Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2016; 5(6): 238-244
Received: 01-09-2016
Accepted: 02-10-2016

Jeannot Bako Razafindrakoto
(1). Ecole Normale Supérieure, Université d'Antananarivo, BP 0, Antananarivo 101, Madagascar
(2). Laboratoire de Chimie des Substances Naturelles et Chimie Organique Biologique (LCSN/COB), Faculty of Sciences, University of Antananarivo, BP 906 Ankatso, Antananarivo 101, Madagascar

Léa Herilala Rasoanaivo
Laboratoire de Chimie des Substances Naturelles et Chimie Organique Biologique (LCSN/COB), Faculty of Sciences, University of Antananarivo, BP 906 Ankatso, Antananarivo 101, Madagascar

Anne Wadouachi
Laboratoire Glycochimie, des Antimicrobiens et des Agroressources CNRS UMR 7378 -Institut de Chimie de Picardie CNRS FRE 3085. Université de Picardie Jules Verne, France

Lalaina Rahajamanana
Laboratoire de bactériologie du Centre Universitaire Mère Enfant Tsaralalana Antananarivo, Madagascar

René Razafindrazaka
Institut Malgache de Recherches Appliquées, BP 3833, Antananarivo 101, Madagascar

Louise Faramalala Rabemandroso
Laboratoire de Chimie des Substances Naturelles et Chimie Organique Biologique (LCSN/COB), Faculty of Sciences, University of Antananarivo, BP 906 Ankatso, Antananarivo 101, Madagascar

Amélie Raharisololalao
Laboratoire de Chimie des Substances Naturelles et Chimie Organique Biologique (LCSN/COB), Faculty of Sciences, University of Antananarivo, BP 906 Ankatso, Antananarivo 101, Madagascar

Correspondence
Léa Herilala Rasoanaivo
Laboratoire de Chimie des Substances Naturelles et Chimie Organique Biologique (LCSN/COB), Faculty of Sciences, University of Antananarivo, BP 906 Ankatso, Antananarivo 101, Madagascar

Antispasmodic effects of sesselin from *Cynanchum ambositrense* on isolated tissues

Jeannot Bako Razafindrakoto, Léa Herilala Rasoanaivo, Anne Wadouachi, Lalaina Rahajamanana, René Razafindrazaka, Louise Faramalala Rabemandroso and Amélie Raharisololalao

Abstract
Cynanchum ambositrense Choux (Asclepiadaceae) is used in Malagasy herbal medicine for diminishing teeth pain, enhancing lactation of childminder, also used against serious cough and for treating intestinal disorders and spasms. The ethanolic extract is efficient on isolated guinea pig trachea pre-contracted with histamine at 2.10^{-5} M with a median effective concentration (EC_{50}) of 0.452 ± 0.127 mg / ml.
Bioassay-guided fractionation using two models guinea pig isolated ileum contraction induced by histamine and isolated rat duodenum contracted with acetylcholine to monitor the activity led to the isolation of sesselin (pyranocoumarin) as the main active constituent from the most promising ethyl acetate extract obtained from partition of ethanolic extract of the aerial part of *Cynanchum ambositrense*. Indeed, sesselin induced a concentration-dependant relaxation on guinea pig ileum contracted by histamine and on rat duodenum contracted by acetylcholine with an IC_{50} respectively of 0.027 ± 0.001 mg/ml and 0.023 ± 0.005 mg/ml. It inhibits the histamine spasm effect on the isolated ileum and the acetylcholine spasm effect on the isolated duodenum in a noncompetitive manner.

Keywords: *Cynanchum ambositrense,* Asclepiadaceae, Madagascar, antispasmodic, sesselin

Introduction
Cynanchum ambositrense Asclepiadaceae, tree origins of the South of Madagascar is popularly known as *vahimavo* [1]. According to our ethnobotanical investigations led with population and traditional healers in the region of Amoron'i Mania, the plant is traditionally used to treat teeth pain, serious cough, intestinal disorders, spasms and enhancing lactation of childminder.
The chemical constituent of many *Cynanchum* species have been studied, sugars [2, 3], steroids [4], triterpenes [5, 6] glycosides [7], alkaloids [8] and phenolic compounds [9] such coumarins [10], have been identified.
The infantile diarrheal diseases are still widespread in Madagascar and are major causes of infant deaths with malnutrition, respiratory infections and malaria [11, 12, 13]. Diarrhoea is often accompanied by intestinal spasms that cause abdominal cramps and pain. It is on account of these data that we investigated *Cynanchum ambositrense* for antispasmodic activity. Prior to our work, no phytochemical or biological studies have been reported on this species. Using three models guinea pig trachea pre-contracted with histamine at 2.10^{-5} M, guinea pig ileum contracted with histamine and rat duodenum contracted with acetylcholine to monitor the isolation of the active product, we identified sesselin (pyranocoumarin) as the most active compound from the most active extract (ethyl acetate). We report in this paper its antispasmodic activity on duodenum and ileum. We tested also extracts and isolated products for their antispasmodic activities.

Materials and methods
Plant material
The aerial part of *Cynanchum ambositrense* Choux (Asclepiadaceae) is collected from Ambositra Fianarantsoa middle part of Madagascar in October 2007. The plant is authenticated by comparison with herbarium sample at Botanic Department from Botany and Zoology Park Tsimbazaza, Antananarivo Madagascar [14].

Extraction and isolation of the constituents
Dried aerial parts of Cynanchum ambositrense were reduced to a fine powder with a mechanical grinder. Air dried aerial parts of *C. ambositrense* (489 g) were soaked at room temperature in ethanol/water (80/20) during one week.

The filtered solvent was evaporated under vacuum to afford an ethanolic extract. The residue was suspended in hot water (40 °C) and filtered in warm to remove the resins. The obtained extractive solution was partitioned by successive extractions three times with different solvents of increasing polarity to yield hexanic, methylene chloride, ethyl acetate, butanol and aqueous extracts. These extracts were used for assessment of the antispasmodic activity.

The methylene chloride extract (2 g) was separated over silica gel column chromatography, eluted with hexane/ethyl acetate solvent system with increasing polarity to afford 90 fractions of 10 ml. These fractions were analysed by thin layer chromatography (TLC). TLC was established using precoated silica gel 60 F254 plates of 0.2 mm thickness, aluminium base Merck. Five microliter of fractions was loaded on TLC plates and it was developed using the adequat eluent, in a chamber saturated for 10 min. The plates was dried, and separated compounds were visualized in UV lamp (254/365 nm) or by spraying with sulphuric acid 50%.The plates were carefully heated at 110 °C for 10 min to obtain an optimal colour development. The behavior of an individual compound in TLC is characterized by a report frontal (R*f*) and is expressed by dividing the distance of the compound travelled from the original position (solvent front) by the distance of the solvent travelled from the original position. The fractions exhibiting an identical form with the same R*f* in TLC were combined in lot and purified to give a pure product.

In the same manner, the ethyl acetate extract (2.6 g) was separated over a silica gel CC eluted with a cyclohexane/EtOAc solvent system with increasing polarity to obtain 150 fractions of 10 ml.

These isolated compounds were used for assessment of the antispasmodic activity.

Pharmacological experiments

Drugs and solubility

Acetylcholine chloride, histamine dihydrochloride were purchased from Sigma (USA). All other chemicals used were of analytical grade available. All of the salts (NaCl, KCl, $CaCl_2$, $MgSO_4$, KH_2PO_4, $NaHCO_3$) as well as D-glucose used to prepare the Krebs-Henseleit or Tyrode solutions were purchased from Prolabo. The various extracts of *Cynanchum ambositrense* and isolated products, were dissolved in DMSO (1‰ final concentration). Control experiments demonstrated that the DMSO used to dissolve products or plant extracts did not affect on various parameters of contractile responses of the isolated tissues at their final bath concentrations.

Animals

Under a protocol approved by the Animal Care and Use Committee of Münster, guinea pigs tri colours of either sex, weighting about 350 g, and Wistar rats weighting about 300 g in the animal house of IMRA (Institut Malgache de Recherches Appliquées) were used. All the animals were kept in air-conditioned rooms, lighting (12h: 12h light-darkness cycle) with free access to normal food and water.

Guinea pig trachea

Guinea pigs tricolour were anesthetized and sacrificed by cervical dislocation followed by exsanguinations. The trachea was removed, washed and placed in Krebs-Henseleit solution, the composition of which in mM is as following: NaCl 122, KCl 5.9, $NaHCO_3$15, $MgCl_2$ 1.25, $CaCl_2$ and glucose 11.

Trachea rings about 3 cm in length were hanged in a 20 ml tissue bath containing Krebs-Henseleit solution, continuously bubbled with a mixture of 95% oxygen and 5% carbon dioxide (carbon gas) and maintained at 37 °C. A preload of 1.5 g was applied to each tissue and kept constant throughout the experiment. The strips were allowed to equilibrate for 90 min and rinsed every 15 min with the survival solution. After equilibration, viability and sensitivity of the rings were tested with 10^{-5} M histamine. Then, the organ was rinsed three times and allowed to relax until the return to the initial tension. Thirty minutes (30 min) after the last rinsing, the organ is pre-contracted again with 2.10^{-5}M histamine. In the contraction board, cumulative and increasing concentrations of the ethanol extract (0.1-2.0 mg/mL), dissolved in a mixture of DMSO (Dimethyl sulfoxide) and distilled water (90/10) are injected in the organ bath in order to highlight the relaxing activity. Such relaxations obtained are evaluated in % in order to calculate the EC_{50} (concentration of the extract which relaxes up 50% the contraction induced by the contracting agent) [15, 16].

Guinea pig ileum

A piece of guinea pig ileum, 10-20 cm long, about 15 cm proximal to the ileo-cecal junction, was removed from the abdominal chamber. The proximal ileum was washed and placed in tyrode solution and the mesenteric residues and adherent fat were eliminated. The composition of Tyrode solution was as follows (mM): KCl 5.9, NaCl 122, $MgCl_2$ 1.25, $NaHCO_3$ 15, $CaCl_2$ 1.25 and glucose 11. The segments of ileum about 2 cm in length were hanged in a 20 ml tissue bath containing Tyrode solution, continuously bubbled with a mixture of 95% oxygen and 5% carbon dioxide (carbon gas) and maintained at 37 °C. A preload of 0.5 g was applied to each tissue and kept constant throughout the experiment. The strips were allowed to equilibrate for 1 h and rinsed every 15 min with the survival solution. At the end of equilibration, the isolated ileum was contracted with 5.10^{-6} M histamine (final concentration in the bath) for 2 min for the viability test. Then the organ was rinsed three times and allowed to release it to return to the initial tension. After washing, the tissues had been pretreated with the extract or the isolated compound and cumulative concentration response curves were recorded, isotonically in the organ bath, for histamine (10^{-9} to 10^{-5} M), in the absence or presence of the following ethanolic extract (0.03 mg/ml and 0.1 mg/ml) [17, 18].

In case of competitive inhibition (the extract enters is competing about the occupation of H1 type histaminic receptors within the intestine smooth muscles of the guinea pig isolated ileum). In case of noncompetitive inhibition, the extract IC_{50} value is the parameter to be evaluated (extract concentration which inhibits up to 50% the maximum contraction induced by the histamine without extract) [17, 18].

Rat duodenum

Wistar rats of either sex were anesthetized and killed by cervical dislocation and exsanguination. A small piece of duodenum, 2 cm long, a part from stomach was dissected out of the abdominal cavity of rat duodenum, cleaned and mounted in 20 ml tissues baths containing Tyrode solution aerated with carbogen and maintained at 37 °C. A tension of 1 g was applied to each tissue and kept constant through the length of the experiment. The strips of duodenum were equilibrated for 1 hour and rinsed every 15 min with the survival solution. After equilibration, the segments were stimulated with 10^{-5} M acetylcholine and then rinsed three times after attaining the maximum contraction for the viability test [17, 18].

After washing, the tissues had been pretreated with the compound and cumulative concentration response curves were recorded, isotonically in the organ bath, for acetylcholine (10^{-8} to 10^{-4} M), in the absence or presence of extracts (0.03 mg/ml and 0.1 mg/ml) [17, 18]. The effect was allowed to reach a steady state at each concentration. The registered contractions are measured and converted into %: it is the acetylcholine effect without extract [17, 18].

Statistical analysis

The response of the rings of trachea was expressed in percentage of reduction of contraction induced by the histamine according to the following formula [19]:

$$r = \frac{100(c - x)}{c - b}$$

c : tonus of stable contraction obtained by precontraction with the histamine
2.10^{-5}M
b : tonus of rest
x = value of tonus induced by the tested substance

The value of relaxation due to the extract was obtained by shielding the value obtained with controls (distilled water or DMSO) from experimental value. The results were expressed in form of median.

Statistical calculations were carried out with Graph Pad Prism 4. The results are expressed as the mean ± standard error of mean (S.E.M.) of (n) independent experiments with individual values. Unpaired student's *t*-test was used for statistical comparison; *p* values < 0.01 or < 0.05 were considered as significantly different from the control.

Results and discussions

Results of extraction and structures of compound isolated

Air dried aerial parts of *C. ambositrense* (489 g) were soaked at room temperature in ethanol/water (80/20) during one week. The filtered solvent was evaporated under vacuum to afford an ethanolic extract noted ASC1 (40 g). The residue (40 g) was suspended in hot water (40°C) and filtered in warm to remove the resins. The obtained extractive solution was partitioned by successive extractions three times with different solvents of increasing polarity to yield hexanic (ASC1 hex, 0.26 g), methylene chloride (ASC1 DCM, 2 g), ethyl acetate (ASC1 AcOEt, 2.6 g), butanol (ASC1 BuOH, 4.2 g) and aqueous extracts. These extracts were used for assessment of the antispasmodic activity.

The methylene chloride and ethyl acetate extracts showing strong antispasmodic activity on guinea pig ileum pre-contracted with histamine and duodenum rat pre-contracted with acetylcholine were subjected to a further separation.

The methylene chloride extract (2 g) was separated over silica gel column chromatography, eluted with hexane/ethyl acetate solvent system with increasing polarity to afford 90 fractions of 10 ml. The fractions with same aspect in TLC were combined in lot and purified. Three of these (D1 to D3) were obtained. D1 (10-17) was isolated with a mixture of hexane/CH_2Cl_2 (94:6), a mixture of D2 and D3 (19-20) with hexane/EtOAc (90:10). D1 (0.15 g, Rf = 0.3), mixture of D2 and D3 (0.1 g, Rf = 0.25).

The ethyl acetate extract (2.6 g) was separated over a silica gel CC eluted with a cyclohexane/EtOAc solvent system with increasing polarity to afford three compounds D4 (0.15 g, Rf = 0.3), D5 (0.15 g, Rf = 0.3), D6 (0.15 g, Rf = 0.3) eluted with (cyclohexane/EtOAc, 60:40). Other lots were mixture of several compounds and could not be separated due to chromatography complexity.

These six compounds (D1 to D6) were used for assessment of the antispasmodic activity with both models.

Antispasmodic activity

Effect of ethanolic on guinea-pig trachea

The incubation of increasing concentrations of ethanolic extract from the aerial part of *Cynanchum ambositrense* (0.1-2.0 mg/mL) for 15 min inhibit the contractile response elicited by histamine on guinea-pig trachea. Reported results on figure 1 show that ethanolic extract (ASC1) relaxes guinea pig isolated trachea in concentration dependent manner with a EC_{50} value equal to 0.452 ± 0.127 mg / ml.

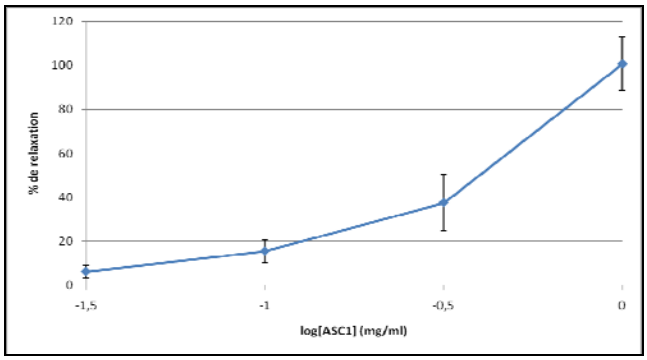

Fig 1: Relaxing effect of ethanolic extract (ASC1) on guinea pig isolated trachea pre-contraced by histamine $2x10^{-5}$M. (n=6) dosed.

Since ethanolic extract from *Cynanchum ambositrense* exhibited relaxing activity of interest in this experimental model, they were also evaluated against other two models.

Effect of ethanolic extract on guinea pig ileum

The first on guinea pig ileum, ethanol extract from *Cynanchum ambositrense* cause a significant inhibitory effect for the contractile response elicited by histamine on guinea-pig ileum in a noncompetitive and concentration- dependent manner, with IC50 values (with 95% confidence limits, mg/mL) of 0.095 ± 0.009 mg / ml (Figure 2).

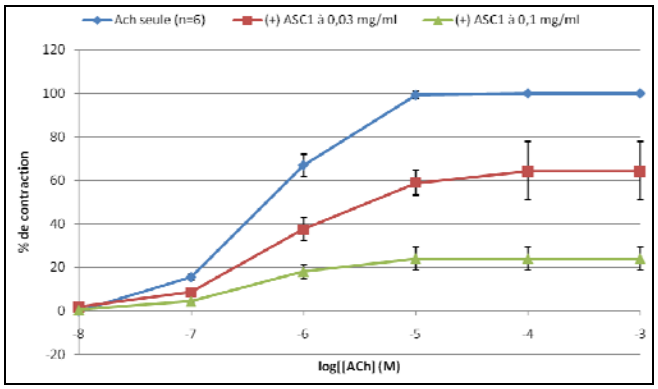

Fig 3: Inhibiting effect of ethanolic extract (ASC1) to the contracting activity of acetylcholine on rat isolated duodenum (n=6).

It is interesting to note that in two tissues preparations, a noncompetitive antagonism was observed, characterized by a clear displacement to the right of the concentration-response curves with a decrease in maximal response above 50%.

Furthermore, the difference between inhibitory response observed on guinea-pig ileum and rat duodenum contracted

respectively by histamine and acetylcholine was also demonstrated in the same preparations by ethanolic extract of *Cynanchum ambositrense,* with IC_{50} values (mg/mL) of 0.095 and 0.053, respectively, which may be due to differences of sensibility of each tissue. The smooth muscle of the guinea-pig ileum undergoes a biphasic mechanical response when exposed to acetylcholine.

Effect of the fractions on guinea pig isolated ileum and rat isolated duodenum

In order to determine the chemical constituents responsible for the effect described, the ethanolic extract (ASC1) was tested for the presence of different groups of chemical constituents and it was found to contain triterpenes and phenolics compounds such coumarins. This extract (ASC1) is separated in polarity with partition liquid-liquid to obtain hexanic (ASC1 hex), methylene chloride (ASC1 DCM), ethyl acetate (ASC1AcOEt), butanolic (ASC1 BuOH) and aqueous extracts (ASC1 aqueous). The effect of the 5 extracts on the histamine induced contraction of guinea pig isolated ileum and the acetylcholine induced contraction of rat isolated duodenum are respectively showed in figures 4 and 5.

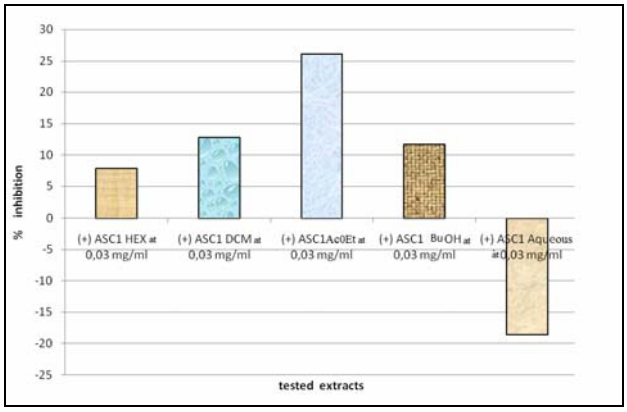

Fig 4: Inhibiting effect of five extracts from *Cynanchum ambositrense* to the contraction induced by histamine on guinea pig isolated ileum.

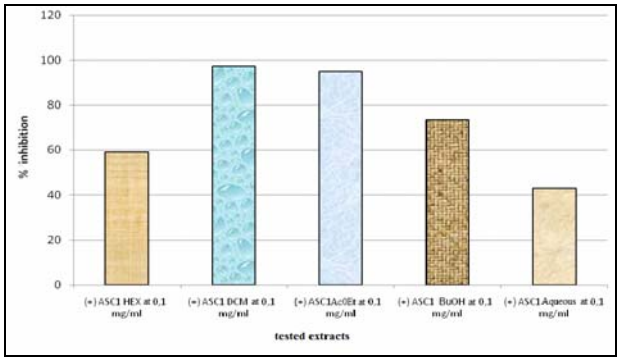

Fig 5: Inhibiting activity of different extracts from *Cynanchum ambositrense* to the contracting activity of acetylcholine on rat isolated duodenum

On isolated guinea-pig ileum contracted with histamine, the ethyl acetate extract was clearly more active than the hexanic, methylene chloride and butanolic extracts, suggesting the presence of a strong antispasmodic agent in this extract (Figure 4). The other (hexanic, butanolic and aqueous) extracts seem to be less interesting.

And figure 5 shows that methylene chloride and ethyl acetate are the most active on the rat duodenum model.

To obtain the most active product, the active extracts methylene chloride and ethyl acetate were separated on column chromatographed on silica gel.

Three products noted D1, D2, D3 were obtained from methylene chloride extract and three products noted D4, D5 and D6 with ethyl acetate extract.

Effect of the isolated products on guinea pig ileum and rat duodenum

This test consists to the identification of the single or numerous most active molecules through bioguided fragmentation. The pharmacological parameter to be studied is the inhibition percentage. The isolated products were tested for antispasmodic activity with the two models rat duodenum contracted with acetylcholine and guinea pig ileum contracted with histamine. Results are shown respectively in figures 6 and 7.

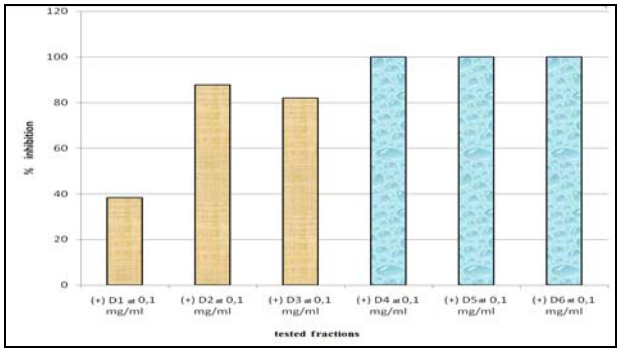

Fig 6: Inhibiting effect of the six products (D1 to D6) isolated from DCM and AcOEt extracts of the contraction induced by acetylcholine on rat duodenum

Of the six compounds (D1 to D6) obtained from ethyl acetate and methylene chloride extracts, D4, D5 and D6 were found to be the most active compounds on inhibition of the contraction induced by acetylcholine on rat duodenum (Figure 6).

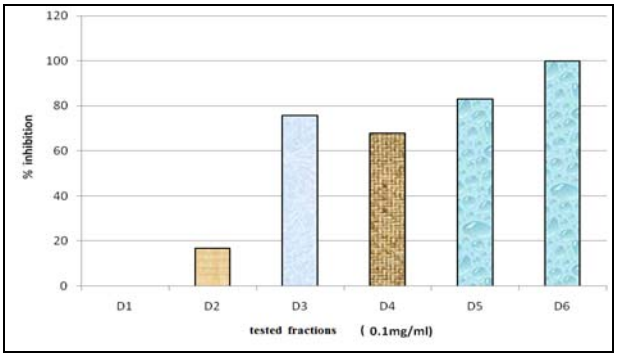

Fig 7: Inhibiting effect of the six products (D1 to D6) isolated from DCM and AcOEt extracts by histamine on guinea pig isolated ileum.

Reported results on figure 7 show that product D6 isolated from AcOEt extract is the most active one.

At 0.1 mg/ml, D6, isolated product from ethyl acetate extract produced a significant inhibition of the contraction induced by histamine on guinea-pig ileum, with a percentage of inhibition of 99.6% ± 0.6 (n=8), The other compounds showed a similar or higher percentage of inhibition of histamine-induced contraction on guinea pig ileum.

Effect of the most active compound on guinea pig ileum and rat duodenum

The effect-concentrations of the two models: the acetylcholine on the duodenum of rat and the histamine on guinea pig ileum in absence and in the presence of various concentrations (0.02 mg/ml and 0.04 mg/ml) of the most active product D6 are determined. Product D6 isolated from ethyl acetate extract of *Cynanchum ambositrense* cause a significant inhibitory effect for the contractile response elicited by histamine on guinea-pig ileum and by acetylcholine on rat duodenum in a noncompetitive and concentration-dependent manner, with IC50 values respectively of 0.027 ± 0.001 mg/ml (Figure 8) and 0.021 ± 0.005 mg/ml (Figure 9).

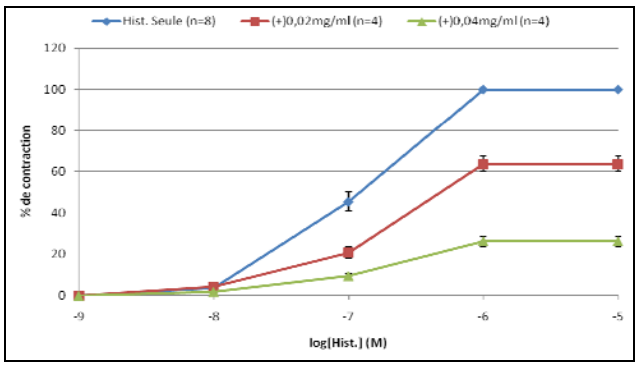

Fig 8: Inhibiting effect of product D6 from AcOEt extract to the contracting activity of histamine on guinea pig isolated ileum.

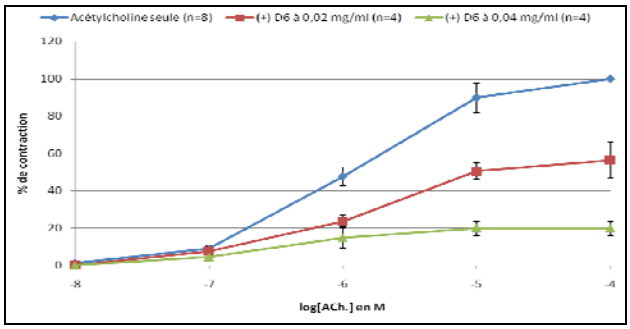

Fig 9: Inhibiting activity of product D6 isolated from ethyl acetate extract to the contracting activity of acetylcholine on rat isolated duodenum.

The results presented demonstrate that D6 significantly inhibited, in a non-competitive and concentration-dependant manner, the contractile response elicited by histamine on guinea pig ileum.

Structure identification of isolated products

Mass analyses were performed on a Bruker Daltonics esquire 6000 1B11E spectrometer. NMR analyses were performed on a Bruker DRX spectrometer operating at 600 MHz for proton ^{1}H and 151 MHz for ^{13}C atoms, and 2D NMR spectra. NMR data were compared to those reported in literature. Structures were assigned by analysis of MS, 1D NMR (^{1}H, ^{13}C) and 2D NMR spectra and by comparison with literature values.

Using MS, ^{1}H NMR spectroscopic analysis, compounds D1, D2 and D3 were identified respectively as lupeol [20], α-amyrine, β-amyrine [21].

D4 and D5 are mixtures containing the same compound D6. By concerted use of one and two dimensional NMR spectroscopy D6, the most active compound was identified as sesselin [22], a pyranocoumarin. All values of chemical shifts of protons and carbons of a pyranocoumarin have been assigned according to the analysis of the spectrum of 1D and 2D: homonuclear correlation COSY and heteronuclear correlations HSQC and HMBC. However, the assignments of two methyl groups in position 2', methines in positions 5 and 6, these protons were not similar to those previously reported [22]. Therefore, we revised the chemical shifts for H-2' (δ_H 1,47 ppm), H-5 (δ_H 7,34ppm, d, J=8,5Hz) and H-6 (δ_H δ 6, 75ppm, d, J=8,5Hz) of sesselin. Results are shown in table 1. Correlations ^{1}H- ^{13}C HMBC observed in sesselin are shown in Figure 10.

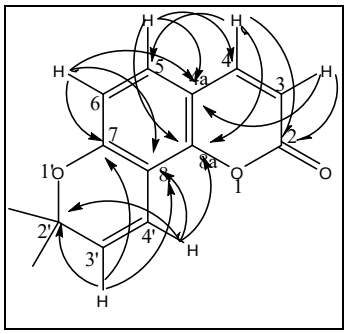

Fig 10: Correlations ^{1}H-^{13}C HMBC observed of a sesselin

Table 1: ^{1}H and ^{13}C-NMR spectral data of sesselin in MeOD

Position	δ_H (multiplicity, *J* in Hz)	δ_C	^{1}H -^{1}HCOSY	^{1}H-^{13}C HMBC
2	-	162.3		-
3	6.24 (*d*, 1H, 9.5Hz)	111.9	7.81	C-2,C-4a
4	7.81(*d*, 1H, 9.5Hz)	145.09	6.24	C-2, C-5, C-6, C-8, C-8a
4a	-	113.2		-
5	7.34(*d*, 1H, 8.5Hz)	128.3	6.75	C-4, C-4a, C-7, C-8,C-8a
6	6.75(*d*, 1H, 8.5Hz)	113.8	7.34	C-4a, C-7, C-8,C-8a
7	-	156.9		-
8	-	109.5		-
8a	-	150.6		-
2'	-	78		-
3'	5.82(*d*, 1H, 10.1Hz)	131.2	6.83	C-2', C-5', C-6', C-7, C-8,
4'	6.83(*d*, 1H, 10.1Hz)	114.5	5.82	C-2', C-5', C-6', C-7, C-8, C-8a
5' 6'	1.47(s,6H)	27.6		C-2', C-5', C-6',C-4',C-3'

Conclusion

This is the first work that describes the spasmolytic effect of *Cynanchum ambositrense.* The ethanolic extract of the aerial part of this species exhibited a significant concentration-dependant relaxation effect (EC_{50} = 0.452 ± 127 mg/ml) on guinea pig trachea pre-contracted with histamine. Our first objective was to identify the bioactive compound(s) responsible for this antispasmodic activity. To this end, bioassay-guided fractionation using isolated guinea pig ileum contracted with histamine and isolated rat duodenum contracted with acetylcholine to monitor the activity led to the isolation of sesselin (pyranocoumarin) as the main active constituent from the most promising ethyl acetate extract. Effectively, this product is effective on isolated guinea pig ileum contracted with histamine with a median inhibition concentration (IC_{50}) of 0.027 ± 0.001 mg/ml. This pyranocoumarin is also active on isolated rat duodenum contracted with acetylcholine. It induced a concentration-dependant relaxation of the acetylcholine with an IC_{50} equal to 0.023 ± 0.005 mg/ml.

We further studied the mechanism of action of sesselin. To this end, we found that the coumarin antagonized histamine (ileum) and acetylcholine (duodenum) in a non-competitive manner.

Phenolics have been previously described as having an effect on intestinal smooth muscle [23]. Some coumarins were described for their spasmolytic effect on gastrointestinal transit. For example, Hasakol apigenin, inhibited guinea-pig ileum induced contractions [24].

Acknowledgements

We also wish sincerely to thank M. René Razafindrazaka (IMRA) for his aid in the biological test of *Cynanchum ambositrense* in 2013.

References

1. Choux P. Catalogue des plantes de Madagascar, *Asclepiadaceae,* 1931, 6-24
2. Yi-Bin Z, You-Mao S, Hong-Ping H, Yan-Mei L, Quan-Zhang M, Xiao-Jiang H. Carbohydrates from *Cynanchum otophyllum*. Carbohydrate Research 2004; 339:1967-1972.
3. Tsukamoto S, Hayashi K, Mitsuhashi H. Studies on the constituents of Asclepiadaceae plants. Further studies on glycosides with a novel sugar chain containing a pair of optically isomeric sugars D- and L- cymarose from *Cynanchum wilfordi*. Chemical Pharmaceutical Bulletin 1985; 33:2294.
4. Liu Y, Qu J, Shi-Shan Y, You-Cai H, Xiang-Zhong H. Seven new steroidal glycosides from the roots of *Cynanchum forrestii.* Steroids, 2007; 72:313-322.
5. Halim AF, Zaghloul AM, Ebaid KA. Lupeol long-chain fatty acid esters and other lipid constituents from *Cynanchum acutum* L. Fam. Asclepiadaceae. Egyptian Journal of Pharmaceutical Science 1990; 31:99-105.
6. Horowitz RM, Gentili BJ. Organic Chemistry 1960; 25:2183. Konda Y, Iguchi M, Harigaya Y, Takayanagi H, Ogura H. Hancokinol, a novel tirterpene from *Cynanchum hancokianum.* Tetrahedron Letters, 1990; 31:5315-5318.
7. Zhang Z, Zhou J, Hayashi K, Mitsuhashi H. Studies on the constituents of Asclepiadaceae plants. The structures of five glycosides, cynatratoside-A, -B, -C, -D, -E, from Chinese drug (pai-wea) *Cynanchum atratum*. Chemical Pharmaceutical Bulletin. 1985; 33:1507.
8. Tian-Ying A, Run-qiu H, Yang Z, Dian-kun Z, Guang-ren L, Yu-cheng Y *et al*. Alkaloids from *Cynanchum komarovii* with inhibitory activity against the tobacco mosaic virus, Phytochemistry. 2001; 58:1267-1269.
9. Lou H, Li X, Zhu T, Li W. Sinapic acid esters and a phenolic glycoside from *Cynanchum hancockianum*. Phytochemistry. 1993; 32:1283-1286.
10. El-Demerdash A, Dawidar AM, Keshk EM, Abdel-Mogib M. Coumarins from *Cynanchum acutum Rev. Latinoamer. Quím.* 2009; 37(1):69.
11. Metz H.C. *Madagascar: a country study.* Washington: GPO for the Library of Congress. 1994.
12. Buchy P. The human intestinal parasites in Mahajanga, West coast of Madagascar. Bulletin de la Société de Pathologie Exotique. 2003; 96:41-45.
13. Ravaoarinoro M, Rafalimanana C, Coulanges P. Viral etiology of diarrheal diseases in Madagascan children. Archives de l'Institut Pasteur de Madagascar. 1986 ; 52:123-130.
14. Markgraff F. Flore de Madagascar et des Comores. 169e famille – Apocynacées. Tananarive : Imprimerie officielle. 1976.
15. Suralkar MM, Sarda PP. Antihistaminic and Antispasmodic Potential of Pongamia pinnata Int. J Pharm. Phytopharmacol. Res. 2014; 4(2):120-122
16. Vogel GH, Vogel WH, Schölkens AB, Sandow J. Günter M., Drug Discovery and Evaluation Pharmacol. Assays, 2002; 2(D):353-354.
17. Gharib Naseri MK, Asadi Moghaddam M, Bahadoram S, Antispasmodic effect of Tecoma stans (L.) Juss leaf extract on rat ileum, DARU. 2007; 15(3):123-128
18. Emendörfer F, Bellato F, Noldin VF, Niero R, Cechinelfilho V, Cardozo AM. Evaluation of the relaxant action of some Brazilian medicinal plants in isolated guinea-pig ileum and rat duodenum. Journal of Pharmacological Sciences 2005; 8:63-68.
19. Irie N'guessan AG, Kouakou Siransy NG, Le Blais V, Champy P, Kablan BJ. Effet antispasmodique induit par les fractions chromatographiques de trois plantes antiasthmatiques de Côte d' Ivoire, *J. sci. pharm. biol*. 2010; 11(1):13-20 19- John T. A and Onabanjo A. O. 2010, Effect of an Aqueous Extract of Entandrophragma utile Bark on Gastric Acid Secretion in Rat and Isolated Ileum Contractility in Guinea pig Afr. J. Biomed. Res. 13: 197 – 206
20. Manitriniaina Rajemiarimiraho, Jean Théophile Banzouzi, Stéphane Richard Rakotonandrasana, Pierre Chalard, Françoise Benoit-Vicale, Léa Herilala Rasoanaivo, Amélie Raharisololalao and Roger Randrianja, Pyranocoumarin and Triterpene from *Millettia richardiana Natural Product Communications.* 2013; 8(8):1099.
21. Léa Herilala Rasoanaivo**,** Anne Wadouachi, Tianarilalaina Tantely, Andriamampianina. Solofoniaina Gabriel Andriamalala, Ernest Jeannot Bako Razafindrakoto, Amélie Raharisololalao, Fanantenanirainy Randimbivololona, 2014. Triterpenes and steroids from the stem bark of *Gambeya boiviniana* Pierre, Journal of Pharmacognosy and Phytochemistry; ISSN 2278-4136; 2013; 3(1):68-72.
22. Tosun A, Ozkal N, Baba M, Okuyama T. Pyranocoumarins from *Seseli gummiferum* subsp. Corymbosum Growing in Turkey. Turk J. Chem. 2005; 29:327-334
23. Harborne JB, Williams CA. Advances in flavonoid

research since 1992. Phytochemistry 2000; 55:481-504.
24. Toshiya Masuda, Nobuyo Kawai, Yukari Muroya, Nobuji Nakatani, Tom J Mabry. Synthesis of Hasakol, an Antispasmodic Coumarin from the fruits of *Citrus hassaku* Natural Product Letters 1994; 4(2):129-132
25. DOI: 10.1080/10575639408044924