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Ancient treatment for lice: a source of suggestions for carriers of other infectious diseases?

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SUMMARY

Louse infestation is one of the oldest contagious pestilential diseases of humankind, which has recently re-emerged in several developing countries as well as in homeless individuals and migrants. The present work provides the first phase of an historical excursus of louse remedies based on the classics of pharmaceutical literature, codes, pharmacopoeia and treatises. The second phase involves a literature search, based on the principal medical databases (SciFinder, Pubmed, Google Scholar, ISI-Web of Science and Scopus), to match ancient raw materials and active principles for the treatment of pediculosis and their possible applications, with other current infectious pathologies transmitted by different carriers. In this regard, *Rhododendron tomentosum* has revealed repellent insect activity, particularly against *Aedes aegypti*, responsible for Dengue fever, Chikungunya, Zika fever, Mayaro, yellow fever and other infectious diseases. *Petroselinum crispum* is an insecticide employed for resistant strains of *A. aegypti*. In the case of *Delphinium staphisagria*, the phytochemical profile was further investigated with the identification of further molecules in addition to delphinine. The latter shows interesting activities against *Trypanosoma cruzi* and *Leishmania*. *Anthemis pyrethrum*, now renamed as *Anacyclus pyrethrum*, although not containing pyrethrins present in several plants of the genus Chrysanthemum, revealed pediculicidal activity but did not produce satisfactory results in antiprotozoal activity.

Keywords: louse, remedies, typhus, mosquito-borne diseases, trypanosomiasis, Chagas disease, leishmaniasis.

INTRODUCTION

Louse infestation, called pediculosis, one of the oldest pestilential diseases of humankind, is very contagious and easily transmitted by close body-to-body contact or contact with infested linen, brushes, or clothes, according to the species of louse. *Pediculosis capitis*, caused by head lice, is the most common louse infestation; it particularly affects school-children 3-11 years of age and its clinical hallmark is scalp pruritus [1]. *Pediculosis corporis*, caused by body lice, represents a major public health concern. It is strongly associated with close body-to-body contact, and occurs only when clothes are not changed or washed regularly. These conditions are more prevalent in individuals living in crowded and unhygienic environments, such as refugee camps or shelters for the homeless and migrants.

Clinical manifestations include generalized pruritus associated with scratching and lesions are typically localized to the neck, thorax, waist, and ankles. Diagnosis is based on a finding of adult lice and, more importantly, eggs in clothing seams.

Three types of louse can affect humans, but only body lice act as vector for human pathogens such as *Rickettsia prowazekii*, *Borrelia recurrentis* and *Bartonella quintana*, which are known to cause epidemic typhus and louse born relapsing fever

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(LBRF) which must be considered in any febrile refugee regardless the country of origin, and trench fever, respectively.
These infections particularly affect populations living in poor-hygiene conditions where body louse infestations are prevalent. Whereas epidemic typhus and epidemic relapsing fever are particularly prevalent in vulnerable populations in developing countries, trench fever is common worldwide, especially in homeless individuals.
Louse-borne diseases are associated with a high prevalence of body louse infestation, and have recently reemerged in jails and refugee camps in central and eastern Africa, Peruvian Andes and in rural louse-infested populations in Russia [2, 3]. LBRF, a neglected and forgotten disease by western physicians has recently reemerged among East African migrants seeking asylum in Europe [4]. Migrants from endemic areas can carry the vector with them; healthcare providers should be aware of this condition to implement adequate diagnostic, therapeutic, and public health measures.
The historical path that has led to today's remedies against pediculosis has been long and laborious, although it is worth recalling that the insecticide action on the common louse can now be exploited against the carriers of severe infectious diseases now rapidly spreading from a continent to another.
In 1939, the dichlorodiphenyltrichloroethane (DDT) came into pratical use revolutionizing the cornerstones of disinfestation although it was banned in 1978 for its adverse health effects. After DDT benefit, treatment of pediculosis has been continued since the late 1970s by the employment of synthetic pyrethroids developed from natural molecules drawn from *Chrysanthemum cinerariaefolium*. Among them, permethrin has shown to be the most effective product. Other synthetic pyrethroids are phenotrine, deltamethrin and sumitrin. A second choice treatment consists of malathion, an organophosphorus pesticide.
The present work provides a first part on an historical excursus of pediculosis remedies based on the classics of pharmaceutical literature, codes, pharmacopoeia and treatises (Figure 1).
A second phase part will involve a literature search, based on the principal medical databases (*i.e.*, through SciFinder, Pubmed, Google Scholar, ISI-Web of Science and Scopus), to match ancient raw materials and active principles for the treatment of pediculosis and their possible applications, with other current infectious pathologies transmitted by different carriers.

Figure 1 - *De Animalibus: Pediculus*, Ortus sanitatis, 1517.

Historical notes

The role of *Pediculus* as a vehicle of epidemic typhus infection was established in 1909 by the Nobel Prize in Medicine Charles Nicolle who discovered that epidemic typhus is transmitted by body lice (*Pediculus humanis corporis*) [5]. The epidemiology of head louse and body louse infestations, and of louse-borne epidemic typhus, indicates that the head lice are potential vectors of *R. prowazekii* in the field [6].

Epidemics occurred during wars and famine and epidemic typhus has been one of the earliest pestilential diseases that have strongly influenced humanity [7].

Paleomicrobiology enabled the identification of the first outbreak of epidemic typhus in the 18th century in the context of a pan-European great war in the city of Douai, France, and supported the hypothesis that typhus was imported into Europe by Spanish soldiers returning from America. *R. prowazekii* was also detected in the remains of soldiers of Napoleon's Grand Army in Vilnius, Lithuania, indicating that Napoleon's soldiers had epidemic typhus [8]. It is estimated that, of the 25,000 soldiers who reached Vilnius, only 3000 survived (Figure 2). The majority of them were infected with louse-transmitted diseases and recently, DNA from soldier's dental pulp gave evidence of infection with either *R. prowazekii* or *B. Quintana* [9, 10].

Figure 2 - A, Map showing the location of Vilnius. The "Grande Armée" retreated from Moscow to Paris. B, General view of the grave in Vilnius (from Raoult D. et al., 2006. Photo by P. Adalian, Centre National de la Recherche Scientifique, Unité Mixte de Recherche 6578) [10].

Remedies

Mercurials

Mercurials have been used in traditional medicine. Mercury and most of its compounds are extremely toxic. They have been used in antiseptics, laxatives and antisyphilitics. Topical mercury treatment was used to treat pediculosis.

In the Lemery's pharmacopoeia (1720) *Unguentum Neapolitanum simplex* made of quicksilver, Venice turpentine and lard is reported. It can be employed for treatment of scabies, phthiriasis, bedbugs, crabs. It is called *Neapolitanum*, and was also used against syphilis [11].

Campana in the *Farmacopea ferrarese* (several editions) recommends mercurial ointment of white or red precipitate of quicksilver [12].

The same remedy is reported in Porati's *Manuale Farmaceutico* (1820): *Unguentum ad pediculos coeruleum* and *Unguentum ad pediculos rubrum* and in Orosi's pharmacopoeia (1856-57): *Pomata Antipedicolare: prec. Rosso o bianco* and lard [13, 14].

In the De Bruc's *Formulario* (1863), including many international pharmacopoeial preparations, simple mercurial ointment, mercurial rose-water and lotion of the bichloride of mercury (*Pomata mercuriale semplice, Acqua contro i pidocchi, Lozione mondificante)* are reported [15].

Brugnatelli in *Pharmacopeia Generale* (1817) cited red precipitate of mercury, also named red nitrated quicksilver, and mercurial creme, *Precipitato rosso e manteca mercuriale* [16].

In *Manuale di Pharmacopeia generale e speciale su Farmacopea Prussiana e lavori Alemanni e stranieri* (1871) Posner recommended the powder of bichloride of mercury mixed with starch and sugar [17].

Stafisagria - Delphinium Staphisagria
The *Ortus Sanitatis*, a treatise published since the end of 15th century, attributed the spread of pediculosis to … *balnei paucitatem* … and indicates hygiene as the main remedy … *precipuum remedium esse dicit frequens ablutio corporis* (Figure 3). As remedies it includes, in addition to mercury, staphisagria with arsenic, as Avicenna said [18].
Antonio Musa Brasavola, in *Examen omnium simplicium medicamentorum, quorum in officinis usus est* (1539), states that it is used by women *ad necandos pediculos*. The name comes from them in *strafusarium*. The name Staphisagria derived from the Greek and means wild raisin. It is the *herba pedicularis* of the Romans [19]. In *Librum quartum Dioscoridis Cap. CL* (*Commentarii*, Latin Edition of *I Discorsi*, 1554) it is illustrated according to Dioscorides *Staphys Agria*: *Trita, & ex oleo illita phthiriasi prodest*. Mattioli points out that *Latini ab effectu, quod pediculos necet, herba pedicularis dicitur.* Oil is effective agaist phthiriasis, Mattioli remembers that it is called by Latini *herba pedicularis* for its effect on lice. It is frequent in Istria, Dalmatia, Apulia and Calabria. It also has harmful effects on humans (Figure 4) [20].
Lemery in *Farmacopea Universale* reports lotion pediculicide *Lotio ad pediculos capitis enecandos (Staphysagria, Seminis contra, assenzio, tanaceti, betonica, centauria minor)*. Hot decoction is used to wash the head. It kills lice and crabs [11].
As reported in Jourdan's *Parmacopea Universalis* (1837) stafisagria is a component in *Pulvis Capucinorum*, made of sabadilla, parsley and tobacco [21].
Orosi proposed an ointment: stafisagria powder mixed with lard in 1:8 ratio [14].
Ferrarini in the *Farmacopea* (1825) for the *Erba de Pidocchi* indicates the habitat in *Linguadocca*, Provence, and in the mountains of Spouthern Europe. He also suggests its use as powder, infusion, ointment [22].
Brugnatelli recommended its use as powder, decoction and ointment. An infusion of staphisagria may be made with vinegar [16].
The *Dizionario delle Droghe semplici e composte* (1831) and *Dizionario classico di Medicina interna ed esterna* (1838) report an ointment (*Pomata*) made of powder of stafisagra (fr. Staphisaigre) and lard [23,24]. Seeds can be macerated in vinegar. The isolation from the seeds of the active component is also cited: Lassaigne and Feneulle report that the seeds contain a basic substance, named *Delfinia*, with an excess of malic acid. The water-soluble fraction is more toxic to animals. Powdery seeds

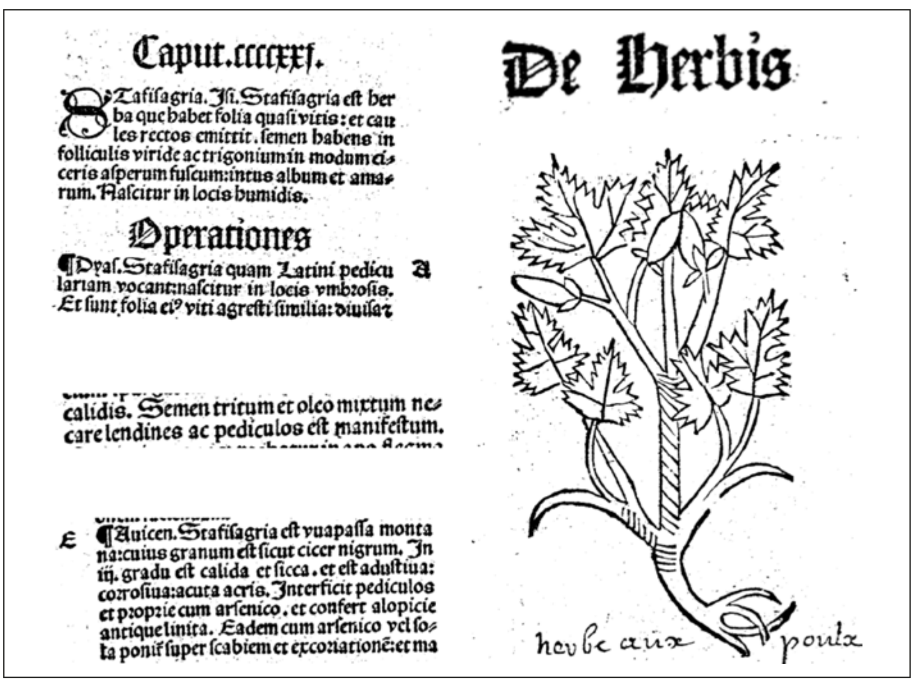

Figure 3 - *De Herbis: Stafisagria*, Ortus sanitatis, 1517.

Figure 4 - *Lib. quartum Dioscoridis Cap. CL: Staphys Agria*, Mattioli P.A., Commentarii, 1554.

are poisonous for ingestion in animals [23].
Campana itself mentions the isolation by Feneulle and Lassaigne of *Delfina*, its characterization and toxicity [12].
Posner suggests its use as powder, decotion and ointment, even in association with mercurials, and report that the seeds contain *Delfinia*, an acre alkaloid similar to *veratrina* [17].
Delfina, whose properties are attributed as a remedy, is described for its tossicity in treatise of Legal Medicin (1840) [25].

Sabadilla - Veratrum sabadilla L.
Sabadiglia *Veratrum sabadilla L.* is recommended in the treatment of pityriasis, as reported in Brugnatelli's pharmacopoeia [16].
The Jourdan's *Farmacopoea Universalis* contains many preparations of Sabadilla. Sabadilla is derived from the seeds of *V. sabadilla*, which grows in Mexico. Jourdan reported that the sabadilla's components isolated by Meissner, Pelletier Caventou are *acido cevadico* and *veratrina o sabadiglina*. The Galenical preparation are: *Pulvis capucinorum, Polvere dei Cappuccin*i, made of *Seminum sabadillae, Staphydis agriae, Petrosellini, Foliorum nicotianae* (seeds of sabadilla, staphisagria, parsley, tobacco); *Unguentum ad s. contra pediculos* (Ointment against lices) made of *Pulveris sabadillae, Synapis, Pyretri, Axungiae preparatae* (sabadilla in powder, mustard, pyrethrum, lard). The *Infusum ad cimices* is also reported, against the bedbugs, *per la distruzione delle cimici, lavando con questo le lettiere* [21].
Pulvis Capucinorum is well-known, reported also in Orosis's *Farmacopea* [14].
Antonio Campana in the *Farmacopea ferrarese* says that *veratrina* is an alkaline substance contained in sabadiglia and also in autumn crocus [12].
Meyer in *Manuale di Farmacologia* (1841) recommends that, if any excoriations exists, care must taken not to use the powder too freel [26].
As reported by Posner, Sabadilla in powder or infusion is a very good preparation. As ointment made mixing powder, lard and an essential oil of *lavandola*, can improve the odour (*Pharm austriaca* is cited). Instead, sabadilla's vinegar, obtained moisten the powder with diluted acetic acid, is not recommended as it causes skin irritation [17].
Galla del Levante, Menispermum cocculus Lin. o Cocculo o Coccola del Levante (fruit, powder) has been a substitute of sabadilla, as claimed by Ferrarini and Campana [12, 22]. Orosi reports that picrotoxin was first isolated by Couerbe and Pelletier and it is extremely toxic. [12]. As pediculicide, it may be applied in the form of ointment, according to *Farmacopea prussiana* [17].

Petrosellino nostrale - Apium petrosellinum L.
A treatise comprehensive of the *Codice Farmaceutico Francese* and the *Farmacopea austriaca* (1838) praises the virtues of the parsley. The *Petrosellino nostrale, Apium petrosellinum*, which grows in Greece and Sardinia, contains *olio etereo* and *canfora*. The seeds were employed to prepare a ointment [27].
The powder is suggested by Brugnatelli and is a component in *Pulvis Capucinorum* previously described [16, 21].

Rosmarino silvestre - Ledum palustre L.
In *Farmacopea ferrarese,* it is reported that decotion of leaves of wild rosemary, Rosmarino silvestre

Ledum palustre Lin., which grows in swamps in North Europe, is also effective [12].

Other compounds

For treatment of phthiriasis, Brugnatelli makes a list of *Vegetabili e loro preparazioni*: Stafisagria (powder), Sabadilla (seeds) and also Colchico autunnale *Colchicum autumnale* L. (*sugo*), Lauro riccio *Laurus nobilis* L. (oil from berries and leave), Pepe nero *Piper nigrum* L. (powder), Tabacco *Nicotiana latifoglia* L. (powder or infusion) [16].

Campana suggested as insecticidal *Colchic Colchicum autumnale* Lin. and oil of *Carapa, Persoonia Guareoides Vild. Carapa oleifera Aubl.* [12].

As reported by Posner, decotion of *Evonimo Europeo*, european spindletree, and powder of seeds of *Datura stramonium* containing *daturina* are effective in the treatment of *phthiriasis* [17].

Throughout history, the treatment of phthiriasis is abundantly simple. Many are the medicines and varied the forms which are used for this purpose. The preparations of mercury, staphisagria and sabadilla are predominantly employed.

A number of these has been considered as homeopathic remedies: *S. staphisagria*, *V. sabadiglia*, *Petrosellinum*, *L. palustre*, *C. autumnale*, *Tabacum*, mercury [28, 15].

The lesson of past remedies for future remedies

The virtues boasted of raw materials conceived in past remedies in popular use or pharmacopoeia, can be certified with today's advanced techniques and tools. The insecticide action on the banal louse can now be exploited against vectors of dangerous infectious diseases, now rapidly spreading from one continent to another and often in a very short time. Some raw materials may cause high toxicity not compatible with their use, but others may reveal unexpected properties.

Mosquito-borne diseases

Mosquitoes are responsible for the transmission of several pathogenic microorganisms to humans, causing mosquito-borne diseases, such as malaria, dengue, yellow fever, West Nile virus (WNV) disease, Chikungunya fever, and Rift Valley fever (RVF). Although there are numerous native mosquito species present and thus able to transmit pathogens in Europe, other mosquito species (*e.g.* Aedes spp.) have recently been introduced and become established in the continent. Among them, the tiger mosquito, *Aedes albopictus*, is probably the major threat to public health in Europe.

The presence of these tropical species means that there is a risk of the appearance of autochthonous mosquito-borne diseases that have previously never or rarely been seen in Europe, acquired after importation from endemic countries [29].

Mosquito-borne diseases, remain a major source of illness and death worldwide, particularly in tropical and subtropical climates [30].

Although surveillance and diagnostic methods are available, control and preventive measures are still limited.

Protection against insect bites is best achieved by avoiding infested habitats, wearing protective clothing and applying insect repellent [31, 32].

Repellents that can be used anywhere at any time are the only feasible measure for preventing arthropod attacks in some situations. Economical and practical insect repellents have, therefore, become a viable and attractive alternative that is widely sold on the market. Commercially available insect repellents can be divided into three categories, synthetic chemicals, botanicals and alternatives such as the combination of synthetic and natural compounds [33].

Therefore, there is an urgent need to develop more efficient surveillance and control tools, and to support coordinated monitoring programmes, particularly in the Mediterranean region, to enable prompt recognition of the threat of potential introduction of some arbovirus into Europe.

Remedies

***Ledum palustre* L.**

***Rhododendron tomentosum* Harmaja**

Insect repellent activity

Rhododendron tomentosum Harmaja (previously: *L. palustre*) is a fragrant evergreen shrub found in peaty soils in northern Europe, Asia and North America, commonly referred to as wild rosemary, marsh tea, marsh rosemary or northern Labrador tea.

At least, since in the eighteenth century it has been used in ethnomedicine for the treatment of various sicknesses, such as rheumatism, cough, cold and insect bites, as well as a repellent. The essential oil of wild rosemary with the rich polyphenolic fraction possesses analgesic, anti-inflammatory, antimicrobial, antiviral, antifungal, promising antidiabetic, antioxidant and anticancer properties. Its insecticidal potential has been demonstrated by *in vivo* and *in vitro* studies.

The ethyl acetate extract of *R. tomentosum* from southern Sweden, significantly reduced biting by *Aedes aegypti* L. mosquito in laboratory tests. The main volatile compounds, collected by solid phase microextraction (SPME) from leaves of wild rosemary, *i.e.* p-cymene, sabinene, and terpinyl acetate, were suggested to be responsible of this effect.

R. tomentosum may be a useful source of chemical compounds of medical, veterinary or agricultural importance for the control of insects [34].

***Apium petroselinum* L.**
***Petroselinum crispum* (Mill.) Fuss**
Insecticidal activity
The increasing and widespread resistance to conventional synthetic insecticides in vector populations, has underscored the urgent need to establish alternatives in the mosquito management system. The study was carried out with the aim to investigate the antimosquito property, larvicidal and adulticidal potential, of plant products against both the pyrethroid-susceptible and resistant strains of *A. aegypti*.

Seventeen plant products, including essential oils and ethanolic extracts, were obtained by steam distillation and extraction with 95% ethanol, respectively. Potential toxicity of the plant candidate was compared with that of synthetic temephos, permethrin, and deltamethrin. The highest efficacy was established from *P. crispum* fruit oil.

The profound larvicidal and adulticidal potential of *P. crispum* oil promises to form a new larvicide and adulticide against either the pyrethroid-susceptible or resistant strains of *A. aegypti*. Consequently, *P. crispum* oil and its constituents can be used or incorporated with other chemicals/measures in integrated mosquito management for controlling *A. aegypti*, particularly in localities with high levels of pyrethroid and organophosphate resistance.

GC-MS analysis of *P. crispum* oil demonstrated that 19 compounds, accounting for 98.25% of the whole oil, were identified as the main constituents such as thymol (42.41%), p-cymene (27.71%), and γ-terpinene (20.98%) [35].

Veratrum sabadilla - Schoenocaulon officinale
Insecticidal activity
Sabadilla is derived from the seeds of plant *Schoenocaulon officinale*, which grows in Venezuela. Sabadilla is one of the least toxic registered botanical insecticides, with mammalian LD50 of 5,000 mg/kg bw. Similar to other botanical insecticides, it has minimal residual activity and degrades rapidly in sunlight and moisture (rainfall). Purified veratrine alkaloids, however, are considered on par with the most toxic synthetic insecticides.

Data from systemic poisoning by sabadilla preparations used as insecticide are rare or nonesixtent [36]. This compound is effective against caterpillars, leafhoppers, thrips, sting bugs and squash bugs. The major insecticidal components of sabadilla are the alkaloids cevadine and veratridine which are inside the seeds. The extracted alkaloids are highly poisonous.

***Nicotiana tabacum* L**
Insecticidal activity
Beside traditional use of botanicals, their commercial use began in the nineteenth century with the introduction of pyrethrum from *C. cinerariaefolium* and also nicotine from *Nicotiana tabacum.* This last is another well-established botanical insecticide. Nicotine analogues also possess insecticidal properties. Nicotine is active against piercing-sucking insects such as *aphids, leafhoppers, whiteflies, thrips,* and *mite*s. However, because of the high mammalian toxicity and detrimental effect on human health, its use as an insecticide has decreased considerably [36].

***Delphinium staphisagria* L**
Trypanocidal properties in Chagas disease
Chagas disease or american trypanosomiasis, is a devastating disease caused by the kinetoplastid protozoan *Trypanosoma cruzi* commonly transmitted to humans and other mammals by an insect vector, the blood-sucking bug of the subfamily Triatominae (family Reduviidae) most commonly species belonging to the *Triatoma infestans* (vinchuca bug), Rhodnius, and Panstrongylus genera. The disease may also spread through blood transfusion and organ transplantation, ingestion of food contaminated with parasites, and from a mother to her fetus. Chagas disease may lead to chronic and systemic stages, which can affect severely heart, esophagus, and colon.

Chagas disease is endemic throughout Latin America and it is the third most widely spread tropical disease after malaria and schistosomiasis according the World Health Organization (WHO). It is estimated that about 100 million people are at risk of infection and from 15 to 20 million are infected, with some 50 000 persons dying yearly from this disease [37].

D. staphisagria is an endemic annual or biennial herb from the Mediterranean Basin. Due to its historical medicinal uses, this plant has probably become widespread in the Mediterranean area. Human-mediated distribution could have promoted few migrant genotypes. The limited genetic variability, the high genetic similarity among populations and the dysploidy of this species, make it worthy of conservation [38].

The molecular structure of major alkaloid isolated from seeds by Lassaigne and Feneulle in 1819, delphinine (Figure 5), was determined and synthesized in 1970 [39].

Diterpenoid alkaloids isolated from the aerial parts of *D. staphisagria* has also been studied extensively [40, 41].

Tissue cell cultures of *D. staphisagria* L. produced dianthramide glucosides. Their formation in cellus tissue of a Delphinium species appears to be unprecedented and may be a response to unknown pathogens. Dianthramides are generally considered to be phytoalexins [42].

Finally, the *in vitro* and *in vivo* trypanocidal activities of nine flavonoids (Figure 6) isolated from the aerial parts of *D. staphisagria,* have been studied in both the acute and chronic phases of Chagas disease [43, 37].

The antiproliferative activity of these substances against *T. cruzi* (epimastigote, amastigote, and trypomastigote forms) exhibited in some cases more potent antitrypanosomatid activity and lower toxicity than the reference drug, benznidazole.

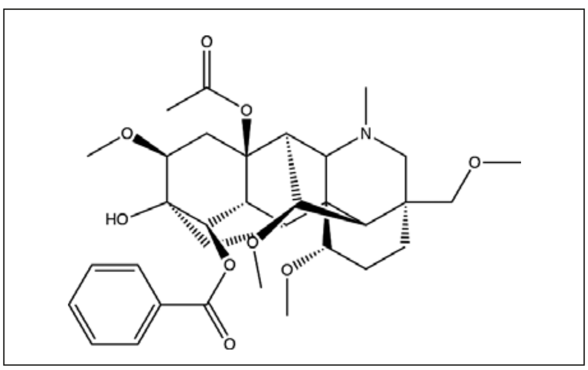

Figure 5 - Delphinine.

In vitro studies using ultrastructural analysis together with metabolism-excretion studies were also performed in order to identify the possible action mechanism of the compounds tested. Alterations mainly at mitochondria level may explain metabolic changes in succinate and acetate production, perhaps due to the disturbance of the enzymes involved in sugar metabolism within the mitochondrion. *In vivo* studies provided results consistent with those observed *in vitro*. No signs of toxicity were detected in mice treated with the flavonoids tested (Figure 6), and the parasitic charge was significantly lower than in the control assay with benzimidazole. The effects of these compounds were also demonstrated with the change in the anti-*T. cruzi* antibody levels during the chronic stage [37].

Leishmanicidal properties in Leishmaniasis

Leishmaniasis is an infection caused by different species of the protozoan genus *Leishmania*, which is transmitted by dipterans of the genera *Phlebotomus* in the Old World and *Lutzomyia* in the New World. Leishmaniasis represents one of the most significant neglected tropical diseases which according to WHO latest report (2013) affects 350 million people in 88 countries. Approximately 12 million individuals are currently infected with *Leishmania spp.* with an estimated 2 million new cases occurring every year (44). Major endemic areas are in the tropics and subtropics; however, leishmaniasis exclusively due to *Leishmania infantum* is also endemic in large parts of southern Europe.

Drug treatment for leishmaniasis has been available since the beginning of the 20th Century, but only a few drugs have been developed for use and there are numerous drawbacks to each of the treatments: antimonials (meglumine antimoniate or glucantime and sodium stibogluconate or pentostan), amphotericin B, paromomycin (aminosidine) and pentamidine isethionate, miltefosine, now used in combination with different classes of azole oral antifungal agents including ketoconazole, juconazole, and itraconazole. In addition to the adverse effects of the drugs, resistance to these treatments is appearing in the parasites.

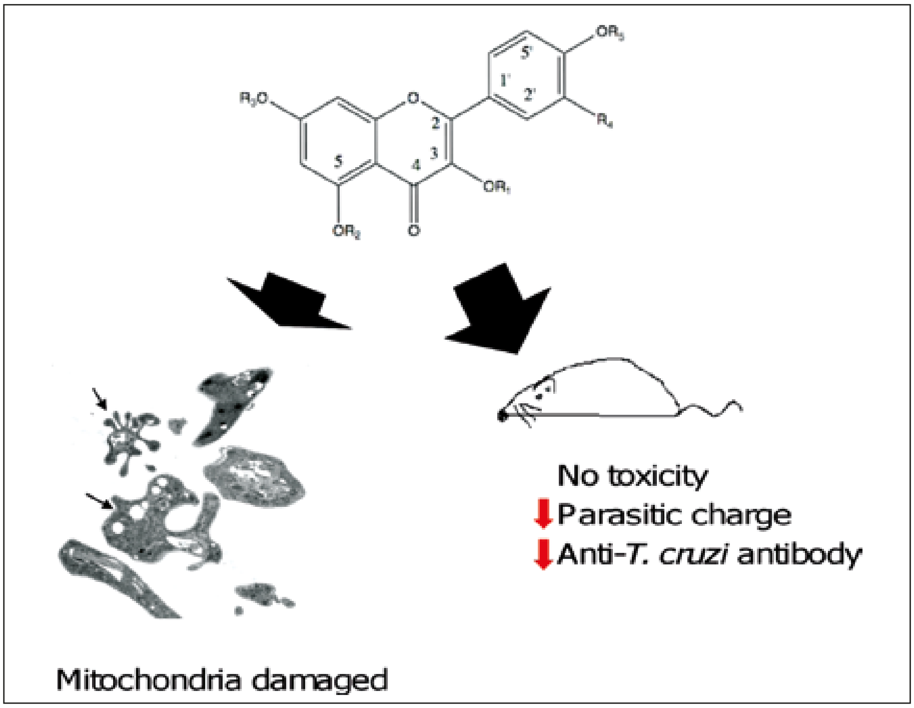

Figure 6 - In vitro and *in vivo* trypanocidal activity of flavonoids from *D. staphisagria* against Chagas Disease [37].

For all these reasons, further novel drug development is necessary to treat these infections. Considerable attention is currently being paid to phytotherapy in the search for new drugs.
Leishmanicidal properties may reside in phytochemicals such as javonoids, which are hence strong candidates for use in combination therapy against these infections.
Flavonoids (Figure 6) from aerial parts of *D. staphisagria* L. (Ranunculaceae) showed leishmanicidal activity against promastigote as well as amastigote forms of *L. infantum* and *Leishmania braziliensis*. These compounds were nontoxic to mammalian cells and were effective at similar concentrations up to or lower than that of the reference drug (Glucantime) [45].

***Anthemis pyrethrum* L.**
***Anacyclus pyrethrum* (L.) Lag**
Antiprotozoan activity
Piretro, Anthemis pyrethrum, is *Anacyclus pyrethrum*. Pyretrum does not contain pyrethrins, which are presents in several plants of the genus Chrysanthemum. The ethnomedicinal use of *Anacyclus pyrethrum* as an antimalarial might be explained by the activity of lipophilic constituents present in roots. Alkamides were isolated and were tested *in vitro* for antiprotozoal activity against *Plasmodium falciparum*, *Trypanosoma brucei rhodiense, T. cruzi* and *Leishmania donovani*. Their overall antiparasitic activity is low [46].

CONCLUSIONS

Natural remedies have served as a source of inspiration in the development of new drugs. This pathway is "experience-driven" and mainly based on traditional uses. Thus, ancient studies may serve as a valuable starting point to develop plant driven discovery of new drugs. This has been proven possible for some important remedies (*i.e. Artemisia annua*) [47]. Starting from existing hystorical proofs, one can envisage a stage of discovery from herbal ingredients, which includes the preparation of extracts, structure/composition elucidation, and *in vitro* bioactivity evaluation.
In the present work, the cross-survey of basic raw materials in the preparation of pastile products in the past and the most used medical database, has led to interesting findings in the fight against pe-

Table 1 - Past and present medical remedies active against lice and other current Infectious Diseases and vectors.

	Activity	*Actual denomination*	*Ongoing studies*
Mercurials	Lice, scabies, bedbugs, crab louse		
Rosmarino silvestre *Ledum palustre* L.	Lice	*Rhododendron tomentosum* Harmaja	Insect repellent *Aedes aegypti*
Petrosellino nostrale *Apium petroselinum* L.	Lice	*Petroselinum crispum* (Mill.) Fuss	Insecticidal against pyrethroid-susceptible and resistant strains of *Aedes aegypti*
Sabadilla *Veratrum sabadilla* L.	Lice, bedbugs	*Schoenocaulon officinale*	Insecticidal caterpillars, leafhoppers, thrips, sting bugs and squash bugs
Nicotiana tabacum			Insecticidal piercing-sucking insects such as aphids, leafhoppers, whiteflies, thrips, and mites
Stafisagria *Delphinium staphisagria* L. Active principle: Delphinine	Lice, crab louse		Trypanocidal and leishmanicidal activities *Trypanosoma cruzi* *Leishmania infantum* *Leishmania braziliensis*
Piretro *Anthemis pyrethrum* L.	Lice	*Anacyclus pyrethrum* (L.) Lag.	Antiprotozoal activity (low) *Plasmodium falciparum* *Trypanosoma brucei rhodiense* *Trypanosoma cruzi* *Leishmania donovani*

diculosis (Table 1). To this end, the search had to be matched with today's denomination.
Therefore, the *L. palustre*, now termed *R. tomentosum*, revealed repellent insect activity, particularly against *A. aegypti*, responsible for Dengue fever, Chikungunya, Zika fever, Mayaro, yellow fever and other infectious diseases. *A. petrosellinum*, today *P. crispum*, is insecticide for resistant strains of *A. aegypti*. In some cases, lately, interest in the insecticide activity of *V. sabadilla*, today *S. officinale*, and *N. tabacum*, has dropped dramatically.
In the case of *D. staphisagria*, the phytochemical profile was further investigated with the identification of additional molecules in addition to Delphinine. The latter have shown interesting activities against *T. cruzi* and *Leishmania*.
Anthemisis pyrethrum, today *Anacyclus pyrethrum*, although not containing pyrethrins present in several plants of the genus Chrysanthemum, had revealed pediculicidal activity but did not produce satisfactory results in antiprotozoan activity.
From the past and from the old louse treatment, seem to emerge therefore cues to combat some of today's diseases and vectors that support them, such as repellents or insecticides, even in cases of resistance.
The use of insecticide-treated nets has become a key malaria control strategy, although more efficacious than in the past. All pesticids have been shown to be toxic.
We need implementation of strategies to minimize potential risk through careful selection and discovery of new products, considering the parasite resistance to current antimalarian drugs and to insecticide by vector mosquitoes [48].
In summary, this context, an approach that takes into consideration past experience, such as those deriving from tradition, can be plentiful of suggestions and may shorten time to discovery, thus reducing overall investment.
Traditional uses still represent the largest clinical study ever conducted by human beings.
Principal drawbacks are related to the preparative methodologies in use in ancient times, which are not easily understandable and do not make clear what part of the activity is linked to original mole-

cules or their by-products that are formed during preparation and storage.
This is the most challenging task in the re-investigation of ancient remedies and ethnobotany preparations in the light of the most recent techniques and equipments [49].

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