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19 - 23 novembre 2018 - Tahiti
Polynésie française

LES ACTES

**Nature
& Culture :**

*De la recherche
à l'innovation,
la valorisation
et/ou la préservation.*

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Lutte contre la transmission des maladies infectieuses : vers un développement durable des insectifuges en Nouvelle-Calédonie

Vector control against infectious diseases: Towards sustainable development of insect repellents in New Caledonia

Abstract

More than 80% of the world's population is threatened by vector-borne diseases with populations in tropical regions most at risk. The World Health Organization (WHO) thus promotes the use of mosquito repellents to prevent infectious diseases. In New Caledonia, the office of Social and Health Affairs Direction (DASS) approved 4 active substances of insect repellents for personal anti-vector protection, among these para-menthan-3,8-diol or PMD. This joint-project between James Cook University (JCU) and the University of New Caledonia (UNC) aimed to identify essential oil bearing plants within the flora of New Caledonia that can supply significant quantities of PMD or its precursor citronellal. The study subsequently identified the essential oil of *Corymbia citriodora* (Hook.) K.D.Hill & L.A.S.Johnson of the Myrtaceae family already produced in New Caledonia as an important candidate for the production of PMD. This oil provides a significant amount of citronellal, which after an acid-catalyzed thermal cyclisation hydration sequence leads to a mixture of cis and trans isomers of PMD. On the basis of a published method, a standard operating procedure for the conversion of citronellal to PMD has been provided to a distillery in New Caledonia. The distillery has since produced and marketed this renewable insect repellent.

Keywords: sustainable development, insect repellent, para-menthan-3,8-diol, citronellal, *Corymbia citriodora*.

Introduction

Blood-sucking insects and especially mosquitoes have a significant impact on life, health and economies in the tropics. Insect-borne diseases such as dengue, Ross River fever, chikungunya, zika or malaria commonly lead to hospitalizations and fatalities. The economic productivity of effected regions is moreover decreased due to sick leaves, the negative impact on tourism and significant financial and staff spending on preventive programs. Allergic reactions and discomfort due to insect bites are also responsible for a decline of the 'tropical outdoor lifestyle' as people prefer to stay indoors. The number of people and regions affected by insect-borne diseases is likely to increase significantly in the future due to climate change and the subsequent spread of mosquito-vectors.

Currently, Oceania is particularly affected by insect-borne diseases with dengue, zika and chikungunya present on many Pacific Islands. Malaria occurs widespread in Papua-New Guinea, the Solomon Islands and Vanuatu, whereas Japanese and Murray Valley encephalitis or Ross River virus are endemics in Australia [1,2]. The burden of insect-borne diseases is significant in New Caledonia. For dengue fever, the Social and Health Affairs Direction recorded about 4,500 confirmed cases and 11 deaths in 2017. Dengue epidemics have been again declared in February and December of 2018. Chikungunya is also present on the island as well as zika with a significant outbreak in 2014 [3].

In order to protect people from infectious diseases, the World Health Organization recommends the use of effective repellants that target mosquitoes. This study thus aimed at the development of an effective repellent from local plants and their essential oils.

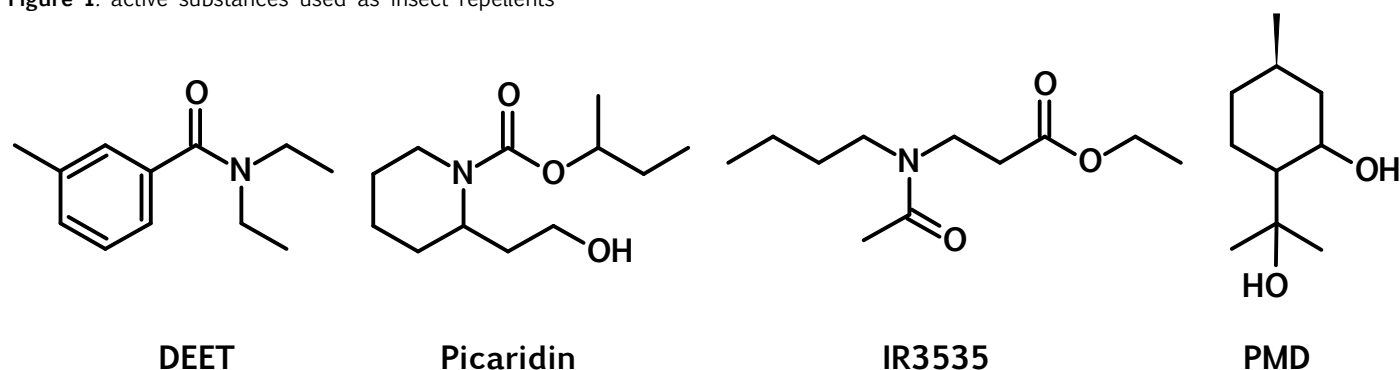
Method

In New Caledonia, the department of DASS has approved 4 active substances that can be used in insect repellent formulations for personal anti-vector protection: DEET, para-menthan-3,8-diol or PMD, picaridin and IR3535 (**Figure 1**) [3]. PMD is the only natural substance approved as a repellent in the United States, Australia and the European Union. PMD has been proven effective in laboratory and field tests against a variety of mosquitos, most importantly the dengue carrier *Aedes aegypti*, with a long efficacy (2-12 h for 20-50% formulation). Moreover PMD is active against midges and ticks and presents no risk to human health (LC50 >2,000mg/kg in rats). PMD also has the pleasant smell and a similar cooling effect to menthol [4]. Despite these benefits, PMD is rare in nature but can be produced synthetically from citronellal. Researchers from James Cook University and the University of New Caledonia have consequently evaluated the flora of New Caledonia to identify potential sources of PMD or its precursor citronellal.

Various essential oils provided by distilleries in New Caledonia have been analyzed by GC-FID-MS. GC-FID-MS analysis was performed using a gas chromatograph coupled with a mass detector (Clarus® 580, Perkin Elmer Inc, Waltham, MA, USA) and a flame

ionization detector (Clarus® 580, Perkin Elmer Inc, Waltham, MA, USA) using helium at 1 mL/min. The capillary column was an elite-5MS (30 m x 0.25 mm, 0.25 µm) (Perkin Elmer Inc, Akron, OH, USA). Analyses were performed using EI mode. The injection temperature was set at 250°C. Analyses were carried out using a temperature program starting from 50°C, with an initial 3 min hold, to 250°C with a 5°C/min heating ramp, and keeping the final temperature stable for 15min. The mass range was set at m/z 40-500. The individual peaks were identified by comparison with mass spectra from libraries as well as the retention indices (RI), which were calculated for all volatile constituents using a homologous series of n-alkanes C8-C32 and were compared with available literature data. Mass Finder 2.3 library, NIST library (Gaithersburg, MD, USA), Wiley library (Hoboken, NJ, USA) were used for mass spectra comparison and identification. We used mainly NIST MS Search 2.2 software, Pherobase [5] and literature data [6] for retention index comparison to identify constituents of the essential oils. Relative percentages of constituents were calculated with the area from the FID GC chromatogram corrected with the number of carbon in the corresponding compound (based on the MS identification).

Figure 1: active substances used as insect repellents



Results & Discussions

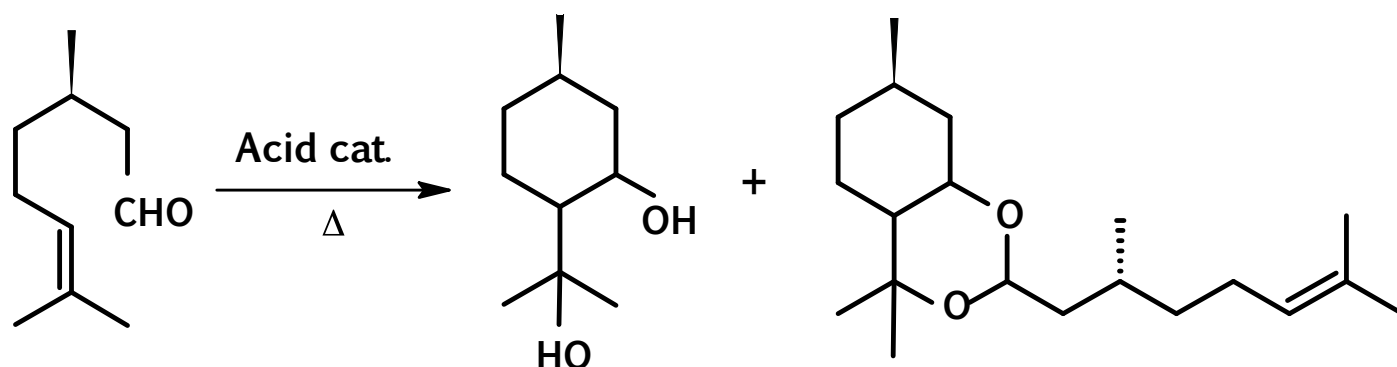
Surveys of the scientific literature and local market data were initially conducted. The two main essential oils produced in New Caledonia are sandalwood and niaouli. *Melaleuca quinquenervia* (Myrtaceae) or niaouli is a native tree and the essential oil of its leaves has several therapeutic and perfumery applications. *Santalum austrocaledonicum* (Santalaceae) which is also a native tree, represents a suitable alternative to Indian sandalwood (*S. album*) for the perfumery industry. However, neither of these two species of endemic plants or their essential oils are known to show repellent activities.

The local market also offers essential oils from introduced species such as lemon eucalyptus or *Corymbia citriodora* (Myrtaceae),

which is endemic to the state of Queensland in Australia but has been introduced in a lot of tropical areas in the past. Wild populations for example exist in Northern New Caledonia and its essential oil is currently produced and sold locally by two distilleries.

Unrefined lemon scented eucalyptus essential oil has a poor repellent activity (22 min for a 20% formulation) but its chemical composition is interesting because of the presence of high amounts of citronellal (50-80%). The latter molecule can be converted to PMD via an acid-catalysed thermal cyclization hydration (**Figure 2**) [7-8]. PMD acetal and isopulegol are commonly formed as minor by-products. Pure PMD is synthesised for commercial production from synthetic citronellal [9].

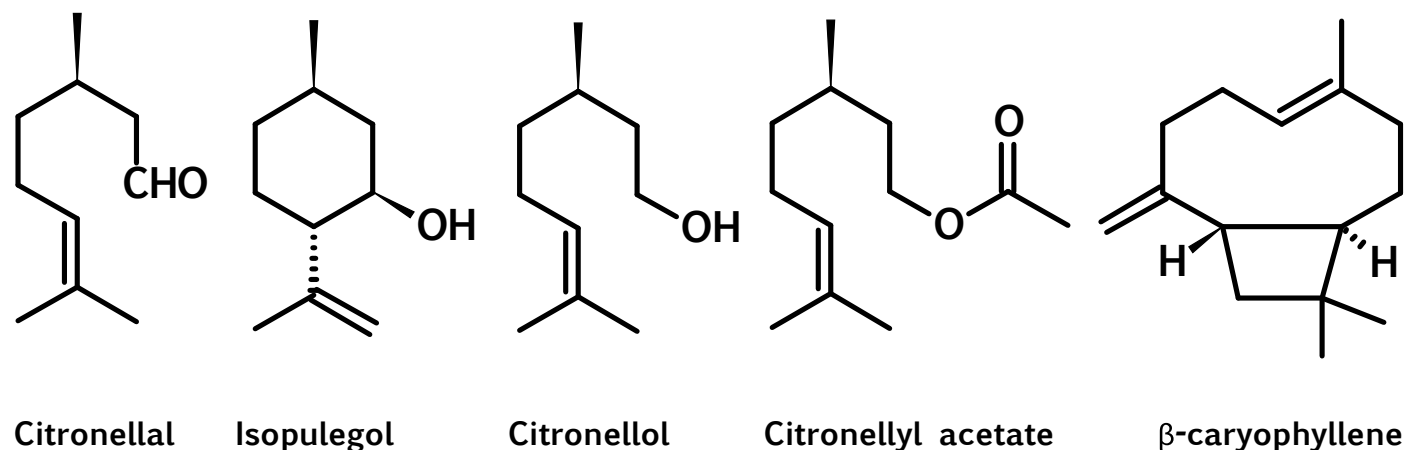
Figure 2: acid-catalysed thermal cyclization hydration of citronellal to PMD



The same conversion can also be performed directly using lemon scented eucalyptus essential oil, as demonstrated by Drapeau et al. using citric acid as catalyst [10]. The product obtained is a PMD rich botanic oil (PMDRBO) containing ca. 80% PMD, which showed complete protection for 5 h against mosquitoes in a 20%

in isopropanol formulation. We thus determined the chemical composition of commercial *C. citriodora* essential oils from local and foreign producers. The main compounds identified were monoterpenes such as citronellal, isopulegol, citronellol, citronellyl acetate, and sesquiterpenes such as β -caryophyllene (**Figure 3**).

Figure 3: Main compounds of commercial *C. citriodora* essential oils



Essential oils obtained from New Caledonia have a lower citronellal content of 46-47% compared to an essential oil obtained from Madagascar with 63% (Table 1). Even higher amounts of citronellal of up to 81% have been found in essential oils from Australia (data not shown). PMD was only found in traces in all of the crude oils. Citronellol and isopulegol were identified as other main components.

An optimised standard operation procedure was subsequently provided to a local distillery. A simple stirred batch reactor with a capacity of

approx. 35L was constructed and utilized for the production of several batches of PMDRBO. Each batch utilized 5kg of locally harvested or important essential oil. Chemical analyses of the locally produced PMDRBO showed incomplete conversions of citronellal to PMD, thus highlighting the need for further optimization. Final formulations of PMDRBO were likewise manufactured using vegetable oil or isopropanol (roll-on/spray) and have proven popular with consumers.

Table 1: chemical composition of commercial *C. citriodora* essential oils

SOURCE	GC composition [%] $\pm 2\%$			
	Citronellal	Citronellol	Isopulegol	PMD
<i>New Caledonia</i> (2018)	47	14	8	traces
<i>New Caledonia</i> (2017)	46	17	14	traces
<i>New Caledonia</i> ('aged', unknown year of harvest)	47	18	14	traces
Madagascar (2017)	63	9	6	traces

Conclusion

The local production of effective insect repellents enables the prevention of infectious diseases and offers attractive opportunities for local industries. This was demonstrated through the synthesis of the proven repellent PMD from a local essential oil. The chemical conversion into PMDRBO was successfully realized in a simple batch reactor although further optimization of the operation procedure is desirable. The project helped a local distillery to find a new market for its essential oils with future potential for new markets, for example in the domestic cattle industry.

For a potential future commercial production, a sustainable large-scale plantation and harvesting is envisaged. This will also require the development of effective quality control measures (in-house or via a service contract) and product registrations.

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