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Epi-betulinic acid from the stem bark of *Anthostema madagascariensis* Baill. (Euphorbiaceae) extracts and its laxative activity in mice

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Abstract
The latex of *Anthostema madagascariensis* is used as a purgative in Malagasy traditional medicine. The study was aimed to investigate the laxative activity of stem barks extracts of *A. madagascariensis*. The hydroalcoholic crude extract of the stem barks from *A. madagascariensis* and the three fractions resulting from the fractionation of the hydroalcoholic extract and the isolated compound from the active extract were tested. The laxative activity was investigated using in vivo models intestinal motility in mice. Among all tested dose, the hydroalcoholic extract more or equal to 1000 mg/kg and ethyl acetate extract at 1200mg/kg produced significant (p<0.05) increase in the intestinal motility in the treated mice when compared to the negative control. The fractionation of the ethyl acetate extract resulted in the isolation of triterpene namely *epi*-betulinic acid. Its structure was established by spectroscopic methods. This compound at dose 10 mg/kg produced significant (p<0.05) increase in the intestinal motility in the treated mice when compared to the negative control. It is for the first time that laxative activity of *epi*-betulinic acid was demonstrated.

Keywords: *Anthostema madagascariensis*, laxative activity, intestinal motility, hydroalcoholic crude extract, ethyl acetate extract, epibetulinic acid

1. Introduction
Anthostema is small genus of three species distributing in Africa. One these occur in eastern Madagascar, Comors and Mayotte: *Anthostema madagascariensis* [1]. This plant, known as vernacular name "mandravoky", is in ample availability in Madagascar and probably suitable for light carpentry, boxes, crates, rotary veneer, and as a component in blockboard and fibre board. The latex may cause eye problems and is used as glue. The smoke from the wood is reportedly used to drive away animals. In traditional medicine a piece of sugarcane dipped into the latex of *A. madagascariensis* is sucked as a very strong purgative [2]. Phytochemical research work has been carried out on this plant, and steroid and triterpene have been identified from the stem bark of this species [3]. In this paper we report the laxative activity of the extracts and the isolation and identification and laxative activity of triterpene from the ethyl acetate extract of the stem bark of *A. madagascariensis*.

2. Materials and Methods
2.1 General
Silica gel (Macherey Nagel, 70-270 mesh) was used for column chromatography and Silica gel 60F254 was used for analytical TLC which was viewed by UV illumination at 254 and 366 ηm and by spraying with vanillin spray reagent (0.1 g vanillin, 28 ml methanol, 1 ml sulphuric acid)[4]. 1D (^{1}H, ^{13}C) and 2D (^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC) NMR spectra were measured on a Bruker Varian 300 NMR and 600 NMR spectrometer. Chemical shifts were internally referenced to the solvent signals[5] in DMSO-d_6 (δ_H 2.49; δ_C 39.70), with TMS as the internal standard.

2.2 Plant material
The stem bark of *A. madagascariensis* was collected in July 2012 in Farafangana Manombo, Vatovavy Fitovinany's region, in South East of Madagascar and was identified by botanists at the Parc National Botanic and Zoologique Tsimbazaza, Antananarivo, Madagascar where a voucher specimen has been deposited in the Herbarium.

2.3 Extraction and isolation

Dried stem barks of *A. madagascariensis* were reduced to a fine powder with a mechanical grinder. The powdered plant material (460g) was extracted by maceration with a mixture of Ethanol-water (90:10) (2000 ml) at room temperature for 7 days. After concentration under reduced pressure, the hydroalcoolic extract (22.24g) was suspended in hot water and then partitioned sequentially using hexane, ethyl acetate and n-butanol furnishing respectively hexanic, ethyl acetate, butanolic and aqueous extract. These extracts were used for assessment of the laxative activity and the active extract was selected to subsequent separation.

The ethyl acetate extract (600mg) was chromatographed over a silica gel column (27g), eluting successively with a gradient solvent system of hexane-ethyl acetate (100:0 → 0:100) and a gradient solvent system of ethyl acetate-methanol (100:0 → 0:100); 280 aliquots of 10ml each were collected and analysed with thin layer chromatography (TLC). Five microliters of aliquots were deposited on TLC plates and eluted with mobile phase hexane-ethyl acetate (50:50). The plates were dried and separated compounds were detected under UV lamp and by spraying with freshly prepared vanillin spray reagent and then heated at 110 °C for 1 min. The spots exhibiting the same R*f* on TLC were combined [6]. The combined fraction F_9 (118 to 131) obtained with hexane-ethyl acetate (40:60) was washed successively with hexane and dichloromethane to give a pure product. Its structure was elucidated by NMR spectral data (1D- and 2D-NMR). This isolated compound was used for assessment of the laxative activity.

2.4 Pharmacological experiments

2. 4.1 Experimental animals

Female Swiss mice (20-25 g) were used for acute toxicity study and Swiss mice (15-17 g) of either sex were used for evaluation of gastro-intestinal motility study. They were provided by IMVAVET (Institut Malgache des Vaccins Vétérinaires) and were acclimatized to laboratory conditions for 3 days before the experiments were conducted.

2.4.2 Acute oral toxicity test

Acute toxicity studies of ethanolic extract of *A. madagascariensis* was carried out using the method discredited by Onifade *et al.*, 2011 [7]. Twelve mice were deprived of food for 12 h and were randomly divided into four groups of three mice. Group 1 received 10 ml/kg of distilled water while groups 2, 3 and 4 received 500, 1000 and 1500 mg/kg of hydroalcoholic extract each. All the mice were observed for general behavioral changes; symptoms of toxicity and mortality after treatment for the first four hours, then over a period of 24 and 48 h.

2.4.3 Effect of *A. madagascariensis* on intestinal transit time

The method described by Vogel G H *et al.*, 2017 [8] was used in this experiment. Fifteen mice were randomly divided into groups of three animals each and were fasted for 18 hours before the experiment. Products were administered orally by gavage (0.2 ml/animal). Group 1 (negative control) received 10% of DMSO in distilled water, group 2 (reference) received lactulose (2 mg/kg), and the other groups received various dosages of extracts or compound. One hour post treatment, standard charcoal meal was administered to all the animals (0.2 ml/animal). The animals were sacrificed 60 minutes post administration of charcoal meal by cervical dislocation and the intestines immediately isolated and ligated at the pyloric sphincter and ileocaecal junction. The small intestinal transit was expressed as percentage of distance travelled by the charcoal meal relative to the total length of the small intestine from the pyloric sphincter to the ileocaecal junction.

2.4.4 Statistical analysis

The results are expressed as mean ± standard error mean. The significance of the differences of the means was evaluated by the test "T" of Student by using the software "Excel 2010". The values of *P* lower than 0.05 were regarded as statistically significant.

3. Results

3.1 Results of extraction and structure of isolated compound

Extraction by hydroalcoholic maceration at room temperature for 7 days of powder from stem bark of *A. madagascariensis* gave 22.24 g of hydroalcoholic extract. Partitioning of EtOH–H_2O extract (20 g) yielded 0.13 g (0.65 %) of hexanic extract, 4.35 g (21.75 %) of ethyl acetate extract and 2.27 g (11.35 %) of butanolic extract. Fractionation of ethyl acetate extract (860mg) gave 280 aliquots grouped in 23 fractions (F_1 to F_{23}). After washing successively with hexane and dichloromethane, the fraction F_9 gave 20 mg of a pure compound.

By concerted use of one and two-dimensional NMR spectroscopy, proton and carbon signals were assigned totally and this compound was identified as an *epi*-betulinic acid [9, 10] (Figure 1). However, the assignment of carbons 1, 2, 12, 15, 16, 18, 19 and 22 was not similar to those previously reported of *epi*-betulinic acid [9].

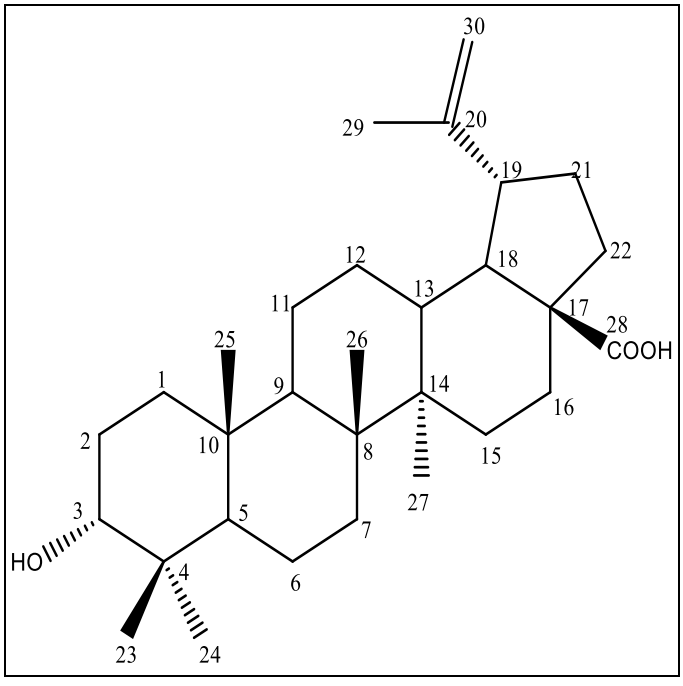

Fig 1: Structure of *epi*-betulinic acid

Therefore, we revised the chemical shifts for C-1 (δ_C 38.33), C-2 (δ_C 27.32), C-12 (δ_C 25.00), C-15 (δ_C 29.26), C-16 (δ_C 31.53), C-18 (δ_C 48.47), C-19 (δ_C 46.53) and C-22 (δ_C 36.53) of *epi*-betulinic acid (Table 1).

Table 1: NMR data of isolated compound (^{1}H 500 MHz, ^{13}C 125 MHz, DMSO-d_6)

Position	δ_C	δ_H	COSY	HMBC Correlation H => C
1	38.33	0.84/1.56	2	
2	27.32	1.44	1, 3	
3	76.58	2.96	2	
4	38.42			
5	54.93	0.63	6	
6	18.00	1.33/1.45	5	
7	33.74	1.32		
8	40.19			
9	49.84	1.25	11	
10	36.68			
11	20.44	1.15/1.36	9	
12	25.00	0.98/1.62	13	
13	37.48	2.22	12, 18	
14	42.00			
15	29.26	1.10/1.39	16	
16	31.53	1.37/2.12	15	
17	55.42			
18	48.47	1.52	13, 19	
19	46.53	2.94	18, 21	
20	150.21			
21	30.32	1.31/1.81	19, 22	
22	36.53	1.43/1.80	21	
23	15.79	0.86		4, 3, 5, 24
24	28.11	0.88		4, 3, 5, 23
25	15.93	0.77		10, 1, 5, 9
26	15.73	0.88		8, 7, 9, 14
27	14.44	0.94		14, 8, 13, 15
28	177.09			
29	18.95	1.65		20, 19, 30
30	109.39	4.57/4.70		

3.2 Acute toxicity

No mortality was observed after the administration of hydroalcoholic extract of *A. madagascariensis* at the tested doses. For all treated animals the pruritus started after one hour to 24h of administration. It was also observed that the treated animals with hydroalcoholic extract at dose 500 mg/kg produced ptosis, poor appetite and decrease in motor activity in first hour to 12 hours of the administration while the animals that received the dose of 1000 and 1500 mg/kg these signs began 15 minutes to 24 hours of administration. At doses 1000 and 1500 mg/kg, the hydroalcoholic extract induced one dyspnea after 10h of administration and persist to 6h. After 48h all the animals were normal.

3.3 Effect of hydroalcoholic extract of *A. madagascariensis* on intestinal transit time

The hydroalcoholic crude extract increase the percentage of distance travelled by the charcoal meal and these effects depend on the dose (Figure 2). Only the dosage at 1000 and 1500 mg/kg showed a significant effect (47.64 ± 4% and 48.45 ± 2% respectively) in comparison to the negative control (20.7 ± 5 %; p-Value < 0.05). In addition, lactulose (2 mg/kg), a known laxative compound, induced also a significant increase (50.89 ± 6%) in comparison to control (p-Value < 0.05).

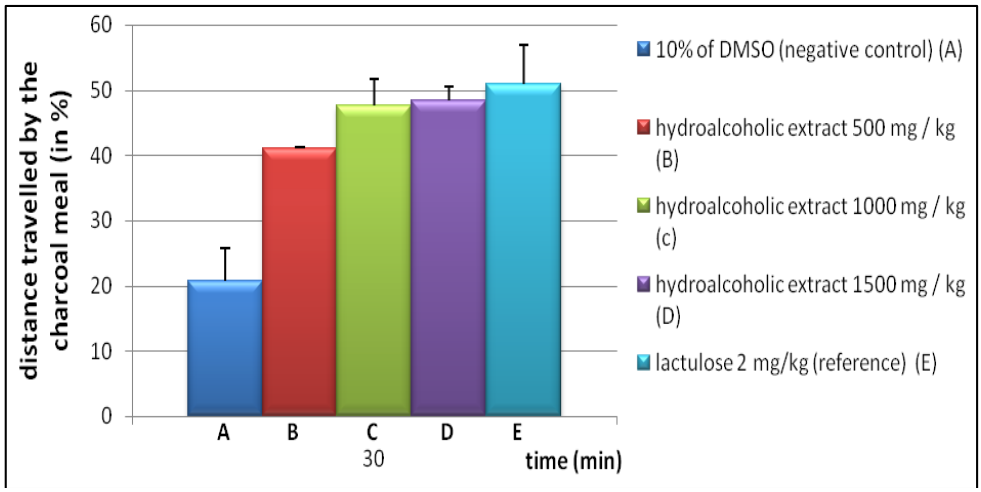

Fig 2: Effect of the hydroalcoholic extract on gastrointestinal motility

3. 4 Effect of the fractions from the hydroalcoholic extract of *A. madagascariensis* on intestinal transit time

Every mice treated with one dose of different extracts including hexanic, ethyl acetate and butanolic extracts, exhibited an increase in the percentage of the small intestine travelled by the charcoal meal (Figure 3). The mice treated with 1200 mg/kg (p.o) of ethyl acetate extract were the most affected with a rate of 68.43 ± 4% which are significant (p-Value < 0.05) in comparison to the control groups (20.7 ± 5 %). In the same conditions, lactulose (2 mg/kg) induced also an increasing (50.89 ± 6%) response which validates these results.

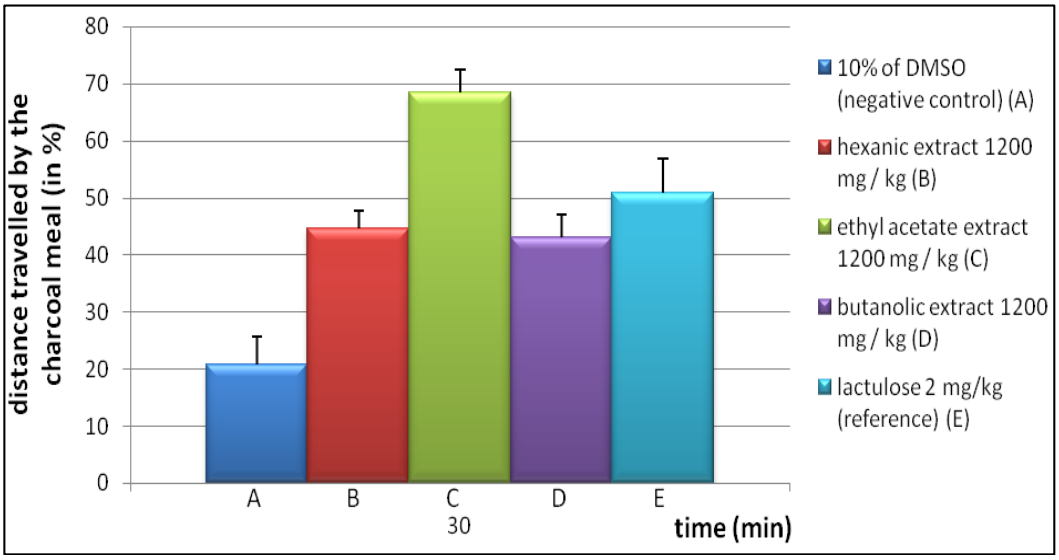

Fig 3: Effect of the fractions of *A. madagascariensis* on gastrointestinal motility

3-5 Effect of *epi*-betulinic acid on intestinal transit time

The *epi*-betulinic acid (10 mg/kg) and lactulose (2 mg/kg) caused the charcoal meal to travel 54.68 ± 3 % and 50.89 ± 6% of the total intestinal length respectively in the treated mice. These effect are significant (p-Value < 0.05) when compared to the negative control (20.7 ± 5 %) (Figure 4).

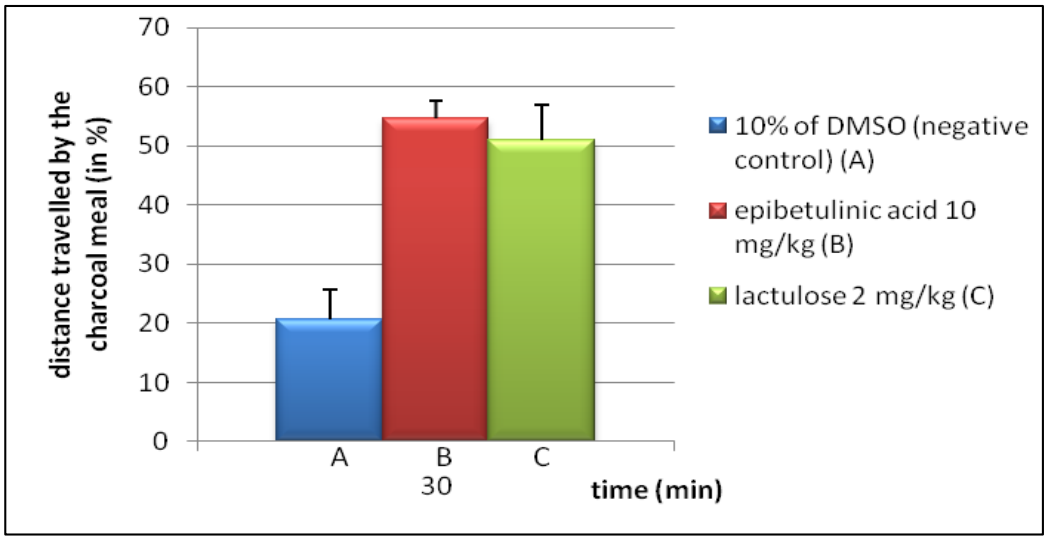

Fig 4: Effect of the epibetulinic acid on gastrointestinal motility

Discussion

The results of this study revealed that the hydroalcoholic extract of stem bark of *A. madagascariensis* showed a significant dose dependant laxative effect at both the tested level of doses in mice (Figure 1). These hydroalcoholic extract exhibited a low toxicity, according the Hodge and Sterner toxicity scale [11].

Fractionation of the ethyl acetate extract having a significant laxative effect (Figure 2) results in the isolation of *epi*-betulinic acid. This product has a significant laxative effect compared to the effects produced by the reference product (Figure 3). This result confirms the laxative effect of plants containing triterpenes [12].

Conclusion

This is the first work that studies the pharmacological activity of *A. madagascariensis*. The results of the present study showed that *A. madagascariensis* has laxative effect and this justifies the traditional use of this plant in the treatment of constipation.

The results of this study also revealed for the first time that the *epi*-betulinic acid has a laxative effect.

References

1. Schmelzer GH. *Anthostema senegalense* A. Juss. I n: Schmelzer, G.H. & Gurib -Fakim, A. (Editeurs). Ressources végétales de l'Afrique tropicale 11(1). Plantes médicinales 1. Fondation PROTA, Wageningen, Pays-

Bas / Backhuys Publishers, Leiden, Pays-Bas / CTA, Wageningen, Pays-Bas, 2008, 112-113.
2. Oyen LPA, Louppe D. *Anthostema madagascariense* Baill. In: Lemmens, R.H.M.J., Louppe, D. & Oteng-Amoako, A.A. (Editeurs). Ressources végétales de l'Afrique tropicale 7(2). Bois d'œuvre 2. Fondation PROTA, Wageningen, Pays-Bas/CTA, Wageningen, Pays-Bas, 2012, 84-85.
3. Rambeloson VHV, Rasoanaivo LH, Wadouachi A, Raharisololalao A. A new triterpene and stigmasterol from *Anthostema madagascariense* (Euphorbiaceae). International Journal of Chemical Studies, 2014; 1(5):42-48.
4. Eloff JN, Famakin JO, Katerere DRP. Isolation of an antibacterial stilbene from *Combretum woodii* (Combretaceae) leaves. Afr. J Biotechnology, 2005; 4(10):1167-1171.
5. Silverstein RM, Basler GC, Morill TC. Identification spectrométrique des composés organiques. 5e édition, Emmanuël Laure, 1991, 261.
6. Kumar S, Jyotirmayee K, Sarangi M. Thin layer chromatography: a tool of biotechnology for isolation of bioactive compounds from medicinal plants. Int. J Pharm Sci Rev Res. 2013; 18(1):126-132.
7. Onifade AO, Ouedraogo M, Ouedraogo M, Zongo FE, Kafando E, Lompo M *et al.* Acute toxicity and anti-inflammatory activity of aqueous ethanol extract of root bark of *Ximenia americana* L. (Olacaceae). African Journal of Pharmacy and Pharmacology, 2011; 5(7):806-811.
8. Vogel HG, Vogel WH, Schölkens BA, Sandow J, Műller G, Vogel WF. Drug Discovery and Evaluation, Pharmacological Assays. 3rd. Berlin, Springer-Verlag, 2007, 1252.
9. Pieroni LG, Rezende FM, Ximenes VF, Dokkedal AL. Antioxidant Activity and Total Phenols from the Methanolic Extract of *Miconia albicans* (Sw.) Triana Leaves. Molecules, 2011; 16:9439-9450.
10. Wang X, Zhang S, Li J, Liu H, Xie X, Nan F. Highly lipophilic 3-epi-betulinic acid derivatives as potent and selective TGR5 agonists with improved cellular efficacy. Acta Pharmacologica Sinica, 2014; 35:1463-1472.
11. Hodge HC, Sterner JH. Determination of substances acute toxicity by DL50. American Industrial Hygien Association, 1943, 10:93-96.
12. Mamyrbekova-Bekro JA, Boua BB, Diaby A, Bekro Y. Screening phytochimique bio guidé et évaluation in vitro des propriétés purgatives d'Anchomanes difformis (Blume) Engl., une plante utilisée en Côte d' Ivoire dans le traitement folklorique de la constipation. Revue « Nature & Technologie ». B- Sciences Agronomiques et Biologiques. 2013; 09:20-26.