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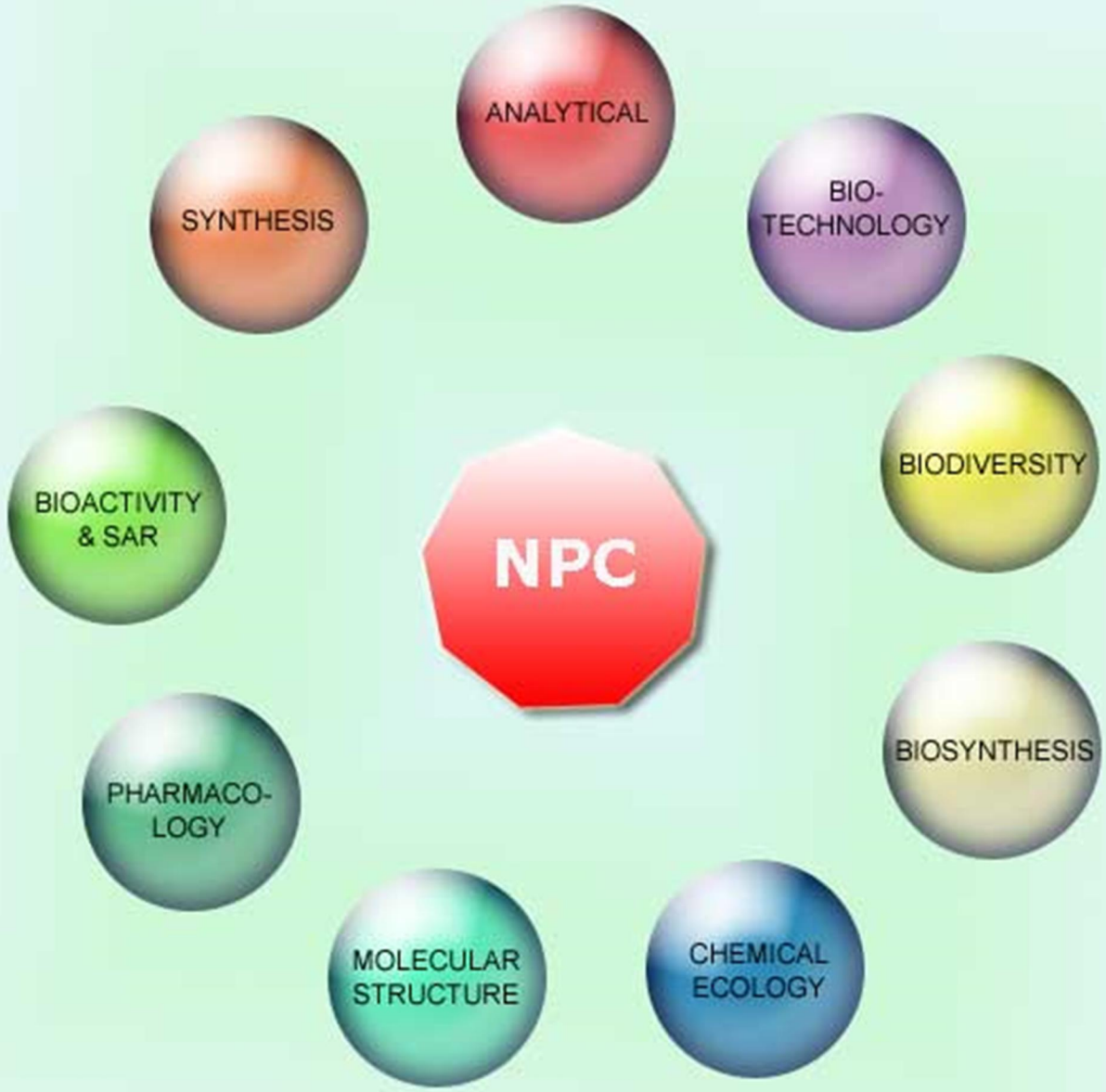

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Vasorelaxant Alkaloids from *Spirospermum penduliflorum* (Menispermaceae), a Plant Used to Treat Hypertension in Malagasy Traditional Medicine

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Spirospermum penduliflorum Thouars (Menispermaceae) is widely used on the eastern coast of Madagascar to treat hypertension. The aim of the present study was to analyse the vasorelaxant properties of different leaf extracts. The activity of the *n*-hexane, dichloromethane and methanolic extracts was tested on phenylephrine-contracted aorta. The dichloromethane extract was shown to be the most effective. Further fractionation of this extract led to the isolation of an active fraction relaxing phenylephrine-contracted aorta with an IC_{50} of 0.18 µg/mL {log IC_{50} (µg/mL) -0.74 ± 0.03} but was much less effective on KCl induced contractions. Bioassay-guided fractionation of this fraction led to the isolation of two aporphinoid alkaloids, neolitsine and dicentrine, which at concentrations of 0.1 µM and 1 µM displaced to the right the phenylephrine concentration-contraction curve. Our results show that *Spirospermum penduliflorum* extracts possess vasorelaxant activity *in vitro* that could be related to the presence of dicentrine in the extracts having an α_1 antagonist activity. This finding is not in accord with the previous studies by Rasoanaivo *et al.* where no alkaloids were detected in the leaves of *Spirospermum penduliflorum*.

Keywords: *Spirospermum penduliflorum*, Menispermaceae, Aporphine alkaloids, Antihypertensive activity.

Madagascar is one of the lands where traditional medicine based on the use of plants has an important place in society. Several plants in the Malagasy flora are alleged to possess therapeutic virtues and are largely used by the local population. Many of these plants are used without pharmacological or phytochemical data or clinical evaluation. Our studies lead us to investigate plants used for the treatment of hypertension. Among these, *Spirospermum penduliflorum* (Menispermaceae) is mentioned to be effective against arterial hypertension. The aim of the present study was to verify the traditional use of the plant and to determine the nature of the bioactive compounds by a bio-guided fractionation approach combining chromatographic methods with vasorelaxant activity tests.

When tested on phenylephrine-contracted aorta (Figure 1), the *n*-hexane, dichloromethane (DCM) and methanol (MeOH) extracts showed significant vasorelaxant activity characterized by log IC_{50} (µg/mL) values of 1.4 ± 0.02, < -0.5 and 0.29 ± 0.01, respectively (respective IC_{50} values were 25 µg/mL, < 0.3 µg/mL and 1.9 µg/mL). Isoprenaline, used as a reference standard, exhibited vasorelaxant activity with an IC_{50} of 1.7 µg/mL.

Fractionation of the dichloromethane extract was then undertaken by preparative TLC giving 4 fractions named RR, RR_1, RR_2 and RR_3. Each fraction was tested at 1 µg/mL for its vasorelaxant activity on rat aorta contracted by phenylephrine (Table 1). RR_1 showed the highest vasorelaxant activity, which was further investigated.

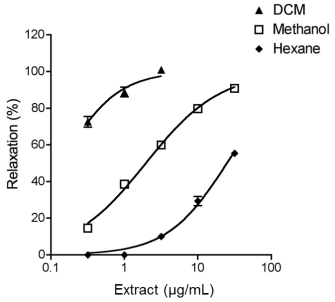


Figure 1: Relaxing effect of different extracts of *Spirospermum penduliflorum* leaves in rat aorta contracted by phenylephrine (1 µM). (n = 6).

Table 1: Vasorelaxing activities of the four fractions of the DCM extract on phenylpephrine-induced contraction of rat aorta at 1µg/mL.

Fractions	RR	RR_1	RR_2	RR_3
Relaxation (%) (*n* = 4)	12.5 ± 0.09	100 ± 8.50	7.2 ± 0.6	37.5 ± 0.4

The relaxation of phenylephrine-induced contraction by RR_1 was tested in rat aorta with (E+) and without endothelium (E-). Figure 2A shows that the activity of RR_1 was not significantly different in either the presence or absence of functional endothelium: the log IC_{50} values (µg/mL) were -0.74 ± 0.03 and - 0.61 ± 0.02 in the presence and absence of endothelium, respectively (IC_{50} values were 0.18 and 0.24 µg/mL) ($p > 0.05$, $n = 6$). To determine the potential role of β-adrenergic receptors in the vasorelaxant effect of RR_1, aorta rings were pre-incubated with propranolol (1 µM) before the contractile response to phenylephrine.

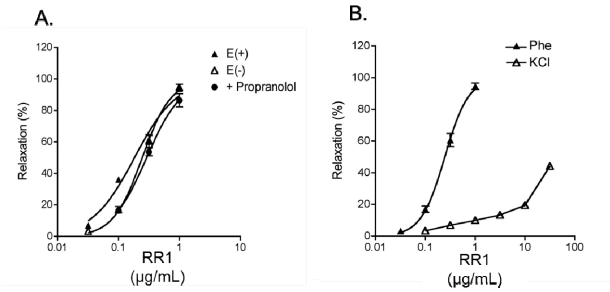


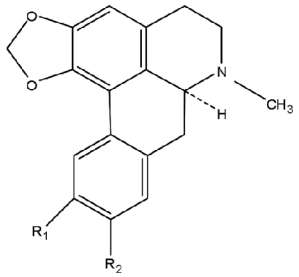
Figure 2: (A) Relaxation evoked by the RR_1 fraction of the phenylephrine-induced contraction of rat aorta with (E+) and without endothelium (E-), and with endothelium in the presence of propranolol (1 µM) (n = 6); (B) Comparison of the relaxant effect of RR_1 in the rat aorta (E-) contracted either by phenylephrine (1 µM) or by KCl (100 mM) solution (n = 6).

Figure 2A shows that propranolol did not affect the vasorelaxant activity of RR_1. Log IC_{50} (µg/mL) of RR_1 in the presence of propranolol was -0.54 ± 0.02 (0.29 µg/mL).

The potential involvement of voltage-dependent Ca^{2+} channels (VDCs) in the relaxing activity of RR_1 was tested by measuring the effect of RR_1 on KCl-induced contraction. When KCl (100 mM) was used to evoke the contraction of the aorta, RR_1 produced a concentration-dependent relaxation. However, RR_1 was less potent in inhibiting KCl-contraction than phenylephrine-contraction (Figure 2B). The IC_{50} value for KCl-contraction was higher than 30 µg/mL (n = 6).

The developed TLC chromatogram of RR1 showed two well separated spots at Rf 0.62 and 0.81, which gave positive reactions with Dragendorff's reagent. Fractionation by successive open column chromatography, followed by purification on preparative TLC led to the isolation of two active compounds, **1** and **2**.

Comparison of our spectroscopic and mass spectrometric data with those in the literature, and with a reference sample, led us to the identification of compound **1** as dicentrine [1,2], and compound **2** as neolitsine [2].



Compounds	Mw	R_1	R_2
Dicentrine (1)	339	O-CH_3	O-CH_3
Neolitsine(2)	323	-O-CH_2-O	

Because of the small amount of neolitsine that could be isolated, the determination of its pharmacological profile was not possible. Dicentrine was tested for its relaxing activity on phenylephrine-induced contraction in endothelium-denuded artery rings. As shown in Figure 3A, preincubation of the aorta with dicentrine shifted the concentration-contraction curve of phenylephrine to the right, in a concentration-dependent manner. The EC_{50} value of phenylephrine was significantly increased from 12 nM to 52 nM and 341 nM in the absence and in the presence of 0.1 µM and 1 µM of dicentrine, respectively (logEC_{50} values were - 7.9 ± 0.02 in control aorta, - 7.14 ± 0.05 and - 6.47 ± 0.03, in the presence of 0.1 µM and 1 µM

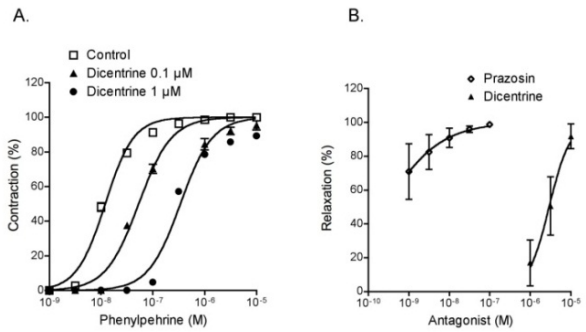


Figure3: Effect of dicentrine on phenylephrine evoked contraction.
(A) Effect of dicentrine on the concentration-effect curves of phenylephrine in endothelium-denuded rat aorta; (B) Relaxation of phenylephrine-precontracted rat aorta by increasing concentrations of dicentrine, compared with the effect of prazosin.

dicentrine, respectively, $p < 0.05$ dicentrine vs control). Dicentrine also relaxed aorta pre-contracted with phenylephrine, with a logIC_{50} (M) value of- 5.53 ± 0.05 (IC_{50} value was 2.95 µM) (Figure 3B).

High blood pressure represents the main cause of death in the world. A recent study in 2009 on the prevalence of hypertension in Antananarivo, Madagascar revealed that 28.0 % of the adult population suffer from this disease with a mean age of 49 years. High blood pressure prevalence increased from 19.1 % in 2000 to 28.0 % in 2009 in Antananarivo [3].

Traditional phytomedical practice is common in Madagascar and *Spirospermum penduliflorum* is used as a traditional medicine to treat hypertension. We showed here that crude extracts of its leaves exhibited significant concentration-dependent relaxation on rat aorta pre-contracted by phenylephrine, the dichloromethane extract being the most effective ($IC_{50} < 0.3$ µg/mL). We further studied the mechanism of action of the most active fraction (RR_1) after a first fractionation of this extract. We found that the vasorelaxant activity was not inhibited by the β-adrenergic antagonist propranolol indicating that the active compound(s) relaxed rat aorta by a mechanism other than the stimulation of the β-adrenergic receptor.

When vasoconstriction was evoked by KCl (100 mM), RR_1 was markedly less active than on phenylephrine pre-contracted aorta. This reduced relaxant activity of RR_1 on KCl-induced contraction indicates that its activity is not related to inhibition of VDCs but to an α_1 adrenergic receptor inhibition. Vasorelaxant activity can also be mediated by an effect of endothelium-released nitric oxide (NO). We observed that the activity on RR_1 was not affected by removing the endothelium, indicating that endothelial NO does not contribute to the effect of RR_1.

Bioguided fractionation of the RR_1 fraction led to the isolation of two aporphine alkaloids identified as dicentrine and neolitsine. Indeed, the Menispermaceae family is known to be a rich source of aporphine alkaloids [4-6]. Previous studies of the root bark of *Spirospermum penduliflorum* have shown the presence of the bisbenzylisoquinoline alkaloids limacine and palmitine, and aporphine alkaloids [7,8]. The authors mentioned that they did not detect alkaloids in the leaves. That was not corroborated by our study. It is well known that production of secondary metabolites such as alkaloids is influenced by numerous factors such as the age of the plant or the leaves, and the nutrients present in the soil [9]. The influence of factors on alkaloid production could be the cause of this discordance, probably linked to the sensitivity of the detection method used.

In agreement with the interaction of the RR_1 fraction with the adrenergic pathway, dicentrine induced a concentration-dependent parallel shift of the phenylephrine concentration-contraction curve, suggesting that it could act as an antagonist of the α_1-adrenergic receptor. This result is in accordance with those reported in the literature. Indeed, a previous study on dicentrine isolated from *Lindera megaphylla* showed that it is an α_1-adrenoreceptor competitive antagonist [10]. Other studies on dicentrine corroborated this allegation [11,12]. Literature data also report that neolitsine is a potent vasorelaxing agent on precontracted rat aorta preparations [13]. The presence of dicentrine and neolitsine may support the use of the plant as an antihypertensive, but standardisation is necessary as it seems that the alkaloid concentration may be highly variable; such a quality control method has recently been described [14]. However, the total safety of the use of the plant is uncertain because dicentrine and neolitsine were shown to be cytotoxic [15-17], and so further toxicological studies are needed.

Experimental

Plant material: Fresh leaves of *Spirospermum penduliflorum* were collected from the east coast of Madagascar. Botanical identification was made by the botanist Benja Rakotonirina. A voucher specimen was deposited at the herbarium of IMRA (n° AML13).

Chemical compounds: The following reagents were purchased from Sigma (St Louis, MO): isoprenaline, acetylcholine, D-glucose, phenylephrine, propranolol, prazosin, dimethylsulfoxyde and *N*-nitro-L-arginine. *L*-Dicentrine was purchased from Sequoia Research Products (Pangbourne, UK). Methanol, ethylacetate, and dichloromethane were purchased from Scharlab (Barcelona, Spain). All the salts (KCl, NaCl, $NaHCO_3$, $MgCl_2$, and $CaCl_2$) were purchased from Prolabo (VWR international, Haasrode, Belgium).

Extraction: The plant material used was in the form of finely ground, dried leaves. Extraction was performed by successive maceration of the plant material (750 g) in increasing polarity solvents, namely *n*-hexane, dichloromethane, and methanol (2.5 L of each). The extracts were filtered and dried under reduced pressure.

Rat aorta: Wistar rats of either sex weighing between 200 to 300 g were used in the study. Animals were sacrificed, the thoracic aorta isolated and cut into rings of 2 mm in length. The rings were mounted under a tension of 2g in organ baths containing Krebs solution (NaCl 122 mM, KCl 5.9 mM, $NaHCO_3$ 15 mM, $MgCl_2$ 1.25 mM, $CaCl_2$ 1.25 mM, and glucose 11 mM). When required, the endothelium was removed from the rat aorta rings by gently rubbing the luminal surface with a cotton rod before mounting the aorta ring in the organ bath. The bath solution was maintained at 37°C and gassed with 95% O_2 and 5% CO_2. Aorta were equilibrated in the medium for 2 h and the bath solution was changed every 30 min. After 1 h of equilibration, the tension was adjusted to 2 g. Contractions and relaxations were recorded with an Ugobasile 7003 isometric force transducer. All experiments were performed in accordance with international guidelines and the local ethics committee.

Pharmacological experiments

Relaxing activity on the contraction induced by phenylephrine: The relaxing activity was tested on phenylephrine pre-contracted aorta either in the absence or presence of endothelium. In both cases, after the equilibration, phenylephrine (1 µM) was added to the organ bath (20 mL) to induce contraction. At the steady-state contraction, acetylcholine (1 µM) was injected into the bath solution to relax the aorta in order to verify the presence of endothelium and its integrity. After 60 min of recuperation time, the artery rings were stimulated with phenylephrine (1 µM) and cumulative concentrations of the extracts (from 0.3 to 50 µg/mL), from a stock solution in DMSO/H_2O (10:90: v/v) were added into the organ bath (20 mL) at the steady-state of contraction to evaluate the vasorelaxant activity. When required, aorta rings were incubated in the presence of propranolol (1 µM) for 30 min before inducing the contraction with phenylephrine.

Relaxing activity on KCl induced contractions: After 2 h equilibration with Krebs solution, the medium was removed and replaced with depolarizing solution containing: NaCl 27 mM, KCl 100 mM, $NaHCO_3$ 15 mM, $MgCl_2$ 1.25 mM, $CaCl_2$ 1.25 mM, and glucose 11 mM to induce contraction. After contraction had reached a maximum, the organ was rinsed 3 times with normal Krebs solution. After 30 min equilibration, the medium was replaced by depolarizing Krebs solution. Cumulative concentrations of the extracts (from 0.03 to 10 µg/mL) from a stock solution in DMSO/H_2O (10:90, v/v) were added to the organ bath (20 mL) at the steady state of contraction.

Phenylephrine concentration-response curves: Concentration-response curves of phenylephrine (from 10^{-9} M to $3x10^{-5}$ M) were realized in endothelium-denuded aorta rings either without or in the presence of different concentrations of dicentrine (0.1 µM and 1 µM from stock solution in DMSO/H_2O (10:90, v/v)). Contraction was expressed as percent of the maximum response obtained in the absence of inhibitor in the same artery ring. The concentration-effect curves were built and the concentrations of phenylephrine producing 50% of the maximum aorta contraction (EC_{50}) were calculated and compared.

Chromatographic methods: TLC was realized on silica gel $60F_{254}$ plates (Merck) using *n*-hexane/ethyl acetate/ethanol/NH_4OH (6/6/2.5/0.01) as mobile phase. The separated components were visualized under visible and ultraviolet light (254 and 365 nm) or using Dragendorff's spray reagent. Fractionation was made by successive open CC using either silica gel 60 (0.063 – 0.200 mm, Merck Darmstadt, Germany) as stationary phase or Sephadex Gel LH-20 (Pharmacia Biotech Healthcare, Diegem, Belgium) using as eluant a mixture of dichlomethane-methanol (1:1, v/v). Final purification was achieved by preparative TLC on Silica Gel 60 GF_{254} plates (Merck Darmstadt, Germany). The mobile phase was a mixture of ethyl acetate/methanol (9:1).

Identification method: Identification of the isolated compounds was performed with a LCQ Advantage Thermo Finnigan (Waltham, MA, USA) mass spectrometer piloted by X-Calibur software. Mass spectra were determined using an APCI source in positive mode, a vaporizer temperature of 455°C, a sheath gas flow rate of 45 (a.u.) and a capillary voltage of 2V. ^{13}C and ^{1}H NMR spectra were recorded on a Bruker Avance DRX-400 spectrometer in $CDCl_3$, CD_3OD or DMSO at 400 MHz (^{1}H) and 100 MHz (^{13}C), at 30°C. A combination of COSY, HMQC, HMBC and ROESY experiments were used when necessary for the assignment of ^{1}H and ^{13}C chemical shifts.

Statistical analysis: The relaxant effect of the tested products was expressed as percent of the steady-state contraction induced by the agonist or the KCl-depolarizing solution. The log values of EC_{50}, which is defined as the concentration producing 50% of the maximum response, or of IC_{50} (concentration inhibiting the contraction by 50%) were determined from the non-linear

regression of the experimental data (Prism, GraphPad) and used for the statistical analysis. Each test was repeated in 3 inter-day experiments. The results are presented as the mean ± S.E.M. of n observations. Values were analyzed using Student's t-test and were considered to be significantly different when $p < 0.05$.

Acknowledgements- Financial support of the Commission Universitaire pour le Devéloppement (CUD), Belgique is gratefully acknowledged. Authors wish to thank A. Randrianirina for technical help. The authors gratefully thank the Belgian National Fund for Scientific Research (FNRS) (FRFC 2.4555.08 and credit for researcher 1.5128.11), the Special Fund for Research (FSR) and the faculty of medicine of UCL.

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H. Rakotoarimanana, H. Rafatro, V. Jeannoda, **G. Raoelison**, RB. Robijaona, S. Ratsimamanga-Urverg. Composé antiplasmodial isolé du Tsilaitra (*Noronhia divaricata* Perr., Oleaceae). *Ethnopharmarcologia* 2008, **41**, 71-73.

Composé antiplasmodial isolé du Tsilaitra (*Noronhia divaricata* Perr., Oleaceae)

H. Rakotoarimanana[1,3], H. Rafatro[1*], V. Jeannoda[3], G. Raoelison[2], R.B. Robijaona[1], S. Ratsimamanga-Urverg[1,2]

Résumé

Cette étude a pour but d'isoler la(es) molécule(s) active(s) des plantes utilisées contre le paludisme appliquant la méthode dite *terrain-labo* mise au point au laboratoire.

La plante a été choisie parmi celles qui entrent dans la recette d'un remède de la médecine traditionnelle pour le traitement du paludisme. Les extraits du matériel végétal étudié ont été préparés et partitionnés. Les fractions obtenues ont été ensuite purifiées par chromatographie sur colonne utilisant successivement le sephadex et la silice. Les tests biologiques ont été réalisés vis-à-vis des souches plasmodiales déterminant la concentration inhibitrice et le pourcentage d'inhibition de la croissance parasitaire.

In vitro, la fraction de la phase dichlorométhane de l'extrait des feuilles exerce une activité antiplasmodiale avec une valeur de concentration inhibitrice de 2,12 µg/mL. De cet extrait, une des molécules biologiquement actives identifiée est l'acide lunularique. *In vivo*, à la dose de 100 mg/kg, cette molécule inhibe de 33,68% la croissance parasitaire.

Mots clés : Acide lunularique, Antiplasmodiale, Plante médicinale, *Noronhia*, Madagascar.

INTRODUCTION

Dans la majorité des articles scientifiques publiés sur le paludisme il y est dit que : la maladie touche actuellement plus de 90 pays dans le monde (Saissy, 2001 ; Gentilini et Duflo, 1986), son incidence est estimée de 300 à 500 millions de cas cliniques par an avec un nombre de décès de 1,5 à 2,7 millions (Saissy, 2001 ; Rogier et al., 2001b). La quasi-totalité de décès due au paludisme est attribuable à *Plasmodium falciparum* (Rogier et al., 2001a). Force est donc de constater que le paludisme reste en ce début de XXIème siècle la première endémie tropicale mondiale. En Afrique subsaharienne, il reste un des problèmes majeurs de santé publique avec 550 millions de personnes vivant en zones impaludées (Pichard, 2001).

L'émergence, la diffusion et l'augmentation de la résistance des souches de *Plasmodium falciparum* aux médicaments antipaludiques conventionnels disponibles sur le marché sont responsables de l'aggravation dramatique de la mortalité par le paludisme (Trape et al., 2001). Ainsi, pour contribuer à la lutte contre ce fléau mondial, une méthode nommée *terrain-labo* a été mise au point au laboratoire. Les résultats décrits dans cet article sont les premiers fruits de l'application de cette méthode.

MÉTHODOLOGIE

Les protocoles expérimentaux appliqués dans cette étude ont été décrits en détail dans l'article publiant nos travaux antérieurs (Rafatro et al., 2006).

À la suite des enquêtes ethnobotaniques, les plantes endémiques de Madagascar, qui sont indiquées et entrent dans la composition (décocté de l'association de plusieurs plantes) des remèdes traditionnels utilisés contre le paludisme, ont été collectées.

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Après séchage et broyage, des extraits bruts hydroalcooliques ont été préparés. En appliquant la méthode classique de fractionnement bioguidé, les molécules actives ont été isolées en deux étapes : tout d'abord, l'extrait brut a été déchlorophyllé par passage au charbon actif, puis a été fractionné par partage liquide-liquide entre eau, d'une part, et des solvants organiques (hexane, dichlorométhane, acétate d'éthyle), d'autre part. Ensuite, les extraits issus de ces fractionnements ont été passés successivement à travers une colonne chromatographique utilisant les phases mobiles le Sephadex LH-20 (Pharmacia Fine Chemicals) et la Silice (40-63 µm, Geduran Si60, Merck). À cette étape, l'extrait a été dilué avec des systèmes de solvants à polarité croissante obtenue par les mélanges de dichlorométhane, d'acétate d'éthyle et du méthanol (VWR, Prolabo). La structure chimique du produit actif isolé de cette plante a été identifiée par la méthode de résonance magnétique nucléaire (RMN).

Les tests antiplasmodiaux *in vitro* et *in vivo* ont été réalisés en appliquant respectivement les méthodes de «micro-test isotopique» (Desjardins, 1979) et de «quatre jours de suppression parasitaire» (Peters, 1975). Les paramètres pharmacologiques qui ont été respectivement déterminés sont la concentration (CI_{50}) et le pourcentage d'inhibition (% I) de la croissance parasitaire par rapport au contrôle non traité sur modèles de *Plasmodium falciparum* FcM29C1(en culture) et de *Plasmodium yoelii nigeriensis* N66 (chez la souris). L'extrait brut hydroalcoolique de *Cinchona* a été utilisé comme produit de référence.

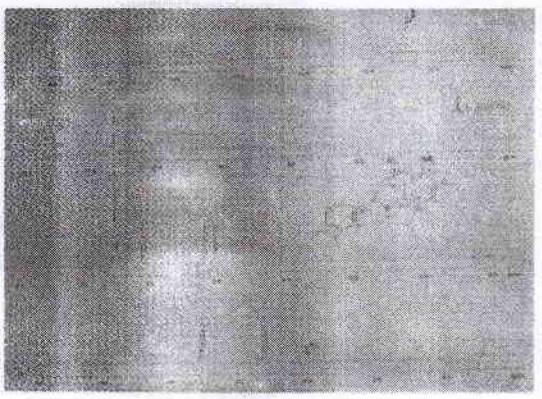

Figure 1 : Spectre RMN pour l'élucidation structurale de l'acide lunularique

RÉSULTATS ET DISCUSSION

Les informations, concernant le «*Isilaitra*», qui ont conduit à la réalisation de cette étude, ont été obtenues par une femme guérisseuse habitant la ville de Toamasina, une province de la région est de Madagascar (Rafatro et al., 2007). Après identification botanique, la plante en question se révèle être le *Noronhia divaricata* Perr. – Oleaceae, une espèce endémique de Madagascar (Perrier, 1952).

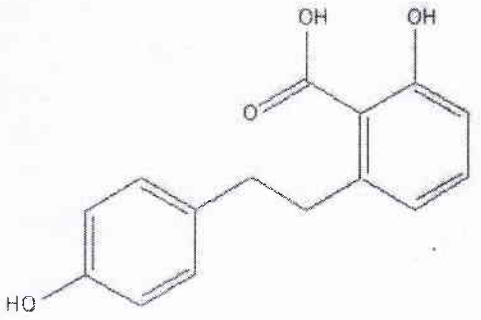

Figure 2 : Structure chimique de l'acide lunularique

A la suite des tests préliminaires, l'extrait brut hydroalcoolique déchlorophyllé des feuilles de cette plante exerce une activité antiplasmodiale *in vitro* avec une valeur de CI_{50} égale à 25,45 µg/mL. Après partage liquide-liquide, l'extrait de la phase dichlorométhane présente une activité antiplasmodiale avec une valeur de CI_{50} de 2,12 µg/mL. En étudiant sur un autre modèle de test antipaludique, *in vivo*, cet extrait hydroalcoolique déchlorophyllé inhibe de 27,74% la croissance parasitaire du *Plasmodium yoelii* à la dose de 100 mg/kg. Pour cette même dose, le produit pur isolé de la phase dichlorométhane de cet extrait brut inhibe de 33,68% la croissance plasmodiale. En effet, l'activité antipaludique est de faible à modérée.

Après étude de la partie chimique, deux molécules pures ont été isolées de l'extrait de feuille de *Noronhia divaricata* dont l'une d'elle est apparentée à l'acide lunularique. Le spectre RMN est illustré sur la figure 1 et l'activité antipaludique *in vivo* est résumée dans le tableau 1. La figure 2 illustre sa structure chimique.

L'acide lunularique est plutôt connu dans le monde végétal, il est surtout distribué dans la vacuole et le cytoplasme des cellules végétales (Imoto et Ohta, 1985). C'est un sous produit issu du métabolisme secondaire lors de la biosynthèse protéinique impliquant la chalcone (Eckermann et al., 2003). Cette molécule est aussi reconnue par sa propriété inhibitrice de la croissance végétale (Yoshikawa et al., 2002). Dans le monde animal, l'acide lunularique est doué d'une activité molluscicide (Wurzel et al., 1990). Tout récemment, il a été découvert que ça pourrait être un nouvel agent potentiel en chimioprévention du cancer (Berti et al., 2004). A notre connaissance, c'est la première fois qu'une telle structure moléculaire, douée d'une activité inhibitrice de la croissance plasmodiale est décrite. Ainsi, en approfondissant cette étude, améliorant la structure chimique des molécules actives, l'acide lunularique pourrait être un médicament potentiel pour contribuer à la lutte antipaludique.

CONCLUSION

Dans la région est de Madagascar, les feuilles de *Isilaitra*, le *Noronhia divaricata* Perr. – Oleaceae, entre dans la composition des remèdes traditionnels d'une femme guérisseuse pour traiter la fièvre et le paludisme. C'est une plante endémique de la grande île. Dans

ces feuilles, après application de la nouvelle méthode nommée *terrain-labo*, l'acide lunularique a, entre autres, été isolé. L'effet antipaludique de l'acide lunularique est considéré comme modéré. Ceci pourrait justifier l'association de l'extrait du *tsilaitra* avec ceux des autres plantes et cet axe d'étude mérite d'être plus approfondi.

Tableau 1 : Activité antiplasmodiale *in vivo* de l'acide lunularique vis-à-vis de la croissance du *Plasmodium yoelii nigeriensis* chez la souris (n=2 à 6)

Lot	Dose (mg/kg)	Moyenne	Test "t" de Student (n=6)
1. Non traité			
% de parasitémie		44 ± 4	
2. Extrait brut de *Cinchona*			
% de parasitémie		24 ± 6	P < 0,05 (n=6)
% d'inhibition	100	46 ± 8	
3. Extrait brut de *Noronhia*			
% de parasitémie		11 ± 1	P > 0,05 (n=2)
% d'inhibition	100	27 ± 3	
4. Acide lunularique			
% de parasitémie		29 ± 4	P < 0,05 (n=6)
% d'inhibition	100	33 ± 11	

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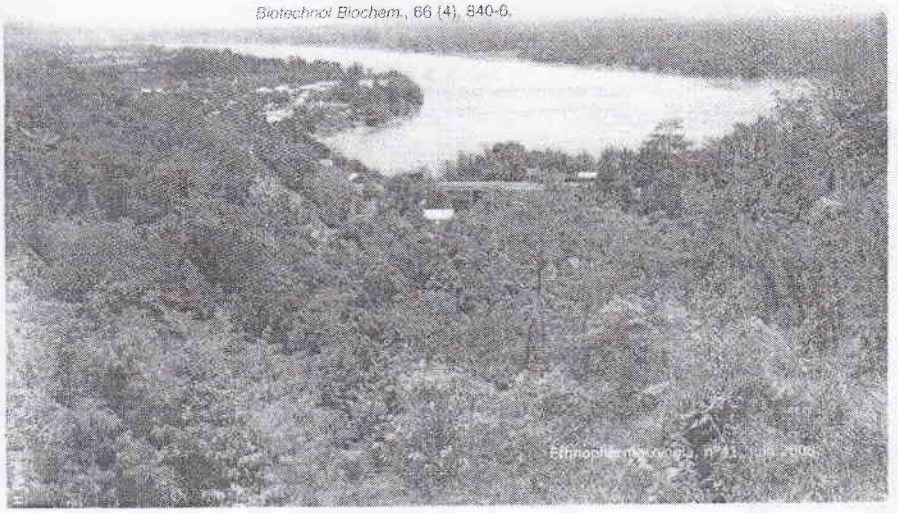

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