Antitussive Property of the Methanolic Extract of *Plecthrantusamboinicus* (Lour.) Spreng for Citric Acid-induced Cough in Sprague-Dawley Rats

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Abstract: Oregano (*Plecthrantusamboinicus* (Lour.) Spreng) is used in the Philippines as a folk medicine for a cough by preparing decoctions from its leaves. Despite being widely used, the extent of its ability to inhibit a cough has not yet been established. This study aims to compare the ability of *P. amboinicus* as an antitussive to that of codeine. Fresh leaves from *P. amboinicus* were macerated for 48 hours in methanol and was concentrated using a rotary evaporator at 40°C.The methanolic extract from *P. amboinicus* produced a yield of 4.26%. Three doses were prepared from the *P. amboinicus* methanolic extract (2,500 mg/kg, 1250 mg/kg, and 625 mg/kg). The antitussive assay was conducted with Codeine as a positive control while distilled water served as a negative control. Twenty (20) Sprague-Dawley rats were divided into five (5) treatment groups (n=4) and were induced with a cough inside an air tight glass chamber (3L) using an aerosol of 17.5% citric acid. The cough responses were recorded using a microphone before and after the treatments. After a 2-week wash period, a second trial was performed for validation. Test for three-way interaction between the two trials and the treatment groups show no significant difference ($p<0.05$). The mean percent reduction in tussive responses for each treatment group was subjected to one-way ANOVA shows a significant difference between the treatment groups ($p<0.001$). Further post hoc analysis using Tukey's HSD test showed that the extract with a concentration of 2500 mg/Kg BW produced the highest mean percent reduction in tussive response, followed by Codeine, the extract with a concentration of 1250 mg/Kg BW, then the extract with a concentration of 650 mg/Kg BW and distilled water having no significant difference ($p>0.05$).

Keywords: antitussive; citric acid; codeine; oregano; plectrantus

1. INTRODUCTION

1.1 Background

A cough is a very common symptom arising from the irritation of the respiratory tract. A cough works as a defensive reflex in clearing the airways. Often, the cough becomes frequent, disturbing, and interferes with day-to-day activities. An unproductive cough or a dry cough is associated with eosinophilic bronchitis, irritation of airways due to several environmental pollutants, airway hyper responsiveness due to infection, gastroesophageal reflux disease and possible without any associated cause, often referred to as idiopathic cough (Gupta et al., 2009).

According to Spina, McFadzean, Bertram, and Page (2009), most cough treatments are designed to target the underlying disease which can cause cough as a symptom. However, there is a range of drugs available which directly target neuronal pathways.

Patel, et al. (2003) state that a cough is one of the common complaints of patients. Antitussives direct its action on the center of the brain, which houses the region that influences the cough reflex. Cough suppressants are prescribed when a person suffers from dry, harsh coughing that normally goes with colds and flu. Lin, Chang, and Wu. (2016) Suggests that based on the physiological mechanism of the medulla oblongata controlling the coughing reflex, centrally acting antitussive drugs, such as codeine and dextromethorphan, were developed, but their clinic utility has been limited by their undesirable and intolerable side effects such as sedation, nausea, addictive potential and constipation. Lin et al. (2016) therefore conclude that the discovery of novel, safe and effective antitussive agents for treating cough could be facilitated by investigating medicinal plants or herb.

Plecthrantusamboinicus (Lour.) Spreng belongs to the Lamiaceaemv family. It is a large succulent, aromatic perennial herb, shrubby below, hispidly villous or tomentose with a life span of three to ten years, and is distributed in Tropical Africa, Asia, and Australia. It is also used as food, additive and fodder, and especially as medicine in treating a wide range of diseases including cough (Lukhoba et. al, 2006).

Lopes et al. (2017) compiled from multiple sources that *P. amboinicus* has been widely used as treatments for asthma, superficial mycosis, cancer, constipation, headache, cough, colds, fever, and digestive diseases.In the Philippines, *P. amboinicus* has been used as a folkloric treatment for a cough. However, the specific active constituent nor the mechanism of action has not yet been established. The present study aims to determine the ability to use *P. amboinicus* extract to significantly inhibit a cough in Sprague-Dawley Rats induced with citric acid. Results will then be compared to the codeine as a positive control.

2. METHODOLOGY

2.1 Plant Authentication and Collection

Plant seedlings were purchased from the Bureau of Plant Industry and were authenticated at the Thomas Aquinas Research Complex (TARC) of the University of Santo Tomas. Seedlings were then cultivated at Silang, Cavite for three months. Five (5) kilograms of the leaves were collected for extraction.

2.2 Plant Extraction

Fresh leaves of *P. amboinicus* were cut and macerated in methanol for 48 hours. The extract was then filtered and concentrated using a rotary evaporator at 40°C. The concentrated extract was stored in an air-tight and light-resistant container at a cool temperature for future use.

2.3 Test Animals

Twenty (20) healthy male Sprague-Dawley rats weighing 150 to 200 g were used for this study. The animal models were purchased from the University of the Philippines Manila and were housed in the Thomas Aquinas Research Complex (TARC) Animal house of the University of Santo Tomas. Test animals were acclimatized for seven days before the experimental procedures. All protocols involving animals were subjected to ethics review by the University of Santo Tomas-Institutional Animal Care and Use Committee.

2.4 Pharmacological Assay

The 20 rats were divided into five groups having four rats each. The first group was treated with distilled water. The second, third, and fourth group were treated with the crude methanolic extract of *P. amboinicus* at 2500mg/kg (1/2LD_{50}), 1250mg/kg (1/4LD_{50}), and 625mg/kg (1/8LD_{50}) Body Weight respectively. The fifth group was treated with Codeine at a dose of 30mg/kg Body Weight.

The antitussive assay followed the method described by Pang et al. (2011). Each rat was placed in a three-liter chamber and exposed to an aerosol of 17.5% (w/v) citric acid for 1 minute followed by a 10-minute observation time to obtain the pre-treatment tussive responses. After one hour, the test substance was given orally using a gavage tube. After a 30-minute rest period, the rats were re-exposed to the aerosol for 1 minute followed by another 10-minute observation time to obtain the post-treatment tussive responses.

The antitussive assay was performed for two trials with a two-week wash period in between the trials.

2.5 Statistical Analysis

The results from the experimentation were evaluated for validity and recorded for future tests and analysis. The data was tabulated and subjected to the two-way repeated ANOVA statistical test. The results were to determine whether there is a significant difference between the two trials and the treatment received by the rats. Post hoc analysis using Tukey's HSD test was used to determine which of the treatments would elicit the highest reduction of tussive responses compared to the positive control (Codeine group).

3. RESULTS AND DISCUSSION

The crude methanolic extract obtained amounted to 213g with a yield of 4.26%. The result from the pharmacologic assay is displayed in Figure 1.

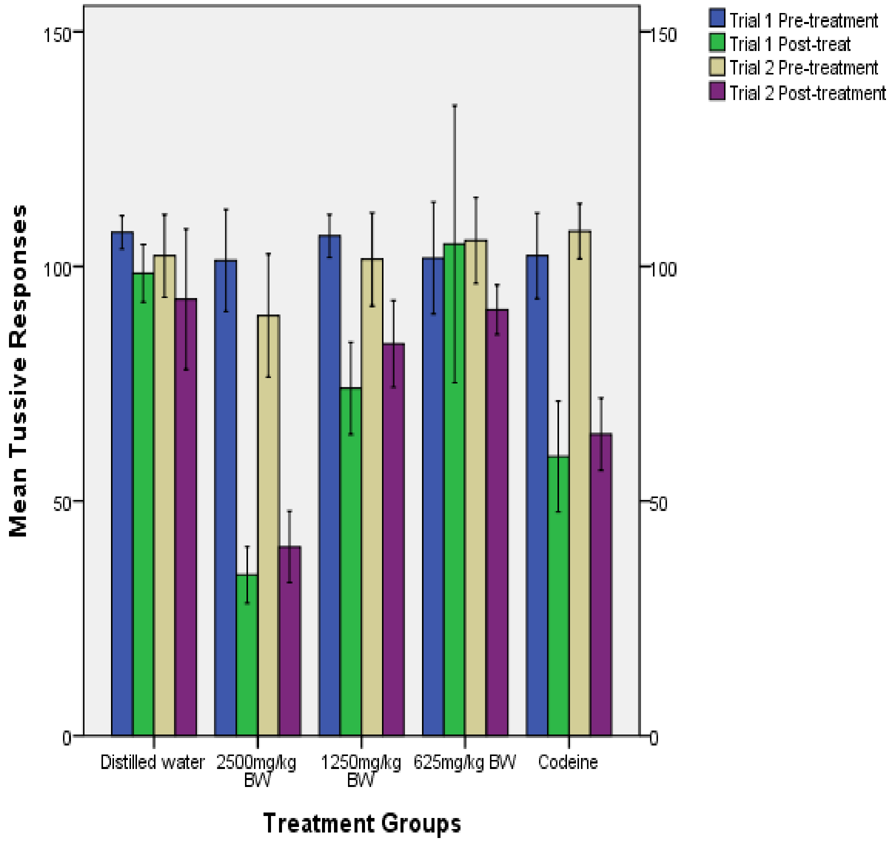

Fig 1. Mean Tussive responses Before and After Treatment for Trial 1 and Trial 2

The raw data for Trial 1, Trial 2 and the Treatment groups were tested for three-way interaction effect. According to the results presented in Table 1, the p-value is 0.001 (<0.05). Therefore, there is a significant difference between the data of Trial 1 to that of the data of Trial 2 to the treatments given.

Furthermore, the raw data of Trial 1 and Trial 2 were tested for the two-way interaction effect. According to the results in Table 1, the p-value is 0.231 (>0.05). Therefore, there is no significant difference between the two trials.

Table 1. Tests of Within-Subjects Effects for Trial 1, Trial 2 and Treatment Groups

Source		**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Trial1 * Treatment groups	Sphericity Assumed	339.825	4	84.956	1.363	.293
	Greenhouse-Geisser	339.825	4.000	84.956	1.363	.293
	Huynh-Feldt	339.825	4.000	84.956	1.363	.293
	Lower-bound	339.825	4.000	84.956	1.363	.293
Trial2 * Treatment groups	Sphericity Assumed	7961.125	4	1990.281	56.104	.000
	Greenhouse-Geisser	7961.125	4.000	1990.281	56.104	.000
	Huynh-Feldt	7961.125	4.000	1990.281	56.104	.000
	Lower-bound	7961.125	4.000	1990.281	56.104	.000
Trial 1 * Trial2	Sphericity Assumed	36.450	1	36.450	1.559	.231
	Greenhouse-Geisser	36.450	1.000	36.450	1.559	.231
	Huynh-Feldt	36.450	1.000	36.450	1.559	.231
	Lower-bound	36.450	1.000	36.450	1.559	.231
Trial 1 * Trial2 * Treatment groups	Sphericity Assumed	804.425	4	201.106	8.603	.001
	Greenhouse-Geisser	804.425	4.000	201.106	8.603	.001
	Huynh-Feldt	804.425	4.000	201.106	8.603	.001
	Lower-bound	804.425	4.000	201.106	8.603	.001

Since there is no significant difference between trials, the two trials' result for tussive responses before and after administration of treatment was compared to determine if there was a significant difference. The results, presented in Table 2, show a p-value of <0.001 suggesting that there is a significant difference between the pre-treatment tussive responses to that of the post-treatment tussive responses to the treatment groups.

Table 2. Tests of Within-Subjects Effects for Pre-treatment and Post-treatment

Source		**Type III Sum of Squares**	**Df**	**Mean Square**	**F**	**Sig.**
Pre-treatment * Post-treatment * Treatment groups	Sphericity Assumed	804.425	4	201.106	8.603	.001
	Greenhouse-Geisser	804.425	4.000	201.106	8.603	.001
	Huynh-Feldt	804.425	4.000	201.106	8.603	.001
	Lower-bound	804.425	4.000	201.106	8.603	.001

Since trial 1 and trial 2 had no significant difference, the means of the two trials were calculated and shown in Figure 2.

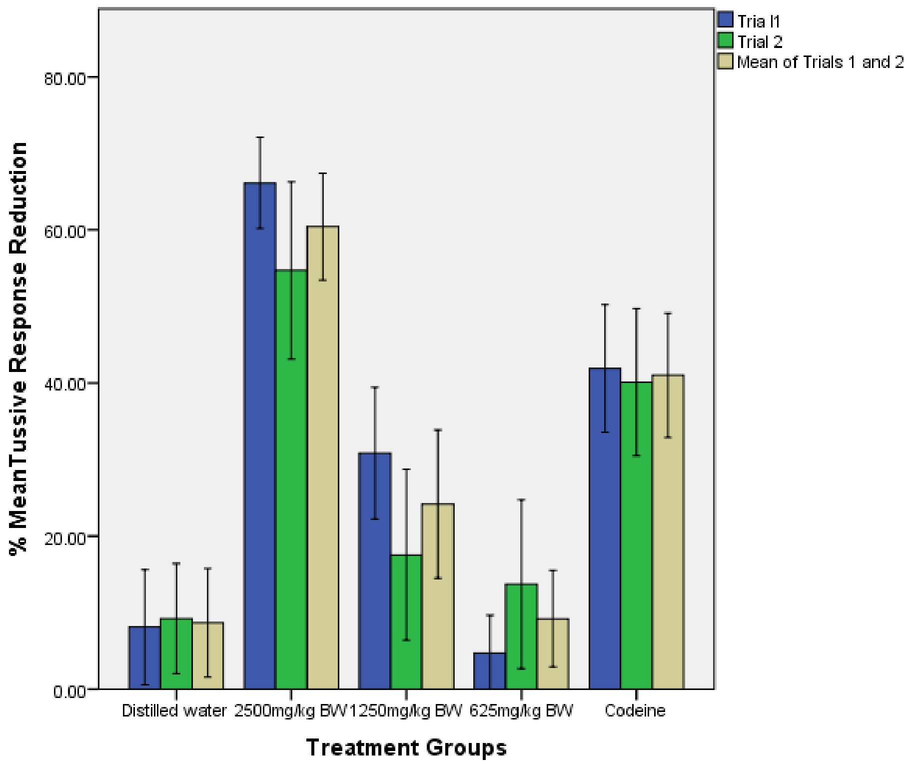

Fig 2. Percent Mean Tussive Response Reduction

Trial 1, Trial 2, and the Mean of the Two Trials

The calculated means of the two trials subjected to One-way ANOVA as shown in Table 3. The result indicates that there is a significant difference between the treatment groups (p<0.001).

Table 3. One-way ANOVA

	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	7836.621	4	1959.155	82.949	.000
Within Groups	354.284	15	23.619		
Total	8190.905	19			

The results of the post hoc analysis using Tukey's HSD testis collated in Table 4. Each treatment group was compared to the other treatments in order to identify if there are significant differences between their percent mean tussive response reduction.

Table 4. Multiple Comparisons

(I) Treatment	**(J) Treatment**	**Mean Difference (I-J)**	**Std. Error**	**Sig.**	**95% Confidence Interval**	
					Lower Bound	**Upper Bound**
Distilled water	2500mg/kg BW	-51.74970*	3.43649	.000	-62.3613	-41.1381
	1250mg/kg BW	-15.51420*	3.43649	.003	-26.1258	-4.9026
	625mg/kg BW	-.53670	3.43649	1.000	-11.1483	10.0749
	Codeine	-32.33585*	3.43649	.000	-42.9475	-21.7242
2500mg/kg BW	Distilled water	51.74970*	3.43649	.000	41.1381	62.3613
	1250mg/kg BW	36.23550*	3.43649	.000	25.6239	46.8471
	625mg/kg BW	51.21300*	3.43649	.000	40.6014	61.8246
	Codeine	19.41385*	3.43649	.000	8.8022	30.0255
1250mg/kg BW	Distilled water	15.51420*	3.43649	.003	4.9026	26.1258
	2500mg/kg BW	-36.23550*	3.43649	.000	-46.8471	-25.6239
	625mg/kg BW	14.97750*	3.43649	.004	4.3659	25.5891
	Codeine	-16.82165*	3.43649	.002	-27.4333	-6.2100
625mg/kg BW	Distilled water	.53670	3.43649	1.000	-10.0749	11.1483
	2500mg/kg BW	-51.21300*	3.43649	.000	-61.8246	-40.6014
	1250mg/kg BW	-14.97750*	3.43649	.004	-25.5891	-4.3659
	Codeine	-31.79915*	3.43649	.000	-42.4108	-21.1875
Codeine	Distilled water	32.33585*	3.43649	.000	21.7242	42.9475
	2500mg/kg BW	-19.41385*	3.43649	.000	-30.0255	-8.8022
	1250mg/kg BW	16.82165*	3.43649	.002	6.2100	27.4333
	625mg/kg BW	31.79915*	3.43649	.000	21.1875	42.4108

*. The mean difference is significant at the 0.05 level.

The homogenous subsets are arranged in Table 5. Tukey's HSD test showed that the extract with a concentration of 2500 mg/Kg BW produced the highest mean percent reduction in tussive response, followed by

Codeine, the extract with a concentration of 1250 mg/Kg BW, then the extract with a concentration of 650 mg/Kg BW and distilled water having no significant difference ($p>0.05$).

Table 5. Homogenous Subsets Tukey HSD[a]

Treatment	N	Subset for alpha = 0.05			
		1	2	3	4
Distilled water	4	8.6681			
625mg/kg BW	4	9.2048			
1250mg/kg BW	4		24.1823		
Codeine	4			41.0039	
2500mg/kg BW	4				60.4178
Sig.		1.000	1.000	1.000	1.000
Means for groups in homogeneous subsets are displayed.					
a. Uses Harmonic Mean Sample Size = 4.000.					

4. CONCLUSION

In conclusion, the present study demonstrates that *P. amboinicus* can be a potential antitussive at a dose of 1250 mg/kg BW and can reduce a cough better than Codeine at a dose of 2500 mg/kg BW. Further studies on *P. amboinicus* extract may be conducted to determine the ED_{50} as well as further bioassay-guided fractionation to isolate the active constituent responsible for the antitussive effect.

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