inability to absorb new information. To assess the memory and learning ability of flies, this study used the odor-taste assay to assess short-term memory of fly larvae. Our results show that BM extract improves memory deficit in AD flies. These results are consistent with our previous studies which indicated that BM has potent to improve memory and cognitive impairment by models of memory impairment in mice models of trimethyltin (TMT)-induced memory impairment and olfactory bulbectomized (OBX) [10],[11].

There are much research works in the world that have proven that BM has the effect of improving memory and cognitive impairment by models of memory impairment in rats caused by aziridinium, colchicine, $AlCl_3$ ions, and mice carrying AD gene (PSAPP mice) [7],[8],[9]. BM extract and its major compounds such as bacoside A, bacoside B act on hippocampus, cerebral cortex, and hypothalamus, leading to increased genes/proteins activity which involve in learning and memory. This extract was also effective in reducing beta-amyloid levels in the brains of PSAPP mice. These evidences suggest that BM improves neurotransmission through synaptic recovery.

Impaired locomotor behavior is a common diagnosis in many neurological diseases, including AD [19]. The locomotor performance showed an association with the severity of the disease [21]. Therefore, we also evaluate the effect of BM extract on locomotor activities in hAPP flies. hAPP flies showed a decrease in the crawling, climbing activities and BM extract treatment mitigated the reduced locomotor activities at a larvae stage and adult stage of AD flies. The aberrant locomotor function observed in hAPP flies in the present study in line withwith a previous study reported by Beg et al (2018) [22].

It has been reported that expressing human APP in fly nervous system produces amyloid β (Aβ) and shorten the life span as well as memory loss [23]. The aggregation of Aβ peptide in the brain, especially Aβ42 is thought to be a pathological hallmark of AD. Therefore, the inhibition of hAPP/Aβ has been used to develop therapeutic drugs for AD. Our results in this study have demonstrated that n-butanol extract from BM markedly suppressed the hAPP expression in adult brain flies. These results suggest that BM extract has improved the deficit of memory and locomotor activities of AD flies partly via a mechanism involving suppression of hAPP protein.

5. Conclusion

Our results of the present study support the evidence that n-butanol extract from *Bacopa monnieri* has a potential improvement of Alzheimer's disease in a fly model, through criteria including short-term memory, locomotor activities and hAPP expression.

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ANTI-MICROBIAL AND EXPECTORANT ACTIVITIES OF LOZENGES AND SYRUP FROM *HEDERA NEPALENSIS*

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Summary

Anti-Microbial and Expectorant Activities of Lozenges and Syrup from *Hedera nepalensis*

Cough is a manifestation of respiratory disease, especially when there is sputum or sore throat caused by the microbes, it is more harmful to health. Hence, it is needed to develop a product which is effective against several respiratory disorders. To avoid the side effects and provide safe medicine, plant products may provide alternative treatments replacing the modern medicine. In this study, we examined the antimicrobial and expectorant activities of two products containing *Hedera nepalensis* extract, a native plant in Vietnam and has been used in traditional medicine for the treatment of cough and inflammation. The syrup and lozenge inhibited the growth of four bacterial strains (*Streptococcus pneumoniae, Streptococcus pyogenes, Haemophilus influenzae, Klebsiella pneumoniae*) with the MIC value of the syrup was 20% (volume/volume) for *Klebsiella pneumoniae,* while it was greater than 20% (volume/volume) for the remaining strains (*Streptococcus pneumoniae, Streptococcus pyogenes, Haemophilus influenzae).* The MIC value of the lozenge was 25 mg/mL for Streptococcus pneumoniae and S. pyogenes, while it was no less than 100 mg/mL for the others (*Haemophilus influenzae, Klebsiella pneumoniae).* Syrup at dose of 16 ml/kg and lozenges at dose of 4 lozenges/kg showed expectorant effects because of a significant decrease (31.15%- 43.56%) on optical absorbance of bronchoalveolar fluid level as compared to normal control. The results of this study could show the potential of this plant and these related products for treating respiratory problems and improving the health of Vietnamese people.

Keywords: *Hedera nepalensis, Anti-Microbial, Expectorant, Syrup, Lozenge.*

1. Introduction

Cough is characterized as a forceful expulsive maneuver, generally against a closed glottis, and is accompanied by a distinct sound [1]. Coughing can be caused by a variety of respiratory tract illnesses, which may need pharmacological therapy for treatment. Most medications are effective for antitussives but there are some side effects and impotence for patients who produce a lot of phlegm. On the contrary, it makes coughing worse. Besides, an upper respiratory infection affects the upper part of your respiratory system, including your sinuses and throat, which can cause more cough and damage your respiratory tract. Therefore, it is necessary to develop a product with contemporary anti-microbial, expectorant, and antitussive activities which are effective against several respiratory disorders.

Because of the limited lack of effective and

safe medication from therapies of modern medicine, it has been suggested that plant products in herbal medicine could provide alternative treatments [2]. *Hedera nepalensis* K. Koch (Araliaceae) is a species of ivy that is distributed from India to East Asia, including Korea, Japan, and Taiwan. In Vietnam, *Hedera nepalensis* has been founded in Lao Cai (Sapa), Ha Giang [3]. Research by Ahmad et al in 2012 showed that chloroform fraction from ivy leaves (*Hedera nepalensis*) has good antibacterial ability against *Staphylococcus aureus* species. Recent studies also show that the triterpene saponins in *Hedera nepalensis* (most of which are hederacoside C and α-hederin) have been shown to have anti-inflammatory, anti-infective, hepatoprotective, and antioxidant effects and hypoglycemia [4],[5]. Hederacoside C was therefore identified and quantified using high-performance liquid chromatography (HPLC) in the European Pharmacopoeia [6].

There are many products from Hedera species on the market, especially for supporting the treatment of cough and other respiratory problems [7],[8]. However, it should be noted that most of these products are mostly used from *Hedera helix* species, which not favorable for cultivation and production on a large scale [9]. In Vietnam, it is very difficult to find this species due to its strict requirements for growth conditions, so there is need for alternatives from local plant materials (*Hedera nepalensis)* to meet the needs of consumers. However, there is little research on the specific pharmaceutical activities of the *Hedera nepalensis* plant extract, especially when taken orally in the form of a syrup or lozenge, the two most popular forms for supporting the treatment of cough. Therefore, the main objective of this study was to formulate a syrup and a lozenge containing the ethanol extracts of *Hedera nepalensis* and to evaluate the anti-microbial and expectorant effects of these products.

2. Material and method

2.1. Raw material and extraction

Leaves of *Hedera nepalensis* were harvested in Ha Giang province, Vietnam. Plant identification was carried out by the National Institute of Medicinal Material (Hanoi, Vietnam). The collected leaves were cleaned, oven-dried at 55°C, and grounded. They were stored under dry and dark conditions at room temperature. Moisture content (7.21%) was determined before further experiments.

Hedera nepalensis leaves were extracted with ethanol 60% at 60 ^{0}C in 2 hours and filter. The process was repeated then combine both the filtrate and evaporate the solvent under reduced pressure to obtain an ethanol extract. The ethanol extract was purification by macroporous resin HPD 100 and evaporate all of the solvent to obtain the dried extract with a yield of extraction of 5.84%.

2.2. Preparation of lozenge

Prepare the lozenge by wet granulation method [10]. The compositions of lozenge are shown in Table 1.

Table 1. The recipe of *Hedera nepalensis* lozenge

Ingredients	Quantity per lozenge
Hedera nepalensis extract	26 mg
Sorbitol	200 mg
Sucrose	400 mg
Maltodextrin	100 mg
Sucralose	10 mg
Orange flavor	20 mg
Milk flavor	20 mg
Magnesium stearate	10 mg
PVP 5%/Ethanol	enough

Dissolve PVP K30 in EtOH 96% to make the binder solution 5%. Blend the sorbitol, sucrose and maltodextrin with *Hedera nepalensis* extract in a mixer. Wet the mixture by slowly adding binder solution and granulate by passing the mass through a 1mm sieve. Dry the resulting granules in an oven at 60°C. Reduce the size of the granules by passing through a 0.85 mm sieve. Add the sucralose, orange flavor, milk flavor and magnesium stearate with the dry granule and compress into lozenges at the predetermined weight (~800mg).

2.3. Preparation of syrup

The compositions of syrup are shown in Table 2.

Table 2. The recipe of *Hedera nepalensis* syrup

Ingredients	Quantity per bottle
Hedera nepalensis extract	700 mg
Sodium benzoate	50 mg
Kali sorbate	100 mg
Citric acid	300 mg
Sorbitol	30 g
Sucrose	20 g
Xanthan gum	50 mg
Peppermint oil	1.5 mg
Water	Enough 100 ml

Sucrose, kali sorbate and citric acid were mixed with water. Adding *Hedera nepalensis* extract in the mixture, stirring and heating at 65 ^{0}C into the uniform mixing. Add xanthan gum in water solution in the mixture. After gum swell up, made up the volume of syrup to 100 mL with water. Then the syrup was bottled and stored.

2.4. Microbial assay

** Preparation of test organism cultures*

Bacterial strains: The antibacterial effectiveness of the lozenge and syrup has been evaluated using six bacterial strains causing respiratory illnesses. Three strains of Gram positive (*Staphylococcus aureus* ATCC® 25923, *Streptococcus pneumoniae* ATCC® 49619 and *Streptococcus pyogenes* ATCC® 12344) and three strains of Gram negative (*Haemophilus influenzae* ATCC® 49247, *Klebsiella pneumoniae* sub sp. *pneumoniae* ATCC® 13883 and *Pseudomonas aeruginosa* ATCC® 27853) bacteria were obtained from The Global Bioresource Center (American Type Culture Collection-ATCC).

** Inoculums preparation*

The agar plates were incubated at 37 °C and the colonies forming on them were counted after 24 hours - Tryptic soy agar for *S. aureus*, *S. pyogenes*, *K. pneumoniae*, and *P. aeruginosa*, blood agar plate for *S. pneumoniae*, Chocolate agar plate for *H. influenzae*. Using a spectrophotometer, the bacterial growth was collected using 5 mL of sterile saline water, and its absorbance was adjusted at 580 nm and diluted such that the turbidity value of the standard of 0.5 MFU (McFarland Units) corresponds to about a culture density of 1.5 x 10^8 cells/mL.

** Qualitative antibacterial activity by disc diffusion assay*

Antibacterial activity of syrup and lozenge was performed using the disc diffusion method. The syrup sample is kept at the original concentration. The lozenges were ground into powder and reconstituted in sterilized double-distilled water to reach a concentration of 500 mg/mL. After that, 200 µL these test samples were loaded over preformed wells (15 mm in diameter) on the top of the agar medium suitable for each strain. The plates were incubated at 37 °C for 24 h. The inhibition zone surrounding the disc was measured by a ruler and was an indication of antibacterial activity: the larger the zone of inhibition, the more potent the bioactivity.

** Quantitative antibacterial activity by minimum inhibitory concentration*

The *in vitro* antibacterial activity of syrup and lozenge of *Hedera nepalensis* extract were done by determination of minimum inhibitory concentration (MIC). The MIC value of the syrup and lozenge was determined as the lowest concentration that completely inhibited bacterial growth after 24 hours of incubation at 37 °C. The MIC value was determined by two-fold serial dilution method in suitable culture media. The syrup sample was mixed with the test medium to form the following concentration range of 0.156-20% (*v/v*). For lozenges, a solution of 500 mg/mL lozenges were prepared with the test medium to form the following concentration range of 0.78-100 mg/mL. 1 µL of bacterial suspension was added to the surface of the agar plate and cultured for 24 hours at 37 °C with a density of ~1 × 10^4 CFU/mL. The agar plates were incubated at 37 °C and observed for counts of colonies growing on agar plates after 24 hours. The MIC is the lowest concentration of antimicrobial agents, which prevented the visible growth of bacteria on the agar plate. Amoxicillin at 0.0312 - 32 µg/mL was used as positive control.

2.5. Expectorant assay

** Preparation of the animals*

Male *Swiss albino* mice weighting 25 ± 2 gram (5-6 weeks) were used for the experiments. They were supplied by the Institute of Vaccines and Medical Biological Products in Nha Trang City. All animal procedures were conducted according to "Guidelines for Preclinical and Clinical Study of Traditional and Natural Medicines" approved by Ministry of Healthy-Vietnam (attached with Decision No.141/QĐ-K2ĐT dated on 27/10/2015). Tap water and standard laboratory food were made available ad libitum. The mice were allowed to acclimatize for at least 5 days prior to the experiment. The oral volume was 10 mL/kg body weight (B.W.).

Before any experience, all animals were kept for 1 week under the same laboratory

conditions of temperature (25 ± 2°C), relative humidity (70 ± 4%), and a 12 hour light/dark cycle and received a nutritionally standard diet and tap water.

** Method [11]*

Mice were randomly divided into the groups (n = 8): Normal control, sample groups (syrup 16 mL/kg, lozenge 4 lozenges/kg), positive control (Ambroxol® 240 mg/kg, Medical Import Export Joint Stock Company DOMESCO, Vietnam).

Mice in the test groups were orally administered the sample once daily for 3 days. One hour after taking the samples on the 3th day, mice injected by (2.5%) phenol red solution (10 mL/kg body weight i.p). Mice were euthanized by cervical dislocation thirty minutes after the injection of phenol red and immediately after the trachea was harvested which had bronchoalveolar fluid. The trachea was put in 2 mL physiological saline which was put into the ultrasonic bath at room temperature for 15 minutes. After, 2 mL natri bicarbonate (5%) added and the absorbence were measured at λ = 558 nm.

** Data analysis*

The data were expressed as mean values: Mean ± SEM (Standard error of the mean) and statistical treatment based on One-way ANOVA-test followed by Student Newman-Keuls test (SigmaStat 3.5). Test results were of the statistical significance of 95% of confidence when $p < 0.05$ compared with the normal control.

3. Results

3.1. Anti-microbial activity

The antimicrobial activity of the syrup and lozenge from *Hedera nepalensis* extract was investigated by the disk diffusion method to determine the antibacterial ability of these lozenge and syrup against some bacterial strains associated with respiratory diseases (Table 3). The results show that the lozenge and syrup inhibited the growth of 4/6 types of bacteria including *S. pyogenes*, *S. pneumoniae*, *H. influenzae,* and *K.pneumoniae.* Moreover, both the syrup and lozenge inhibited the growth of four bacterial strains at a moderate to strong level with inhibition zone diameter values ranging from 17.17 to 22.17 mm and from 16.83 to 23.00 mm, respectively.

Table 3. Antibacterial activity of syrup and lozenge from *Hedera nepalensis* extract on several strains of bacteria related to respiratory diseases

Microorganism		Zone of inhibition (mm)		
		Control	Syrup	Lozenge
Gram (+)	*S. aureus*	-	-	-
	S. pneumoniae	-	22.17 ± 0.44	23.00 ± 0.29
	S. pyogenes	-	18.33 ± 0.17	18.50 ± 0.29
Gram (-)	*H. influenzae*	-	17.17 ± 0.17	16.83 ± 0.33
	K. pneumoniae	-	17.33 ± 0.33	17.17 ± 0.44
	P. aeruginosa	-	-	-

Note: "-" has no antimicrobial activity; the inhibition zone diameter, including the diameter of the agar well is 15 mm. n = 3, Mean ± SEM.

Furthermore, the MIC values provided evidence for the inhibitory ability at low concentrations of the products (Table 4). The lower the MIC, the more sensitivity of bacteria react to tested extract. The MIC value of the syrup was 20% (v/v) for *K. pneumoniae,* while it was greater than 20% (v/v) for the remaining strains. The MIC value of the lozenge was 25 mg/mL for *S. pneumoniae* and *S. pyogenes,* while it was no less than 100 mg/mL for the others. Overall, among the bacteria tested, the *Gram* (-) group was less sensitive than the *Gram* (+) group to the lozenge. On the contrary, the effect of syrup against *Gram* (-) group was better but not really significant. This might be related to changes in bacterial characteristics, such as cell walls, the proportion of peptidoglycan, and the type of cross-linking effect of bacterial activity [12].

Table 4. MIC values of syrup and lozenge from *Hedera nepalensis* extract for several strains of bacteria related to respiratory diseases

Microorganism		MIC		
		Syrup (% *v/v*)	Lozenge (mg/mL)	Amoxicillin (µg/mL)
Gram (+)	*S. aureus*	>20	>100	0.125
	S. pneumoniae	>20	25	0.0312
	S. pyogenes	>20	25	<0.0312
Gram (-)	*H. influenzae*	>20	>100	2
	K. pneumoniae	20	100	>32
	P. aeruginosa	>20	>100	>32

3.2. Expectorant effect

The expectorant effect was evaluated by the optical absorbance of bronchoalveolar fluid mice and the percentage of increasing phenol red of the sample groups compared with normal control. Ambroxol® administered mice at dose of 240 mg/kg exerted a significant increasing effect (52.46%) on optical absorbance of bronchoalveolar fluid level as compared to normal control (*p=0.008*). Syrup at dose of 16 mL/kg and lozenges at dose of 4 lozenges/kg exerted a significant decrease (31.15%- 43.56%) on optical absorbance of bronchoalveolar fluid level as compared to normal control (*p= 0.031, p= 0.035, respectively*). All these are showed expectorant effect similar as ambroxol (*p= 0.168, p= 0.595, respectively*) (Table 5).

Table 5. Expectorant effect of syrup and lozenge from *Hedera nepalensis* extract

Groups (*n*=8)	Absorbance	Percentage of increasing phenol red (%)
Normal control	**0.053 ± 0.006**	-
Syrup 16 mL/kg	0.070 ± 0.004*	31.15
Lozenge 4 lozenges/kg	0.077 ± 0.006*	43.56
Ambroxol® 240 mg/kg	0.081 ± 0.009**	52.46

***p< 0.01: compared with the normal control; *p< 0.05: compared with the normal control.*

4. Discussion

Coughing up significant amounts of phlegm is a typical symptom of respiratory disorders. High sputum production may irritate the respiratory mucosa, resulting in coughing. [13]. The blocks of bronchioles will not only cause asthma, which results in further damage of the respiratory tract, but also increase the risk of respiratory infections. Therefore, it is necessary for researching the treatment with simultaneous anti-microbial, expectorant, and antitussive activities which can defensive against various respiratory disorders.

In this study, the pharmacological activity of the syrup and lozenge was confirmed by the antimicrobial activity of the products against a range of respiratory pathogens *S. pyogenes, S. pneumonia, K. pneumoniae* and *H. influenzae*. Respiratory pathophysiology was used to select pathogens. Although many of the pathogens investigated in this study are not the only causes of respiratory infections, most of them are known to cause respiratory infections in persons with weakened immune systems. *S. aureus* can cause otitis media and is also known to induce secondary infection in influenza patients, which can lead to death [14]. The antibacterial activity of the plant could be due to the presence of saponins [15],[16], with active compounds such as hederacoside C in particular [17],[18]. While the products showed a potential antibacterial activity against a variety of tested bacteria, they only had a negligible amount of antibacterial activity against the test bacteria, as determined by their MIC values. Further antimicrobial evaluation with the mixture of other plants for synergistic effect for the treatment of respiratory infections will be done in the future.

Sputum tenacity, which results in adhesiveness and cohesiveness of sputum, has a significant impact on sputum clearance. An expectorant is a medication that is supposed to promote mucus or periciliary fluid hydration. Although increasing sputum hydration will not increase clearance in the event of depleted airway surface liquid, increasing hydration of this surface fluid may separate secretions from the epithelium, lowering tenacity and boosting sputum transportability and clearance [19]. Syrup and lozenge, which contained *Hedera nepalensis* extract, significantly increased tracheal secretion. These products showed expectorant effects for mice in the test with the dose 16 mL/kg for syrup and 4 lozenges/kg for lozenge. These expectorant effects of these products are most likely due to their major components in the *Hedera nepalensis* extract such as saponin, in which hederacoside C and α-hederin are the most common major compounds [9],[20]. Recently, α-hederin has been shown to inhibit the inactivation of β2 receptors in the lungs and bronchi [21]. Additionally, it is known that hederacoside C is actively metabolized in the body and generates the action of α-hederin. The metabolic route of hederacoside C was discovered using a novel analytical technique, and the α-hederin biotransformation was detected *via* deglycosylation processes including the elimination of sugar moieties [22].

5. Conclusion

In the paper, we showed that the syrup and lozenges with the main ingredient from the extract of *Hedera nepalensis* has good antimicrobial and expectorant activity in the lab test. The results of this study could show the potential of this plant and these related products for treating respiratory problem and improving the health of Vietnamese people. However, further study in human population should be followed.

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EFFECT OF SOME MEDICINAL PLANT EXTRACTS AGAINST *SCLEROTIUM ROLFSII* CAUSING BASAL ROT OF CHINESE FOXGLOVE (*REHMANNIA GLUTINOSA*)

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Summary

Effect of some Medicinal Plant Extracts Against *Sclerotium rolfsii* Causing Basal Rot of Chinese Foxglove (*Rehmannia glutinosa*)

Four medicinal herbs (*Artemisia annua*, *Croton tonkinensis*, *Cinnamomum camphora*, and *Datura metel*) were extracted with 96% ethanol in a rate 1:10 (w/v). These dry extracts can be used at various concentrations (from 0.01% to 3.0%) *in vitro* in the PDA medium to inhibit the growth of *Sclerotium rolfsii* Sacc., which causes the basal rot of Chinese Foxglove *(Rehmannia glutinosa* Libosh.) in Bac Giang province. The results demonstrated that the extracts of *C.camphora* and *A. annua* at a concentration of 1.5% inhibited the development of *S. rolfsii*. Similarly, the extract from the *D. metal at* a concentration of 3% on the PDA medium, totally inhibited the *S. rolfsii* after 15 days of inoculation. Finally, a 3% concentration of the *C. tonkinensis* extract significantly inhibited the development of *S. rolfsii* in the PDA medium with the percentage of inhibition reaching over 71%; however 10 days after inoculation, the fungus stopped growing.

Keywords: *Extract, Artemisia annua, Croton tonkinensis, Cinnamomum camphora, Datura metel, Inhibition*

1. Introduction

Rehmannia glutinosa Libosh. is common medicinal plant which planted in some northern areas of Vietnam such as Bac Giang, Vinh Phuc, Phu Tho, and so on. *Rehmannia glutinosa* suffered from some severe diseases such as stem rot (*Sclerotium rolfsii*), root rot *(Rhizoctonia solani),* and leaf blight *(Phomopsis* sp.), in which stem rot was the most common disease in Bac Giang province [1].

Worldwide, *Sclerotium rolfsii* Sacc. is a serious soil-borne pathogenic fungus and causes severe damaged to many economically valuable crops in most of the tropical and subtropical regions [2]. *S. rolfsii* has a wide host range and has been referred to as an almost antipathogenic organism. In addition, *S. rolfsii* can maintain its continuity of generation under adverse conditions by forming sclerotia [3]. Hence, it is very difficult to control this disease even with the use of chemical fungicides.

There are numerous research using plant extracts to control *S. rolfsii* all over the world. Plants extract such as garlic, onion, ginger, neem leaf, and Allamanda leaf were tested for antifungal activities against *S. rolfsii*. The result indicated that the above plant extracts (the ratio of distilled water to plant parts was 200ml to 100g) was effectively produce percent mycelial growth that inhibits the growth of *S. rolfsii* in