Malays. Appl. Biol. (2020) 49(3): 43–51

INVESTIGATION OF TOXICITY, ANTIMICROBIAL ACTIVITY AND COUGH TREATMENT OF PRODUCTS PRODUCED FROM *Pouzolzia zeylanica* PLANTS GROWING IN VIETNAM

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Accepted 21 September 2020, Published online 25 October 2020

ABSTRACT

Pouzolzia zeylanica (L.) Benn is one of the medicinal plants that possess a lot of bioactive compounds with potential for therapeutics. These plants have usually been consumed in traditional way despite its efficacy and safety proven. The objectives of this study were to evaluate the toxic and pharmacological effects of concentrated and spray-dried extract of *Pouzolzia zeylanica*. The plant is popular for its auxiliary substances in the treatment of cough; however, the information about the safety of their usage need to be explored. In the study, the antibacterial ability of respiratory, acute oral toxicity, effects of reducing sputa and cough symptom were evaluated. The antibacterial ability of the test product was assessed on 5 strains of respiratory tract pathogenic bacteria by minimum inhibitory concentration (MIC) method. The acute oral toxicity, effect to reduce sputum and cough treatment was tested in mice through capsaicin model following standardized methods. It was observed that both products were not causing acute oral toxicity. *Pouzolzia zeylanica* spray-dried powder had antibacterial activity on *Streptococcus pyogenes* with MIC of 50 mg/mL. Dose, which used (6 g/day converted in humans) showed effect on reducing sputa and cough symptom due to stimulation of cough model with capsaicin. In contrast, the concentrated product did not show antimicrobial activity in tested bacterial strains. Dose at 4 g/day converted in humans showed effect on reducing sputa in experimental mice that were affected with capsaicin. Nevertheless, the effect of it on cough symptoms caused by the capsaicin model was not typical in comparison with spray dried powder. Result showed that spray-dried powder did not exhibit acute oral toxicity, had antibacterial properties, reduced sputum and treated cough. This promises that it can be developed into a convenient instant tea product, supporting good health for consumers in preventing diseases.

Key words: *Pouzolzia zeylanica*, concentrated and spray-dried products, toxicity, pharmaceutic, experimental mice

INTRODUCTION

Plants have been used in traditional medicine for several centuries. From ancient times, people have been exploring and using various plants and plant products to cure many different diseases. Several plants are currently undergoing investigation to determine their therapeutic efficacy and many plant species are traditionally used for respiratory illness treatment. Respiratory diseases can be caused by several reasons, such as the presence of micro-organisms or toxins in the environment, which generally attack weak organisms with nutritional deficiencies. The most common related respiratory illness symptoms are tonsillitis, bronchitis, pneumonia and influenza. The main symptoms of these disorders are often very similar and are manifested in the following ways: flushing, cough, fever, headache and sore throat (Shah *et al*., 2015).

Many plants are used in phytotherapic preparations without standard quality control. Some studies have showed the presence of secondary metabolites with toxic and carcinogenic potential when used chronically (Ernest, 2004; Rietjens *et al*., 2005). The toxicological studies on effects of medicinal plants on human health have been conducted deliberately (Simão *et al*., 2014).

Pouzolzia zeylanica is a medicinal plant, which is located in tropical and subtropical regions. It has been used as one of the components in herbal remedies for treating various diseases. In Vietnam, this plant was popularly cultivated in Mekong Delta region. The plant is commonly used as fresh or dried plant, decoction drunk to treat cough up phlegm,

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pulmonary tuberculosis, sore throat, enteritis, dysentery, diuretic, anti-inflammation, urinary infections, galactopoietic, pulmonary disease, etc (Vo Van Chi, 2012). In addition, people from many Asian countries have used it to treat various kinds of diseases by traditional method such as poultice to bone fractures (Ratnam & Raju, 2008), boils and to relieve stomachache (Yusuf *et al*., 2006), diabetes (Mondal *et al.,* 2013), cancer (Sandhya *et al.,* 2013), treat eyes injuries (Purkayastha *et al.,* 2007), itching, dysentery and loose stools of infant (Bhattacharjya & Borah, 2008), cure stomach ailments, preventive radiation and confirmed the therapeutic value of polyphenols contained in the leaves (Li, 2006). In modern medicine, *Pouzolzia zeylanica* is also combined with other herbs that have ability to fight cancer cells, against tuberculosis and good effect on lungs (Le, 2007).

The objectives of this study were to determine antibacterial ability of respiratory, acute oral toxicity, effect of reducing sputa and cough symptom of two products (concentrated and spray-dried) made from extraction of *Pouzolzia zeylanica* plants.

MATERIALS AND METHODS

Preparation of products for test

Pouzolzia zeylanica was collected from experimental area of An Giang University, Vietnam in March 2015 and harvested after one and a half month of cultivation, with 20-30 cm in height. The whole plant was then cleaned with tap-water, dehydrated by sun drying until the final moisture content about 12%, cut into small pieces about 2-3 cm long. The optimal extraction conditions of dried plants were performed at 81°C for 30 min and water/material ratio of 27/1 v/w. The hot *extract* was *filtered* through cotton *cloth* and determined their volumes. After that, the extract was blended with citric acid of 0.29%, 20°Brix by sucrose and 0.29% carboxymethyl cellulose; vacuum cooking at 600 mmHg for 40 min, the final product was 60°Brix (concentrated product). The extracted compound was blended with 9% maltodextrin and 0.08% arabic gum. Later, spray dried process was conducted at inlet drying temperature of 179°C, feed flow rate of 18 rpm, the moisture of obtained powder was 6.5% (spray-dried product). They were packaged and stored at room temperature for further experiments.

Source of experimental organisms

The bacterial strains selected for this study were *Staphylococcus aureus (ATCC12600), Streptoccocus pyogenes (ATCC12344), Streptococcus pneumoniae (ATCC27336), Klebsiella pneumoniae (ATCC70063), Pseudomonas aeruginosa (ATCC9027).* These bacterial strains were stored at Medicine and Pharmacy University in Ho Chi Minh City, Vietnam. Male and female white mice, Swiss albino strains, average weight 20 ± 2 g were provided by Nha Trang Institute of Vaccines and Medical Biologicals, Vietnam. Mice were raised with pelleted foods, drinking water sufficiently and were kept stable for at least a week before the test.

Experimental designs

Quantify the antibacterial capacity of the product by the Minimum Inhibitory Concentration method

Experimental bacteria were cultivated on Mueller Hinton Agar (MHA) medium at 37°C for 24 hr. About 3-4 colonies of bacteria transferred into a test tube containing 10 mL of Mueller Hinton Broth (MHB), incubated for 4-6 hr at 37°C until the absorbance at 625 nm was measured with a cuvette (1 cm) to reach 0.08-0.10; corresponding to about 10^8 CFU/mL. This bacterial suspension was diluted to a density of 10^7 CFU/mL with sterile buffer solution. After dilution, the bacteria suspension was proceed for further testing within 15 min. Two test products were diluted in MHA medium, which were melted and allowed to cool to about 50°C. Accurately weighed 500 mg of the test product was put into a sterile test tube No.1. Subsequently, about 10 mL MHA was added into the mixture and then strongly vortexed to mix the mixture well and to obtain a test product concentration of 50 mg/mL. Later, approximately 5 mL was taken from tube No.1 and transferred to test tube No.2 containing 5 mL of MHA medium. serial dilution 1/2 was continued as above to test tube number 10 to obtain a range of medium containing diluted test product 1/2 gradually. Test tubes containing MHA was poured into petri dishes, cooled to solidify. When medium solidified, about 1 µl of the above prepared bacterial suspension was streaked onto the surface of all agar plates. The plates were then incubated at 37°C for 24 hr. The trace of bacteria growing at the implantation point and MIC was recorded (Patel *et al*., 2015).

Oral route toxicity investigation

Male and female white mice were starved for 16 hr and divided into similar groups. Mice in the same batch would receive the same dose of the survey. The volume of oral dose was 20 mL/kg of mouse weight and no more than 0.5 mL/rat/time. The assessment based on the reactive or incapacitated (life or death) was found in each mouse in the group after 72 hr. The mice were followed up after 14 days of drinking to recognize abnormal symptoms (if any) (Do, 1996; National Institute of Medicinal Materials, 2006).

Survey reducing ability of phlegm

White male mice with Swiss albino strains, 5-6 weeks of age, were randomly divided into 6 experiments group (n = 8-10) as follows: batches of control (drinking distilled water), test batches 1 and 2 were given drinking concentrated product with dose equivalent to 1/10 and 1/20 D_{max}, test batches 3 and 4 were given drinking spray-dried powder samples equivalent to 1/10 and 1/20 D_{max} and batches of reference (positive) drinking ambroxol (capsule Ambroxol ®, containing ambroxol HCl 30 mg, DOMESCO Pharmaceutical Joint Stock Company (Vietnam), lot number 0050316, expecting March 5, 2019) dose of 240 mg/kg. Samples and ambroxol were dissolved in distilled water and given to mice with an oral volume of 10 ml/kg of mouse weight, continuously for 3 days and given once a day. One hour after the final drink, mice were injected with 2.5% phenol red solution in physiological saline. After 30 min of injection with phenol red, the mice were euthanized and the trachea was separated, immediately placed in the test tube containing 2 mL of physiological saline and placed in the ultrasonic tank for 15 min. Then 2 mL of 5% sodium bicarbonate solution was added and the mixture was measured absorbance at 558 nm. Expectorant effect was assessed by increasing the optical density in the test plots or the lot compared to the control batch by the formula: Percentage of phenol red secretion (%) = $[(Dt - D_0)/D_0 \times 100)]$, where Dt is the optical density measured in the test lot, D_o is the optical density measured in the control lot (Engler & Szelenyi, 1984; Dapaah *et al*., 2016).

Investigation of cough suppressant effects

White male mice with Swiss albino strains, 5-6 weeks of age, were randomly divided into 6 experiments group (n = 8-10) as follows: batches of control (drinking distilled water), test batches 1 and 2 were given drinking concentraed product with equivalent to 1/10 and 1/20 D_{max}, batches 3 and 4 were given drinking spray dried powder sample with doses equivalent to 1/10 and 1/20 D_{max} and comparison batches (positive) Neo-codion® tablets (film tablets containing codeine camphosulfonate 25 mg equivalent to codeine 14.93 mg, sulfogaiacol 100 mg and 20 mg Grindélia extract) dose of 1 tablet/kg. The test and positive samples were dissolved in distilled water and given to the mice orally at dose of 10 mL/kg of mouse weight once daily for 3 days. Two hours after the last drink, mice were placed in glass jars (similar in size to desiccators, vented) and exposed to capsaicin (Sigma-Aldrich, USA, mixed in ethanol 10% +Tween-80 10%) aerosol form at a concentration of 100 mM for 10 min with an injection volume of 0.25 mL/min. The potential for cough, number of coughs and behavioral symptoms of the mouse (sniffing-scratching the muzzle, jumping up by strong stimulation) for 10 min were identified. Later, mice trachea were separated and the phenol red assay was performed similar as content of survey reducing ability of phlegm (Nieto *et al*., 2003; Tanaka & Maruyama, 2005; Canning, 2008; Zhang *et al*., 2009; Satia *et al*., 2017).

Statistical analysis

The results obtained in the tests are indicated by the average (Mean ± SEM) of replication (n = 8-10) and statistical analysis of data based on the T-test or One-way ANOVA tests followed by Student-Newman test- Keuls, with 95% reliability ($p<0.05$) with SigmaStat® ver software. 3.5 (SYSTAT Software Inc., Richmond, CA, USA).

RESULTS AND DISCUSSION

The antibacterial capacity of two test products

Survey of antimicrobial activity of medicinal plants can be used in many different assessment methods. But the method of minimum inhibitory concentration (MIC) and agar well diffusion (AWD) are the two most commonly used methods (Anyanwu & Okoye, 2017). MIC assessment is a quantitative method of measuring antibacterial activity based on the principle of contact between bacteria with a range of different diluted test substances concentrations. MIC is the lowest concentration of antimicrobial substances that prevent bacterial growth under known conditions (Nasir *et al.*, 2015). AWD method is used because it is possible to indicate plant extract concentration that affects the structural and functional morphology of the test bacteria, based on the principle of contact between test bacteria and equal volume of test substances. Each of the test substances had different concentrations pumped into wells of equal depth on agar medium. Differences in the data collected using MIC assessment may be influenced by factors such as the concentration of the test substance, the type of development medium of bacterium, the incubation time and the method of preparation of the test substance (Balouiri *et al.*, 2016).

Test results of samples diluted with a concentration from 50 to 0.1 mg/mL on bacterial strains in Table 1 showed that most of the bacteria were not inhibited (–) at the concentrations used, except for *Streptococcus pyrogenes* (+) at the concentration of spray dried powder 50 mg/mL (Figure 1). This could be explained as the survey concentration of the sample was not enough to inhibit bacteria because both spray dried and concentrated products made from aqueous extract, concentration of bioactive compounds in the product was relatively low.

Table 1. The results determine the antibacterial activity of two test samples

Bacterial strains	Control	Concentrate product (mg/mL)									
		50	25	12.5	6.25	3.13	1.56	0.78	0.39	0.20	0.10
S. aureus	–	–	–	–	–	–	–	–	–	–	–
S. pyogenes	–	–	–	–	–	–	–	–	–	–	–
S. pneumoniae	–	–	–	–	–	–	–	–	–	–	–
K. pneumoniae	–	–	–	–	–	–	–	–	–	–	–
P. aeruginosa	–	–	–	–	–	–	–	–	–	–	–
Bacterial strains	Control	Spray-dried powder (mg/mL)									
		50	25	12.5	6.25	3.13	1.56	0.78	0.39	0.20	0.10
S. aureus	–	–	–	–	–	–	–	–	–	–	–
S. pyogenes	–	+	–	–	–	–	–	–	–	–	–
S. pneumoniae	–	–	–	–	–	–	–	–	–	–	–
K. pneumoniae	–	–	–	–	–	–	–	–	–	–	–
P. aeruginosa	–	–	–	–	–	–	–	–	–	–	–

Note: (–) no antimicrobial activity; (+) shows antibacterial activity.

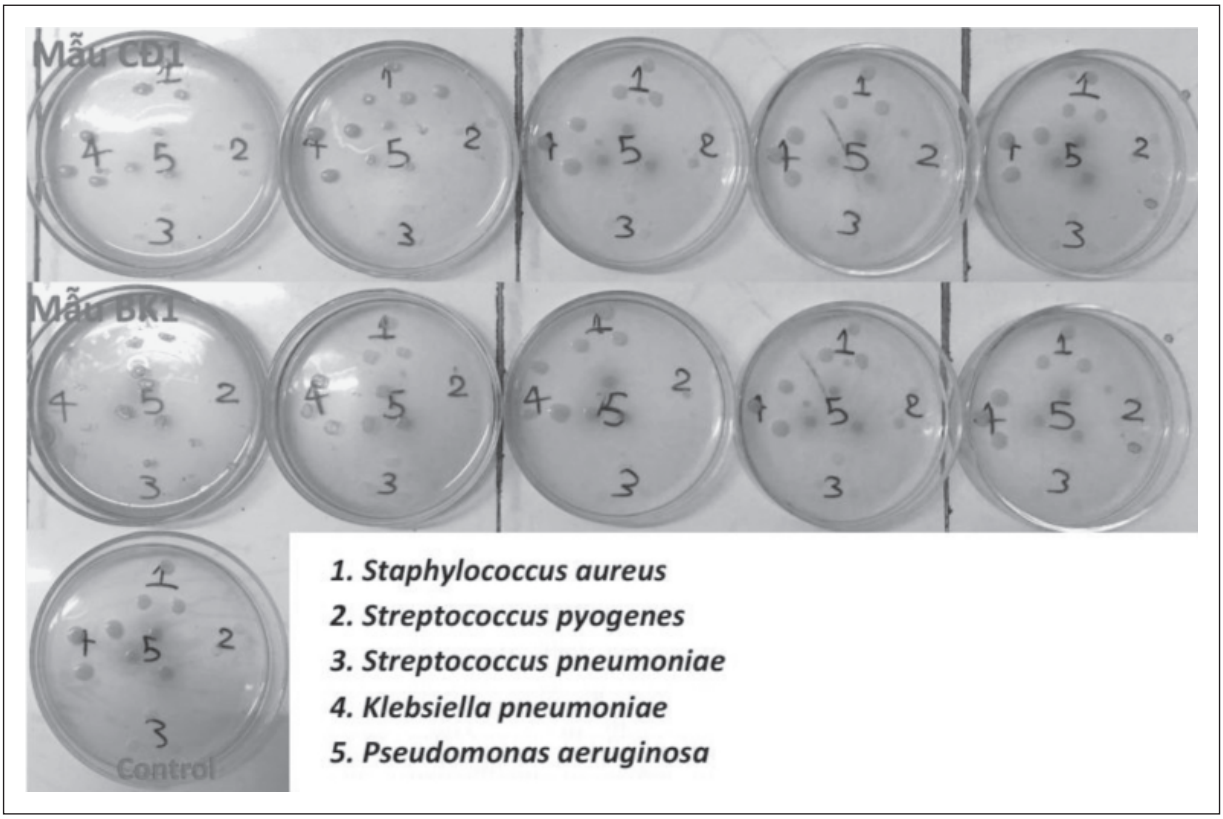

Fig. 1. Antibacterial test samples by MIC method.

In reported studies, the low concentration of the test sample could inhibit the activity of bacteria. Foe example aqueous extract from leaves of *Amaranthus hybridus* with MIC of 6.33 mg/mL (Nduche *et al.*, 2016); and methanol and ethanol extract of *Allium ascalonicum, Terminalia glaucescens, Allium cepa*, and ethanol extract of *Securidaca longepedunculata* with MIC of 50 mg/mL (Adeleye *et al.*, 2008). Research of Bello *et al.* (2011) showed aqueous extract from *Pavetta crassipes* with antibacterial activity of *P. aeruginosa, S. pyogenes, K. pneumoniae* and *N. gonorrhoeae* with MIC from 6.25–12.5 mg/mL. Antimicrobial activity (*P. aeruginosa, S. aureus, E. coli, K. pneumoniae*) and mold (*A. niger, C. albicans*) of ethanol extract from *C. alata* Linn (Leguminosae) seed had the inhibitory concentration of 6.25–50 mg/mL (Okwu & Nnamdi,

Table 2. MIC of two test samples in survey condition

Baterial strains	MIC (mg/mL)	
	Concentrate product	Spray-dried powder
Staphyloccocus aureus	–	–
Streptoccocus pyogenes	–	50
Streptoccocus pneumoniae	–	–
Klebsiella pneumoniae	–	–
Pseudomonas aeruginosa	–	–

Note: (–) not determine activity.

2011). The methanol extract from *G. latifolium* leaves had an inhibitory MIC of *P. monteilli* of 75 mg/mL (Ikegbunam *et al.,* 2014). The ethanol extract of *Evolvulus alsinoides* had MIC from 16 mg/mL to 512.5 mg/mL; lowest in *S. typhi* and highest in *B. cereus* and *S. aureus* (Omogbai & Eze, 2011). Extracts from *Uritca dioica* at a concentration of 100 mg/mL showed antimicrobial activity but no antifungal activity (Modarresi-Chahardehi *et al.,* 2012). Minimum inhibitory concentration values of plant extracts inhibit bacteria with different concentrations depending on the kind of various plants, the solvent used and the test strain (Van-Vuuren, 2015).

The results in Tables 1 and 2 showed that the spray-dried powder sample with MIC on the Streptoccocus pyogenes strain was 50 mg/mL. The test samples did not exhibit antimicrobial activity on other bacterial strains.

Oral route toxicity of two test products

After 72 hr of testing, the maximum recommended dose of the test sample (concentrated product) on white mice was 20 g/kg of mouse weight, with a death rate of 0% and LD_{50} could not be determined. Therefore, the highest dose mouse can drink without causing the death (D_{max}), D_{max} = 20 g/kg body weight of mouse could be determined. The maximum dose of the test sample (spray dried powder) on white mice was 30.12 g/kg of mouse weight, with a death rate of 0% and could not be determined LD_{50}. Therefore, D_{max} = 30.12 g/kg of mouse weight could be determined.

Test samples (concentrated product and spray-drying powder) did not exhibit oral acute toxicity on white mice; all mice were still eating and living normally during 72 hr of observation. Mice were followed up after 14 days of drinking and abnormal symptoms were not noticed. The results of general anatomy of internal organs of liver, heart, lungs, stomach, kidneys showed no abnormal signs (Table 4). Based on the results of the oral toxicity assessment, the safe therapeutic dose range for pharmacological tests was determined (Table 3).

Table 3. Dose selected for pharmacological tests

Test samples	Dose (g/kg)	
	1/10 D_{max}	1/20 D_{max}
Concentrate product	2.0	1.0
Spray-dried powder	3.0	1.5

Reducing ability of phlegm of two test products

The ambroxol is a metabolite product which has active bromhexine, acts to destroy mucus that accumulates in the bronchial wall and increase their excretion through the active function of villi. Ambroxol alters the structure of bronchial secretions by reducing and cutting mucopolysaccharide fibers, reducing synthesis sulfomucin of cells, so the phlegm spitting becomes easier. The concentrated product (dose of 1.0 g/kg) and spray-dried powder from the *Pouzolzia zeylanica* extract (dose of 1.5 g/kg) all showed the effect of increasing the red phenol secretion in the white mouse trachea, reaching statistical significance ($p<0.05$) compared to the control group and similar to the effect of ambroxol (Table 5). In high concentration of concentrated product (2.0 g/kg) and spray-dried powder (3.0 g/kg) from the *Pouzolzia zeylanica* extract did not show the effect of increasing phenol red secretion in the white mice trachea ($p>0.05$). It might be due to reverse inhibitory reaction. Therefore, dose of 1.0 g/kg and 1.5 g/kg of test samples were selected for the study of the effect of two *Pouzolzia zeylanica* products on capsaicin cough models.

Besides, results in Table 5 showed that capsaicin reduces phenol red secretion in the white mice trachea of the untreated group (capsaicin control group), achieving statistical significance compared to the physiological control group. Neo-codion® drugs increased phenol red secretion of mice trachea that caused cough with capsaicin, with increasing 54,55% and statistical significance compared to the batch of capsaicin, indicating the effect of reducing phlegm. The ingredients in Neo-

Table 4. Results of assessing the behavior of mice in the 14 days of survey after drinking the test samples in maximum dose D_{max}

Organ systems	Observe and test	General signs
Central nervous system and movement nervous system	Behavior, movement	Mouse movement and normal eating activities in the cage. Do not record states of agitation or loss of reflexes, sleep, or coma
Vegetative nervous system	Brain and spinal cord reactions Muscle strength Eye-nose	No abnormal expression Normal Normal
Respiratory system	Characteristics and speed	Normal breathing, not recognized symptoms of dyspnea or convulsions trachea
Cardiovascular system	Cardiovascular signs	Not noted abnormalities in heart rhythm
Stomach, intestines	The symptoms Abdominal shape Stiffness and color of stool	Not recognized symptoms of diarrhea, constipation. Not recognized symptoms of abdominal twists Normal mouse droppings.
Genital	Penis	Pinky and puffed up
Skin and fur	Color, status	Silky white hair. No redness symptoms on the skin or ruffling
General condition	Weight gain	In normal physiological threshold

Table 5. The evaluation result of percent ratio increased the phenol red secretion of normal mice group and mice group caused cough using a capsaicin model

Experimental group (n = 8–10)	Oral dose (g/kg)	Optical density	*p*-value	% increased phenol redness
Distilled water control	–	0.083 ± 0.005	–	–
Ambroxol	0.24	0.166 ± 0.027	0.004	99.70
Concentrate product	1.0	0.150 ± 0.021	0.026	80.96
	2.0	0.102 ± 0.013	0.409	23.01
Spray-dried powder	1.5	0.164 ± 0.022	0.008	97.47
	3.0	0.102 ± 0.014	0.682	22.13
Experimental group (n = 9)	**Oral dose (g/kg)**	**Optical density**	***p*-value**	**% increased phenol redness**
Physiological control	–	0.086 ± 0.004	–	–
Capsaicin control	–	0.060 ± 0.007	0.008*	↓ 30.27
Neo-codion®	1 tablet/kg	0.093 ± 0.010	0.016#	↑ 54.55
Concentrate product	1.0	0.093 ± 0.013	0.043#	↑ 55.29
Spray-dried powder	1.5	0.099 ± 0.010	0.006#	↑ 65.68

*Notes: *: compare with physiological control; #: compare with pathological control.*

codion® formula all work to treat coughs. Codeine is an alkaloid of opium fruit, an anti-cough drug which was effective on central nerous system and inhibition of respiratory center. Sulfogaiacol has the effect of secretions dilution in the respiratory mucosa and had effect on reducing phlegm. Ethanol extract Grindélia has anti-cough effect. Concentrated product (dose of 1.0 g/kg) and spray-dried powder (dose of 1.5 g/kg) all showed the effect of increasing red phenol secretion in the trachea of white mice caused by capsaicin, with an increase of 55.29% and 65.68%, reaching statistical significance compared to the batch of capsaicin and not significantly different from the physiological control group. The effect of reducing phlegm of the samples of *Pouzolzia zeylanica* is similar to the effect of Neo-codion®.

Cough suppressant effects of two test products

Capsaicin (8-methyl-N-vanillyl-6-nonenamide), a spicy ingredient in chilli, has long been used to stimulate coughing in a safe way, depending on the dosage used to show different levels of cough, this can reflect the reaction of nerve fibers (C-fiber) in the lining of the respiratory tract. The results in Table 6 have shown that powder sample (1.5 g/kg dose) prolonged the occurrence of cough symptoms in mice groups, which caused cough using capsaicin model, significantly different from the batch of control capsaicin. The occurrence time of cough symptoms in mice group using Neo-codion® or concentrated product (dose of 1 g/kg) was not statistically significant difference ($p>0.05$) compared to the capsaicin control group.

The results in Table 7 showed that Neo-codion® drug reduced the number of sneezing and brushing feather at the muzzle of mice using capsaicin model, which was statistically different from the batch of control capsaicin. But the number of sneezing and combing feather at the muzzle of mice drinking concentrated product (dose of 1 g/kg) or spray-dried powder (dose of 1.5 g/kg) had a reduced, which did not reach statistical significance compared to the control capsaicin experimental group.

The results in Table 8 also showed that the spray-dried powder (1.5 g/kg dose) had similar effect of Neo-codion® drug reduced the number of jumps due to stimulation caused using capsaicin coughing model, statistically significant difference from the batch of control capsaicin. However, the number of jumps due to stimulation in rats taking concentrated product (dose of 1 g/kg) was not statistically significantly from the control capsaicin experimental group.

CONCLUSION

The two tested products did not exhibit oral tract acute toxicity when evaluated in white mice. At the same time, the two products did not have antibacterial activity against the 5 selected strains of bacteria (*Staphylococcus aureus, Streptoccocus pyogenes, Streptococcus pneumoniae, Klebsiella pneumoniae, Pseudomonas aeruginosa),* which were related with diseases of respiratory tract except, spray-dried powder.

The spray-dried powder had antibacterial activity on strains of *Streptococcus pyogenes* with a minimum inhibitory concentration (MIC) of 50 mg/mL; at dose of 6 g/day converted in humans,

Table 6. The evaluation result of the time that appear cough symptoms of mice group caused cough using a capsaicin model

Experimental group (n = 8)	Oral dose (g/kg)	Time (seconds)	*p*-value
Capsaicin control		1.63 ± 0.26	–
Neo-codion®	1 tablet/kg	1.75 ± 0.25	0.736
Concentrate product	1.0	2.38 ± 0.26	0.063
Spray-dried powder	1.5	2.88 ± 0.52	0.049

Table 7. The evaluation result of the number sneezing and brushing feather at muzzle of mice group caused cough using a capsaicin model

Experimental group (n = 8)	Oral dose (g/kg)	Times number	*p*-value
Capsaicin control		126.0 ± 13.90	–
Neo-codion®	1 viên/kg	78.75 ± 5.44	0.007
Concentrate product	1.0	102.38 ± 9.25	0.179
Spray-dried powder	1.5	115.13 ± 13.11	0.578

Table 8. The evaluation result of the number jumps due to stimulation of mice group caused cough using a capsaicin model

Experimental group (n = 8)	Oral dose (g/kg)	Number of jumps	*p*-value
Capsaicin control		21.38 ± 4.02	–
Neo-codion®	1 tablet/kg	10.13 ± 2.89	0.039
Concentrate product	1.0	12.0 ± 3.53	0.101
Spray-dried powder	1.5	9.25 ± 3.10	0.032

it had effect on reducing sputa and cough symptom due to stimulation of cough model with capsaicin.

The concentrated product did not show antimicrobial activity in bacterial strains related with respiratory tract disease; at a dose of 4 g/day converted in humans, it had effect on reducing sputa in experimental mice affected with capsaicin, but the effect of it on cough symptoms caused by the capsaicin model was not typical in comparison with spray-dried powder.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Research Centre of Ginseng and Medicinal Material, Department of Microbiology, Medicine and Pharmacy University in Ho Chi Minh City, Vietnam that supported the implementation of this research.

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