Natural products and their application to the control of head lice: An evidence-based review

Jörg Heukelbach¹,², Richard Speare² and Deon Canyon²
¹Department of Community Health, School of Medicine, Federal University of Ceará, Brazil; ²School of Public Health, Tropical Medicine and Rehabilitation Sciences, James Cook University, Townsville, Australia

Abstract

Head lice are insects that have been parasitizing human beings for thousands of years. Chemical pediculicides have been used extensively for the treatment of infestations since the early 20th century. Resistance to these pediculicides, such as permethrin, is constantly increasing and there is a need for the development of new effective compounds killing adult lice and eggs. Natural products have been used in traditional medicine since long and have been more
and more the issue of interest, since the costs are usually lower and they are in general considered less toxic. However, toxicology information on natural products is poor. Here we present an evidence-based review on plant compounds for the treatment of head lice infestations. An overview is given on published evidence on the use of pediculicides and ovicides in in vitro studies and clinical trials to cure pediculosis. Additionally, the available data on deterrent compounds are reviewed. We conclude that large numbers of plant products, such as neem and tea tree oils, offer promise for new compounds to treat head lice infestation. The number of clinical studies is very limited, and there is an urgent need to increase research assessing the effectiveness and safety of promising compounds. Over the counter natural products should be supported by in vitro data and adequately designed controlled trials that evaluate cure rates and safety. Providing that a new family of synthetic insecticides is not discovered, it is possible that plant products will replace chemical insecticides on the market in future.

Introduction

Head lice, *Pediculus humanus* var *capitis*, are wingless insects that have been obligate parasites of humans for thousands of years. Since the oldest archaeological record is 8,000 years BC, some authors have proposed that lice were associated with pre-hominoid ancestors and were dispersed globally when humans migrated [1]. Prior to modern existence, humans have removed head lice using physical techniques or attempted to kill them using various substances applied to the hair. Mutual grooming with capture of lice by hand is a common practice still today in many countries. Archaeological discoveries of lice in coprolites indicates that captured lice were often eaten. Combs with narrow teeth containing entrapped head lice have been found commonly in the archaeological record [2,3].

Topical treatments applied to the hair using plant parts and plant derivatives, also known as ‘natural products’, would have been used in these times, but the archaeological record is inadequate. It is likely that these botanicals contained insecticidal phytochemicals, predominantly secondary compounds, produced by plants as anti herbivory agents. However, there is little other than anecdotal, traditional or cultural evidence on this topic [4,5].

Having head lice is called pediculosis and this is subdivided into active pediculosis and inactive pediculosis. People with active pediculosis have live lice motile in the hair of the head or live embryos in viable eggs attached to hair shafts. People with inactive pediculosis have no live lice even in eggs, but hatched or dead eggs are present on hair shafts. Inactive pediculosis is historical evidence of past infection and although it is not an infection risk, it often is a cosmetic, social, mental and economic problem. Distinguishing
between these two states is unfortunately not often undertaken, but is critical since a person with active pediculosis is an infection risk, in contrast to a person with inactive pediculosis.

The child who has black hair and inactive pediculosis with prominent pale hatched eggs visible on the hair shaft is more likely to be sent home from school than the child with blond hair and active pediculosis whose eggs and lice are more difficult to see. The former is not an infection risk while the latter is. For active pediculosis lice and viable eggs need to be killed or removed. For inactive pediculosis cosmetic measures only are needed and removal of eggs becomes the major goal.

**History of pediculicides and current pediculicide situation**

In the twentieth century treatments based on chemical insecticides began to be used in developed countries. Dozens of natural and synthetic compounds were used, and hundreds were tested by the military during World War I & II in a search for an effective treatment for body lice [6], before DDT was discovered in 1939 and used towards the end of World War II to control lice [7].

Then in the late 1940’s, lindane, an organochlorine, was found to be a more effective pediculicide than DDT [8] and gradually replaced it in many countries [6]. Lindane is still used today in some countries but there remain concerns about its safety. As resistance to DDT emerged a decade later in Israel [9] and in the United Kingdom [10], organophosphates such as malathion were investigated as alternatives. Other chemicals in this group and carbamates were found to be highly effective against lice, but malathion’s safety record and lack of significant resistance until recently, have rendered it a permanent fixture in pharmacies even today. Carbaryl, a carbamate, was initially sold over the counter in the United Kingdom, but was subsequently restricted to doctor’s prescription in an attempt to limit resistance [11]. However, resistance to carbaryl is now developing in some centres in the United Kingdom [12].

In some countries carbaryl, lindane and benzyl benzoate are still available over the counter to treat pediculosis. Repeated or prolonged application of lindane shampoo is known to have caused seizures and deaths, but also in rare cases following a single application [13].

Casida (1980) [14] and Taplin and Meinking (1987) [15] wrote excellent reviews on pyrethrins and pyrethroids and their use as ectoparasite insecticides. It is certain that extracts from chrysanthemum flowers, *Chrysanthemum cinerariaefolium* in particular, have been used as insecticides for centuries since industry records date back to 1886. It was only around 1940
when pyrethrin, the active compound, was identified, and currently six natural pyrethrins are known. Pyrethrin is still popular today, despite numerous synthetic analogues on the market. Some assume that this is due to the public’s desire for natural pesticides, but the fact is that they still work in many situations. Natural pyrethrins have a number of advantages over other insecticides. They are very active, have very low mammalian toxicity, are biodegradable and have an important property, fast knockdown: the ability to rapidly disable an insect. After almost 30 years of research into synthetic analogues a suitable product, bioresmethrin, was discovered in 1967 by Michael Elliott at Rothamsted. Today, a large number of synthetic pyrethrins have been developed and they have largely replaced natural pyrethrin because, in general, they are over 1,000 times more active than natural pyrethrum I, and have extremely low mammalian toxicities (average LD$_{50}$ = 8,000 mg/kg). The mode of action of pyrethrins is similar to that of organochlorines in that they interfere with the permeability of sodium ion channels across nerve membranes. It could be argued that the high cost of producing these complex structures is offset by the very low application rates required. However, emerging resistance coupled with rather toxic effects on bees and aquatic life means that it has become imperative for new classes of insecticides to be discovered or created.

Currently in many countries, the mainstay of head lice treatments are topical chemical insecticides, such as permethrin, allethrin, deltamethrin, lindane or benzyl benzoate [16]. However, in vitro and clinical resistance to pediculicides containing insecticides, particularly to permethrin, has been increasing [12,17-25]. Permethrin resistance is particularly frequent in countries where head lice are common and where chemical insecticides are intensively used, mainly developed countries such as the United States, United Kingdom, France, Israel and Australia, but also in less developed countries, such as Argentina.

There is thus an urgent need for the development of new effective pediculicidal products on the market. One reason that natural products are being increasingly looked at is that there is a general perception that they kill lice using alternative mechanisms to manufactured chemicals. However, the evidence for this belief is very limited, owing to lack of research on the way natural products kill head lice. Most known insecticides target neurotransmitter receptors, ion channels and membrane transport processes [22], and natural product modes of action are not expected to be different. For instance, the synergist, pipronyl butoxide, which slows the biotransformation of pyrethrum in insects, works in the same way with natural products like neem [26]. This suggests that the identification of alternative pathways may be more difficult than first thought.
Additional reasons that are prompting researchers to investigate natural products more thoroughly are prohibitive costs of synthetic pyrethrins and pyrethroids, environmental and food safety, unacceptable toxicity of many organophosphates and organochlorines, besides the worldwide decreasing susceptibility [27].

**Life cycle and attributes of the ideal pediculicide**

Understanding how the ideal pediculicide would work requires an evidence-based approach and thorough knowledge of the head lice life cycle (Figure 1). Points of the life cycle relevant to treatment strategies are that eggs take about seven days to hatch and that lice become mature at about day 10 after hatching. Hence, if a treatment protocol does not kill embryos in eggs, it needs to be repeated after lice have hatched (i.e. seven or more days), but before the earliest hatched lice (i.e. those that hatched on day 1 after treatment) have become mature, mated and started to produce a new crop of eggs (i.e. at least 10 days). There is a narrow window of treatment opportunity (day 7 to 10) to cure an infection, assuming no reinfection. Most manufacturers, therefore, recommend retreatment at day 7, as a week makes biological sense and consumers find it easier to remember. It may, however, mean lice in eggs that were 1 or 2 days old when the first treatment was given and were slower to hatch, actually emerge after the second treatment.

![Figure 1. Life cycle of the head louse (courtesy of Nitpickers, http://www.nitpickers.com.au).](image-url)
The ideal topical head lice product has the following attributes:

- 100% kill of lice
- 100% kill of embryos
- removes all eggs from hair shafts (solving the cosmetic problem)
- repels lice (solving the reinfection problem)

This ideal product has not yet been developed! Research on topical treatments for head lice has focused on achieving 100% insecticidal and ovicidal effect. The former has been achieved with many chemicals and natural products, but the latter has not. Research has been scanty on repellents, and little research has been done on compounds to remove eggs from the hair shafts.

Natural products
Increasing demand for natural products
In 2006, the Organic Trade Association [28] reported that organic foods have a market share of 2.5% and have experienced annual growth rates of 15% to 21% since 1997. In addition, sales of organic personal care products was USD 282 million, an annual rise of 28% in 2005 alone. In contrast, Pamela G. Marrone, chairman and founder of AgraQuest, a biotechnology company specializing in the development of safe and environmentally friendly pest management products, estimated that 26 billion dollars are spent on synthetic pesticides worldwide per year, while only 300 million is spent on biological pesticides [29], indicating a market share of only 0.1%. So, we can expect significant growth in public demand for organic or natural products in all areas including insect control. Possibly consumer demand for organic pediculicides is leading this growth.

Are natural products a safer alternative?
If one of the main reasons for increased interest in ‘natural’ insecticides is the concern about safety, and there is a paucity of toxicology information on the topic, then one should look laterally for guidance. Food production requires that insecticides are to be applied to products that are eventually ingested. This is similar to pediculicides that are applied to the scalp except that the route of exposure is different.

Interestingly, the ‘natural’ tag does not really mean that much in reality. For instance, organic foods can be produced using approved synthetic pesticides and ‘natural’ biopesticides, and the United States Department of Agriculture (USDA) makes no claims that organically produced food is safer or more nutritious than conventionally produced food [30]. Some
biopesticides, such as the fungicide sulphur, are even more toxic than many synthetic alternatives [29].

Natural biopesticides are derived from materials from animals, plants, bacteria and minerals and can be classed as follows:

- **Microbial pesticides:** A pesticide consisting of a living bacterium, a virus or a fungus as the active agent. Instead of poisoning the target, a microbial pesticide infects a pest and then kills or inhibits it. Pesticidal chemicals extracted from microorganisms are called antibiotic pesticides. There are no microbial pesticides approved for use in or on humans.

- **Plant-Incorporated-Protectants (PIP):** Modified plants that have been enhanced by the addition of genetic material known as PIPs are able to produce pesticide substances internally.

- **Biochemical pesticides:** These are naturally occurring substances, such as pheromones, that can be used to manipulate or control pests by non-toxic action.

- **Natural pesticides:** Pesticides or growth modifiers consisting of a non-synthetic biochemical active ingredient or some other naturally occurring substance.

- **Organic pesticides:** From a natural products perspective, this is a misnomer. Organic chemicals consist mainly of a carbon skeleton plus other elements such as hydrogen, oxygen, nitrogen, sulphur, phosphorus or chlorine, so most pesticides are organic and not natural.

Natural products are usually considered less toxic than synthetic insecticidal compounds and culturally more acceptable. This mistaken perception is perpetuated in part by the fact that natural products are not required to undergo the rigorous toxicological testing required for standard pharmaceuticals.

In most countries natural products are listed, and are not required to be registered by national drug licensing authorities [30,31]. Listing is a lower category and usually can be achieved without demonstration of efficacy in clinical trials. Registered products have to demonstrate efficacy and safety in clinical trials. In Ellis et al (2004) [32], the United States Food and Drug Administration was reputed to have said that holistic products are not regulated as closely as pharmaceutical products, so false advertising and unfounded claims are common. Ignorance also plays a role, since it is not widely known that pyrethrin products are contraindicated for patients with contact allergy to ragweed or turpentine [33].

Although this is economically beneficial to companies marketing these products, it is unfortunate for safety since few, if any, scientists will deny that
many natural products (organic secondary compounds) have striking adverse affects on mammals and are more toxic than some synthetic insecticides [34-36].

Although decreased susceptibility to natural products has not been reported in any significant way, this is no reason to believe that resistance is less likely to develop if usage increases to the levels experienced by synthetic compounds.

Many products consist of a mixture of different plant products, but synergistic and even additive effects are not a standard expectation and antagonistic effects are possible. Synergistic mixtures would act to reduce the risk of possible resistance development in the future.

**Natural product insecticides**

Plant-based compounds or phytochemicals have been used in traditional medicine throughout the world to treat a variety of conditions and diseases, including ectoparasitic infestations, such as scabies and pediculosis. There have been extensive laboratory and applied investigations into the insecticidal activity of many phytochemicals against a wide variety of pathogens and arthropods [37-39]. As early as 1947, Roark listed over 1200 plant species from previous studies known to have insecticidal qualities. Biochemical insecticides are not simple compounds and often contain a wide spectrum of bioactive fungicides, nematicides, acaricides, insecticides and carcinogenic inhibitors.

Nash (2003) [40] stated incorrectly that “Herbal treatments (including tea tree oil) and aromatherapy are sometimes used to treat head lice. No studies have evaluated their efficacy or possible toxicity.” Many natural products have been evaluated in *vitro* against body lice and head lice and some in clinical trials against pediculosis (Table 1). Many of the products available commercially use combinations derived from several natural sources. In some instances this is to achieve synergy. However, in such combinations the active ingredient is difficult to identify unless the components are evaluated individually. One reason manufacturers adopt the approach of combining several source plants or products is that many of the combinations are not patented and could be copied by competitors. However, by using combinations the active ingredient can be disguised to some extent.

In this review we have included data from published in *vitro* or clinical trials only and have omitted records of natural product pediculicides based on historical or anthropological reports.

**In *vitro* efficacy**

The *in vitro* assessment of insecticidal activity is typically done prior to clinical use. Head lice (*P. humanus* var *capitis*) or captive bred body lice (*P.*
humanus var corporis) are used for in vitro studies, the latter much more frequently than the former. The reason for this is that captive bred body lice show no insecticide resistance and can be provided at uniform life stages in suitable numbers when desired. Head lice are usually collected off heads prior to use (posing a logistical problem in certainty of numbers available), are always of different sex, stages and ages, and may be insecticide resistant. Head lice have been raised in captivity, but not as a continuous captive strain unlike body lice [41].

It is assumed that the killing effect on body lice is the same for head lice although this has not been demonstrated comprehensively across a range of products. Interestingly, a study using wild-caught head and body lice showed significant differences in responses to a range of insecticides [42]. In vitro testing techniques for assessment of the killing effect of chemicals and products on head lice are not standardized, making comparison across techniques difficult [43-45]. The criteria for mortality used in laboratory-based studies differ from study to study and are difficult to compare; in many cases, the definition of ‘mortality’ is not sufficiently strict. For example, head lice may resurrect after being depressed for some hours and thereafter show normal activity again [22,43-45]. As a consequence, several compounds such as mayonnaise, petroleum jelly and olive oil have been described to kill head lice, but in reality caused a transient knock-down period; after some hours the head lice show normal activity [22,45,46]. In vitro tests need to be done with negative and positive control groups using standardised criteria for mortality and the lice have to be observed for a prolonged period. We have not attempted here to differentiate between published results based on the techniques used.

The most comprehensive in vitro study of natural products comprised 54 essential oils against adult female head lice collected from children in South Korea and compared mortality against pyrethrum and δ-phenothrin [47]. Sixteen of the 54 essential oils had efficacy equal to or greater than pyrethrum and a further six were slightly less active. Yang et al (2004) [47] also noted differences in activity within plant genus indicating that finding an active essential oil derived from one species does not imply other species in the same genus are also active. These authors noted that the most active essential oils had a fumigant activity due to volatile compounds as well as a contact activity on head lice, whereas pyrethrum and δ-phenothrin had contact activity only.

Pyrethrins

The in vitro activity of pyrethrins is well known [15] and pyrethrum has been used as a standard to screen other natural products for activity against head lice [47-49]. Resistance of head lice to pyrethrins has also been shown using in vitro tests [20,50], the prevalence of resistance varying from site to site in both studies.
Tea tree oil

Tea tree oil is distilled by steam from the leaves of the tea tree, *Melaleuca alternifolia* and is a complex mixture of over 100 hydrocarbons and terpenes [51]. Veal (1996) [52] obtained 94% mortality against body lice with 1% emulsion of tea tree oil in water, although efficacy varied between replicates. In another study, tea tree oil killed head lice but less effectively than δ-phenothrin and pyrethrum [47]. An *in vitro* study against head lice showed that 1% tea tree oil caused no mortality after 2 hours while 10% resulted in 86% mortality [51]. We have shown a 97% mortality of head lice after four hours with an emulsion of 5% tea tree oil in 20% ethanol, but <50% mortality with concentrations of 1% and 2% tea tree oil (J. Heukelbach, personal observation). However, since some extracts of tea tree oil are ineffective in killing head lice *in vitro* (R. Speare, personal observation), careful characterization of the extract is indicated.

Four compounds from tea tree oil showed markedly different efficacies against head lice *in vitro* [51] although all failed to produce significant mortality at 1%. The most active compounds were terpinen-4-ol, terralin and α-terpeninol that were each 100% effective at 10% concentration. Walton et al (2004) [53] showed that although tea tree oil was effective at killing the scabies mite *Sarcoptes scabiei* *in vitro*, the killing efficacy of components of tea tree oil varied markedly. Some of the compounds in tea tree oil, 1,8-cineole and terpinen-4-ol, have an inhibitory effect on acetylcholinesterase [54], an enzyme targeted by the organophosphate insecticides, and this action may play a role in killing head lice.

A mixture of tea tree oil (*Melaleuca alternifolia*) and cinnamon leaf oil (*Cinnamomum zeylanicum*) (1:1) at 1% with 40% ethanol had a 100% efficacy against adult body lice after a 17 hr exposure to the oil mixture [52]. Exposure to a 0.1% oil-vinegar emulsion resulted in only a 2% mortality.

Neem

Neem (*Azadirachta indica*) extract killed head lice slowly *in vitro* in a filter paper test and was less effective than malathion, permethrin and benzyl benzoate [55]. However, the units of concentration of active ingredient reported in the paper are confusing. In the same study lemon (*Citrus medica*) extract had less effect on head lice than neem extract, and *Aloe vera* extract had no killing effect after 3 hours. A neem based shampoo was highly active *in vitro* against head lice that were partly resistant to a 1% permethrin product [44]. The neem extract in this shampoo is evidently derived from the residue after extraction of the oil.

About 150 compounds have been isolated from neem tree products, for example isoprenoids and its derivatives (such as nimbin, salanin and azadirachtin), sulphur compounds, flavonoids and polyphenolics [56]. Some
active compounds have been shown to be insecticidal and to have insect repellent activity, such as 22,23-dihydrnimocinol extracted from leaves and azadirachtin, extracted from seeds [56].

**Other plant products**

Of the several plant essential oils identified as highly potent by Yang et al (2004) [47,48], the most active was *Eucalyptus globulus* oil, followed by pennyroyal (*Mentha pulegium*), marjoram and rosemary oil that were more active than δ-phenothrin. Other essential oils, such as cade, cardamone ceylon, clove bud and leaf, coriander, cypress, *Eucalyptus citriodora*, myrtle, peppermint, spearmint, rosewood, basil and sage oil were more active than δ-phenothrin [47]. A previous study had demonstrated essential oils of clove bud and leaf had *in vitro* activity against head lice comparable to δ-phenothrin and pyrethrum and that this was probably due to the ingredients eugenol and methyl salicylate [49]. These latter compounds also had fumigant activity against head lice while δ-phenothrin and pyrethrum did not.

Red thyme (*Thymus vulgaris*), oregano and aniseed oils at 1% for 17 hrs followed by a 0.1% rinse each resulted in 100% mortality against adult body lice [52]. Cinnamon oil tested in a similar technique had 86% mortality [52] and was also shown to be effective against head lice in another study [57]. Mixtures of oils of 1. red thyme and rosemary (*Rosmarinus officinalis*) and 2. peppermint (*Mentha piperita*) and nutmeg (*Myristica fragrans*) at 1% concentration for 17 hr resulted in 87% and 100% mortality in body lice [52].

Essential oil from the African tea bush, *Lippia multiflora*, killed head lice *in vitro* but was less effective than kerosene and more effective than benzyl benzoate [42]. Activity against wild-caught body lice was comparable. The composition of the essential oil of *Lippia multiflora* varies with location highlighting the potential problem of variability in composition of essential oils with source [58].

Four combinations of paw paw (*Asiminia triloba*), thymol (*Thymus vulgaris*), and tea tree oil (*Melaleuca alternifolia*) in a shampoo base were trialled *in vitro* against head lice to select the most effective combination [59]. The most effective combination was then used in an open clinical trial (Table 1). The authors proposed that the product worked by depleting ATP levels in lice, but provided no evidence to support this statement.

Priestley et al (2006) [60] in a very comprehensive *in vitro* study using body lice examined 28 compounds found in essential oils. The compounds fell into high and low efficacy groups, with mono-oxygenated compounds being most active against body lice. Substances with flat compact molecules also showed most activity. The top seven most active compounds had a methyl group [60].
**Table 1.** *In vivo* clinical studies on the efficacy/effectiveness of plant products against head lice (studies testing pyrethrins have not been included).

<table>
<thead>
<tr>
<th>Name</th>
<th>Active compounds</th>
<th>Type of trial</th>
<th>Number of subjects included</th>
<th>Efficacy/Effectiveness</th>
<th>Comments</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cream based on custard apple seed extract</td>
<td>Custard apple (<em>Annona squamosa</em>) 20%</td>
<td>Unblinded, controlled</td>
<td>37 (treatment groups) 6 (negative control)</td>
<td>Killed &gt;90% of lice</td>
<td>Traditional medicine</td>
<td>[61]</td>
</tr>
<tr>
<td>Paw Paw Lice Remover Shampoo</td>
<td>Shampoo based on paw paw (<em>Asimina triloba</em>), thymol (<em>Thymus vulgaris</em>), and tea tree oil (<em>Melaleuca alternifolia</em>)</td>
<td>Unblinded, uncontrolled</td>
<td>16</td>
<td>100%</td>
<td>Patients carried out 3 treatments 8 days apart.</td>
<td>[59]</td>
</tr>
<tr>
<td>Chick-Chack</td>
<td>Coconut oil, anise oil, ylang ylang (<em>Cananga odorata</em>) oil</td>
<td>Unblinded randomized comparative trial</td>
<td>70 (treatment group), 73 (comparative group with permethrin and malathion)</td>
<td>92.3% (treatment group) 92.2% (control group)</td>
<td>Parents of infested children carried out the treatment</td>
<td>[83]</td>
</tr>
<tr>
<td>Wash-Away™ Louse</td>
<td>Neem (<em>Azadirachta indica</em>) seed extract</td>
<td>Unblinded, uncontrolled</td>
<td>60</td>
<td>100%</td>
<td></td>
<td>[67]</td>
</tr>
<tr>
<td>Quassia tincture</td>
<td>Jamaica quassia (<em>Picrasma excelsa</em>)</td>
<td>Unblinded, uncontrolled</td>
<td>160</td>
<td>98%</td>
<td>Assessed 1 week after a single treatment</td>
<td>[84]</td>
</tr>
</tbody>
</table>
Custard apple (*Annona squamosa*) has been highly efficacious in killing head lice in several studies (cited in [61]). An extract of custard apple seeds in coconut oil killed 98% of head lice within two hours, and a petroleum ether extract of 20% *A. squamosa* seeds achieved a mortality of 93% (cited in [61]). In these studies, the seed extracts showed higher killing rates than leaf extracts.

**Natural products from micro-organisms**

Recently an insecticidal compound, spinosad, produced by the bacterium *Saccaropolyspora spinosa*, has been shown to kill permethrin resistant head lice *in vitro* [62]. Spinosad has a low mammalian toxicity (3783-5000 mg/kg in the rat and may be suitable as a topical treatment for head lice [63].

**Clinical trials**

**Pyrethrins**

Several clinical trials, including blinded randomized comparative and controlled trials have been conducted on head lice products based on pyrethrins [14,15,64,65]. Resistance to pyrethrins is more common in the USA than in Panama, a developing country [66].

**Neem**

A shampoo based on a neem tree seed extract (*Azadirachta indica*) was effective in an open clinical trial [67]. Interestingly, this shampoo (Wash Away Louse™), which is commercialized in Germany, is made from the fibrous residue remaining after the oil is extracted, material which is usually regarded as waste and thus cheap to obtain.

Formulations based on neem have been extensively used for more than a thousand years in traditional Indian medicine due to its insecticidal, repellent and pesticidal properties [56]. Neem-based products used in agriculture are biodegradable, and the mammalian toxicity is considered low [56]. However, it is toxic to fish and aquatic invertebrates which limits its agricultural use close to aquatic environments [56].

There are many publications on the acute, subacute and chronic toxicity of *A. indica*, indicating that the therapeutic topical use of neem products on humans can be considered as safe [56,68]. An open trial with a topical compound based on neem and turmeric achieved a 97% cure rate in scabies [69]. However, there are single cases of intoxication in children after oral ingestion of Margosa oil, which contains neem [68]. In fact, the crude oil can induce toxic effects similar to the symptoms observed in the children having ingested Margosa oil, when administered orally to animals [68,70,71].

**Tea tree**

Tea tree oil is occasionally used alone in natural products for pediculosis, but typically it is used in combination with other essential oils and is arguably one of
the most common ingredients in natural products. However, adequately designed clinical studies are similarly overdue as for most other plant-based products. A clinical trial which evaluated a shampoo containing paw paw, thymol and tea tree oil was an open study and evaluation criteria were poorly described [59].

The topical use of tea tree oil can be considered as safe. However, allergic contact dermatitis has been observed after the use, and in higher doses it can cause skin irritation [72-79]. Toxic effects occurred in children after accidental oral ingestion of small amounts of the oil [80-82].

Other natural products

A preparation containing 20% custard apple (Annona squamosa) seed extract was shown to be highly effective against head lice infestations in a small controlled trial [61]. More than 90% of lice were dead three hours after application of the product to infested school children, as compared to 60% dead lice in the control group, treated with 25% benzyl benzoate emulsion. Custard apple has been used in traditional Thai medicine against pediculosis since long [61].

A product containing coconut oil, anise and ylang ylang applied every five days was highly effective (92% cure rate) in an unblinded comparative therapeutic trial with clearly defined assessments [83]. Interestingly, in an in vitro study, ylang ylang did not show any significant effect on the survival of head lice [47], consistent with our studies in Australia that showed no killing effect against head lice in vitro (R. Speare, personal observation).

A tincture prepared from Jamaica quassia (Picrasma excelsa) used to wash the hair and then air-dried in an open study had a 98% cure rate a week after a single treatment [84].

Natural product ovicides

Topical treatments are used to kill lice and if effective, will kill 100% of the motile lice, but typically kill less than 50% of the embryos in eggs. One reason for this is that most effective insecticides are neurotoxins and for young embryos (two days and less), the nervous system is too immature to be affected. Another reason is that penetration of products into the egg is apparently inadequate, and killing doses are not attained.

Products are usually evaluated for their insecticidal effect (ability to kill lice) and their ovicidal effect (ability to kill embryos). Since most products do not claim a 100% ovicidal action, typically manufacturers recommend a second application seven days after the first to kill lice which have hatched over the intervening days.

In vitro efficacy

One of the earliest reports of ovicidal efficacy was published by King (1954) [85], who demonstrated some ovicidal efficacy when using dodecyl
alcohol, a component of coconut oil. Until the 1990s, studies on pediculicides that have included an investigation into ovicidal properties have been lacking.

In an interesting study by Veal (1996) [52] on three blended oils (1: red thyme & rosemary, 2: peppermint & nutmeg, 3: tea tree & cinnamon) with and without an oil-vinegar-water rinse the procedure was as follows: eggs were exposed to the oil in solvent, returned to the incubator overnight, washed in shampoo, rinsed with the oil/vinegar/water mixture, and returned to the incubator for about a week. After all the lice from the control gauzes had emerged and died, the numbers of hatched, half-hatched, dead, and undeveloped eggs were noted for each of the replicates. Ovicidal efficacy, expressed as percentage mortality, without the rinse stage was not impressive (Blend 1: 3.7%, 2: 12.6%, 3: 6%), however the rinse was surprisingly effective (Blend 1: 39.4%, 2: 82.4%, 3: 96.2%) and did not work without prior exposure of eggs to the oils. When seven essential oils (rosemary, pine, tea tree, oregano, cinnamon, aniseed, red thyme) were tested independently with an ethanol rinse, mortalities ranged from 83% to 100%.

The only other significant work has been conducted by Yang’s team in Seoul National University, Korea. In 2003, they investigated Eugenia caryophyllata bud and leaf oil-derived compounds against louse eggs [49]. Methyl salicylate and eugenol were found to be highly effective at 0.25 and 1.0 mg/cm², respectively, whereas little or no activity at 5 mg/cm² was observed with the other test compounds as well as with δ-phenothrin and pyrethrum. In 2004, Yang et al. [48] investigated the toxic effects of Eucalyptus globulus leaf oil-derived monoterpenoids (1,8-cineole, l-phellandrene, (-)-α-pinene, 2-β-pinene, trans-pinocarveol, γ-terpinene, and 1-α-terpineol) and the known Eucalyptus leaf oil terpenoids (β-eudesmol and geranyl acetate) on eggs and females of the human head louse. At 1.0 mg/cm², (-)-α-pinene, 2-β-pinene, and γ-terpinene exhibited moderate ovicidal activity, whereas little or no ovicidal activity was observed with the other terpenoids and with δ-phenothrin and pyrethrum. In these studies, ovicidal activity was noted to be compound- and dose-dependent, but the exact adulticidal and ovicidal mode of action of 1,8-cineole, (E)-pinocarveol, and 1-α-terpineol remains unknown.

Finally, in 2005, Yang et al. [57] conducted a study on essential oils from cinnamon bark and found that they could be useful as insect control fumigants or ovicides for lice. Ovicidal effects from a direct contact bioassay were measured by reduced hatching and were compared with those of δ-phenothrin and pyrethrum. After 24 h of exposure, cinnamon reduced hatching to 3% at 0.25 mg/cm², to 27% at 0.125 mg/cm² and to 55% at 0.063 mg/cm². Component analysis showed no hatching with 0.063 mg/cm² salicylaldehyde, 0.125 mg/cm² benzaldehyde, 1.0 mg/cm² cinnamaldehyde and 1.0 mg/cm² benzyl cinnamate. Salicylaldehyde and benzaldehyde gave 13% and 17% hatching at 0.031 and 0.063 mg/cm², respectively.
Cinnamaldehyde and benzyl cinnamate caused 7 and 18% hatching at 0.5 mg/cm², respectively. Little or no ovicidal activity at 1.0 mg/cm² was observed with benzyl alcohol, cinnamyl acetate, δ-phenothrin and pyrethrum compared with the acetone control.

Priestley et al (2006) [60] evaluated in vitro ovicidal activity against eggs of body lice for 19 compounds found in essential oils. Mono-oxygenated monocyclic terpenoids had high ovicidal activity. Citronellic acid and nerolidol were highly ovicidal, but had no activity against adult lice.

Clinical trials

Abramowicz (1995) [86] stated that pyrethrins must be applied twice, separated by a week because clinical observations have shown them to be not ovicidal. To enhance activity, most pyrethrins are now formulated with a synergist, commonly piperonyl butoxide [6], but these still lack ovicidal potency. Cordero and Zaias (1987) [87] conducted a clinical evaluation on 40 children into the ovicidal efficacy of two pyrethrin-piperonyl-butoxide formulations. They found reasonable efficacy with the shampoo formulation being significantly better than a lotion. Following this, Burgess (1994) [88] published laboratory findings and a clinical trial involving a quick-break foam mousse that appeared to overcome egg surface tension problems encountered by other insecticides. Although it was claimed that only a single application was required to kill all louse eggs, it must be noted that the eggs were removed from the head after treatment to assess ovicidal activity which may have skewed results.

Natural lice deterrents

Thinking that there are merely insecticides and repellents is a misconception. Dethier’s (1947) [89]definition of a repellent, which states that a repellent must cause ‘oriented movements away from its source’, does not come close to describing the plethora of possibilities open to deterring lice. Deterrents function by the following mechanisms:

- Mechanical – a product can deter transmission by rendering hair slippery or clothing and hairstyle can constitute significant physical barriers.
- Odorant – a product can contain chemicals that bind to odor receptors to create a behavioral response.
- Irritant – a product that contains chemicals that repel insects by produce an excito-repellent effect.
- Toxic – a product containing toxic compounds that are detectable or whose effects are undesirable which leads to aversion of a treated area.
Canyon and Speare (2006) [31] stated that the following outcomes of deterrents are important to consider:

- **Tropotaxis** (avoidance) - refusal of lice to walk onto repellent-treated hair.
- **Tropotaxis** (hesitation) – lice do not avoid repellent-treated hair, but do so with some hesitation and/or reversing and leaving the treated area.
- **Orthokinesis** – the speed of progress of lice is negatively affected while walking on the treated portion.
- **Klinokinesis** (confusion) – lice experience disorientation while on the treated hair.
- **Antifeedance** – lice move onto treated areas, but are disinclined to blood feed.

**In vitro efficacy**

Although Burgess (1993, 2004) [43,90] cited the first documented research into lice repellents as being the evaluation of lavender for use against clothing lice in World War II [91], several observations preceded this work. Twenty eight years earlier, three notable researchers first made mention of the detrimental effects of various oils used as hair dressing on head lice [92-94]. They made it fairly clear that some of the oils used as dressing for the hair acted as deterrents to head lice transmission.

Twenty years later, Buxton (1938) [95] noted that hair samples originating from Ceylon often contained very large numbers of first stage head lice nymphs that were dead when the collections were made. He hypothesized that the use of coconut oil on the hair had coated the eggs and rendered them so sticky that the first stage larva failed to emerge from the egg. Subsequent trials using dodecyl alcohol on body lice demonstrated complete knock-down within 24 hours and all exposed body lice were killed [85]. In 1969, Pence and Viray [96] reported that dodecyl alcohol, a component of coconut oil, caused atrophy and developmental abnormality in several species of insects.

Piperonal has an interesting history as a deterrent, but its story starts when researchers evaluated its usefulness in controlling human lice in North America [97] and in Australia [98]. Its repellent qualities remained unreported until Peock and Maunder (1993) [99] tested a 2% piperonal spray in the lab and found it to exhibit consistent high repellency after 30 minutes, dropping slightly over 24 hours. This spray was found to be twice as effective as N,N-diethyl-3-methylbenzamide (DEET). In what appears to be a rehash of this research, Burgess (1993) [90] repeated the results and avidly supported piperonal. This research, however, was vigorously refuted by Ibarra and
Williams (1994) [100] who had valid safety concerns and believed that it was ineffective. In their dialogue, Ibarra and Williams went on to state that anyone who used DEET, formerly known as N,N-diethyl-m-toluamide, betrayed a misunderstanding of its mode of action. Despite this, it has been used in a comparative way in several studies to evaluate the efficacy of various natural product deterrents. In truth it is not a very useful control since it failed to significantly outperform various essential oils and terpenoids applied to hair substrates in vitro and skin [31,101]. The insecticidal qualities of terpenoid compounds that are commonly found in essential oils, are well known [102]. Oladimeji et al (2000) [42] evaluated the pediculicidal efficacy of Lippia multiflora Moldenke, synonym Lippia adoensis Hochst (Verbenaceae), a shrub found growing in West Africa Savannah that contains terpenoid chemicals. The major constituents were linalool (26.7%), geraniol (20.4%) and limonene (15.4%). Minor constituents terpineol (2.9%), a-pinene (1%) and b-pinene have known insecticidal properties and have been shown to kill body lice [102,103].

Canyon and Speare (2006) [31] tested tea tree (Melaleuca alternifolia, lavender, peppermint, coconut oil, a rosemary-bay-cedarwood-patchouli-ylang ylang-jojoba-apricot mixture (Mix 1), a coconut-neem-citronella mixture (Mix 2), a tea tree-lavender-rosemary-citronella-neem mixture (Mix 3) and a neem-citronella mixture (Mix 4) (Figure 2). This study found that all substances tested were non-efficacious as head lice repellents with only a marginal level of activity demonstrated. Tea tree was shown to be the superior repellent and an antifeedant. Over half of the head lice transferring to a tea tree-treated head would thus be expected to transfer out as soon as possible and failing that would be less inclined to survive since blood meals would be inhibited. Although all oils reduced transmission to a treated hair by 33%-65%, most of this effect was due to the slippery nature of treated hair as demonstrated by the response to KY-Jelly, which was used as a 'slip' and irritant control. This was thought to partly explain why head lice are more prevalent in clean hair heads than in greasy hair heads.

The value of the irritant response was questionable since most lice progressed forward despite showing some tropotaxis except with coconut. Neem and citronella were not significant irritants and reduced the irritancy of coconut when combined.

Lavender, a common ingredient in head lice preventative preparations, is only useful in limiting blood feeding, but may help to tone down the strong smells of the other substances.

Any application of a repellent or other preventative in infested individuals may act to increase the dispersal of head lice to other contacts in social groups or family. Head lice are thought to view people's heads as 'rooms' in their 'home' rather than viewing a head as a permanent abode (D. Canyon, personal
Natural products against head lice

Figure 2. Comparative summary of overall test substance efficacy against head lice in various experimental conditions involving transmission, irritancy, repellence and antifeedance.

observation). Core social groups in classrooms probably facilitate this behaviour. Increased dispersal within the core group would thus cause a more rapid change of rooms with greater potential to infest subsidiary contact groups. So, application of preventatives to all subsidiary groups and family would be beneficial if combined with identification and thorough treatment of all core group members.

These results supported those obtained earlier by Mumcuoglu et al (1996) [101]. In a study on several essential oils, such as lavender, rosemary, piperonal, eucalyptus, D-limonen and citronella, they found that apart from citronella oil and its active component citronella, all elicited less than 50% repellency. Repellency was greatly affected by volatility resulting in limited efficacy after a few hours.

Clinical trials

Following their promising in vitro results, Mumcuoglu et al (2004) [104] went on to conduct a randomized, double-blind, placebo-controlled clinical trial to evaluate the efficacy of a citronella formulation as a louse repellent. The study was conducted over four months in four elementary schools in which 103 children were treated with the test formulation and 95 received a placebo. Evaluations every two months resulted in a significant difference between treated children (15.4% infested) and controls (55.1% infested).

In what may be an end to the piperonal saga, CM Brown and IF Burgess (unpublished in Burgess 2004 [43]) evaluated piperonal in a six month long, double-blind, cross-over, clinical field trial, involving forty families. Although this study is yet to be published, the author stated that piperonal failed to demonstrate clear effectiveness.

Despite a distinct lack of impressive results in in vitro studies and little in the way of clinical evidence of efficacy, home remedy deterrents are gaining
favour as a way of addressing resistance issues, mistreatment, and reinfection. In action on the part of the scientific community in identifying an effective compound may be changing due to the increasing global prevalence of pediculosis and accompanying increased awareness, which has resulted in a dramatic increase in the number of over the counter head lice repellents and preventatives that are not accompanied by quality assurance or regulation.

Natural products able to remove head lice eggs

There are two reasons for needing to remove head lice eggs. The first was discussed in the above ovicide section which described how few pediculicides are effective as ovicides leading to the necessity to retreat after eggs have hatched following initial treatment. The second is that empty or dead eggs can lead to people being falsely accused of harbouring an infestation.

Louse eggs are laid at the base of hairs close to the scalp and are attached firmly by means of a quick-hardening secretion from the ovipositing female. Eclosure occurs from 6 to 10 days later. Empty eggshells (nits) and dead eggs usually remain attached to the hair for as long as 6 months and sometimes over a year. Since human hair grows at a rate of about 1 cm per month, nits slowly become more visible as the hair they are on grows away from the scalp. The presence of nits commonly results in misdiagnosis and they can cause social and mental disturbance.

Mechanical removal of lice and lice eggs is common in many developing countries and while it is often used to enhance social bonding, it is a time consuming method. Since many eggs are often nonviable, their removal is often impractical and unjustified [105,106]. Removal using fine toothed nit combs is common and can be facilitated by wetting the hair or applying a slicking agent.

Although the ultimate egg removal product would aim to dissolve the secretion that binds the egg to the hair shaft, this has not been achieved yet and most chemical and natural products assist in the removal of eggs by making the hair more slippery. Several have been tried such as the use of 8% formic acid [107], acid shampoos (pH 4.5–5.5), 5% acetic acid, vinegar (diluted 1:1 with water), conditioners and vegetable oils which are claimed to be effective in detaching eggs and nits from the hair [105].

There are no chemical or natural remedies for nit removal on the market that have been clinically evaluated.

Conclusions

Large numbers of plant products offer promise for new compounds to treat head lice both as essential oils and as individual compounds identified as active ingredients in the effective essential oils. A major challenge for manufacturers of natural products will be maintaining a commercial product of
consistent composition owing to the potential variation in composition of essential oils with location, cultivar, season and over time.

The number of new commercially available natural products for head lice has expanded over the last decade to a much greater extent than products containing defined chemical insecticides. However, the evidence on the efficacy of these new products based on published results of in vitro and clinical trials is markedly deficient. Evidence on safety is also deficient. All over the counter natural products should be supported by in vitro data and well designed comparative therapeutic trials using head lice derived from the populations for whom the product is intended.

Since the prevalence and degree of insecticide resistance of head lice to pyrethrin, permethrin and malathion is expected to increase, alternative topical therapies for pediculosis are needed. It is possible that, on the long run, plant extracts, or their constituent compounds, will replace chemical insecticides on the market.

References
10. Maunder, J.W. 1971, Resistance to organochlorine insecticides in head lice, and trials using alternative compounds, Medical Officer, 125, 27.
Natural products against head lice

76. Khanna, M., Qasem, K., and Sasseville, D. 2000, Allergic contact dermatitis to tea tree oil with erythema multiforme-like id reaction, Am. J. Contact Dermat., 11, 238.
94. Nuttall, G.H.F. 1918, Combating lousiness among soldiers and civilians, Parasitology, 10, 411.
95. Buxton, P.A. 1938, Studies on populations of head lice (Pediculus humanus capitis, Anoplura), Parasitology, 30, 85.
103. Windholz, M. 1983, The Merck Index, Merck, Rahway, USA.