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REVIEW

Nature helps: from research to products against blood-sucking arthropods

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Abstract Today, there is a trend in research to return to plant extracts as remedies against endo- and ectoparasites. Many daily appearing papers describe the efficacy of different plant extracts. However, the second step, to develop a product for the market (that could settle obvious needs), is in general not done. Thus, many results will be forgotten soon and the work was done in vain. The present review shows in examples that very efficacious biocidal and repellent products were developed from extracts of the plants *Vitex agnus castus*, *Azadirachta indica*, and from others which produce etheric oils. Of course, it is needed that the extracts have to be tested seriously for their activity, non-toxicity, tolerability, and user compliance. However, the selected examples show that it is worthwhile to consider plants in the fight against endo- and ectoparasites.

Introduction

Before 1900, when the later (1908) Medicinal Nobel Prize winner Paul Ehrlich (1854–1915) and other international colleagues started to introduce chemotherapeutics, all medicaments and medicinal remedies had been developed from plant extracts (Brown 1996; Leung 1985). Some of those plants had been used for many centuries, and even today many of them are known and used as "medicinal plants" (Schmutterer 2002; Fajimi and Taiwo 2005; Bäumler 2007). Since long ago, such extracts also have been used against endoparasites (e.g., worms and ectoparasites such as mosquitoes, ticks, mites, flea, bugs, etc.) of humans or animals (Athanasiadou et al. 2007; Amer and Mehlhorn 2006). With respect to skin-penetrating or blood-sucking ectoparasites, many plant extracts have been screened either for a defined biocidal (killing efficacy) or for strong repellency activity (prohibiting arthropods from landing on a host). In increasing numbers, papers appear daily that describe hundreds of plant extracts, which are more or less well characterized. However, in most cases, this knowledge remains at a rather theoretical level since the authors do not try to develop a product from their findings (Oladimeji et al. 2000; Michaelakis et al. 2009; Mumcuoglu et al. 2004).

Such a process is often blocked just by legal reasons:

1. Research results cannot become basis for a patent, when their publication had appeared before a patent was submitted. Exclusivity, however, is needed since no trade company will develop and distribute a product which can be produced easily by another company.
2. The regulations of the European Community have placed the repellents onto the list 19 and, in addition, limited the number of compounds on this list. Therefore, it is rather difficult and very costly for newly detected plant ingredients to become produced and officially registered among those already known compounds within a reasonable time.

List 18 contains compounds which act as insecticides or acarizides—their number is also reduced.

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However, intense research activities on the efficacy of some plant extracts showed that there are legal possibilities to develop plant extracts for the use as biocides or medicinal products against a broad spectrum of ectoparasites of humans and house animals. Some examples of the transfer of scientific results into ready-to-use products were presented in this review. Of course, only such compounds were used, which had proven their activity in a dose-dependent manner and which were non-toxic and did not harm the user's skin. The results presented herein underline the fact that it is worthwhile to develop natural products, since arthropods build up resistances much easier against chemical compounds with a single active ingredient than against natural products with multiple ingredients.

Materials and methods

Parasites

The tests were done with the following parasites:

(a) laboratory-bred specimens of mosquitoes: *Aedes aegypti* and *Culex quinquefasciatus*
(b) ticks: *Ixodes ricinus* (from forest catches) and laboratory-bred species such as *Dermacentor reticulatus* and *Rhipicephalus sanguineus*
(c) head lice: *Pediculus humanus capitis* obtained by combing children in Egypt and Germany
(d) mites: *Dermanyssus gallinae* obtained from chicken stables

Products

(a) Extracts of neem seeds (*Azadirachta indica*) are contained in products like "Lice Stop" (Fa. OTC Pharma, Gorinchem, The Netherlands) and "Wash-Away Louse" (Fa. Alpha-Biocare, Düsseldorf, Germany and Fa. DEEF, Saudi Arabia) (1–3 in Fig. 1).
(b) Extracts of the seeds of the plant *Vitex agnus castus* (4–5 in Fig. 2) are contained in the products "Viticks-Cool" and "Viticks-Cool Plus" (Fa. Alpha-Biocare).
(c) Extracts of acidified *Eucalyptus citriodora* oil are contained in products like "Picksan Tekenstop", "Tick Stop", "Picksan Muggenstop", and "Mosquito Stop" (Fa. OTC Pharma, Gorinchem, The Netherlands) (6 in Fig. 2).
(d) Etheric oils of cinnamon, lavender, cat mint, lemongrass, tea tree, and laurel leaves are contained in a concentration of 6% in the products "Picksan Muggenstop doosje" and "Mosquito stop box" (Fa. OTC Pharma).

Procedures

The tests on repellent effects against mosquitoes were done in two different ways:

1. Skin repellency was tested when product-treated hands were introduced into cages with hungry *Aedes* or *Culex* mosquitoes for a period of 3 min beginning 1 h after the application of the product. This exposition was repeated at intervals of 1 h for 5–6 h ("Picksan Muggenstop", "Viticks-Cool", and "Viticks-Cool Plus"). The touchdowns and biting actions of the mosquitoes were recorded.
2. Skin repellency of ticks was tested by placing ticks at intervals of 1 h on treated human legs. The drop downs of the ticks were recorded as sign of the repellent activity of the product, while ticks that stayed on treated legs were registered as failure.
3. General repellency of the combination of the repellent different etheric oils was tested by placing an open box of "Picksan Muggenstop doosje" between a cage filled with mosquitoes and an empty one or placed between two connected rooms so that the mosquitoes had the chance to move from one cage or room to the other. The box used was just opened or after being opened for 14 days.
4. Lice tests were done in vitro (immediately after combing children). Then the lice were incubated for 10 or 20 min in the neem seed extracts "Picksan Luizenstop" or "Wash-Away Louse" or in combination with other plant extracts, respectively. Then the lice were washed for 2 min with clear tap water before being placed onto a dry filter paper being observed for up 24 h (at intervals of 5, 10, 20, 30, 60 min, etc.). In vivo tests were also done when incubating hair of children for 10–20 min with the different neem-containing shampoos. This washing was repeated after 10 days.
5. *Dermanyssus galllinae* mites were tested in the laboratory and in chicken hatcheries and egg production facilities, when the neem seed extract was diluted 1:33 with tap water and sprayed onto the mites. The activity of the product was observed in the laboratory by light microscopes and in the stables by magnification glasses.

Results and discussion

Neem seeds

The tree *Azadirachta* (syn. *Melia*) *indica* (trivially called neem, niem, or margosa) that grows up to 20 m high originates from India but is now found worldwide, being often placed into fruit plantations due its efficacy to repel insects that might harm the fruits (Fig. 1). In India, the

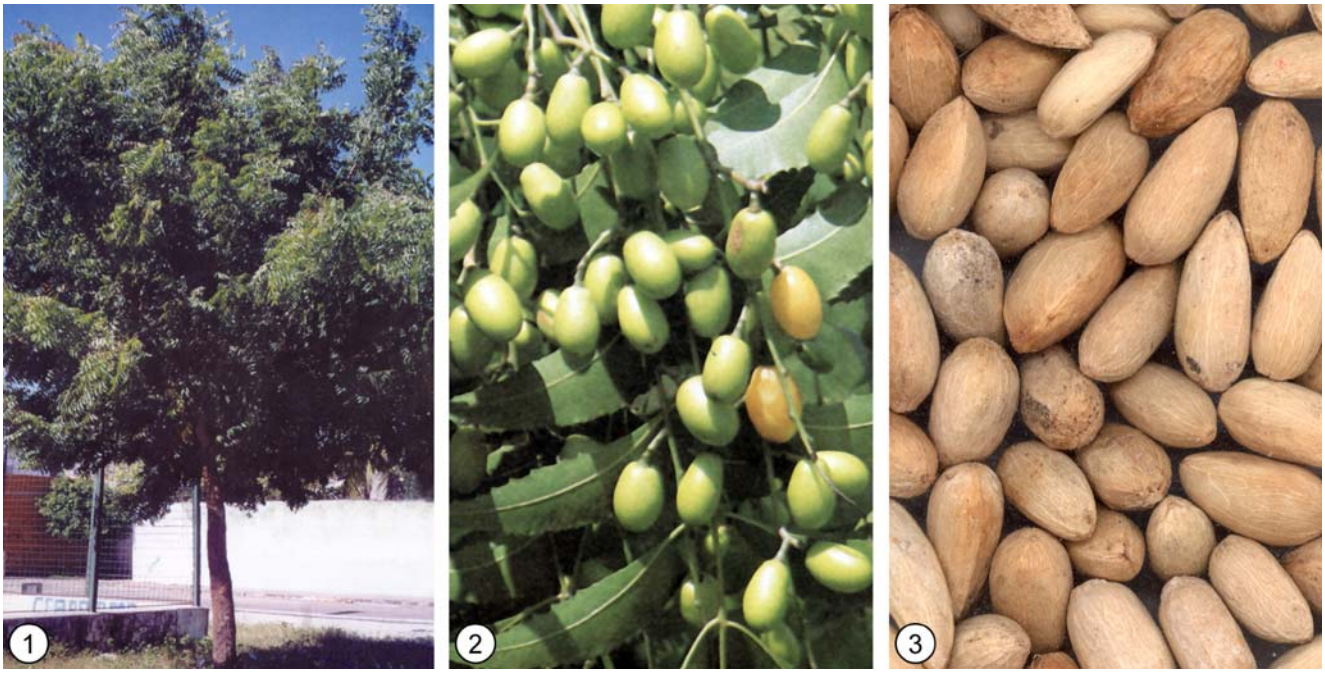

Fig. 1 *Azadirachta indica*: tree (*1*), seeds on tree (*2*), and peeled seeds (*3*)

seeds, leaves, and many different extracts are used in natural medicine for thousands of years (Schmutterer 2002). Many claimed properties turned out to be wrong when studied by modern methods. However, many others were found to be true (Schmutterer 2002). The ovoid, about 15-mm-long seeds of this tree appear nut-like (2 and 3 in Fig. 1). Patented di-basic-ester (DBE) extracts have been proven in several in vivo and in vitro tests to eliminate infestations with head lice (*P. humanus capitis*; Heukelbach et al. 2006; Abdel-Ghaffar and Semmler 2007), scabies mites (*Sarcoptes scabiei*; Abdel-Ghaffar et al. 2008b), or red chicken mites (*D. gallinae*; Abdel-Ghaffar et al. 2008a, 2009). Also, it has been documented that such extracts have significant killing effects on mallophaga (*Werneckiella*

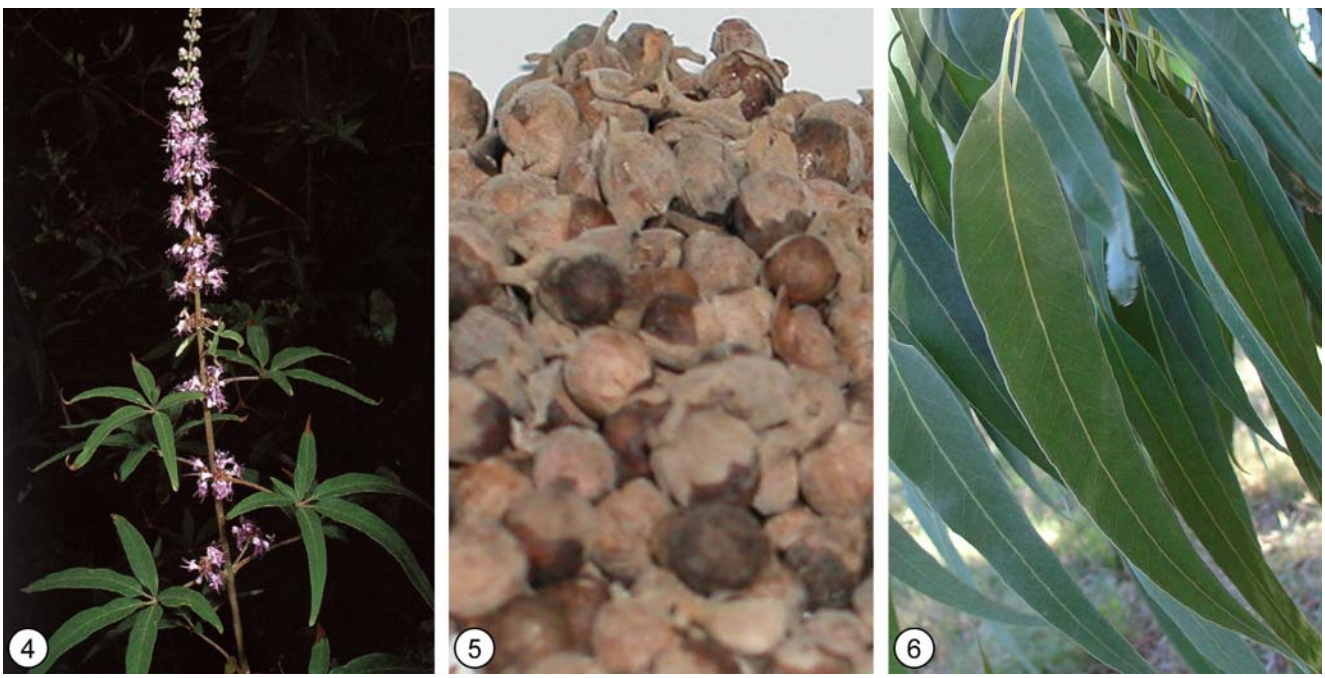

Fig. 2 *Vitex agnus castus*: flower (*4*) and seeds (*5*). Typical *Eucalyptus* leaves (*6*)

species) in horses (Mehlhorn 2009) or on grass mites (*Neotrombicula autumnalis*; Mehlhorn 2008). The neem extracts were used in different dilutions: 10% concentration in anti-louse shampoos, 1:20 diluted with tap water for skin care of horses, 1:30 diluted with tap water against chicken mites, or 1:60 diluted with tap water against grass mites (*N. autumnalis*). These extracts were proven not only to be highly efficacious but also extremely skin-friendly (Pitterman et al. 2008). Furthermore, neem seed remnants can legally be mixed in a dose of up to 40% into food of chicken or cattle. When such DBE–neem extracts were used as active compounds in anti-lice shampoos such as "Wash-Away Louse", "Picksan Luizenstop", or "Wash-Away Dog/Cat", they turned out to be highly effective when they were used twice at an interval of 10 days, since the louse larvae hatch from nits within this period. In cases when the neem extract is diluted 1:30 with tap water, it was also proven to be highly efficacious against the red chicken mite *D. gallinae* (Abdel-Ghaffar et al. 2008a, 2009). It can be sprayed on chicken and on the floor at the same time and has the advantage—in contrast to true insecticides like the organophosphorous compound phoxim—that eggs laid during the spraying process must not be thrown away and that there is no waiting time for the consumption of chicken meat after the chicken had been treated (Abdel-Ghaffar et al. 2008a, 2009). Head lice, mallophaga, and mites die due the effect that the combination of the neem extract and the selected shampoo components cover the surface at the end of the tracheoles (fine channels of the breathing system of arthropods), thus blocking the oxygen transmission from the tracheoles into the cells. Due to this effect, the products "Wash-Away Louse", "Picksan Louse Stop", and "Mite Stop" have several advantages (e.g., no arising resistance) compared to commonly used products, the number of which just recently was legally restricted due to toxicity (Abdel-Ghaffar et al. 2009; Mehlhorn et al. 2009).

Vitex agnus castus

The spherical 3–4-mm-sized seeds (4 and 5 in Fig. 2) of *V. agnus castus* (plant family Verbenacea) was trivially named "monk's pepper" since in medieval times they were used as spice, while the seeds of the "true" pepper had been too expensive at this time—even for monks in rich monasteries. The plant reaches as a bush a height of 3 m and thus may appear tree-like when fully grown. All parts of this plant contain substances which have repellent activities against blood-sucking insects, ticks, and mites. While *V. agnus castus*—in contrast to *V. rotundifolia* or other *Vitex* species—has only slight repellent effects against mosquitoes, its activity against ticks and mites is very strong—especially when using extracts from seeds (Mehlhorn et al. 2005, 2006).

Such effects against blood-sucking arthropods are rather astonishing, since the seeds and leaves of this plant are mainly attacked by plant-eating insects. Nevertheless, patented extracts produced by CO_2 extraction or by help of PEG-40 hydrogenated castor oil were successfully used to develop the very effective Viticks® and Picksan® product series of repellents, which keep away ticks and insects for 5–6 h and thus prohibit the transmission of agents of diseases such as those of borreliosis or of several severe virosis. Extracts of *Vitex* seeds have the advantage that they do not contain chlorophyll and their extracts thus can be sprayed onto shoes and clothes without damage in addition to the regular skin treatment. Such ways of application are obligatory, since ticks attack humans via shoes and/or trousers. Extracts of *V. agnus castus* are also shown in clinical tests to be harmless to human skin (test system of the Clinic for Dermatology at Düsseldorf University, Germany). Furthermore, other extracts of this plant are successful components of medicaments commonly used to decrease menstruation pain (e.g., 14 registered products of such type are available in Germany).

Para-menthane-3,8-diol

At first look, this name does not seem to describe a plant compound, but indeed it does, since small portions of *para*-menthane-3,8-diol (PMD) are contained in oils of lemon-scented eucalyptus (*E. citriodora*) leaves (6 in Fig. 2). This tree, which often reaches a height of 20 m, contains inside its leaves an oil that was already used as repellent in the "Old Chinese Empire" (Leung 1985). However, this oil only develops its full activity when natural acids were added, introducing a natural chemical reaction that increases the amount of PMD components inside repellents. These effects of the natural plant extract are used when PMD was introduced as essential component in the product series "Picksan Tekenstop" and "Picksan Muggenstop" (tick stop, mosquito stop). Abdelkrim Amer and Mehlhorn (2006) had shown that the *E. citriodora* oil repels females of *Anopheles* and *Culex* mosquitoes for up to 8 h and keeps away surely the most aggressive specimens of *A. aegypti* for 3 h.

General remarks

Of course, in any case, it is important how the repellent-containing plant extract is fixed on the skin and/or on the clothes. Furthermore, it is clear that sweating will dilute the active compounds on the skin. Thus, the tests on the general efficacy of a plant extract is only the first step of research; the second step are the tests on toxicity and tolerability (e.g., in smelling). The final decision on the success of the transfer of a scientific result (e.g., high repellency activity) into a product

will be done by research for a stable product which offers the full efficacy of a compound as long as possible without harming the skin or destroying clothes even under unfavorable conditions (sweating, etc.). Therefore, in cases of the use of repellents on the skin and on clothes, efficacy studies alone are not sufficient to develop a product. However, the development of an efficient "off-body product" is much easier when looking at the problems described above but poses new and other ones (e.g., to avoid the quick evaporation of active compounds, which unfortunately is characteristic for etheric oils).

Open-air use of repellents

Abdelkrim Amer and Mehlhorn (2006) showed that, while testing oils of 41 plants and comparing them with DEET and Bayrepel® (Icaridin®, Saltidin®), the oils of cinnamon, lavender, cat mint, lemon grass, etc. have high repellent activities against mosquitoes. These oils are included besides tea tree oil and laurel oil in the product "Picksan Muggenstop doosje", the boxes of which have to be opened in the evening after being placed inside of open windows or beside the bed and which have to be closed after use in the morning. Intense laboratory tests have shown that the 6% mixture of these oils prohibits, e.g., mosquitoes from leaving one cage or room in order to fly into another. Unfortunately, these etheric oils are highly volatile. Therefore, the product "Picksan Muggenstop doosje" (mosquito stop box) is used as basis for a sophisticated mixture of water, alcohol, and agar that preserves the volatile oils for weeks if the box is closed after use. Tests showed in addition that the repellent effects are even present if the boxes had been open constantly for 14 days.

Conclusions

Thousands of older and recent publications show that plants contain thousands of components that have repellent or biocidal activities, which made and make it possible for plants to survive for millions of years the attacks of arthropods, nematodes, annelids, and even those of plant-eating mammals. During the times prior to chemotherapy, such plant components were used as essentials in remedies against human, animal, and plant diseases. However, in modern times, this knowledge has passed so that today many details have to be rediscovered. This way is hard and long. However, if there is no will and activity to develop products from these scientific test results—as it was done in the examples presented herein—the newly discovered or rediscovered results will be forgotten again in the future. Thus, this review should stimulate other groups to accept the help of nature in order to develop efficacious, non-toxic, and user-friendly products.

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