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Antitussive properties of aqueous extracts of *Sterculia setigera* Delile (Sterculiaceae) and mixture of *Aframomum melegueta* K. Schum (Zingiberaceae) - *Citrus aurantifolia* (Christm. and panzer) Swingle (Rutaceae)

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Abstract
Sterculia setigera, *Aframomum melegueta* and *Citrus aurantfolia* are plants commonly used in African traditional medicine and especially in Benin to treat cough and asthma. The Phytochemical analysis of aqueous extracts of these plants, based on methods of precipitation and differential staining, revealed the presence of alkaloids, tannins, flavonoids, anthocyanins, leuco-anthocyanins, mucilages and reducing compounds. The presence of essential oils is found only in the mixture. The orally administration of aqueous extracts before induction of cough by inhalation of vapor 10 mL of 20% citric acid in *Cavia porcellus* (Guinea Pigs) inhibits the cough significantly with dose compared to the control and reference : Neo-codion ®. The extract of mixture *Aframomum melegueta* - *Citrus aurantifolia* (AMCA) was more active than *Sterculia setigera*'s (SS). These results confirm that aqueous extracts of *Sterculia setigera* and mixture *Aframomum melegueta* - *Citrus aurantifolia*, used in traditional medicine in Benin, have antitussive properties.

Keywords: *Sterculia setigera*; *Aframomum melegueta*; *Citrus aurantifolia*; extracts; antitussive properties

1. Introduction
The data for recent decades in United States of America have shown, in general, that the consultation for cough and upper respiratory tract infections prevail in a remarkable way on any other consultation [1]. Since the 1970s, observed a return to therapies considered soft and natural. Seeking a greater quality of life and a return to basics has led to the emergence of herbal medicine as opposed to chemotherapy [2]. The World Health Organization [3] confirms this by a newspaper article in which it is stated that in northern countries, patients are increasingly using alternative treatment is preventive or curative [4]. In countries such as China, North Korea and South Viet Nam, traditional medicine is integrated into the national health policy. Unfortunately, many African countries are slow to standardize and enhance these natural resources, although more than 80% of their populations have used this practice [5]. This lack of recovery of such traditional knowledge seriously undermines the protection of rare species in our countries. Thus, the scientific exploration of local flora is necessary to demonstrate their efficacy, safety, and especially the standardization of their use.
So, would we emphasize that in Benin and accurately in large cities such as Cotonou, increasing air pollution caused an increase in certain diseases including respiratory diseases [6], particularly cough and asthma. To heal, people do use modern medicines becoming increasingly expensive, but the alternative of treatment plants available to them. Through a multidisciplinary approach drawing on social anthropology and ethnobotany in interviews with farmers, traditional healers and midwives, we were able to identify many herbal preparations widely used in the treatment of cough and asthma. Two of these preparations are the subject of our attention. These are the aqueous extracts of *Sterculia setigera* Delile (Sterculiaceae) and the mixture *Aframomum melegueta* K. Schum (Zingiberaceae) + *Citrus aurantifolia* Swingle (Rutaceae). Our focus is on these extracts because of their frequency of use by people to validate the use of traditional medicine. Using an animal model for *Cavia porcellus* (Caviideae) commonly known as Guinea Pig, this study was to evaluate the antitussive properties of these traditional preparations.

2. Material and Method
2.1 Material
2.1.1 Plant material
The study material consists of dried and ground into powder of *Aframomum melegueta* seeds purchased in Adjara (departement of Oueme, south Benin) in June 2010, leaves and fruits of *Citrus aurantifolia* collected in Cotonou (Littoral departement, south Benin) in March 2011 and leaves of *Sterculia setigera* Delile Okpara (North Benin, departement of Borgou) harvested in July-August 2011. All the plants are identified by the National Herbarium of Abomey-Calavi University where voucher specimens were deposited respectively at the number AA6374/HNB, AA6375/HNB and AA6376/HNB.

2.1.2. Animal material
It consists of 102 male and female guinea pigs (*Cavia porcellus*, Caviideae), 72 guinea pigs for the study of antitussive properties and 30 for the study of toxicity and of motor, weighing between 250 and 300 g. These animals were acclimated to the conditions of that animal breeding (12 h of luminosity and 12 h of darkness, average temperature 29 °C). They have free access to water and food. The food that of the breeder, consists mainly of corn bran.

2.2 Methods
2.2.1.Preparing and obtaining hot aqueous extracts
The first aqueous extract was obtained with the decoction of 125 g of leaves and 290 g of whole fruits of *Citrus aurantifolia* for 30 min in 1 L of distillated water. The powder of *Aframomum melegueta* seeds was added to the decoction at a dose of 125 g for 1 L. After 05 hours of agitation, the mixture was filtered, centrifugated and lyophilized. The second aqueous extract was obtained by the decoction of 125 g of leaves powder of *Sterculia setigera* in 1 L of distillated water boiled for 30 min and then, filtered centrifugated and lyophilized. Both aqueous extracts were stored in vials in a refrigerator at 4° C.

2.2.2. Phytochemical Screening
The experimental method adopted for the phytochemical screening is the same for both plant extracts. This is the method of Houghton and Raman (1998) [7] based on differential reactions of precipitation and coloring supplemented and improved.

2.2.3. Constitution of experimental groups
To perform the tests, twelve (12) batches of six (06) animals each were formed to study the antitussive properties. The animals were randomly selected for the constitution of batches. Thus we have: a batch of animals are treated with distilled water and represent the control group. The animals of group 2 were treated with 10 mg/kg *po* Neo-Codion ® (codeine camphosulphonate, sulfogaiacol, alcoholic extract of Grindelia) as reference. Animals of groups 3 and 3 ', 4 and 4', 5 and 5 ', 6 and 6', 7 and 7 'are treated respectively by 1000, 1500, 2000, 2500 and 3000 mg/kg orally extracts respectively *Sterculia setigera* and the mixture *Aframomum melegueta + Citrus aurantifolia*.

2.2.4. Antitussive activity
The experimental phase is relative to the *in vivo* study of the antitussive effect of aqueous extracts. The induction of the cough is conducted using the method of Vogel and Vogel (1997) [8] modified. The animals are fasted for about 24 hours before manipulation day, but they had water *ad libitum*. The day of the experiment, each animal is placed in a cylindrical glass jar (20 cm in diameter and 32 cm in height). The cough is caused by inhalation of 10 mL of citric acid solution to 20% vaporized in the closed jar at a pressure of 550 mm Hg using an air pump brand Büchi Vac V-500 ® . After induction of cough, the chest is observed by counting the number of contractions of the intercostals muscles during 10 min.

2.2.5. Traction and acute toxicity
Traction and acute toxicity of the extracts were tested at doses 1000, 2000 and 3000 mg/kg body weight. Three groups of 5 guinea pigs each were formed. The method of Martin *et al* (1990) [9] slightly modified was used to verify the motor. Thirty minutes after oral administration of substances, an animal is placed in a rectangular cage of 60 cm long by 40 cm wide. The floor of the cage is divided into 24 equal squares. Activity is assessed by the number of squares crossed by each animal in 10 min. For acute toxicity, each animal of each group receives a single dose in the corresponding dose. We note the number of deaths within 24 hours and animal behavior at least for 14 days.

2.3 Statistical Analysis
The results processed by the SPSS 12.0 for Windows are averages plus or minus the standard error of the mean (M ± SEM). Student's test compared to the control group was used to evaluate the difference significant at probability $p < 0.05$.

3. Results and Discussion
3.1 Results
3.1.1. Phytochemical Screening
The search for chemical groups in the extracts of *Sterculia setigera* and mixture *Aframomum melegueta + Citrus aurantifolia* gave the results shown in Table 1 below. These results show that both extracts contain the same chemical groups with the exception of catechin tannins and triterpenoids present in the extract of *Sterculia setigera* Delile (Sterculiaceae) and not in the mixture and the opposite in regard to essential oils.

3.1.2. Results of the antitussive activity
Analysis of Table 2, we note, at a dose of 10 mg/kg body weight of New Codion ®, a very highly significant inhibition of 63% of intercostal contractions compared to control. To extract *Sterculia setigera* (SS), it is from a dose of 1500 mg/kg of body weight that we obtain a significant inhibition of contractions of 3.2% ($p = 0.0237 < 0,05$). It is from 1000 mg/kg of the mixture (AMCA) a significant inhibition of intercostal contraction of 4.62% was obtained. At equal dose of 1500 mg/kg body weight, the extract of the mixture revealed four times more effective than SS. For 3000 mg/kg, mixture AMCA appears to be more effective than the Neo-codion ® at 10 mg/kg with inhibitory rate of 73.70% against 63.20%. By cons SS appears to be effective with 50.70%. The antitussive activity of both extracts is dose dependent and AMCA is more active than SS.

3.1.3. Results of tests of motor and acute toxicity
No torpor was observed in guinea pigs in batches of doses 1000 and 2000 mg/kg body weight, animal traction is maintained. For batch of dose 3000 mg/kg body weight, there was a torpor after 15 min with loss of traction. 30 min after the animals regained their appetite and motor skills. No deaths occurred during at least 14 days following the experiment.

4. Discussion

The method used is that of Vogel and Vogel (1997) [8] cited by Hosseinzadeh and Ghenaati (2006) [10]. The latter studied the antitussive properties of ethanolic and aqueous extracts of flowers and stigmas of *Crocus sativus* and its active principles safranal and crocin in sealed glass (20 x 20 x 40 cm^3) with 5 mg/kg intraperitoneal codeine. In the chamber, 10 mL of citric acid 20% are sprayed under constant pressure of 500 mm Hg. As part of this work, we used a cylindrical glass chamber (D = 20 cm, H = 32 cm) with an orifice entry of air, an air pump and a spray that vaporizes directly citric acid in the jar. Instead of 5 mg/kg intraperitoneal codeine, we used 10 mg/kg *per os* of Neo-Codion ®, by gavage. The recording of respiratory contractions was performed for 10 min and then the visual average was calculated.

Hosseinzadeh and Ghenaati (2006) [10] studied the antitussive properties of ethanolic and aqueous extracts of stigma and petals of flowers of *Crocus sativus* and its components safranal and crocin. He assessed these properties by using nebulized citric acid to 20% on guinea pigs. Extracts and active ingredients were injected intraperitoneally with codeine in reference (5mg/kg) in this study, the specialty was Neo-Codion and used orally. Hosseinzadeh and Ghenaati (2006) [10] obtained the ethanolic extracts (100-800 mg/kg) and safranal (0.25 to 0.75 mL/kg) significantly reduced the number of intercostal contraction and the aqueous extracts and crocin lacked antitussive properties. Here, it has been studied only aqueous extracts because they are used by people and in *per os* and in addition, we are part of the validation of the practice population. At doses of extracts in this work (1000 to 3000 mg/kg), they appear high compared with doses of ethanol extract of *Crocus sativus*. This can be explained by the fact that *per os*, extracts suffer the action of digestion and first-pass effect, which decreases the bioavailability of active components.

Kiyoshi *et al*. (2006) [11] here studied three cough preparations of the Sino-Japanese medicine. They used a new model of guinea pig developed by Tanaka and Maruyama (2003) [12]. This animal model has the advantage of direct stimulation of cough receptors in the larynx by microinjection, bypassing the disappointments of a direct inhalation of citric acid by spraying. Indeed nebulization stimulates all the receptors of the bronchial tree, including those of coughing and sneezing (which is hardly distinguishable from the cough). Moreover, the method of nebulization is not repeatable because of tachyphylaxis [13] and the number of intercostal movement also depends on the ventilation and air pressure. Here the water extracts were made orally. Guinea pigs in this work, were acclimated to laboratory temperature for 10 days. They were fed only water and corn bran. Sampling for the constitution of lots was reasonable because of their differences in weight from 253 g to 296 g for a formation of homogeneous batches. They were of both sexes. Hosseinzadeh and Ghenaati (2006) [10] did the same in the sample and the constitution of batches. But Kiyoshi *et al*. (2006) [11] used only for males, according to him, avoid gender bias. After inhalation of citric acid vapor, dry cough was installed as the intercostal muscle contractions became more intense than before inhalation. These muscle contractions are probably due to irritation of RARs and inflammation of the airway cells. This irritation is due to release of histamin and the formation of other mediators [14 15]. The histamin causes the allergy, bronchoconstriction, vasodilation and increased capillary permeability [16].

Analysis of Table 2, we note that oral administration of the Neo-Codion ® and extracts caused significantly lower frequency of contractions of the intercostal muscles induced by citric acid compared to the control group who received only distilled water. This could lead us to say that these extracts contain chemicals that would inhibit the mediators that affect the process of triggering the cough at the center of the cough. The inhibition rate of frequency of contractions with extracts of the mixture is higher than AMCA extracts of SS. This shows that the extract of the mixture was significantly more effective against coughs than SS. From the perspective of phytochemistry, the difference in antitussive activity of two extracts could be explained by the presence of essential oils in the mixture AMCA. The antitussive components would then in larger amounts in the mixture. Since codeine (morphine derivative, an alkaloid) allows concomitant analgesic and antitussive and is partially metabolized into morphine and dihydrocodeine [17], one could speculate that the receptors of derivatives of morphine could be involved in the inhibitory activity of these extracts containing alkaloids found in chemistry during the screening [18]. Phytochemical analysis of these two extracts and properties accorded to different chemical groups found, we believe that the antitussive properties, local anesthetics, vasoconstrictors, sympathomimetics, anti-allergic, anti-inflammatory, emollient, soothing and calming of alkaloids, flavonoids, tannins, saponins, mucilages and essential oils [19] can be utilized in reducing the frequency of contractions of the intercostal muscles of guinea pigs.

Table 1: Results of tests to identify chemical families in extracts of *Sterculia setigera* and the mixture *Aframomum melegueta* + *Citrus aurantifolia*

Families sought	Extracts	
	Sterculia setigera	***Aframomum melegueta - Citrus aurantifolia***
Alkaloids	+	+
Catechin tannins	+	-
Gallic tannins	+	+
Flavonoids	+	+
Anthocyanins	+	+
Leucoanthocyanes	+	+
Quinone derivatives	+	+
Saponin	-	-
Triterpenoids	+	-
Steroids	-	-
Mucilages	+	+
Anthracene compounds free	-	-
Reducing compounds	+	+
Coumarins	-	-
Cardenolides	-	-
Essential oils	±	++

Presence of compounds : (+, ++), absence of compounds : (-), trace of compound : (±)

Table 2: Frequencies and rates of inhibition of contractions caused by intercostal aqueous extracts of *Sterculia setigera* and the mixture *Aframomum melegueta* + *Citrus aurantifolia*

Administrative dose (mg/kg, *p.o*)	Substance used	Frequency of contractions per minute	Rate of inhibition
4 mL	Sample Distilled water	50,92± 0,32	-
10	Reference (Néo-Codion®)	18,71± 0,37***	63,2%
1000	SS	50,41 ± 0,31	1%
	AMCA	48,55 ± 0,64*	4,65%
1500	SS	49,29 ± 0,17*	3,2%
	AMCA	44,30 ± 0,36*	13%
2000	SS	40,23 ±0,32**	21,%
	AMCA	35,44 ± 0,31**	30,40%
2500	SS	32,59 ±0,42**	36,0%
	AMCA	30,86 ± 0,35**	39,41%
3000	SS	25,10 ± 0,16***	50,70%
	AMCA	13,39 ± 0,17***	73,7%

SS: *Sterculia setigera*; AMCA: mixture *Aframomum melegueta* K. Schum + *Citrus aurantifolia* Mean ± SEM (n = 6), ANOVA and t-test of Student with respect to the witness: * Significant difference ($0.05 < p < 0.001$),
** Highly significant difference ($0.0001 < p < 0.001$), *** Very highly significant difference ($p < 10^{-5}$)

5. Conclusion

The results we have achieved in our work had shown that citric acid causes irritation and contrition of bronchial cells characteristic of the cough. Extracts of *Sterculia setigera* Delile (Sterculiaceae) and the mixture *Aframomum melegueta* K. Schum. (Zingiberaceae) and *Citrus aurantifolia* Swingle (Rutaceae) decrease the frequency of cough induced by inhalation of citric acid. Pharmacologically, these results demonstrate at least partially, the use of these extracts against cough in traditional medicine in Benin would be justified. In terms of toxicology, no animals died from the inhalation of citric acid, which shows the non-toxicity of such use. Similarly, no animals died following administration of the extracts. One could say that these two extracts have no acute toxic effects, but we can not conclude. This study could be complemented by in vitro studies on the trachea and the diaphragm to determine the mechanism of action of this plant. This study could also be prosecuted on other extracts and other plants.

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