Cytotoxic, Anti-inflammatory and Antioxidant Activities of *Heliotropium indicum* Extracts

Jitpisute Chunthorng-Orn MSc*, Bhanuz Dechayont MSc*,
Pathompong Phuaklee MSc*, Onmanee Prajuabjinda MSc*,
Thana Juckmeta MSc**, Arunporn Itharat PhD *.**

** Department of Applied Thai Traditional Medicine, Faculty of Medicine, Thammasat University, Pathumthani, Thailand*
*** Center of Excellence on Applied Thai Traditional Medicine Research (CEATMR), Faculty of Medicine, Thammasat University, Pathumthani, Thailand*

Background: *Heliotropium indicum Linn., or 'Indian heliotrope' is very common in India with a long history of traditional medicinal uses in many countries in the world. In Thailand, the plant has been traditionally use to cure various diseases such as fever, insect bites, stings, diarrhea, skin rashes, menstrual disorder and urticaria. In addition, the plant is commonly used by Thai folk doctors as a component in remedies for treatment of lung cancer.*
Objective: *In the present study, we investigated cytotoxicity against two types of lung cancer cell lines (A549 and NCI-H226), anti-inflammatory effect and antioxidant activity of Heliotropium indicum extracts.*
Material and Method: *The water and ethanolic extracts of Heliotropium indicum were tested. The cytotoxic activity against two types of human lung cancer cell lines (A549 and NCI-H226) was evaluated by sulforhodamine B (SRB) assay. The anti-inflammatory effect was investigated on lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages. LPS-induced nitric oxide (NO) production was determined by Griess reagent. The antioxidant activity was performed by 1, 1-diphenyl-picrylhydrazyl (DPPH) radical scavenging method.*
Results: *The ethanolic extract showed cytotoxic activity only against NCI-H226 (IC_{50} = 51.90±2.35 μg/ml) whereas the water extract had no cytotoxic activity against both A549 and NCI-H226 (IC_{50} >100 μg/ml). For anti-inflammatory effect, the results revealed that the ethanolic extract exhibited the most potent inhibitory activity on nitric oxide production (IC_{50} = 24.17±2.12 μg/ml), followed by Indomethacin (positive control) with an IC_{50} value of 34.67±6.23 μg/ml while water extract was apparently inactive (IC_{50} >100 μg/ml). For antioxidant activity, the ethanolic extract showed high antioxidant activity (EC_{50} = 28.91±4.26 μg/ml) but the water extract showed no antioxidant activity (EC_{50} >100 μg/ml).*
Conclusion: *These results can support using Heliotropium indicum Linn. for component in lung cancer remedy by Thai folk doctors. However, more studies are required.*

Keywords: *Cytotoxicity, Anti-inflammatory, Antioxidant, Heliotropium indicum Linn*

J Med Assoc Thai 2016; 99 (Suppl. 4): S102-S109
Full text. e-Journal: *http://www.jmatonline.com*

Heliotropium indicum Linn., commonly known as 'Indian heliotrope' is very common in India and some parts of Africa and Bangladesh, but also found in other countries. *Heliotropium indicum* has been used in different traditional and folklore systems of medicine for curing various diseases. In Thailand, the dried inflorescence is believed to produce permanent sterilization when taken orally in females. One gram of the dried and powdered inflorescence mixed with milk or water is used for three days beginning with the fourth day of menses to achieve the desired result[1]. Other folk remedies include use of decoction of the leaves for treatment of fever[2], insect bites, stings, diarrhea, skin rashes, menstrual disorder and urticaria[3]. The decoction of the leaves is also credited to be useful in curing insect stings (macerated with sugar cane juice), scorpion stings and as abortive in large dose and emmenagogue in small dose[3]. Different extracts of *Heliotropium indicum* have been studied for possible biological activities in various animal models and reported to possess significant antimicrobial, antifertility, anti-tumor, anti-tuberculosis, anti-inflammatory, histogastroprotective, anti-cataract, analgesic and wound healing activities[4]. The anti-inflammatory activity of different extracts of *H. indicum* has been reported using egg white[5], carrageenin-induced acute paw edema model and cotton pellet granuloma subacute inflammation models[6].

Correspondence to:
Itharat A, Department of Applied Thai Traditional Medicine, Faculty of Medicine, Thammasat University, Rangsit Campus, Klongluang, Pathumthani 12120, Thailand.
Phone: +66-2-9269749, Fax: +66-2-9269705
E-mail: iarunporn@yahoo.com

Chloroform extract of *H. indicum* was investigated for anti-inflammatory and antinociceptive activities in experimental animal models. The extract (150 mg/kg body weight) showed maximum inhibition (80.0%) and (82.79%) antinociceptive activity on carrageenan-induced raw paw edema and hot plate model in male Swiss albino mice, respectively[7]. The extracts have also been reported to produce significant anti-inflammatory effect in both acute and subacute animal models of inflammation. The methanol extract of the dried roots of *H. indicum* was investigated for its possible antinociceptive, cytotoxic and diuretic activities in animal models. The extract produced significant writhing inhibition in acetic acid-induced writhing in mice[8]. For anti-tumor activity, the methanolic extracts of stem and leaf of *Heliotropium indicum* possessed a good amount of anticancer activity and IC_{50} for both the extracts found to be 200 µg/ml whereas stem extracts exhibited excellent activity up to 64.5% at 200 µg/ml and followed by leaf extract up to 49.67% at 200 µg/ml[9]. The anti-tumor activity of different extracts of *Heliotropium indicum* showed significant activity in several experimental tumor systems. The active principle was isolated and found to be N-oxide of the alkaloid, indicine[10]. Indicine-N-oxide has reached Phase 1 clinical trials in advanced cancer patients[11]. In early clinical studies, the possibility of using indicine N-oxide for the treatment of leukemia and tumors was discussed[11]. But severe toxic side-effects showed that a therapy with indicine-N-oxide was not justified. Most of the alkaloids are hepatoxic, and therefore, internal use of *Heliotropium* species is not recommended[10]. Ethanolic extract of *Heliotropium indicum* showed significant anti-proliferative activity against SKBR3 human breast adenocarcinoma cell line using MIT assay[12]. Nevertheless, there are no reports on cytotoxicity (lung cancer), anti-inflammatory effect by nitric oxide inhibitory assay of *Heliotropium indicum*. In addition, this plant is a commonly used component in a lung disease remedy by Thai folk doctors. Thus, in the present study, we investigated cytotoxicity of two lung cancer cell lines (A549 and NCI-H226), anti-inflammatory effect on inhibition of nitric oxide production and antioxidant activity of *Heliotropium indicum* extracts.

Material and Method

Plant materials

The whole part of *Heliotropium indicum* was collected from Pathumthani. The voucher specimen was identified and kept in Southern Center of Thai Medicinal plant at Faculty of Pharmaceutical Science, Prince of Songkhla University, Songkhla, Thailand.

Preparation of the plant extracts

Plant material was washed, sliced thinly, dried in an oven at 50°C, powdered and extracted similar to those practiced by Thai folk doctors, i.e. dried plant material was boiled in distilled water for 30 minutes, filtered and dried using a lyophilizer to obtain water extract. For maceration, dried plant material was macerated in 95% ethanol for 3 days, 3 times, filtered and dried using an evaporator to obtain ethanolic extract. The percentage yield of water and ethanolic extracts was calculated.

In vitro assay for cytotoxicity

Preparation of sample solution

The water extract was dissolved in sterile water and filtered whereas the ethanolic extract was dissolved in sterile dimethylsulfoxide (DMSO). We prepared serial dilutions of the extract to determine IC_{50}.

Human cell lines

Two types of lung carcinoma cell lines such as human lung adenocarcinoma epithelial cell line (A549) and human lung squamous carcinoma cell line (NCI-H226) were used. A549 and NCI-H226 cells were cultured in RPMI 1640 medium with 10% heated fetal bovine serum, 1% of 2 mM 1-glutamine, 50 IU/ml penicillin and 50 µg/ml streptomycin. All cancer cell lines were incubated at 37°C in a 5% CO_2 atmosphere with 95% humidity.

Cytotoxicity assay[13,14]

In the first step of the assay, all cancer lines were washed with phosphate-buffered saline (PBS) and cells detached with 0.025% trypsin-EDTA to make a single cell suspension. Then, RPMI 1640 medium added to the volume of 5 ml in flask to stop for working trypsin-EDTA. The viable cells were counted by trypan blue exclusion in hemocytometer. Single cell suspension was diluted with RPMI 1640 medium to give determined optimal densities of A549 and NCI-H226 were determined to be $1x10^3$ and $1x10^3$. One hundred microlitre per wells of these cell suspensions were seeded in 96-well microplates and incubated at 37°C in at 5% CO_2 atmosphere with 95% humidity for 24 hours. Second step, 100 µl of the sample solution was added and control solvent was added with 2% DMSO solution by mixture with medium. Cells in 96-well plate were

incubated at CO_2 incubator for exposure time at 72 hours. Third step, at the end of each exposure time (72 hours) the medium was removed and washed with medium. The 200 µl of fresh medium were added. Then, incubated for recovery period (72 hours) and the survival percentage were measured calorimetrically by using SRB assay and IC_{50} values were calculated by mean of Prism program.

***Sulphorhodamine B (SRB) assay*[13]**

The anti-proliferative assay, SRB (sulphorhodamine B) was performed to assess growth inhibition by a colorimetric assay which estimated cell number indirectly by staining total cellular protein with the dye SRB. Cells culture was fixed by 100 µl of ice-cold 40% trichloroacetic acid (TCA) per well. Cells were incubated at 4°C for 1 hour, after which plates were washed five times with tap water, the excess water drained off and the plates left to dry in air. After that, SRB solution 50 µl (0.4% w/v in 1% acetic acid) was added to each well and left in contact with the cells for 30 minutes, and thereafter the plates were washed with 1% acetic acid, rinsed four times until only dye adhering to the cells were left. After well plates were dried, addition of 100 µl of 10 mM Tris base pH 10.5 was done (Tris (hydroxyl methyl) aminomathane, Sigma). The absorbance (OD) of each well was read at 492 nm. Cell survival was measured as the percentage absorbance compared to the control (non-treated-cell). The IC_{50} values were calculated by the Prism program obtained by plotting the percentage of survival versus the concentrations, interpolated by cubic spine. According to National Cancer Institute guidelines extracts with IC_{50} values <30 µg/ml were considered active.

***Anti-inflammation by nitric oxide (NO) inhibitory assay*[15]**

Inhibitory effects on NO production by murine macrophage-like RAW 264.7 cells were evaluated by the following method. The RAW 264.7 cells were cultured in RPMI-1640 medium supplemented with 10% FBS and 1% P/S (penicillin-streptomycin) in 96-well plates with $1x10^5$ cells/well for 1 h. The cells were stimulated with 5 µg/ml LPS together with test samples at various concentrations for 48 hrs. NO production was determined by measuring the accumulation of nitrite in the culture supernatant using the Griess reagent. Cytotoxicity was determined using the MTT colorimetric method. The absorbance at 570 nm was measured. The inhibition of NO production was calculated using the following equation and IC_{50} values was calculated from the Prism program.

% Inhibition = [(A - B)/(A - C)] x 100

[A: LPS (+), sample (-); B: LPS (+), sample (+); C: LPS (-), sample (-)]

***DPPH radical scavenging assay*[14,16]**

Scavenging effect of extracts on DPPH radical were examined based on the method of Yamasaki[5]. The antioxidant activity testing based on chemical testing. Butylated hydroxytoluene (BHT) was used as reference standard and positive control. Samples for testing were prepared by dissolution in absolute ethanol for ethanolic extract and dissolution in sterile water for water extract. Samples were assayed at various concentrations ranging 1-100 µg/ml (100, 50, 10 and 1 µg/ml). A portion of sample solution (0.1 ml) was mixed with the same volume of 6×10^{-5} M DPPH in absolute ethanol. After the mixture had been allowed to stand for 30 minutes at room temperature, its absorbance was measured at 520 nm using a spectrophotometer. All tests were determined in triplicate.

The values were reported as means $\pm$ SEM of three determinations. The percentage of inhibition was calculated as follows: % Inhibition = $[(OD_{control} - OD_{sample})/OD_{control}] \times 100$

The EC_{50} value (effective concentration of sample required to scavenge DPPH radical by 50%) was obtained by linear regression analysis of dose-response curve plotting between % inhibition and concentrations by Prism program.

Results and Discussion

The percentage yields of water and ethanolic extracts from all parts of *Heliotropium indicum* were 23.23% and 9.82%, respectively. Table 1 shows the cytotoxic activity of the two extracts against two types of lung cancer cells: human lung adenocarcinoma epithelial cell line (A549) and human lung squamous carcinoma cell line (NCI-H226). The water extract had no cytotoxic activity against two types of lung cancer cells (IC_{50} >100 µg/ml) but the ethanolic extract showed cytotoxic activity against NCI-H226 (IC_{50} = 51.90$\pm$2.35 µg/ml) and it had no cytotoxic activity against A549 (IC_{50} >100 µg/ml). However, its ethanolic extract exhibited less activity because NCI[13] indicated active cytotoxic activity that IC_{50} must less than 30 µg/ml. This result relates with the previous results in that its ethanolic extract from aerial part only showed great activity against breast cancer cell lines and leukemia[11,12]. The present study on lung cancer A549 and NCI-H226 cell lines is the first report and support

the use of the plant by Thai folk doctors to treat lung cancer patients. However, the good cytotoxic activity of previous report used only the aerial part to test cytotoxicity but the present study used all parts including root and stem. Thus, each part of the plant should be studied and compared in terms of its cytotoxic activity. The active compound was is N-oxide of the alkaloid, indicine[10], which indicine-N-oxide has already researched in Phase 1 clinical trials in advanced cancer patients[11] and clinical studies of indicine N-oxide for the treatment of leukemia and tumors were also researched[11]. But this compound had severe toxic side-effects such as hepatoxic, thus it is not recommended to use this single plant[10]. In Thai traditional medicine, this plant was used in combination with many plants for toxic reduction. Thus, the next research should continue to study the remedy and compare it with a single plant.

For anti-inflammation by determination inhibitory effect of nitric oxide production on RAW246.7 cells was shown in Table 2. The ethanolic extract showed the most potent inhibitory activity on nitric oxide production (IC_{50} = 24.17±2.12 µg/ml). Surprisingly, indomethacin as positive control showed less inhibitory effect than its ethanolic extract with IC_{50} value of 34.67±6.23 µg/ml. The ethanolic extract showed 1.43 times higher, better anti-inflammatory activity than indomethacine. Its water extract was apparently inactive (IC_{50} >100 µg/ml). These results are consistent with those in previous studies in which animal models were tested for their anti-inflammatory activity by using egg white[5], carrageenin-induced acute paw edema model and cotton pellet granuloma subacute inflammation models[6]. Another report used chloroform extract of *H. indicum* and found that this extract (150 mg/kg body weight) showed maximum inhibition (80.0%) and (82.79%) antinociceptive activity on carrageenan-induced raw paw edema and hot plate models in male Swiss albino mice, respectively[7]. The previous report used the methanol extract of the dried roots of *H. indicum* which also exhibited possible antino-ciceptive activities in animal models and this extract also produced significant writhing inhibition in acetic acid-induced writhing in mice[8]. However, the present study is the first report for in vitro anti-inflammatory activity of the plant by studying the inhibitory effect on NO release on the RAW 246.7 cells model.

For antioxidant activity, the results are calculated Table 3. The ethanolic extract showed high antioxidant activity (EC_{50} = 28.91±4.26 µg/ml) while

Table 1. Cytotoxic activity of water and ethanolic extracts from *Heliotropium indicum* against human lung adenocarcinoma epithelial cell line (A549) and human lung squamous carcinoma cell line (NCI-H226) (n = 3)

Extracts	% inhibition ± SEM at various concentration (µg/ml)				IC_{50} ± SEM (µg/ml)
	1	10	50	100	
Water					
A549	-	-	0±2.45	-	>100
NCI-H266	-	-	41.16±2.08	-	>100
Ethanolic					
A549	-	-	0±2.69	-	>100
NCI-H266	10.65±8.55	8.46±6.61	46.00±3.68	97.52±0.41	51.90±2.35

Table 2. Anti-inflammation by nitric oxide inhibitory assay of water and ethanolic extracts from *Heliotropium indicum* (n = 3)

Extracts	% inhibition ± SEM at various concentration (µg/ml)/(% cytotoxic against RAW246.7)				IC_{50} ± SEM (µg/ml)
	1	10	50	100	
Water	-	-	0±8.83	-	>100
Ethanolic	0±4.90	22.37±4.81	62.20±8.56	66.96±6.15	24.17±2.12
Indomethacin	26.10±7.86	29.41±6.82	64.16±5.82	79.31±2.93	34.67±6.23

the water extract showed no antioxidant activity (EC_{50} >100 μg/ml). However, the antioxidant activity of the ethanolic extract was 2.21-fold lower than that of BHT. These results are consistent with the previous studies showing that callus of *Heliotropium indicum* had high contents of total flavonoids and total phenolic compounds[17]. These compounds showed antioxidant activity. However, there is no report for antioxidant activity in whole plant.

Conclusion

The present study demonstrated that the ethanolic extract of whole part of *Heliotropium indicum* showed selective, moderate cytotoxic activity against only the human lung squamous carcinoma cell line NCI-H226, which is the most common type of lung cancer. Its ethanolic extract also exhibits high anti-inflammation especially inhibition of NO release in chronic inflammation. This activity of the plant can indicate it for cancer treatment because NO release or inflammations facilitate cancer progression. The antioxidant activity by reducing ROS also showed antioxidant activity. The ethanolic extract of this plant exhibits more anti-inflammatory than antioxidant activities and less cytotoxic activity against squamous lung cancer cells. Thus, action of this plant in Thai traditional remedy showed anti-inflammatory and antioxidant activities, which can reduce progression of cancer cell growth. The method of extract have to macerate in ethanol, its water extract had no activities at all. It can be concluded that the function of the extract of the whole part of *Heliotropium indicum* acts indirect against cancer because of its less cytotoxic activity, but it showed high anti-inflammation and antioxidant activities.

What is already known on this topic?

The decoction of the *Heliotropium indicum* leaves is credited to be useful in curing insect stings (macerated with sugar cane juice), scorpion stings and as abortive in large dose and emmenagogue in small dose[1]. The alcoholic extract of *H. indicum* was found to be having antimicrobial activity by agar cup plate diffusion method in a dose dependent manner (100 μg/ml, 1 mg/ml, 50 mg/ml and 100 mg/ml) against all the test organisms[2]. The petroleum ether extract of the entire plant is reported to possess significant antifertility activity when studied in rats[3]. The anti-inflammatory effect of *H. indicum* leaf was found to posses significant in carrageenan-induced hind paw edema and cotton pellet granuloma models of inflammation[4]. Chloroform extract of *H. indicum* was investigated for anti-

Table 3. Antioxidant activity of water and ethanolic extracts from *Heliotropium indicum* by DPPH assay (n = 3)

Extracts	% inhibition ± SEM at various concentration (μg/ml)				EC_{50} ± SEM (μg/ml)
	1	10	50	100	
Water	1.24±1.61	14.27±3.34	33.90±4.73	43.34±1.54	>100
Ethanolic	0.42±3.30	19.27±4.38	74.85±7.16	95.63±0.67	28.91±4.26
BHT (Butylated hydroxytoluene)	7.96±1.62	41.21±0.36	76.05±0.28	86.36±0.93	13.08±0.29

Table 4. Percentage yield, cytotoxic, anti-inflammation and antioxidant activities of water and ethanolic extracts from *Heliotropium indicum*

Samples	Percentage yields of extracts	Cytotoxic activity [IC_{50} (μg/ml) ± SEM]		Anti-inflammation by nitric oxide inhibitory [IC_{50} (μg/ml) ± SEM]	Antioxidant activity [EC_{50} (μg/ml) ± SEM]
		A549	H226		
Water extract	23.23	>100	>100	>100	>100
Ethanolic extract	9.82	>100	51.90±2.35	24.17±2.12	28.91±4.26
Indomethacin*	-	-	-	34.67±6.23	-
BHT**	-	-	-	-	13.08±0.29

* Positive control for anti-inflammation activity, ** Positive control for antioxidant activity

inflammatory and antinociceptive activities in experimental animal's models[5]. The volatile oil from the aerial parts of *H. indicum* was isolated by hydrodistillation and analyzed by a combination of gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS). The major constituent of the volatile oil were phytol, 1-dodecanol and β-linalool and shows significant anti-tuberculosis activity against *Mycobacterium tuberculosis* H37Ra in the Alamar blue assay system with an MIC of 20.8 μg/ml[6]. The methanolic extracts of stem and leaf of *H. indicum* possessed a good amount of anticancer activity[7]. The anti-tumor activity of different extracts of *H. indicum* showed significant activity in several experimental tumor systems. The active principle was isolated and found to be N-oxide of the alkaloid, indicine[8]. Indicine-N-oxide has reached Phase 1 clinical trials in advanced cancer patients[9]. But severe toxic side-effects showed that a therapy with indicine-N-oxide was not justified. Most of the alkaloids are hepatoxic and therefore internal use of *Heliotropium* species is not recommended[8]. Ethanolic extract of *H. indicum* showed significant anti-proliferative activity against SKBR3 human breast adenocarcinoma cell line using MIT assay[10]. Twelve plant species including *H. indicum* traditionally used in Benin for the treatment of malaria was evaluated in order to validate their use. The results showed that extracts of *H. indicum* did not reveal any antiplasmodial activity in this study. As this plant is used for hyperthermias or colics, which are two symptoms of malaria, this could explain its use as adjuvant in mixture remedies[10]. The ethanolic leaf extract of *H. indicum* was found to be having anti cataract activity. The results showed that, in the groups of the extract and vitamin E treated animals there was significant increase in the lens glutothione, soluble protein and water content as compared to galactose control[11]. Alcoholic extract of *H. indicum* was studied for wound healing properties in a rat model. This study suggests that the extract of *H. indicum* possesses wound healing activity[12]. The n-butanol crude extract from *H. indicum* leaded to the isolation and identification of two alkaloids: Pestalamide B and Glycinamide, N-(1-oxooctadecyl) glycyl-Lalanylglycyl-L-histidyl. These compounds presented an excellent wound healing activity[13]. The histo-gastroprotective activity of the aqueous extracts of the dried leaves of *H. indicum* was evaluated in Wistar rats, where ulceration of the gastric mucosa was induced via the oral administration of 80 mg/kg/bodyweight of Indomethacin. The histo-gastroprotective potential of the aqueous extract of the dried leaves of *H. indicum* against indomethacin-induced ulceration in rats might in part be due to its tannins, alkaloids and saponin constituents[14]. The aqueous and ethanolic extracts of *H. indicum* (30-300 mg/kg) dose-dependently inhibited both the first and second phases of the formalin-induced nociception. Oral doses of the aqueous extract (1-5 g/kg) in imprint control region mice were well tolerated in acute toxicity studies; however, a 14-day oral administration of 1-2 g/kg of the extracts in Sprague Dawley rats produced pathologic effects on the heart, kidney, liver and lungs. Therefore, although the aqueous and ethanol extracts have analgesic activity, it could have a cumulative toxic effect hence prolonged and continuous use is not advised[15].

What this study adds?

Different extracts of *Heliotropium indicum* have been studied for possible biological activities in various methods and reported to possess antibacterial, anti-tumor, uterine stimulant effect, antifertility, wound healing, anti-inflammatory, antinociceptive and diuretic activities. A few chemical investigations have been performed on this plant, as for example, pyrrolizidine alkaloids and other chemical compounds like Indicine-N-Oxide, Tannins, Saponins and Heliotrine were also isolated from this plant[16]. Nevertheless, there are no reports on cytotoxicity (lung cancer), anti-inflammatory effect by nitric oxide inhibitory assay and antioxidant activity of the water and ethanolic extracts of the entire *Heliotropium indicum* plant. Thus, in the present study, we investigated cytotoxicity of two lung cancer cell lines (A549 and NCI-H226), anti-inflammatory effect on inhibition of nitric oxide production and antioxidant activity of the water and ethanolic extracts of the entire *Heliotropium indicum* plant.

Acknowledgements

The authors thank Miss Phatcharanut Jittripluem and Miss Ustanisa Prasertsup for assistance with cytotoxicity, anti-inflammation and antioxidant activities and thank the Faculty of Medicine, Thammasat University, Thailand, for financial support.

Potential conflicts of interest

None.

References

1. Panthong A, Kanjanpothi D, Taylor WC. Ethnobotanical review of medicinal plants from Thai traditional books, Part-I: Plants with

antiinflamatory, antiasmatic and antihypertensive properties. J Ethnopharmacol 1986; 18: 213-28.
2. Giron L, Freira V, Alonzo A, Caceres A. Ethnobotanical survey of medicinal flora used by the Caribs of Guetmala. J Ethnopharmacol 1991; 34: 173-87.
3. Duttagupta S, Datta PC. Pharmacognostic study of the leaf of *Heliotropium indicum* Linn. (Boraginaceae). J Crude Drug Res 1977; 15: 141-51.
4. Anupam R. Pharmacological activities of Indian Heliotrope (*Heliotropium indicum* L.): A review. J Pharmacol Phytochem 2015; 4: 101-4.
5. Shalini S, Kaza R, Firoz S. Study on the anti-inflammatory activity of *Heliotropium indicum.* J Innovative Trends Pharm Sci 2010; 1: 43-6.
6. Srinivas K, Rao MEB, Rao SS. Anti-inflammatory activity of *Heliotropium indicum* Linn. and *Leucasaspera* Spreng. in albino rats. Indian J Pharmacol 2000; 32: 37-8.
7. Betanabhatla KS, Jasmin Sajni R, Karthik R, Raamamurthy J, Christina AJM, Sasikumar S. Anti-inflammatory and anyinociceptive activities of *Heliotropium indicum* Linn. In experimental animal models. Pharmacologyonline 2007; 3: 438-45.
8. Rahman MA, Mia MA,Shahid IZ. Pharmacological and phytochemical screen activities of roots of *Heliotropium indicum* Linn. Pharmacology online 2011; 1: 185-92.
9. Sivajothi V, Shruthi DS, Sajini Jasmin R. Cytotoxic effect of *Heliotropium indicum* extracts on Hela cell line. Int J Pharm Pharmaceut Sci 2015; 7: 412-4.
10. Dash GK, Abdullah MS. A review on *Heliotropium indicum* L. (Boraginaceae). Int J Pharm Sci Res 2013; 4: 1253-8.
11. Ohnuma T, Sridhar KS, Ratner LH and Holland JF. Phase 1 study of indicine N-oxide in patients with advanced cancer. Cancer Treat Rep 1982; 66: 1509-15.
12. Goyal N, Sharma SK. Bioactive phytoconstituents and plant extracts from genus Heliotropium. Int J Green Pharm 2014; 8:217-25.
13. Prommee N, Prajuabjinda O,Itharat A. In vitro cytotoxic, antioxidant and antimicrobial activities of *Derris scandens*. GS-NETT 2012; Sci-Health 008: 1-8.
14. Ruangnoo S,Itharat A. Antioxidant and cytotoxic activities of Thai medicinal plants named Hua-Khao-Yen. Thai J Pharmacol 2010; 32: 115-8.
15. Makchuchit S, Itharat A. Nitric oxide inhibitory activity of Thai medicinal plants called Kod-Tung-Ha. Thai J Pharmacol 2010; 32: 188-91.
16. Yamazaki K, Hashimoto A, Kokusenya Y, Miyamoto T, Sato T. Electrochemical method for estimating the antioxidative effect of methanol extracts of crude drugs. Chem Pharm Bull (Tokyo) 1994; 42: 1663-5.
17. Kumar MS, Chaudhury S, Balachandran S. In vitro callus culture of *Heliotropium indicum* Linn. for assessment of total phenolic and flavonoid content and antioxidant activity. Appl Biochem Biotechnol 2014; 174: 2897-909.

ฤทธิ์ความเป็นพิษต่อเซลล์มะเร็งปอด ฤทธิ์ต้านการอักเสบ และฤทธิ์ต้านอนุมูลอิสระของสารสกัดหญ้างวงช้าง

จิตพิสุทธิ์ จันทร์ทองอ่อน, ภาณัฐ เดชะยนต์, ปฐมพงษ์ เผือกลี, อรมณี ประจวบจินดา, ธนา จักษ์เมธา, อรุณพร อิฐรัตน์

ภูมิหลัง: หญ้างวงช้างหรือ 'Indian heliotrope' เป็นพืชที่มีการใช้ในการรักษาโรคมายาวนานในประเทศอินเดียและในอีกหลายประเทศทั่วโลก สำหรับประเทศไทยก็มีการนำหญ้างวงช้างมาใช้ในการรักษาโรคหลายโรค เช่น ไข้ แมลงสัตว์กัดต่อย ท้องเสีย ผื่นผิวหนัง ประจำเดือนผิดปกติ และผื่นลมพิษ นอกจากนี้หมอพื้นบ้านของไทยนิยมนำหญ้างวงช้างมาใช้เป็นส่วนประกอบในตำรับยารักษาโรคเกี่ยวกับปอดอีกด้วย

วัตถุประสงค์: เพื่อทดสอบฤทธิ์ความเป็นพิษต่อเซลล์มะเร็งปอด 2 ชนิด (A549 และ NCI-H226) ฤทธิ์ต้านการอักเสบ และฤทธิ์ต้านอนุมูลอิสระของสารสกัดหญ้างวงช้าง

วัสดุและวิธีการ: สารสกัดที่ใช้ทดสอบคือสารสกัดชั้นน้ำ และชั้นเอทานอล การทดสอบความเป็นพิษต่อเซลล์มะเร็งปอดด้วยวิธี SRB การทดสอบฤทธิ์ต้านการอักเสบโดยการยับยั้งปริมาณไนตริกออกไซด์ที่ถูกกระตุ้นด้วย LPS และการทดสอบฤทธิ์ต้านอนุมูลอิสระด้วยวิธี Scavenging of DPPH free radical

ผลการศึกษา: สารสกัดชั้นเอทานอลมีฤทธิ์ความเป็นพิษต่อเซลล์มะเร็งปอดเพียง 1 ชนิด คือ H226 (IC_{50} = 51.90±2.35 ไมโครกรัม/มิลลิลิตร) ในขณะที่สารสกัดชั้นน้ำไม่มีฤทธิ์ความเป็นพิษต่อเซลล์มะเร็งปอดทั้ง 2 ชนิด (A549 และ H226) (IC_{50} >100 ไมโครกรัม/มิลลิลิตร) ฤทธิ์ต้านการอักเสบพบว่า สารสกัดชั้นเอทานอลมีฤทธิ์ยับยั้งปริมาณไนตริกออกไซด์ดีที่สุด (IC_{50} = 24.17±2.12 ไมโครกรัม/มิลลิลิตร) รองลงมาคือสารมาตรฐาน Indomethacin (IC_{50} = 34.67±6.23 ไมโครกรัม/มิลลิลิตร) ในขณะที่สารสกัดชั้นน้ำไม่มีฤทธิ์ยับยั้งปริมาณไนตริกออกไซด์ (IC_{50} >100 ไมโครกรัม/มิลลิลิตร) สำหรับฤทธิ์ต้านอนุมูลอิสระพบว่า สารสกัดชั้นเอทานอลมีฤทธิ์ต้านอนุมูลอิสระ (EC_{50} = 28.91±4.26 ไมโครกรัม/มิลลิลิตร) แต่สารสกัดชั้นน้ำไม่มีฤทธิ์ต้านอนุมูลอิสระ (EC_{50} >100 ไมโครกรัม/มิลลิลิตร)

สรุป: จากผลการทดลองดังกล่าวสามารถสนับสนุนการนำหญ้างวงช้างไปใช้เป็นส่วนประกอบในตำรับยารักษาโรคมะเร็งปอดของหมอพื้นบ้าน แต่อย่างไรก็ตาม ควรจะต้องศึกษาเพิ่มเติมในฤทธิ์ที่เกี่ยวข้องกับมะเร็งปอด ฤทธิ์ต้านการอักเสบ และฤทธิ์ต้านอนุมูลอิสระ