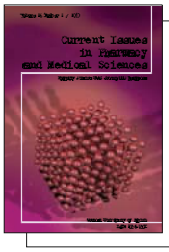

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# Acaricidal effect of some plants on *Ixodes ricinus* – a pilot study

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**ABSTRACT**

In the search for natural antiticktide substances, 23 raw materials belonging to 16 plant species were tested. In the study, the behavior of ticks after stimulation with chloroform and methanol plant extracts was observed. All the tested extracts have shown toxicity against *Ixodes ricinus*. The most toxic appeared to be methanol extracts from *Helichrysum arenarium* (L.) Moench., *Centaurea cyjanus* L., *Taraxacum officinale* Web., *Tanacetum vulgare* L., *Chelidonium maius* L. and *Armoracia rusticana* Gilib. which caused the death of ticks within 1 hour in the concentrations 0,6; 4,4; 6,1; 6,8; 7,2 and 7,6 mg/mL respectively.

**Keywords**: Acaricidal effect, *Ixodes ricinus*, plant extracts

## INTRODUCTION

Ticks are small arachnids that are external parasites of vertebrates. They occur throughout the world in various habitats. Ticks can be divided into two families: hard ticks (*Ixodidae*) and soft ticks (*Argasidae*). The common tick, *Ixodes ricinus* is the most frequently recorded species of tick in Poland [14]. It is commonly known that ticks cause diseases to humans and animals through out the world. On the territory of Europe including Poland, the *Ixodes ricinus* is found to be a major vector of *Borrelia burgdorferii sensu lato*. Depending on the developmental stage of ticks they take blood from different animals such as rodents, birds, wildlife and livestock which are reservoir of spirochetes. Complex *Borrelia burgdorferii sensu lato* includes eighteen species and this group contains *B. burgdorferii sensu stricto*, *B. afzelii* and *B. garinii* which are species most frequently isolated from humans. The three species are especially dangerous because they cause multi-system disease called Lyme disease [12,13].

*Ixodidae* are of the great importance not only as vectors (germ carriers) of numerous pathogens, including those of great medical importance, but also they appear to be vectors of multiple virus, bacterial and protozoa diseases [3,16],

In addition to the usual consequences of an attack of a tick such as skin lesions, and polymorphic changes, tick can cause more serious diseases such as tick paralysis [4,8], or even anaphylactic shock [5,6].

Currently ticks are considered the second after the mosquitoes vector of pathogens causing human diseases throughout the world [9].

No wonder that for a long time people have been trying to get effective repellents or ticktides. The first repellents were based on natural components, but since 1929 (discovery of synthetic repellent - DMP) synthetic repellents have gained popularity. Worth mentioning are repellents such as EH (ethyl heksanodiolu), DEPA (N,N-diethyl-phenylacetamide) or DEET (N,N-diethyl-m-toluamide) [1,2]. Despite their effectiveness some doubts have appeared about their harmful effects on natural environment [7] and humans and animal health [1,2]. Due to the high frequency of using synthetic repellents many species of ticks show a growing resistance to them [5]. That is why, natural products should be considered. In many laboratories screening tests are being performed to search for natural plant substances that could be used in the prevention of infectious tick borne diseases in humans and animals. Plant active substances against ticks have been reported by variety of authors as an alternative to synthetic preparations [11].

The aim of this study was to search for plants showing potential tickcidal effect against *Ixodes ricinus*.

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## MATERIALS AND METHODS

*Plant material*. The different plant organs such as leaves, inflorescences, stems, herb, and roots from selected species of Polish flora were used in this study (Table 1). Samples were collected from natural habitat at the end of June (herb), July (inflorescence) and in September (roots) 2011, near Lublin, Poland (Table 1). Voucher specimens were deposited at the Chair and Department of Pharmaceutical Botany, Medical University of Lublin.

Table 1. Plant material used in the study

| No | Plant material (systematic name) | Family | Plant material (English and Polish common name) | Part of plant | Place of collection |
|---|---|---|---|---|---|
| 1 | *Asarum europaeum* L. | *Aristolochiaceae* | *European wild ginger / Kopytnik pospolity* | leaves | Motycz Leśny |
| 2 | *Artemisia abrotanum* L. | *Asteraceae* | *Southernwood / Bylica Boże drzewko* | herbs | Botanical Garden UMCS |
| 3 | *Artemisia absinthium* L. | *Asteraceae* | *Absintha wormwood / Bylica piołun* | herbs | Motycz Leśny |
| 4 | *Artemisia vulgaris* L. | *Asteraceae* | *Common wormwood / Bylica pospolita* | herbs | Botanical Garden UMCS |
| 5 | *Centaurea cyanus* L. | *Asteraceae* | *Cornflower / Chaber bławatek* | inflorescence | Surroundongs Łęczna |
| 6 | *Centaurea cyanus* L. | *Asteraceae* | *Cornflower / Chaber bławatek* | herbs | Surroundings Łęczna |
| 7 | *Helichrysum arenarium* Moench | *Asteraceae* | *Dwarf everlast / Kocanki piaskowe* | inflorescence | Motycz Leśny |
| 8 | *Helichrysum arenarium* (*L.*) Moench | *Asteraceae* | *Dwarf everlast / Kocanki piaskowe* | herbs | Motycz Leśny |
| 9 | *Tanacetum vulgare* L. | *Asteraceae* | *Tansy / Wrotycz pospolity* | inflorescence | Motycz Leśny |
| 10 | *Tanacetum vulgare* L. | *Asteraceae* | *Tansy / Wrotycz pospolity* | herbs | Motycz Leśny |
| 11 | *Taraxacum officinale* Web. | *Asteraceae* | *Dandelion / Mniszek lekarski* | roots | Motycz Leśny |
| 12 | *Taraxacum officinale* Web. | *Asteraceae* | *Dandelion / Mniszek lekarski* | herbs | Motycz Leśny |
| 13 | *Berberis vulgavis* L. | *Berberidaceae* | *Barberry / Berberys pospolity* | Blossoming twig | Ciecierzyn |
| 14 | *Armoracia lapathifolia* Gilib. | *Brassicaceae* | *Hrseradix / Chrzan pospolity* | leaves | Samoklęski |
| 15 | *Chenopodium ambrosioides* L. | *Chenopodiaceae* | *Jesuits 's tea / Komosa piżmowa* | roots | Botanical Garden UMCS |
| 16 | *Chenopodium ambrosioides* L. | *Chenopodiaceae* | *Jesuits 's tea / Komosa piżmowa* | herbs | Botanical Garden UMCS |
| 17 | *Juglans nigra* L. | *Juglandaceae* | *Walnut / Orzech czarny* | leaves | Botanical Garden UMCS |
| 18 | *Mentha piperita* L. | *Lamiaceae* | *Peppermint / Mięta pieprzowa* | roots | Samoklęski |
| 19 | *Mentha piperita* L. | *Lamiaceae* | *Peppermint / Mięta pieprzowa* | herbs | Samoklęski |
| 20 | *Chelidonium maius* L. | *Papaveraceae* | *Celandine / Glistnik jaskółcze ziele* | herbs | Motycz Leśny |
| 21 | *Polygonum cuspidatum* Sieb. et Zucc. | *Polygonaceae* | *Japanese knotweed / Rdest japoński* | leaves | Botanical Garden UMCS |
| 22 | *Polygonum cuspidatum* Sieb. et Zucc. | *Polygonaceae* | *Japanese knotweed / Rdest japoński* | stem | Botanical Garden UMCS |
| 23 | *Ribes nigrum* L. | *Saxifragaceae* | *Blackcurrant / Porzeczka czarna* | leaves | Samoklęski |

Plant material was cleaned from dirt (pollutants). The aboveground and underground parts of plants were separated and cut into small pieces. The air -dried and powdered plant material was prepared in accordance with Polish Pharmacopeia IV.

*Extraction*. The chloroform extracts were obtained from 1 gram of plant material (1/10 w/v), extracted twice in supersonic water bath for 15 minutes and after that filtered. The combined extracts were concentrated under rotary evaporator with reflux to dryness. The residue of the extract was dried under nitrogen.

The water-methanol extracts were prepared from 2 grams of plant material. The 50% methanol was used for extraction in 1/10 w/v ratio. The rest of the extraction and sample preparation was carried out the same way as for chloroform extracts.

*Tick test.* One-hundred-and-ninety adult ticks of the *Ixodes ricinus* were collected from the natural places (different field sites in forest areas near Zalew Zemborzycki) in the period between 11.05-14.08.2012. The ticks were kept in glass bottle for no more than 1-2 hours and then used in test.

The tested procedure was as follows: Petri dishes with an inner diameter of 6 cm were provided with roundlet filter paper. The stimulation of ticks was carried out in petri dishes with discs of filtration paper inside. The appropriate amount of the test extract sufficient to moisten the filter paper was applied (about 0.25 mL,) Next 2 ticks were placed in the centre of the paper and the petri dish was covered unhermetically. The ticks were observed in specified periods of time after 30, 60, 90, 120 minutes and 6, 24, 48, 72 hours.

## RESULTS

Behavior of the ticks was measured based on the following symptoms: moving about, moving limb of tick, reaction to light pressure of pincers. The test was performed in duplicate. In the study, the behavior of ticks being in contact with the tested plant extracts was observed. As a result, more or less intense reactions of the ticks were noted. They involved the ticks leaving the filter paper and remaining on the walls of the scale pan or staying in the same place with or without visible limb movements.

It was noted that in most cases, shortly after the start of stimulation with the studied extracts, the ticks moved in the pan to the places (edges of the pan or the lid) which did not contain the tested solution. This demonstrates the repellant activity of these extracts. In the case of the extracts from *Artemisia absinthium* [7,15] and Tanaceum vulgare [10] the present study confirms the previous findings.

The toxicity of chloroform extracts convert into the concentration of dry extract in probe is shown in Table 2. The investigations were made on specimens collected in a game purchasing centre. It was observed that the time from the moment of stimulation with the extracts to their death ranged from 6 to 72 hours. In the series of probe

Table 2. The results of this study referring to the time within which the ticks died after the exposition with the examined extracts

| Plant material No | Chloroform extracts (l g/mL) (n=96 ticks) | | Methanol extracts (0.4 g/mL) (n=94 ticks) | |
|---|---|---|---|---|
| | Concentration of dry extract [mg/mL] | Average of time exposition [h] | Concentration of dry extract [mg/mL] | Average of time exposition [h] |
| 1 | 16 | 48 | 53 | 2 |
| 2 | 45 | 72 | 60 | 2 |
| 3 | 20 | 72 | 62 | 4 |
| 4 | 15 | 72 | 79 | 2 |
| 5 | 14 | 72 | 44 | 1 |
| 6 | 12 | 72 | 46 | 2 |
| 7 | 9 | 48 | 62 | 3 |
| 8 | 4 | 72 | 6 | 0.75 |
| 9 | 8 | 72 | 73 | 4 |
| 10 | 22 | 48 | 67 | |
| 11 | 6 | 72 | 75 | 12 |
| 12 | 24 | 48 | 61 | 1 |
| 13 | 8/24* | 6/1-2* | 75 | 3 |
| 14 | 15/45* | 6/1-2* | 78 | 1 |
| 15 | 6 | 72 | 46 | 2 |
| 16 | 4 | 72 | 61 | 2 |
| 17 | 11 | 72 | 49 | 2 |
| 18 | 9 | 48 | – | – |
| 19 | 10 | 72 | 34 | 2 |
| 20 | 21 | 48-72 | 72 | 1 |
| 21 | 9 | 72 | 24 | 3 |
| 22 | 1 | 48 | 16 | 2 |
| 23 | 13 | 72 | 72 | 3 |

* chloroform extract (3 g/mL)

control the ticks lived for over 72 hours. The most active extracts were the extracts of *Berberis vulgaris* and *Armoracia lapathifolia*, under the influence of which the death occurred after 6 hours. For these species, the test was performed by changing the concentration of the extract into 3g/mL based on dry raw material. The increase in the concentration of extracts resulted in the death of ticks in a period of 1 to 2 hours.

To the activity test of aqueous- methanol extracts, solutions at concentrations of 0.8 and 0.4 g/ml were used. Stimulation by the extracts at the concentration of 8 g/mL resulted in the death of the examined ticks within 2 hours. Extracts at the concentration of 4 g/mL caused the death of ticks within 45 minutes – 4 hours. The comparison of toxic effect for the analyzed extracts is presented in Table 2. The relatively highest activity was observed in the case of the extract from the herb of *Helichrysum arenarium* which resulted in the death of ticks after 45 minutes. The death of the tick stimulated by 10% ethanol solution of DEET followed after the same time. The extracts from five other species caused the death of ticks during the first hour of the exposition.

The data obtained in this work suggest that all the investigated plants containing chloroform and methanol extracts have a potential anti-acaricidal effect against Ixodes ricinus. The toxicity of the extracts depended upon concentration and exposure time. The tested dry extracts in 6-78 mg/ml concentration, resulted 100% mortality of ticks within 45 minutes – 72 hours.

On the basis of the research results, it can be stated that the following plant extracts deserve special attention: methanol extracts from herb and inflorescences of *Centaurea cyanus*, herb of *Taraxacum officinale*, leaves of *Armoracia rusticana*, herb of *Tanacetum vulgare* and stem of *Polygonum cuspidatum* also chloroform extract from blossoming twig of *Berberis vulgaris*.

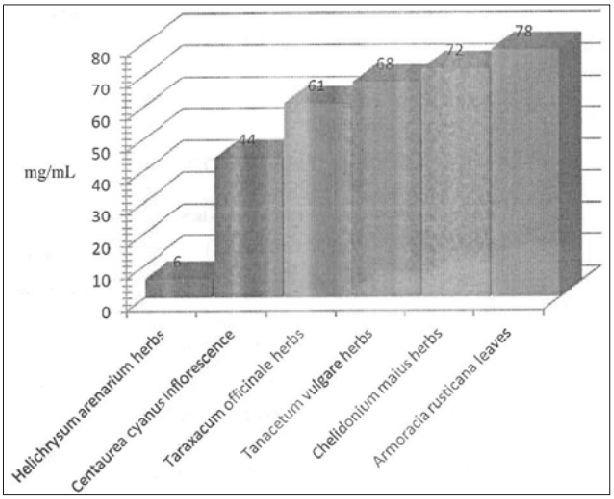


Fig. l. The concentration (mg/mL) of dry methanol extracts which caused the death of the ticks during the first hour of stimulation

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