Section 4

14

Pharmacology

Management of Convulsion and Migraine by Inhalation Therapy

Madagascar

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Summary

The smoke of dry leaves *of Myrothamnus moschatus* is commonly used to treat convulsions and migraines in Madagascar. We have subjected this culturally-accepted empirical treatment to an examination of its scientific value and therapeutic applications. To this end, assuming that the active compounds are volatile constituents, we extracted the essential oil by steam distillation, determining its chemical composition using chromatography. We then evaluated its anticonvulsive activity, observing and quantifying the reduction and inhibition of seizures provoked by convulsing agents in rats. Administered subcutaneously at 0.4ml/kg, the essential oil completely inhibited pentylenetetrazole-induced convulsions, with neither toxicity nor sedative effects. Thereafter, we comparatively analyzed the chemical composition of the smoke and the dry leaves using a solid phase micro-extraction technique. The striking difference was the presence of significant quantities of limonene in the smoke although present in only small amounts in dry leaves and essential oil. *Citrus* species are good sources of limonene, which has been reported to display anticonvulsant and neuroprotective effects, prompting us to evaluate the neuroregenerative effect of the essential oil of *M. moschatus* on an SHSY5Y neuroblastoma cell line culture. At higher concentrations, clear neuronal cell growth was observed. After basic toxicity and neurotoxicity evaluations, we successfully carried out clinical observational studies on a pharmaceutical composition including *M. moschatus* and *Citrus* sp. essential oil blends, with 31 convulsive patients and 38 migrainous patients being enrolled in the study. We mainly targeted patients unable to afford conventional drugs, and those refractory to, or complaining about toxicity of existing medications. Additionally three unexpected emergency convulsion cases and one very severe migraine case were treated with positive outcomes. Administration was by inhalation. Three therapeutic failures in convulsions and two in migraines were recorded. We manufactured two patented phytomedicines respectively under the tradename Fanalarofy® for convulsions and Fanalanendo® for migraines.

Background and Justification

Epilepsy, convulsive seizures and headaches are the most common chronic neurologic disorders affecting respectively 5 to 10 out of every 1,000 persons worldwide for epilepsy and 15 to 30 out of every 1,000 persons for migraines, with a heavy socioeconomic impact in terms of both lost productivity and burden to healthcare systems. Nearly 80% of people with these diseases reside in developing countries, where it remains a major

public health problem, not only because of the health implications but also for the social, cultural, psychological and economic consequences. The prevalence of epilepsy in sub-Saharan Africa seems to be higher than in other parts of the world, with 10 million people directly affected according to the World Health Organization (WHO) estimates. Patients are of all ages, but especially within childhood, adolescence and the ageing population. In the Central Highlands of Madagascar, an epidemiological study on epilepsy showed that the prevalence of the disease is estimated as 27 per 1,000 persons. In addition to environmental and genetic factors, the effects of central nervous system infections are the main cause of seizures and acquired epilepsy in Sub Saharan Africa. However, little public health attention has been paid to the neurological burdens associated with infectious diseases. Regarding migrainous headaches, this disease has been and continues to be underestimated in scope and scale, and headache disorders remain under-recognized and under-treated everywhere. Africa has an estimated 56 million people sufferers. In Madagascar, the prevalence of migraine was reported to be 19% with specific rates of 26.8% for women and 9.4% for men. By virtue of it mainly afflicting people of working age (22-55 years old), migraine not only causes high medical disability but also great socioeconomic consequences. One serious problem in low-income countries is the poor availability and high cost of medication. The epilepsy and convulsive seizures treatment gap are defined as the proportion of people who require but are not receiving treatments. With an average gap of approximately 75% for low-income countries and the poorest in Africa having a gap of more than 90%, the situation is quite alarming. In Madagascar, the treatment gap was estimated as 92%. However, given the sociological, economic and sanitary conditions of the country, the true treatment gap may be even higher.

Epilepsy is traditionally believed to be caused by a supernatural power, be it a god or a demon or an ancestral spirit, because of transgression of taboos or punishment for sins, or attributed to possession by evil spirits. It is also thought to be due to witchcraft, or poisoning, and often taken to be contagious. A fear of contagion results in isolation of the convulsive patients and unwillingness to intervene in preventing injury. Profound psychological and physical disability may thus occur. Headache is believed to result from excess blood in the brain. And to remove the excess blood, the healer hits gently the head until blood comes out through the nose. In Madagascar, and probably elsewhere Sub-Saharan Africa, traditional treatments for epilepsy are mainly prayers and exorcisms. The danger of such practices has been raised, but it is difficult to counteract deeply-rooted traditional beliefs. With the active participation of local populations, we learned from traditional healers that the smoke of *Myrothamnus moschatus* (Baillon) (family *Myrothamnaceae* as 'resurrection plant') is used to treat epilepsy and migraines. We postulated that volatile compounds might represent the active constituents of the plant.

A review paper reported the anticonvulsant effects of essential oils extracted from 30 plant species and 30 isolated constituents. Little has been done to bring this knowledge into drug/phytomedicine development and therapeutic use. As far as migraines are concerned, to the best of our knowledge, no review paper with scientific validation has been published on the use of essential oils for the management of migraines and headaches. However, many anecdotal reports have been found in current text, journal articles, Masters or PhD dissertations, and internet resources have been utilized as important sources of information about the successful use of essential oils in headache management. We therefore decided to investigate *Myrothamnus moschatus* with the aim of translating this culturally-accepted empirical treatment into a validated scientific finding with therapeutic applications.

Description

To translate the traditional recipe into therapeutic applications, we first set out to learn the learning ethnomedical uses and particular botanical features of *M. moschatus* in our bioprospecting programme. Dried leaves are smoked like a cigar and the smoke is gathered in a rice bag. The patient then inhales the fumes to expel bad spirits entering the body of a convulsive patient. We also learned that the dried leaves are used similarly for the treatment of migraines with nose bleeds. The Myrothamnaceae family comprises one genus, *Myrothamnus*, and two shub-like species, *Myrothamnus flabellifolius* indigenous to Southern Africa and *Myrothamnus moschatus* endemic to Madagascar.

Figure 1: *Myrothamnus moschatus* in dry (left) and rainy (right) seasons.

We assumed that the bioactive substances would be volatile in nature. Consequently, the essential oil was extracted by steam distillation and its chemical composition determined by chromatographic techniques.

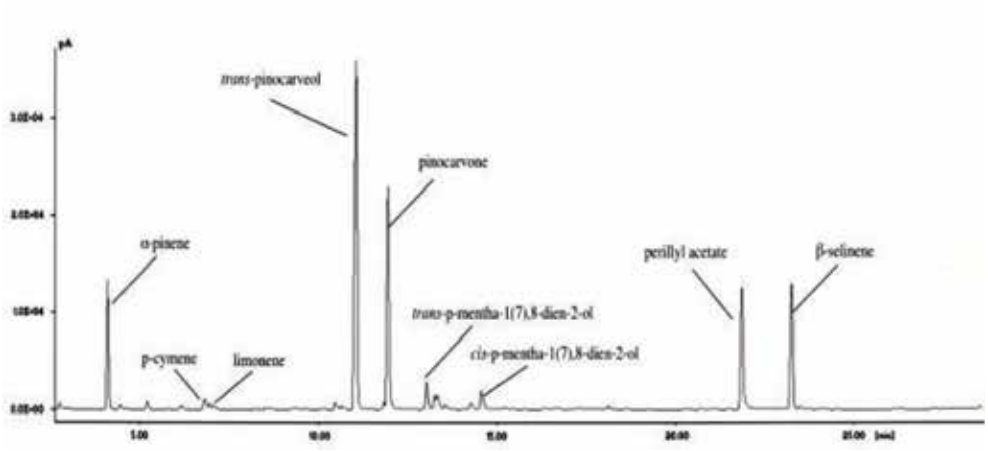

Figure 2: Chromatogram profile of the essential oils of *Myrothamnus moschatus*. Note the relatively low proportion of limonene present.

The chromatogram profile is shown in Fig. 2.

We then tested the anticonvulsant effects of the crude essential oil in rats against seizures induced by the chemo-convulsing agent pentylenetetrazole. We used subcutaneous injection using decreasing dosages of the oil (0.8 ml/kg, 0.4 ml/kg, 0.2 ml/kg). Diazepam at the dose of 1 mg/kg was used in intra-peritoneal injection as a positive control. Animals that did not convulse within 60 min were considered as protected. In unprotected animals, the latency to first convulsion and the durations of convulsions were recorded. The animals were observed for mortality for 24 hours after administration of pentylenetetrazole.

We observed a clear dose-response effect. At 0.8 ml/kg and 0.4 ml/kg, the convulsive effects induced by pentylenetetrazole were completely inhibited, without any toxicity or sedative effect. At lower doses, the essential oil demonstrated a significant increase in seizure latency and a significant reduction in seizure duration compared with the control group. No deaths were recorded in treated animals.

At this stage, we had to decide whether to proceed to the elucidation of the detailed mechanism(s) of action and the isolation of the active principle(s), or explore what else the traditional recipe might bring. To this end, we gained important points from a critical review (Loscher, 2011) that confirmed:

- the efficacy of new antiepileptic drugs (AEDs) is at best similar to that of older AEDs;
- the major goal should be more effective treatments for the AED-resistant epilepsy patients;
- new AEDs have clinically important usefulness for disorders other than epilepsy; and
- that current strategies of preclinical AED development need to be radically overhauled.

We therefore oriented our studies towards the analysis of the smoke of *Myrothamnus moschatus*.

We performed the analysis of the chemical composition of dry leaves and active smokes of *M. moschatus* using the solid phase micro-extraction (SPME) technique. The most striking difference between the chemical composition of smokes, dry leaves and extracted essential oil was the unexpected significant presence of limonene in the smoke. The comparative percentage of the main constituents from essential oils, dry leaves and active smoke is summarized in Table 1.

It was reported that limonene displayed anticonvulsant and neuroprotective effects. This prompted us to evaluate the neuroregeneration activity of the essential oil of *M. moschatus*.

Component	Essential oil (dry season)	Essential oil (rainy season)	Dry leaves at 37°C	Smoke
α-Pinene	5.8	10.5	1.3	4.8
p-Cymene	0.9	1.6	-	4.6
Limonene	0.4	2.3	0.5	6.6
trans-Pinocarveol	36.3	35.6	35.5	12
Pinocarvone	19.8	20.0	30.5	2
trans-p-Mentha-1(7),8-dien-2-ol	2.8	3.3	5.9	15.1
cis-p-Mentha-1(7),8-dien-2-2.3 2.1 ol	2.3	2.1	-	-
Perillyl acetate	12.7	6.0	2.6	0.6
β-Selinene	8.5	8.5	5.2	1.3

Table 1: Comparative percentage of the main constituents from essential oils, dry leaves and active smokes of *Myrothamnus moschatus*.

Smell reception occurs in the brain's limbic system, particularly the temporal lobe that is the site of temporal lobe epilepsy. Essential oils stimulate smell receptors that send chemical messages to the limbic system. The result is the rapid onset of action at very low dosages. Accordingly, the potential therapeutic applications of the essential oil by inhalation therapy may be:

- Prevention of oncoming convulsion or migraine as a simple self-help technique; or
- First management of emergency cases of convulsion.

Inhalation therapy could offer benefits such as less invasiveness, site-specificity, ease and speed to reach the brain, avoidance of the first hepatic passage, less systemic exposure and adverse effects.

We then proceeded to evaluate the neuroregeneration effect of the essential oil of *M. moschatus* by assessing the effect of the essential oil on the neurite outgrowth of neuronal culture of SHSY5Y neuroblastoma cell line (Fig. 3).

Neuronal regeneration was observed at different concentrations of the essential oil of M. moschatus. Untreated control cells clearly have a polygonal shape, with no or very few cell protrusions (neurites). By contrast, cells treated with the extract show a typical neuronal morphology with an increased number of protrusions. In fact, cells developed a huge number of neurites that connect cells to one and other, thus forming a neuronal network. This effect was dose-dependent and became most prominent with higher concentrations (1:40,000 and 1:80,000).

Based on this result, the potential therapeutic application of the essential oil may be the adjunct therapy to existing drugs for the chronic treatment of convulsion and migraine by neuro-protective effects.

Optimizing the anticonvulsant activity

The unexpected presence of limonene in the active smokes prompted us to explore the possibility of combining essential oil of *M. moschatus* with essential oils rich in limonene

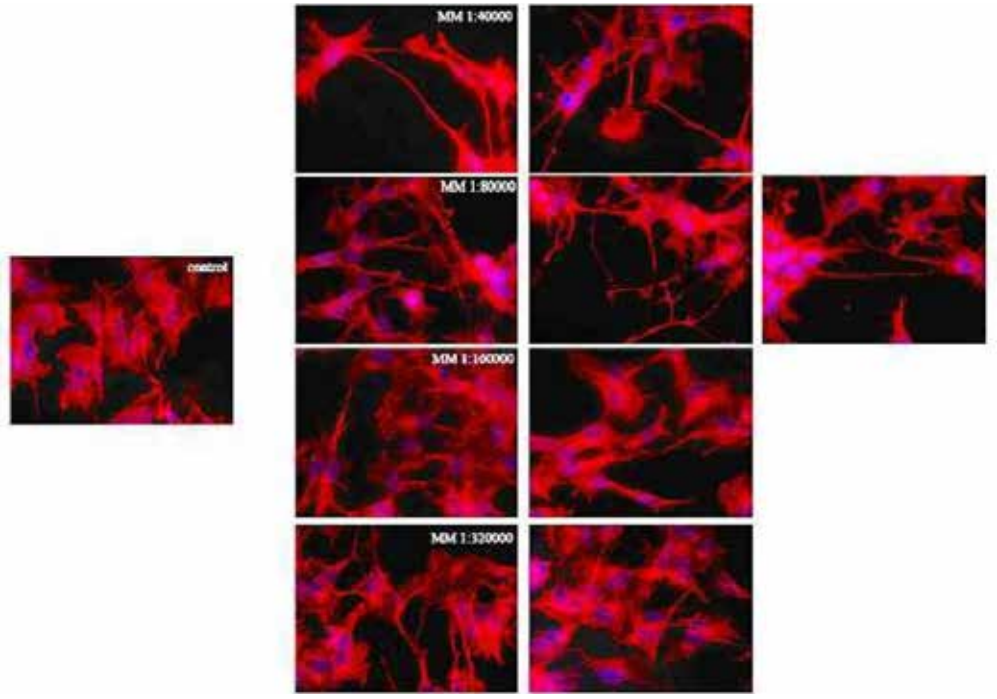

Figure 3: Neuronal regeneration observed at different concentrations of the essential oil of *M. moschatus.*

with the aim of optimizing the biological activity. *Citrus* species are good sources of limonene. Consequently, we extracted essential oils from different *Citrus* species and found that a wild species widely available had the highest amount in limonene (92% yield). Thereafter, we blended the two essential oils in different ratios and tested each combination in pentylenetetrazole-induced convulsions in rats. We found that the 50/50 blend had the highest comparative anticonvulsive effects.

Evaluating the natural abundance of M. moschatus

Prior to commercializing a new therapeutic agent, it is important to ensure a sufficient supply of the ingredients. Fig. 4 shows the geographical distribution of *M. moschatus*, and indicates that this species is found growing wild in different ecological regions of Madagascar. We therefore investigated the presence of chemical variability of the volatile composition of the species depending on phytogeographic origin of the samples (represented in red circles in the map), along with the traditional uses. Five distinct chemotypes were identified. Essential oil composition of *M. moschatus* is therefore very susceptible to ecological factors, with significant qualitative and quantitative variations. Interestingly, the variation of the chemical composition is followed by variations in the ethnobotanical uses of the samples.

Figure 4: (A): Map of Madagascar showing geographical distribution of *Myrothamnus moschatus* and locations where samples were collected (red circles). (source: MBG Vahinala project). (B) Collecting in dry season. (C) Collecting in rainy season.

Performing clinical observational studies

The diversity of anticonvulsant targets has been exploited for their utility in disorders other than epilepsy (Moch, 2010). This is called polypharmacology and drug repositioning. Interestingly, smoke of *M. moschatus* is also traditionally used to attenuate and arrest migraine. This prompted us to include migraine in our clinical observational studies. After general toxicity and neurotoxicity evaluations, we carried out clinical observational studies for patients suffering from convulsions and/or migraines with a pharmaceutical composition comprising essential oils of *M. moschatus* and *Citrus*. Each patient confirmed their informed consent.

We targeted two types of patients: patients unable to afford conventional drugs (essential oils as primary treatment), and those refractory to, or complaining about the toxicity of existing medications (essential oils as adjunctive treatment). Overall, 31 patients suffering from convulsions and 38 patients with migraines were enrolled in the trials. Additionally, unexpected emergency cases also received treatments. The route of administration was by simple inhalation. Results are outlined in Table 2.

CONVULSIONS: number of patients = 31; age: 18 months-55 years				
	Number of cases	Primary treatment	Adjunctive treatment	Therapeutic failure
Symptomatic epilepsy with generalized seizures	25	19	6	2 (cysticercosis)
Epilepsy with absence	2	0	2	0
Epilepsy with partial seizures	3	2	1	0
Generalized seizure accompanied by strange possessive behaviour	1	0	1	1 (unexplained)
MIGRAINES: number of patients = 38; age: 12-51 years				
Migraines without aura	31	19	12	2
Migraines with aura	2	0	2	0
Migraines with convulsions	5	3	2	0

Table 2: Clinical observational studies of a pharmaceutical composition comprising blend essential oils of *M. moschatus* and *Citrus* in convulsive and/or migraineous patients.

Twenty-one convulsive patients did not receive any conventional treatment. The seizures were successfully managed with the essential oil as a primary preventive treatment just before convulsions occurred. ten patients were refractory to, or complaining about the toxicity of existing conventional drugs. We progressively decreased the dose of conventional drugs while using the essential oil as adjunctive therapy. Overall, we observed a significant reduced frequency and duration of convulsions. Three therapeutic failures were observed, two with patients diagnosed with cysticercosis and one with unexplained causes despite repetitive EEG analysis and use of several conventional drugs. Some patients reported that the phytomedicines, once used, lost their effect after two months. As regards migraines, twenty-two patients did not receive any conventional treatment. They successfully used the essential oil as preventive medication. Sixteen patients were refractory to conventional drugs, or complained about their toxicity. In these cases, the essential oil was used as adjunctive therapy. Two treatment failures were observed.

In addition to these case studies, we used the essential oil in three emergency cases. On our way back home, we unexpectedly found a man of about 20 years old who presented severe convulsive seizures right on a public road, without nearly healthcare facilities. We immediately put the flask containing the essential oil into his nostril for one minute. The seizures stopped and he recovered consciousness. The second case concerned a baby aged 18 months who suddenly presented with a severe convulsive generalized tonic seizure. Her mother brought the baby to the clinical department of an institute. While waiting for diazepam that had to be bought from the closest pharmacy, we put the essential oil in her nostril. After a few minutes, the seizures ceased (in fact it took 45 minutes to get the diazepam). The third case concerned a 28-year old patient who came for consultation in a private clinic. She was in full convulsive crisis with hypotension and in very bad general state. Before transferring her to the hospital, the doctor applied a drop of the oil to her nostril. The doctor thought that she would die. Fortunately, her family said that her seizures ceased *en route to* the hospital. After intensive healthcare, she left the hospital in good condition. One case of very severe and unexpected migraine of a colleague was also successfully managed.

Patenting and commercialization

A national patent was granted: Philippe Rasoanaivo, Emmanuel Randrianarivo, Solofo Razafimahefa. *Composition pharmaceutique comprenant l'huile essentielle de Myrothamnus moschatus pour la prévention ou le traitement des désordres du cerveau*. OMAPI Patent N° 613, 20th October 2014.

Subsequently, two phytomedicines were manufactured, respectively, under the tradename Fanalarofy® (*fanala* = which remove, and *rofy* = chronic diseases) and Fanalanendo (anendo = headache) (Fig. 5). They are commercialized through the distribution channels of SOAMADINA, the commercial branch of the *Institut Malgache de Recherches Appliquées*.

Figure 5: Packaging of Fanalarofy® and Fanalanendo® - two products to emerge from this research now commercially available in Madagascar.

Partnerships

Internationally, we collaborated with collagues at School of Pharmacy, University of Camerino, Camerino, Italy, and the Department of Environmental Biology, *La Sapienza* University, Rome, Italy.

Nationally, we partnered with public and private health care departments as well as SOAMADINA, the commercial branch of IMRA.

Impact

One significant indicator of the success of this experience that can be quantified is the good monthly sales revenue received from sales of the two products.

Local production is also sustainable thanks to available technical and commercial infrastructures.

Replicability

Madagascar has a very rich ethnomedical heritage. Nearly 4,000 plants possess medicinal uses. Most of research on medicinal plants in Madagascar has been focused on the isolation of the active constituents and little consideration has been put on the holistic approach. Many diseases are not caused by the disregulation of a single molecular target but have a multi-factorial pathogenesis, and the observed effects often cannot be clearly assigned to specific chemical compounds. We therefore need a paradigm shift from the reductionist method to the holistic approach in investigating medicinal plants. At this point, our case study may serve as a model.

Lessons Learned

Some medical doctors have been reluctant to prescribe phytomedicines. To overcome this, a conference was delivered at the Malagasy Academy in April 2015 to explain the therapeutic usefulness of phytomedicines. A brochure has also been widely distributed.

Wild harvesting has limitations for the sustainable sourcing of the raw materials. Large scale *in situ* cultivation is needed for scaling up production.

Future Plans

Most of pharmacological tests used to validate the anticonvulsant activity of essential oils are phenotypic assays. It is necessary to move to the elucidation of the mechanism(s) of action of the essential oil of *M. moschatus* and some isolated compounds. This is under way.

While commercializing the phytomedicines, post-marketing surveillance will be carried out.

A big challenge is the large scale *in situ* cultivation of *M. moschatus*. The plant grows only in rocky areas, and its chemical composition is susceptible to variations following environmental factors. Work is being done to tackle this issue. The therapeutic usefulness of the pharmaceutical composition will be explored in other brain disorders, in the context of polypharmacology and drug repositioning.

Publications

- P. Rasoanaivo *et al*. (2012). Journal of Essential Oil Research, 24: 299-304.
- M. Nicoletti *et al*. (2012). Natural Product Research, 26(24): 2291-2300.
- E. Randrianarivo *et al*. (2013). Chemistry & Biodiversity, 10: 1987-1998.
- E. Randrianarivo *et al*. (2015). *Bulletin de l'Académie Malgache*, XCV/1: 235-248.
- E. Randrianarivo *et al*. (2016). Asian Pacific Journal of Tropical Biomedicine, 6(6): 501-505.

References

- Loscher, W.(2011). Critical review of current animal models of seizures and epilepsy used in the discovery and development of new antiepileptic drugs, Seizure 2011, 20: 359-368.
- Moch S. (2010). Therapeutic uses of antiepileptic drugs in non-epileptic disorders, South African Pharmaceutical Journal 2010; 77(5): 18-27.