's formula (Abbott 1925). Probit analysis of dose-mortality data was conducted to estimate the LD_{50} and LD_{90} values and associated 95% confidence limits for each treatment (Finney 1971).

RESULTS

Mean counts of individuals of individual development stages of *T. urticae* found on the plants on day 10 after application of the dose 50 g/l, along with the determined efficacy compared to the control, are provided in Table 2.

It was discovered that there was a significant reduction in adult counts on the plants after application of all extracts. Of the 28 extracts, 24 showed an efficacy higher than 50%; of these, 16 extracts caused a reduction in the count of adults on the plants by more than 90% compared to the control.

The counts of nymphs and eggs showed significant differences depending on the extract used. Only 13 extracts showed an efficacy higher than 50%, and only 2 extracts (obtained from *Ammi visnaga* and *Saponaria officinalis*) caused more than 90% efficacy. Twenty extracts resulted in eggs reduction of more than 50% compared to the control; of these, 6 extracts (from *A. visnaga*, *G. glabra*, *J. palmata*, *L. carthamoides*, *O. majorana*, *S. officinalis*) exhibited an efficacy higher than 90%.

Taking the total count of all development stages found in the bean plants as the main criterion for determining the biological efficacy of the extracts, nine of the 28 tested extracts showed an efficacy higher than 80%; of these, only 2 extracts (obtained from *Ammi visnaga* and *Saponaria officinalis*) caused an efficacy higher than 90%. On average, 0.1 adults, 0.0 nymphs, and 4.9 eggs were found on plants treated with the extract from *S. officinalis* roots, which are the lowest counts of all the tested extracts, while the control showed 8.1 adults, 21.6 nymphs, and 104.5 eggs (Table 2).

The effect of selected most efficient extracts in terms of acute toxicity for *T. urticae* adults is shown in Table 3. By comparing the confidence intervals (CI_{95}) for individual LD_{50} values, extracts obtained from *Saponaria officinalis* roots and *Ammi visnaga* seeds were found to provide the significantly highest efficacies, and lethal doses (LD_{50}) of 10.3 g/l

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Table 2. Mean counts of individuals of individual development stages of *T. urticae* found on the plants on day 10 after application of the dose 50 g/l, along with the determined efficacy compared to the control

Plant	Mean number/leaf*				Efficiency (%)**			
	adults	nymphs	eggs	sum of all stages	adults	nymphs	eggs	sum of all stages
Ammi visnaga	0.0 ± 0.0^{a}	0.5 ± 0.1^{b}	7.9 ± 3.5^{ab}	8.4 ± 2.6^{a}	100.0^{e}	97.7^{e}	92.4^{f}	93.7^{f}
Angelica archangelica	0.0 ± 0.0^{a}	13.9 ± 2.8^{ef}	23.5 ± 8.6^{d}	37.4 ± 5.9^{d}	100.0^{e}	35.6^{c}	77.5^{ef}	72.1^{de}
Capsicum annuum	0.0 ± 0.0^{a}	5.1 ± 1.3^{c}	15.8 ± 2.8^{c}	20.9 ± 2.8^{c}	100.0^{e}	76.4^{de}	84.9^{ef}	84.4^{e}
Cinchona officinalis	2.3 ± 0.3^{d}	26.8 ± 6.8	122.5 ± 18.6	151.6 ± 19.3	71.3^{c}	–24.1^{a}	–17.2^{a}	–13.0^{a}
Cinnamomum verum	5.1 ± 0.9^{ef}	2.8 ± 0.5^{c}	15.1 ± 3.9^{c}	23.0 ± 3.8^{cd}	36.3^{a}	87.0^{e}	85.6^{e}	82.8^{e}
Curcuma longa	0.5 ± 0.2^{bc}	21.3 ± 5.5^{fg}	41.7 ± 5.8^{e}	63.5 ± 4.9^{e}	93.8^{cd}	1.4^{b}	60.1	52.6^{d}
Cymbopogon citratus	0.3 ± 0.1^{b}	10.2 ± 2.1^{e}	19.6 ± 2.7^{cd}	30.1 ± 5.9^{d}	96.3^{de}	52.8^{d}	81.2^{e}	77.6^{e}
Eucalyptus globulus	0.4 ± 0.2^{b}	21.9 ± 4.5^{fg}	63.8 ± 7.2^{ef}	86.1 ± 10.8^{fg}	95.0^{de}	–1.4^{b}	38.9^{cd}	35.8^{c}
Foeniculum vulgare	0.7 ± 0.3^{b}	8.7 ± 1.7^{d}	25.4 ± 5.9^{d}	34.8 ± 6.8^{d}	91.3^{cd}	59.7^{d}	75.7^{ef}	74.0^{e}
Glycyrrhiza glabra	1.1 ± 0.3^{c}	12.3 ± 1.8^{de}	5.6 ± 1.8^{a}	19.0 ± 2.8^{bc}	86.3^{cd}	43.1^{cd}	94.6^{f}	85.8^{ef}
Illicium vernum	3.1 ± 0.3^{de}	15.9 ± 5.2^{ef}	33.3 ± 12.5	52.3 ± 12.3^{e}	61.3^{b}	26.4^{c}	68.1^{de}	61.0^{de}
Jateorhiza palmata	1.9 ± 0.4^{cd}	9.5 ± 2.5^{de}	9.1 ± 2.5^{bc}	20.5 ± 2.2^{c}	76.3^{c}	56.0^{d}	91.3^{f}	84.7^{ef}
Laurus nobilis	0.1 ± 0.1^{ab}	12.5 ± 3.2^{ef}	53.3 ± 5.4^{e}	65.9 ± 6.9^{ef}	98.8^{de}	42.1^{cd}	49.0^{d}	50.9^{d}
Lavandula angustifolia	3.8 ± 0.8	7.5 ± 2.4^{d}	18.2 ± 7.5^{cd}	29.5 ± 6.3^{cd}	52.5^{b}	65.3^{d}	82.6^{ef}	78.0^{e}
Leuzea carthamoides	0.5 ± 0.2^{bc}	19.9 ± 6.7^{f}	9.7 ± 2.3^{bc}	30.1 ± 4.8^{cd}	93.8^{d}	7.9^{b}	90.7^{f}	77.6^{de}
Lycium chinense	3.8 ± 0.5^{d}	20.2 ± 3.9^{fg}	75.2 ± 8.3^{ef}	99.2 ± 7.9^{fg}	52.5^{b}	6.5^{bc}	28.0^{c}	26.0^{bc}
Mentha arvensis	5.3 ± 0.7^{ef}	13.2 ± 2.6^{ef}	49.8 ± 13.2^{e}	68.3 ± 12.1^{ef}	33.8^{a}	38.9^{c}	52.3^{d}	49.1^{d}
Mentha × piperita	0.1 ± 0.1^{ab}	5.3 ± 1.1^{cd}	12.8 ± 5.3^{c}	18.2 ± 6.5^{bc}	98.8^{de}	75.5^{de}	87.8^{f}	86.4^{e}
Origanum majorana	0.0 ± 0.0^{a}	9.3 ± 2.3^{d}	4.2 ± 3.1^{a}	13.5 ± 2.7^{b}	100.0^{e}	56.9^{cd}	96.0^{f}	89.9^{e}
Petasites hybridus	4.6 ± 0.9^{d}	25.3 ± 4.8^{g}	85.5 ± 9.5^{f}	115.4 ± 8.6^{g}	42.5^{ab}	–17.1^{a}	18.2^{bc}	13.9^{b}
Peumus boldus	4.2 ± 0.2^{de}	18.6 ± 2.6^{f}	59.3 ± 5.8^{e}	82.1 ± 12.3^{f}	47.5^{ab}	13.9^{bc}	43.3^{d}	38.8^{c}
Saponaria officinalis	0.1 ± 0.0^{a}	0.0 ± 0.0^{a}	4.9 ± 2.6^{a}	5.0 ± 2.1^{a}	98.8^{d}	100.0	95.3f	96.3^{f}
Silybum marianum	0.3 ± 0.1^{b}	13.5 ± 3.8	17.3 ± 5.8^{cd}	31.1 ± 4.8^{d}	96.3^{d}	37.5^{cd}	83.4^{de}	76.8^{de}
Syzygium aromaticum	0.3 ± 0.1^{b}	13.5 ± 2.8	91.8 ± 6.5^{fg}	105.6 ± 10.5^{g}	96.3^{de}	37.5^{c}	12.2^{b}	21.3^{bc}
Thymus vulgaris	0.1 ± 0.1^{ab}	10.5 ± 2.1^{e}	45.8 ± 10.5	56.4 ± 11.6^{e}	98.8^{d}	51.4^{cd}	56.2^{de}	57.9^{de}
Tilia cordata	1.2 ± 0.1^{c}	8.8 ± 2.2^{d}	18.8 ± 5.2^{cd}	28.8 ± 5.1^{cd}	85.0^{cd}	59.3^{d}	82.0^{ef}	78.5^{e}
Valeriana officinalis	1.8 ± 0.6^{cd}	3.4 ± 0.3^{cd}	12.6 ± 3.9^{c}	17.8 ± 2.8^{b}	77.5^{c}	84.3^{de}	87.9^{ef}	86.7^{ef}
Zingiber officinale	0.2 ± 0.1^{b}	21.2 ± 3.5^{fg}	86.9 ± 18.5	108.3 ± 12.9^{fg}	97.5^{d}	1.9^{b}	16.8^{b}	19.2^{bc}
Control	8.1 ± 1.2^{f}	21.6 ± 5.8^{fg}	104.5 ± 26.1^{g}	134.1 ± 21.8^{g}				
ANOVA *F; P****	84.22; 0.0001	120.62; 0.0001	54.12; 0.0001	165.35; 0.0001	79.55; 0.0001	98.52; 0.0001	95.72; 0.0001	67.88; 0.0001

*mean numbers of different developmental stages 10th day after application (± S.E) within a column follower by the same letter do not differ significantly according to the least significant difference (Turkey's HSD test, $P < 0.05$); **numbers represent the average efficiency (in %) of the extract compared with the control; ***ANOVA parameters: *F*-value, *P*-significantly level

and 12.5 g/l, respectively, were estimated for them. However, when also comparing the doses resulting in the reliable death of at least 90% of *T. urticae* adults, an LD_{90} value lower than 50 g/l was estimated for only 4 extracts (*Ammi visnaga*, *Capsicum annum*, *Origanum majorana*, and *Saponaria officinalis*) (Table 3). The contents of substances dissolved in the application liquid, obtained by extracting 50 g of plant material in 1 litre of water, were determined for selecting extracts with the highest efficacies (Table 4). The highest content of substances dissolved in the extracts was found for extracts obtained from *Saponaria officinalis* (2.87%), *Glycyrrhiza glabra* (2.18%), *Ammi visnaga* (2.11%), and *Valeriana offi-*

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Table 3. The effect of selected most efficient extracts in terms of acute toxicity for *T. urticae* adults

Plant	LD_{50} [a] (CI_{95})[b]	LD_{90} [a] (CI_{95})[b]	χ^{2c}
Ammi visnaga	12.5 (11.8–13.6)	38.7 (36.5–43.9)	0.453
Capsicum annuum	32.3 (29.7–36.8)	48.5 (45.9–58.6)	0.251
Cinnamomum verum	72.8 (69.5–78.9	> 100	2.252
Glycyrrhiza glabra	45.2 (39.9–48.6)	75.6 (72.8–89.6)	1.854
Jateorhiza palmata	48.2 (42.7–52.7)	81.9 (76.5–89.7)	0.528
Mentha × piperita	39.8 (33.3–42.8)	53.7 (49.5–68.7)	0.785
Origanum majorana	36.8 (32.3–41.8)	48.2 (46.5–52.6)	0.256
Saponaria officinalis	10.3 (8.3–12.6)	41.1 (35.1–51.6)	3.727
Valeriana officinalis	42.7 (39.9–48.5)	65.5 (52.8–79.6)	2.528

[a]doses LD_{50} (LD_{90}) in µg/cm^2 causing 50% (90%) mortality of adults *T. urticae,* the lethal dose was not possible to calculated due to the low mortality (the calculation was inaccurate); [b]CI_{95} – 95% confidence intervals, essential oils activity is considered significantly different when the 95% CI fail to overlap; [c]Chi-squared value, significant at $P < 0.05$ level

cinalis (1.98%). The content of dissolved substances was about 1% (w/w) in the other extracts.

DISCUSSION

The study tested 28 plant species used both in folk medicine and in the preparation of various meals (Table 1). Water was used to prepare simple extracts that were applied on plants infested with *T. urticae*. With the exception of the extract from *Cinchona officinalis*, all extracts showed an efficacy against *T. urticae*; nevertheless, significant differences were found in the efficacies of individual extracts.

All extracts caused a significantly lower incidence of adults on the treated plants at the time of evaluation compared to the control. No adults were found on plants treated with extracts from *A. visnaga*, *A. archangelica*, *C. annum*, and *O. majorana*. Given that all plants were initially infested with the same counts of adults, and that the experiment was undertaken under identical conditions, several modes of activity of the extracts can be estimated based on our results.

(*i*) Some extracts (from *A. visnaga*, *C. annum*, *C. verum*, *M. × piperita*, *O. majorana*, *S. officinalis*, *V. officinalis*) caused adult mortality immediately after application, and thus only a few nymphs and eggs were found on the treated plants, which were probably oviposited before the application. This hypothesis was also confirmed by subsequent toxicity tests, where $LD_{50(90)}$ values could be estimated for 48 h from application, providing evidence of the acute

Table 3. The effect of selected most efficient extracts in terms of acute toxicity for *T. urticae* adults.

Plant	LD_{50} [a] (CI_{95})[b]	LD_{90} [a] (CI_{95})[b]	χ^{2c}
Ammi visnaga	12.5 (11.8–13.6)	38.7 (36.5–43.9)	0.453
Capsicum annuum	32.3 (29.7–36.8)	48.5 (45.9–58.6)	0.251
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toxicity of the substances contained in the extracts (Ratan 2010).

(*ii*) Another probable mechanism of action was found for extracts obtained from *C. officinalis*, *C. longa*, *E. globosus*, *I. vernum*, *L. nobilis*, *S. aromaticum*, and *Z. officinalis*. In these cases, although a significantly lower count of adults was found on day 10 from the application, the counts of eggs or nymphs differed only slightly compared to the control (with efficacy < 50%). We can thus believe that the substances contained in the extracts lowered the longevity of the adults but had no effect on their fertility. Such a manifestation can be attributed to the inhibitory effect of the substances on growth and food intake (Attia *et al*. 2013).

(*iii*) Some extracts (from *A. archangelica*, *C. stratus*, *F. vulgare*, *L. angustifolia*, *L. carthamoides*, *S. marianum*, *T. cordata*) did result in a significant reduction in the counts of all development stages, but their overall efficacies were < 80%. It is thus likely that these extracts resulted in the mortality of the weakest individuals and had a general effect on the vitality of all development stages. Although it caused a relatively high mortality, in practice the application would have to be repeated several times to achieve the required efficacy. Such an efficacy can be attributed to the antifeedant and repellent activities of the substances contained in the extracts (Pavela 2010). However, the exact mechanisms of action cannot be specified at the moment, and further, more specific studies will have to be carried out.

It is difficult to compare our results to those of other authors because until now, all studies focused on the acaricidal efficacies of plant extracts have been based on efficacies of extracts obtained using organic solvents, where different substances may be present (Flamini 2006). A lot of studies focus on the acaricidal efficacy of essential oils, including their active substances (Attia e*t al*. 2013). As far as we know, this study is the first to focus on the efficacy of aqueous extracts on *T. urticae*.

Wanting to compare acute toxicity based on the estimated LD_{50} values, we only choose two out of all the tested extracts, i.e. those obtained from *Ammi visnaga* and *Saponaria officinalis*. Their LD_{50} values were the lowest compared to other extracts; however, wanting to also compare LD_{90} values, we chose 5 extracts (*A. visnaga*, *C. annum*, *M.* × *piperita*, *O. majorana*, and *S. officinalis*) whose CI_{95} intervals overlapped ($P \leq 0.05$). As we found earlier, methanol extracts from *A. visnaga* seeds contain the furanochromones visnagin and khellin as their major ingredients with acaricidal effects (Pavela 2015). Both these substances were responsible for biological efficacy; nevertheless, visnagin showed a significantly higher efficacy compared to khellin. Extracts obtained from *C. annum*, containing capsaicin, have already found their application in plant protection (Miresmailli & Isman 2014). The insecticidal effects of extracts and essential oils isolated from *M.* × *piperita* and *O. majorana* are known as well (Koul 2008; Pavela *et al.* 2014), including their acaricidal effects (Attia *et al*. 2013). Since all these plants are used in the food industry, they can be expected to be safe and their extracts should not raise any concerns, which is a fundamental precondition for BSs registration pursuant to Article 23 of the Regulation (EC) No. 1107/2009 (SANCO 2015).

As far as we know, the acaricidal efficacy of aqueous extracts obtained from *Saponaria officinalis* roots was newly discovered. These extracts have been known to contain a relatively high percentage of saponins, which have been used in medicine and cosmetics (Budan *et al*. 2014). According to Budan *et al.* (2014), the content of saponins in *S. officinalis* roots can range between 224.0 and 693.8 mg/g of dry matter. The soapwort extract itself contains 11.6–19.6% total saponin, which increases the importance of soapwort (Battal 2002). It can thus be expected that, similarly to saponins from other plants (Ding *et al.* 2013), the saponins from *S. officinalis* will also be responsible for acaricidal activity. Nevertheless, this hypothesis will have to be confirmed in further tests. The properties of saponins contained in extracts from the roots of this plant have been traditionally used for the production of Turkish Delight and Halvah (Sezgin & Artik 2010). Baylan (1990) determined the total saponin of Tahini Halvah by thin layer chromatography, and the total saponin content of Tahini Halvah was reported as 119–266 mg/kg. Such a relatively high content of saponins in traditionally produced foods can lead us to expect that any residues of extracts applied on the plants will have no negative effects on human and animal health.

CONCLUSIONS

To conclude, we can note that although focused only on basic screening of the acaricidal efficacy of plant extracts, this study has succeeded in choosing several plants that could be considered for future registration as BSs per applicable European regulations, thanks to their traditional use in the food

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industry. Taking into account the efficacy and the fundamental condition for registration – use of the BSs as foods – the extract from *Saponaria officinalis* roots can be viewed as the one that offers the best prospects. The efficacy of aqueous extracts from *S. officinalis* on *T. urticae* was more than 96%, with an estimated $LD_{50(90)}$ value of 10.3 (41.1) g/l, respectively. Expressing the estimated lethal doses using the content values of the dissolved substances or their dry mass, respectively, in the application liquid, we see that with a dry mass content of 2.87% (w/w), LD_{50} 0.29 g and LD_{90} 1.18 g of dissolved substances are contained in 1 litre of water. Moreover, *S. officinalis* is a European plant that can also be grown in Central Europe, which makes this plant even more promising.

Nevertheless, we are aware that further tests will be needed concerning extract stability, maceration time, any phytotoxicity, and impacts on other, both target and non-target organisms.

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