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Antitussive and antibacterial activity of *Trompang Elepante* (*Heliotropium indicum* Linn.)

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ABSTRACT

Heliotropium indicum is used in folkloric medicine for many diseases. In this present study, we established the antitussive and antibacterial activities of ethanol extract of H. indicum leaves and to elucidate the phytochemical components. The antitussive effect of the extract was evaluated in mice in the citric acid saturated chamber while antibacterial activity was evaluated using disc diffusion assay. Results revealed that the 100% of extract syrup of H. indicum significantly recorded the lowest number of cough of 2.0 which statistically comparable with dextromethorphan. However, mice treated with 50% extract syrup had a mean cough number of 4.67. H. indicum ethanol extract also showed antibacterial activity against K. pneumoniae and S. pyogenes having zone of inhibitions of 9.55 mm and 9.81 mm, respectively. Air-dried leaves of this plant contain alkaloids, saponins, cardiac glycosides and terpenoids. These significant findings suggest that H. indicum is another promising plant source of important compounds with antibacterial and antitussive properties.

Keywords: *H. indicum*, antitussive, antibacterial, phytochemicals, *trompang elepante*

INTRODUCTION

Plants have been applied and most used for medicinal and pharmacological for a long period of time. Several medicinal plants have been used for treatment of several diseases and have been extensively reported in Ayurvedic system. These include *Aloe vera*, *Azadirachta indica*, *Carica papaya*, *Celosia argentea*, *Centella asiatica*, *Cinnamomum zeylanicum*, *Curcuma longa*, *Nelumbo nucifera*, *Ocimum sanctum*, *Phyllanthus emblica*, *Plumbago zeylanica*, *Pterocarpus santalinus*, *Terminalia arjuna*, and *Terminalia chebula* [1]. They have been considered as valuable resource of natural products and bioactive compounds which could play important roles in maintaining healthy life style.

Heliotropium indicum, belong to Family Boraginaceae, is an annual, erect, robust, succulent herb with branched hairy stem and opposite or alternate, ovate to oblong dense leaves that can grow to a height of about 15 to 50 cm. This plant is also known as *trompang elepante*, *buntot-leon*, *higad-higaran* in Tagalog, *kambra-kambra* in Bisaya, and *pengnga-pengnga* in Ilocano. In folkloric medicine, *H. indicum* is highly valued in treating dermatitis, venereal diseases, insect bites, scabies, skin pruritis, menstrual disorder, malaria, abdominal pain, fever, urticaria, and sore throat. Its crude extract is applied to cure rheumatism and skin infections while its decoction is used as diuretic and

kidney stone remedy [2][3]. However, studies showed that *H. indicum* extract possess significant anti-inflammatory, diuretic, abortifacient, wound healing and antitumor activities [4].

Antitussive is any agent that suppresses coughing or that is effective against cough. A number of different cough medications include benilyn, sudafed, robitussin, vicks and among others that contain active ingredients such as antihistamines, expectorants, decongestants and antipyretics [5]. In the Philippines, lagundi (*Vitex nigundo*) is one of the most common plants which found effective to relief cough due to common cold, flu and mild to moderate bronchitis and cough of bacterial origin. Plemex lagundi is commercially available in the market. However, the searching for agents or supplements derived from antibacterial plants have also accelerated. Because it is believe that plants are natural source of phytochemicals such as terpenoids, alkaloids, tannins, and flavonoids, which exhibits antimicrobial properties.

In this present work, we established the antitussive effect in citric acid cough-induced mice, antibacterial activity against *Klebsiella pneumonia* and *Streptococcus pyogenes*, and phytochemical attributes of *H. indicum* with our intention to provide alternative resource of plant-derived properties with promising human healthful benefits.

MATERIALS AND METHODS

2.1 Plant Collection and Preparation:

H. indicum leaves were collected from Bongabon, Nueva Ecija, sealed in polypropylene plastics and brought in the laboratory for processing. The collected plants samples were air-dried for 5 days, pulverized using mortar and pestle and prepared for ethanol extraction.

2.2 Ethanol Extraction:

The active components of the milled plant sample (100 g) were extracted in 1 L of 95% ethanol for 48 h. After which, the extract was obtained through filtration using Whatman filter paper and concentrated in rotary evaporator at the Chemistry Laboratory of the Philippine Rice Research Institute, Maligaya, Science City of Munoz, Nueva Ecija. The extract was used for antitussive and antibacterial evaluation.

2.3 Experimental Animals:

Four-week old female mice weighing 21-26 g were used in this study. Mice were housed in cages, fed with standard pellet diet and water ad libitum, maintained under standard conditions (12 h light and 12 h dark regime; 25°C). They were acclimatized in this condition for 7 days prior to antitussive evaluation.

2.4 Antitussive Effect:

Acclimatized mice were exposed to citric acid by enclosing the animals in a cough chamber. They were nebulized and allowed citric acid to saturate cough chamber for 5 minutes. The number of cough in 10 min was recorded. After which, mice were orally administered with 0.5 ml of 50% and 100% extract syrup (30 grams of sucrose was dissolved in 50 ml ethanol extract), dextromethorpan (positive control), and distilled water (placebo). Triplicate mice were administered per treatment. The number of cough for 10 min was again recorded. Evaluation was done in two trials.

2.5 Antibacterial Activity:

The antibacterial activity of *H. indicum* extract was determined following the paper disc diffusion method of Bauer et al. [6]. *K. pneumoniae* and *S. pyogenes* were cultured in 9 ml of nutrient broth (NB) medium and incubated at 37 °C. After 24 hours, the turbidity of each bacterial culture was adjusted to equal that of 0.5 McFarland standard, which approximated 1.5×10^8 ml^{-1}. The bacterial suspension was spread using a sterile cotton swab on nutrient agar plate. Six millimetre diameter paper discs impregnated with ethanol extract and streptomycin as standard were placed equidistantly on the medium. Plates were incubated at 37 °C, and the zones of inhibition were measured after 24 hours. Each test was done in triplicate.

2.6 Phytochemical Analysis:

The chemical screening of the extract of *H. indicum* were carried out following the procedures described by Edeoga et al. [7]. The extract was screened for the presence of tannins, alkaloids, flavonoids, saponins, terpenoids, and cardiac glycosides. Results were determined based on the color/intensity of the reaction and compared with distilled water as control. Three replicates were laid out for each test parameter.

2.7 Statistical Analysis:
Data were analyzed using Analysis of Variance (ANOVA) in SAS Statistical Program. Treatment means were compared using Least Significant Difference (LSD) at 5% level of significance.

RESULTS AND DISCUSSION

3.1 Antitussive Effect of *H. indicum* extract:
There are several mechanisms that cause cough including chemicals, inflammatory mediators, mechanical and neurotransmitter stimulation of normal afferent system [8]. Some causes of cough are due to common cold, flu and mild to moderate bronchitis and cough of bacterial origin. In order to establish the antitussive effect of *H. indicum* ethanol extract, this study was necessarily carried out. Table 1 presents the number of cough of mice before and after oral administration of extract syrup of *H. indicum* in a citric acid chamber for 10 min. It can be seen that before oral administration, the baseline number of cough was ranged from 6.33 to 6.67. However, significant difference was observed after treatment administration. Among the two extract syrup concentrations, 100% extract syrup-treated mice significantly recorded the lowest number of cough of 2.0 which statistically comparable with dextromethorphan-treated mice with a mean of 1.67. Mice treated with 50% extract syrup had a mean cough number of 4.67 which significantly comparable with placebo mice. Interestingly, these results suggest that the pure extract syrup of *H. indicum* has a very promising effect in relieving cough. However, although the antitussive effect of extract of this plant was comparable to that of dextromethorphan, the mechanism(s) of effect of the extract cannot be concluded yet based from the results of the present work. Thus, further study on the mechanism of effect is highly recommended.

Table 1. Number of cough of mice before and after oral administration of extract syrup of *H. indicum* in a citric acid chamber for 10 min

Treatment	Number of Cough of Mice	
	Before oral administration	After oral administration
100% extract syrup	6.67	2.00[b]
50% extract syrup	6.33	4.67[a]
Dextromethorpan	6.67	1.67[b]
Distilled water	6.33	6.33[a]

In the mean column, means having the same letter of superscript in the same column are not significantly different from each other using LSD at 5% level of significance.

Morphine and codeine are generally considered to be the most potent and effective antitussive drugs that inhibit cough through suppression of cough center in the CNS [9]. In natural resource, some plants have also been found effective as cough inhibitor. For example, boiled extract of *Portulaca oleracea* L. showed significant reduction of cough numbers of guinea pigs [10]. The mixture of *Hedera helix* and *Rhizoma copditis* extracts in a 1:1 concentration at a dose of 200 mg/kg showed a potent effect on phenol red secretion and cough inhibition in mice and guinea pigs [11]. In addition, *Althaea officinalis* polysaccharide rhamnogalacturonan inhibits cough reflex in dose-dependent manner in unsensitized guinea pigs [12]. These antitussive mechanisms of these plants including *H. indicum*, may be due to the suppression of neurotransmitter release or may be due to its relaxant effect or bronchodilator activity, but the exact mechanism must be considered in future studies.

3.2 Antibacterial Activity of *H. indicum* Extract:
To study further the biological activities of *H. indicum*, the antibacterial activity of the ethanol extracts of *H. indicum* leaves against the two human pathogenic microbes, *K. pneumoniae* and *S. pyogens*, was investigated. The mean diameter zone of inhibitions of the extract of *H. indicum*, streptomycin (positive control) and distilled water (negative control) against *K. pneumonia* and *S. pyogens* is shown in Table 2. Based on the results of the bioassay test, ethanol extract of *H. indicum* showed zone of inhibitions in both bacteria. Ethanol extract of *H. indicum* had the diameter zone of inhibition of 9.55 mm against *K. pneumonia* and 9.81 mm against *S. pyogens*. These diameters were found statistically differed from distilled water showing no activity. Although, significant difference was found between the extract and streptomycin, the presence of zone of inhibition strongly indicate that the ethanol extract of the plant tested has a promising potential as antibacterial agent against *K. pneumonia* and *S. pyogens*. This antibacterial activity of *H. indicum* ethanol extract could be explained by their phytochemical properties which were reported previously for their applications as antimicrobial with diverse micro-community and as natural antibiotics for microbial infections in plants, animals and human.

A wide variety of plants have been evaluated for antibacterial activity. Among the twelve Philippine medicinal plants evaluated for antibacterial activity against multidrug-resistant bacteria, *Psidium guajava*, *Phyllanthus niruri*, *Ehretia microphylla* and *Piper betle* showed antibacterial activity against the methicillin-resistant *S. aureus* and vancomycin-resistant *Enterococcus*. *P. betle* had the greatest potential value against multidrug-resistant bacteria [13]. Moreover, four out of eleven medicinal plants from Cordillera Region, Philippines namely *Agathis dammara*, *Eupatorium triplenerve*, *Citrus aurantifola* and *Tithonia divserifolia* showed antibacterial properties against *B. subtilis*, *S. aureus* and *P. vulgaris* [14].

Table 2. Diameter zone of inhibition of *H. indicum* ethanol extract against *K. pneumonia* and *S. pyogens*

Treatment	Diameter zone of inhibition (mm) against	
	K. pneumoniae	*S. pyogens*
Ethanol extract	9.55[b]	9.81[b]
Streptomycin	29.10[a]	31.02[a]
Distilled water	0.00[c]	0.00[c]

In the mean column, means having the same letter of superscript in the same column are not significantly different from each other using LSD at 5% level of significance.

3.3 Phytochemical Composition:

The phytochemical screening of the *H. indicum* leaves was also conducted in the present study. Table 3 shows the phytochemical components of *H. indicum* leaves. It can be noticed that among the six phytochemicals screened, four were detected in traceable amounts that include saponins, terpenoids, cardiac glycosides and alkaloids. Phytochemicals are known to exhibit different functionalities. Saponins reduce the uptake of certain nutrients including glucose and cholesterol at the gut through intra-lumenal physicochemical interaction and can inhibit the growth of cancer cells, lower cholesterol, boost immune system and energy, act as natural antibiotic, anti-inflammatory, and anti-oxidant [15].

Table 3. Phytochemical compositions of the leaves of *H. indicum*

Parameter	Results
Tannins	Negative
Saponins	Positive
Flavonoids	Negative
Terpenoids	Positive
Cardiac glycosides	Positive
Alkaloids	Positive

Terpenoids have been found to be useful in the prevention and therapy of several diseases including anti-tumor, cancer, and also to have antimicrobial, antifungal, antiparasitic, antihyperglycemic, anti-inflammatory, antiimmunomodulatory properties [16]. Terpenoids can be used as protective substances in storing agriculture products as they are known to have insecticidal properties [17]. On the other hand, cardiac glycosides are primarily involved in the treatment of cardiac failure. It increased cardiac output and used in treatment of congestive heart failure and cardiac arrhythmia. Thus, the presence of cardiac glycosides shows medicinal activity. The pharmaceutical properties of plants may also be recognized to the presence of alkaloids which has been reported to have a stimulating effect, act as topical anaesthetic in ophthalmology, powerful pain reliever, antipuretic action among other uses [18]. The presence of alkaloids in the plant extract explains antibacterial activity.

In conclusion, *H. indicum* leaves ethanol extract contains alkaloids, saponins, cardiac glycosides and terpenoids which are important and valuable compounds that exhibit antibacterial activity against *K. pneumoniae* and *S. pyogens* and antitussive effect in mice cough induced by citric acid. However, further studies on the mechanism of action of these bioactive components must take into consideration.

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