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Expectorant and Antitussive Effect of *Hedera helix* and *Rhizoma coptidis* Extracts Mixture

Kee Jae Song,[1] Young-June Shin,[2] Kang Ro Lee,[3] Eun Jung Lee,[4] Yun Suk Suh,[4] and Kyung-Su Kim[4]

[1]Department of Otorhinolaryngology, International St. Mary's Hospital, Incheon;
[2]Research & Development Center, Ahngook Pharmacy Co. Ltd., Suwon;
[3]Natural Products Laboratory, School of Pharmacy, Sungkyunkwan University, Suwon;
[4]Department of Otorhinolaryngology, Yonsei University College of Medicine, Seoul, Korea.

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Corresponding author: Dr. Kyung-Su Kim,
Department of Otorhinolaryngology,
Gangnam Severance Hospital,
Yonsei University College of Medicine,
211 Eonju-ro, Gangnam-gu,
Seoul 135-720, Korea.
Tel: 82-2-2019-3463, Fax: 82-2-3463-4750
E-mail: ydrhinol@yuhs.ac

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Purpose: This study aims to investigate the additive effect of the *Hedera helix* (HH) and *Rhizoma coptidis* (RC) extracts mixture on antitussive and expectorant activities in animals. **Materials and Methods:** The expectorant assay was performed with phenol red secretion in mice trachea. Mice or guinea pigs were randomly divided into groups of 8 each, including negative and positive control groups. After gastric administration of the test extracts in mice, 2.5% phenol red solution (0.2 mL) was intraperitoneally injected. Trachea was dissected and optical density of tracheal secretion was measured. After gastric administration of the test extracts in guinea pigs, the antitussive activities were assessed using a citric acid-induced cough measurement. **Results:** The extracts of HH and RC significantly increased tracheal secretion and inhibited cough. The mixture of HH and RC extracts in a 1:1 concentration at a dose of 200 mg/kg showed a more potent effect on phenol red secretion (25.25±3.14) and cough inhibition (61.25±5.36) than the individual use of each extracts [phenol red secretion; HH 13.39±4.22 (p=0.000), RC 20.78±2.50 (p=0.010), cough inhibition; HH 9.89±4.14 (p=0.010), RC 30.25±7.69 (p=0.000)]. A 3:1 ratio mixture of HH to RC demonstrated an optimal expectorant effect (p<0.001), and this mixture showed expectorant and antitussive effects in a dose-dependent manner. **Conclusion:** This study provides evidence for antitussive and expectorant effect of a 3:1 mixture of HH and RC, which may be a useful therapeutic option for respiratory diseases.

Key Words: *Hedera helix*, *Rhizoma coptidis*, expectorant, antitussive agent

INTRODUCTION

Even though coughing is a natural protective reflex to remove airway secretions and pathogens from the respiratory tract, it is one of the common symptoms associated with chronic inflammatory disease of the respiratory tract such as asthma, chronic bronchitis, pneumonia and postnasal drip syndrome.[1] At present, a cough can be regulated by medications such as codeine and dextromethorphan, which are

associated with side effects including drug dependency or drowsiness. Sputum tenacity, which results in adhesiveness and cohesiveness of sputum, considerably influences the clearance of sputum.[2] An expectorant is a drug that is thought to increase the hydration of either mucus or the periciliary fluid.[3] Although increasing hydration of the sputum itself will not improve clearance in the case of depleted airway surface liquid, improving the hydration of this surface fluid may detach secretions from the epithelium, thus decreasing tenacity and improving transportability and clearance of sputum.[4,5] Therefore, there is an increasing demand for drugs that promote antitussive and expectorant activities with fewer adverse effects.

Hedera helix (common ivy, English ivy, European ivy, or just ivy: HH) has been used for centuries to treat diverse diseases as folk medicine in Europe. Decoctions made from the leaves of HH were used during the 19th century to treat catarrh of the respiratory tract. Nowadays the dry extract of HH is commercially formulated, and many positive studies have been published to prove that this extract is an effective and well-tolerated therapeutics in both children and adults suffering from respiratory diseases.[6] Sieben, et al.[7] reported that the secretolytic effects of HH extract are due to its saponin contents, particularly α hederin, as an inhibitor of the β2 receptors endocytosis. Moreover, the same group also elucidated that α hederin has bronchodilatory effect in bovine trachea via the same mechanism.[8]

In traditional medicine, many natural products have been used to treat cough and sputum for thousands of years, and have shown minimal side effects.[9] Thus, it is valuable to search for effective treatments for both cough and sputum among natural products because of their low toxicity and fewer side effects. We reported that berberine suppresses the expression of *MUC5AC* gene, one of the major mucin producing genes in chronic inflammatory airway diseases,[10] and Sánchez-Mendoza, et al.[11] showed that berberine has bronchodilatory activity. Through these works, we postulated that medicinal plants of which major constituent is berberine, and which have been prescribed for respiratory inflammatory diseases in traditional Chinese medicine, may show expectorant effect and antitussive effect. In this study, therefore, *Rhizoma coptidis* (RC) was selected, and we investigated expectorant and antitussive effect of each HH and RC extract. If any positive effects were observed in individual extract, additive effects of HH and RC extract mixture were investigated.

MATERIALS AND METHODS

Plant materials and chemicals

Dried leaf of HH and dried RC were purchased at the Kyungdong herbal market in Seoul and were identified by Prof. Kang Ro Lee (Sungkyunkwan University, Suwon, Korea). The voucher specimens of HH and RC were preserved under the reference number SKKU-NPL 1226 and 1227 at the Herbarium of Natural Products Laboratory, School of Pharmacy, Sungkyunkwan University. Ambroxol, phenol red, theobromine, and citric acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Preparation of the extracts

Dried leaf of HH was percolated with 30% ethanol for 6 hours. Then, the ethanol extract was spray-dried. RC was refluxed twice with water-saturated butanol at 80°C, each for 5 hours and 3 hours. Subsequently, the percolating material, such as ethanol or butanol, was removed via spray-drying or vacuum evaporization technique. The final solid extract was obtained via freeze-drying technique. The yields of dried extracts as a percentage weight of HH and RC were 14.5% and 15.7%, respectively.

Expectorant assay using phenol red secretion in mice trachea

Eight week-old male ICR mice were purchased from Orient Bio Inc. (Gapyeong, Korea). The animals were housed at room temperature (23±3°C) with constant humidity (55±15%) under a 12 hour light-dark cycle with food and water available ad libitum. After 3–5 days of adaptation, the eligible animals were randomly divided into groups of 8 mice each, including negative and positive (ambroxol) control groups. All procedures involving animals were conducted at facilities of the Gyeonggi Bio-Center (Suwon, Korea), and all protocols were approved by the Institutional Ethical Committee of the Gyeonggi Bio-Center. The procedures were carried out as described previously.[12,13] Briefly, each natural product extract was administered via the gastric route. Thirty minutes after gastric administration of the test extracts, 2.5% phenol red solution (0.2 mL) was intraperitoneally injected. Then, 30 minutes after the application of phenol red, the mice were sacrificed. Trachea was dissected and immediately placed into 1 mL of normal saline. After the trachea was washed, 0.1 mL of 1 M NaOH was added to the saline and the optical density was measured at 546

nm using a microplate reader (Molecular Devices, Sunnyvale, CA, USA). Data were expressed as a percentage of the optical density of each experimental sample compared to that of the negative control (being derived from a mouse trachea not treated with any agent). The expectorant activities were assessed by the increase of the optical density in terms of that in negative control groups by using the following equation: the percentage of increase=$[(D_t-D_0)/D_0\times100]$ (D_0: the optical density of negative control, D_t: the optical density of the treatment group). Thus, data represent a percentage of increased sputum clearance by each agent. The data were analyzed by Kruskal-Wallis test and Mann-Whitney test for significance among groups. Statistical analyses were performed by using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA).

Antitussive assay using citric acid-induced cough in guinea pig

Six week-old Hartley male guinea pigs, weighing 360–380 g, were purchased from Daehan Biolink Co. (Daejeon, Korea). The animals were housed at room temperature (23±3°C) with constant humidity (55±15%) under a 12 hour light-dark cycle with food and water available ad libitum. After 3–5 days of adaptation, the eligible animals were randomly divided into groups of 8 guinea pigs each, including negative and positive (theobromine) control groups. An aerosol delivery system (Buxco Electronics, Wilmington, NC, USA) was used for this experiment. The cough reflex was induced as described previously with modification.[14] Briefly, 60 minutes after gastric administration of drugs, the guinea pigs were exposed to a nebulized solution of 0.2 M citric acid under conscious condition for 10 minutes. Then, each guinea pig was placed in a 2 L glass chamber with a plethysmograph system and the number of coughs was counted for 15 minutes. The antitussive activities were expressed as the percentage of inhibition of the number of coughs in terms of that in control groups by using the following equation: the percentage of inhibition=$[(C_0-C_t)/C_0\times100]$ (C_0: the number of coughs of the negative control, C_t: the number of coughs of the treatment group). Thus, the data represent a percentage of cough inhibition by each agent. The data were analyzed by Kruskal-Wallis test and Mann-Whitney test for significance among groups. Statistical analyses were performed by using SPSS version 18.0.

RESULTS

Expectorant and antitussive effect of each extract

In the expectorant and antitussive assay, each extract was administered via gastric route at a dose of 100 and 200 mg/kg. The expectorant and antitussive effect of HH and RC was compared with the negative control and the results are listed in Table 1. No serious adverse event, such as liver failure or renal failure which leads to death, was found during the study.

Additive effect of the mixture of HH and RC extracts on expectoration and cough inhibition

To examine their additive effect, a single treatment was compared to a mixture of HH and RC extracts for expectoration and cough inhibition. In the expectorant assay, a mix-

Table 1. Effect of *Hedera helix* (HH), *Rhizoma coptidis* (RC) Extracts and the Mixture of HH and RC on Phenol Red Secretion and Cough Inhibition According to Dose

Group	Increase of phenol red secretion (%)	Cough inhibition (%)
Positive control		
Ambroxol (250 mg/kg)	25.80±2.41	-
Theobromine (50 mg/kg)	-	53.22±5.66
100 mg/kg		
HH	5.33±6.79	30.00±11.62
RC	8.84±3.17	24.58±9.12
HH+RC (1:1 mixture)	14.81±4.45	46.97±5.49
200 mg/kg		
HH	13.39±4.22	39.89±4.14
RC	20.78±2.50	30.25±7.69
HH+RC (1:1 mixture)	25.25±3.14	61.25±5.36

All data were compared to the negative control group and were shown as the mean±standard deviation. For the phenol red secretion experiment, each group was composed of 8 mice and 64 mice were used in total including negative control. For the cough inhibition experiment, each group was composed of 8 guinea pigs and 64 guinea pigs were used in total including negative control.

ture of HH and RC extracts in a 1:1 concentration significantly increased phenol red secretion in a dose-dependent manner, and this increase was much more than that in the use of the extracts individually (at a dose of 200 mg/kg; p=0.000 compared to HH, p=0.010 compared to RC). However, phenol red secretion of 1:1 mixture of HH and RC showed no significant difference compared the positive control at a dose of 200 mg/kg (p=0.721) (Table 1, Fig. 1A). In the antitussive assay, a mixture of HH and RC extracts in a 1:1 concentration significantly inhibited cough in a dose-dependent manner, and this inhibition was also much more than that in the use of the extracts individually (at a dose of 200 mg/kg; p=0.010 compared to HH, p=0.000 compared to RC) (Table 1, Fig. 1B). Furthermore, cough inhibition of 1:1 mixture of HH and RC showed more potency than the positive control at a dose of 200 mg/kg (p=0.038).

Optimal mixing ratio and dose of HH and RC extracts

Since 200 mg/kg of the 1:1 mixture of HH and RC extracts showed an additive effect, we attempted to find an optimal mixing ratio for additive expectorant and antitussive effect. Thus, multiple mixing ratios of HH and RC (200 mg/kg) were examined. At a dose of 200 mg/kg, a 3:1 ratio of HH to RC showed a maximal expectorant effect and was statistically significantly more potent than the positive control (p<0.001) (Table 2, Fig. 2). Thus, this 3:1 ratio was used in the following experiments.

To determine an optimal dose of a 3:1 ratio mixture of HH

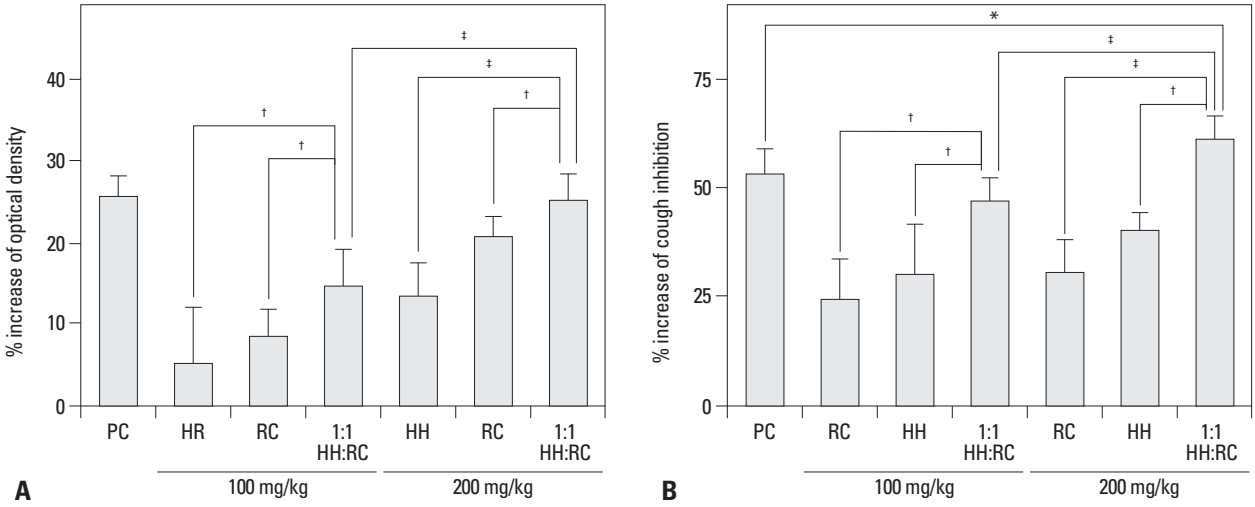

Fig. 1. Effect of *Hedera helix* (HH), *Rhizoma coptidis* (RC) extracts and the mixture of HH and RC on phenol red secretion and cough inhibition according to the dose. (A) The expectorant assay using phenol red secretion. A mixture of HH and RC extracts in a 1:1 concentration significantly increased phenol red secretion in a dose-dependent manner, and this increase was more significant than the use of the extracts individually. (B) The antitussive assay using citric acid-induced cough. A mixture of HH and RC extracts in a 1:1 concentration significantly inhibited cough in a dose-dependent manner, and this increase was also more significant than the use of the extracts individually. PC, the positive control group. *p<0.05, †p<0.01, ‡p<0.001.

Table 2. Effect of the Mixture of *Hedera helix* (HH) and *Rhizoma coptidis* (RC) Extracts on Phenol Red Secretion According to Various Mixing Ratios

Group	Increase of phenol red secretion (%)
Positive control (ambroxol 250 mg/kg)	25.55±2.06
HH 200 mg/kg	13.20±5.23
RC 200 mg/kg	21.87±2.31
HH:RC mixture 200 mg/kg	
1:1	22.35±2.81
2:1	23.34±2.28
3:1	27.48±4.45
4:1	19.74±2.92
5:1	15.19±2.73

All data were compared to the negative control group and were shown as the mean±standard deviation. Each group was composed of 8 mice and 72 mice were used in total including negative control.

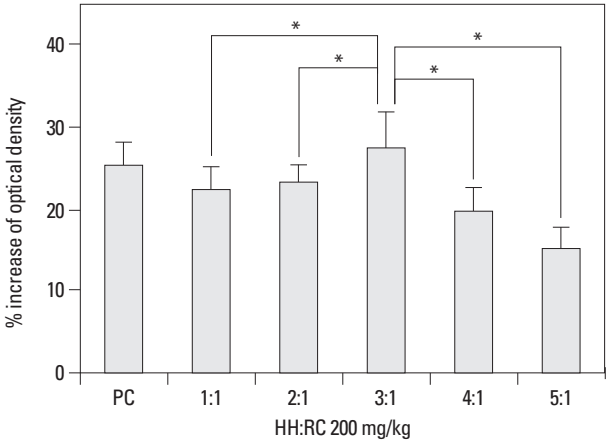

Fig. 2. Effect of mixture of *Hedera helix* (HH) and *Rhizoma coptidis* (RC) extracts on phenol red secretion according to various mixing ratios. A 3:1 ratio of HH to RC showed a maximal expectorant effect. PC, the positive control group. *p<0.001.

and RC extracts, various doses were examined. The mixture of HH and RC extracts showed a dose-dependent increase of phenol red secretion and inhibition of cough (p<0.001). Even though there was no significant difference from the positive control (p>0.05), the effect of this mixture on expectoration was as much potent as that of the positive control at a dose of 200 mg/kg (Table 3, Fig. 3A), while the effect of this mixture on cough inhibition was statistically more potent than that of the positive control at a dose of 200 mg/kg (p<0.001) (Table 3, Fig. 3B). Therefore, the optimal dose of a 3:1 ratio mixture of HH and RC extracts for both expectorant and antitussive effect was determined as 200 mg/kg.

DISCUSSION

There has been a great effort to utilize natural products as medications that have been traditionally used for thousands of years. Currently, dried leaf of HH extract is commercially used as an antitussive agent. Nevertheless, there is an increasing demand for drugs with more potency and fewer side effects.[6] In the present study, therefore, we investigated the interaction between HH and RC.

Each extract showed significant cough inhibition and expectorant effects. We investigated whether an HH and RC mixture (at a 1:1 concentration) shows a more potent expectorant and antitussive effect than the use of extracts individually. The results showed that the HH and RC mixture had more potent effects in the expectoration and antitussive assays compared to the extracts individually in a dose-dependent manner. Therefore, the HH and RC mixture seemed to be a strong candidate in developing an effective expectorant and antitussive agent. As for the optimal mixing ratio of HH and RC extracts, a 3:1 mixture of HH and RC showed a potent effect at a dose of 200 mg/kg.

These expectorant and antitussive effects of HH and RC are most likely due to their major components such as saponin and berberine, respectively. Saponins (hederacoside C and α hederin), flavonoid (rutin), and phenolic derivatives (chlorogenic acid) are the main components of the dry

Table 3. Effect of the 3:1 Ratio Mixture of *Hedera helix* (HH) and *Rhizoma coptidis* (RC) Extracts on Phenol Red Secretion and Cough Inhibition According to Dose

Group	Increase of phenol red secretion (%)	Cough inhibition (%)
HH+RC (3:1 mixture)		
25 mg/kg	13.64±3.81	34.86±12.37
50 mg/kg	11.59±5.43	35.80±11.74
100 mg/kg	14.81±5.39	58.64±5.85
200 mg/kg	27.48±4.45	73.02±4.02

All data were compared to the negative control group and were shown as the mean±standard deviation. For the phenol red secretion experiment, each group was composed of 8 mice and 40 mice were used in total including negative control. For the cough inhibition experiment, each group was composed of 8 guinea pigs and 40 guinea pigs were used in total including negative control.

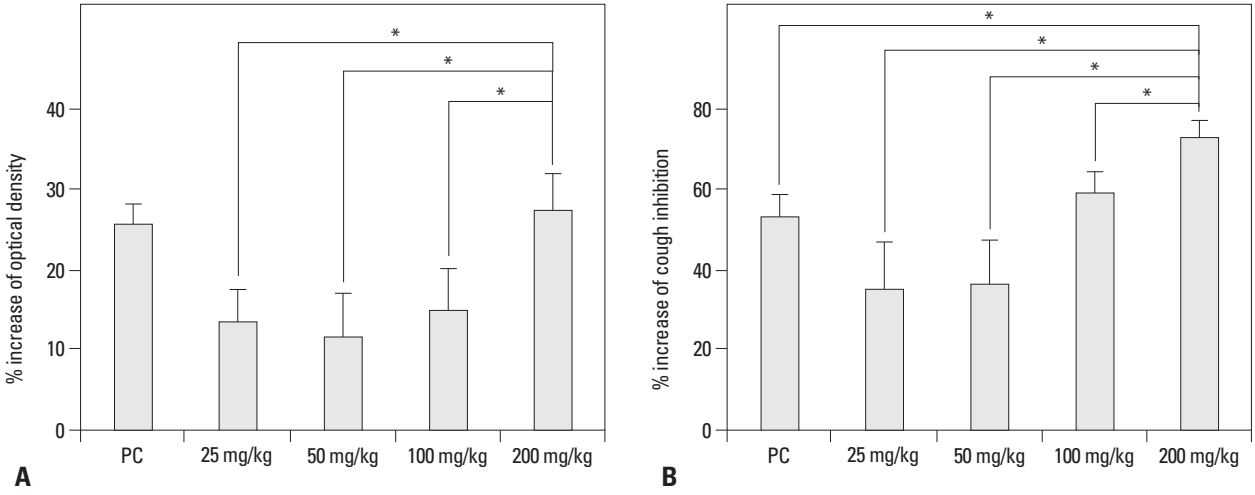

Fig. 3. Effect of 3:1 ratio mixture of *Hedera helix* (HH) and *Rhizoma coptidis* (RC) extracts on phenol red secretion and cough inhibition according to dose. (A) In the expectorant assay using phenol red secretion, a mixture of HH and RC extracts in a 3:1 concentration showed dose-dependent increase of phenol red secretion. (B) In the antitussive assay using citric acid-induced cough, a mixture of HH and RC extracts in a 3:1 concentration significantly inhibited cough in a dose-dependent manner. PC, the positive control group. *p<0.001.

extract of HH. Approximately 10% of the HH extract consists of a complex mixture of saponins, and the expectorant activity of saponins had been thought to be mediated by the gastric mucosa, with reflex stimulation of the bronchial mucous glands via the parasympathetic pathway. Recently, α hederin, one of the major components of HH saponins, has been shown to inhibit the inactivation of β2 receptors in the lungs and bronchi. It is, therefore, likely that more functioning receptors are present on the surface of the cell and the adrenaline present can have a greater effect.[7] HH saponins have also been shown to have spasmolytic, bronchodilatory and antibacterial activities.[8,15,16]

RC is a widely used traditional Chinese medicine. Many studies have demonstrated that RC has a wide range of pharmacological properties, such as analgesic, anti-inflammatory and antibacterial activities.[17-20] RC has various alkaloids constituent, such as berberine, coptisine, palmatine and epiberberine.[21] Among them, berberine is the most important and potent constituent. It has been reported that berberine has bronchodilatory and antimicrobial activities.[11,22] An additional action of berberine is to inhibit the mucin component of sputum. Through the ERK- and p38 MAPK-dependent signal transduction pathways, berberine has been shown to suppress IL1β-induced *MUC5AC* gene expression in human airway epithelial cells,[10] suggesting that berberine can inhibit mucin production in sputum as well as having expectorant and antitussive effects. These mechanisms are most likely involved in both expectorant and antitussive effects of the extracts of HH and RC.

In the present study, we showed that HH and RC extracts mixture had good expectorant and antitussive effects in animal models and the optimal mixing ratio was 3:1 ratio of HH and RC. The present study is expected to provide convincing evidence for the 3:1 HH and RC mixture to be used as one antitussive and expectorant medicine. However, further study in human population should be followed.

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