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Original Article

Anti-Ulcer Activity of Spray-dried Powders Prepared from Aerial Parts Extracts of *Ampelopsis cantoniensis*

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ABSTRACT

Introduction: *Ampelopsis cantoniensis* is called "Che day" in Vietnam and has been used as a traditional treatment for inflammatory diseases. **Methods:** Plant material and hydroalcoholic extract were processed and analyzed for their physicochemical characteristics. A method using HPLC was validated to quantify ampelopsin. Hydroalcoholic extract was spray dried and the powder obtained was characterized in terms of its physicochemical parameters and potential for antiulcerogenic activity. **Results:** The analytical method proved to be selective, linear, accurate, sensitive. *A. cantoniensis*s pray dried extract (ACP) was obtained using colloidal silicon dioxide as adjuvant and was shown to possess 25.94 % ampelopsisn. It showed significant antiulcer activity in a model of an indomethacin-induced gastric lesion in rats and also produced a gastroprotective effect.

Key words: *Ampelopsis cantoniensis*, *Vitaceae*, Spray drying, Antiulcer.

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INTRODUCTION

Gastric ulcer is one of the most common digestive system diseases with high incidence and prevalence which affect millions of population worldwide, being a major public health burden in the present century.[1,2] Its harmful effect resulted from the imbalance between the protective and damaging in the stomach.[3] There are various factors that could cause gastric ulcers such as *Helicobacter pylori*, pepsins, non-steroidal anti-inflammatory drugs, bile acids, stress, smoking and alcohol.[4] Numerous studies have shown that alcohol is one of the major factors in the formation of gastric ulcer.[5] Several medications such as antibiotics, antacids, proton-pump inhibitors and H2 receptor antagonists been used in the treatment of gastric ulcers.[6] However, there are numerous side effects associated with these agents used in the treatment of ulcers, including gynecomastia, hypoacidity, impotence, osteoporotic bone fracture, hypergastrinaemia and cardiovascular disease.[7-10] Therefore, new drug candidates which could provide high efficacy and low toxicity are needed valuable for the prevention and treatment of gastric ulcer.

Ampelopsis cantoniensis (Hook. & Arn.) K. Koch is a medicinal medicine plant which belongs to the family *Vitaceae*. It is wild distributed in India, China, Japan, Taiwan, and Vietnam.[11,12] In traditional systems of medicine, the plant has been used to treat rheumatic-arthritis, hepatitis, dermatitis, and gastritis.[11] Previous phytochemical investigations on this plant reported the presence of various types of meroterpenoids and flavonoids,[11,12] some of which exhibited cytotoxic, antioxidant, anti-inflammatory, anti-fatigue, and anti-cancer,[12] antitumor, antibacterial, antiviral, and anti-obesity effects.[11-14] In this study, the spray drying technique was applied to process a hydroalcoholic extract from the aerial parts of *A. cantoniensis* and ampelopsin content, physicochemical properties, and anti-ulcer potential were evaluated.

MATERIALS AND METHODS

Plant material

The *A. cantoniensis* leaves were collected from Lao Cai Province, Vietnam and identified by Dr. V.H. Do from Department of Plant Resources, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology.

Validation of the method to quantify ampelopsin by High Performance Liquid Chromatography

Chromatographic analysis was performed using a Waters high-performance liquid chromatography (Milford, MA, USA), equipped with a diode array detector and chromatographic column Eclipse XDB-C18, 5µm, 4.6 × 150 mm (Agilent, USA). A modified version of the method described by Park *et al.* was used to quantify ampelopsin.[15]It consisted of a mobile phase of methanol: water: phosphoric acid (22:78:0.1), flow rate of 1.0 mL/min, isocratic mode, oven temperature 25°C, injection volume of 10 µL, and 292 nm wavelength. System suitability parameters were evaluated and the following parameters were investigated in the validation study: selectivity, linearity, accuracy, and limits of detection and quantification.[15]

Preparation and characterization of the hydroalcoholic extract

Ultrasound assisted extraction of hydroalcoholic extract of *A. cantoniensis* was performed in an ultrasonic bath (Model 2510, Branson Ultrasonics Corporation, Connecticut, USA), using ethanol 70% (v/v; Duc Giang Chemicals Group, Gia Lam, Ha Noi, Vietnam) as extractor solvent, and the process was

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monitored by TLC. The plant/solvent ratio was set at 1:10 (w/v) and the ultrasound equipment operated at a frequency of 40 kHz and at room temperature. The extraction solution obtained was filtered and concentrated in a rotary evaporator (evaporator Buchi R220, Germany) until reaching the soluble solid of 15^0 Brix and was characterized by measuring pH, viscosity, and determining ampelopsin content by HPLC (Waters high-performance liquid chromatography - Milford, MA, USA)

Preparation of the A. cantoniensis spray dried extract

The concentrated of *A. vulgaris* extract was mixed with colloidal silicon dioxide at 6%. The mixture was spray dried in Mini Spray Dryer B-290 (Buchi, Flawil, Switzerland). The four drying air inlet temperatures were 130^{0}C, 140^{0}C, 150^{0}C, and 160^{0}C. The atomization air volumetric flow rate, feed volumetric flow rate, and drying air volumetric flow rate were constant at400 l/h, 3,0 ml/min 35 m^3/h. After the drying process, the ACP were collected and packed in a zip-lock bag wrapped with aluminum foil and immediately stored in a desiccator at room temperature for further analysis.

Characterization of the A. cantoniensis spray dried extract

The process yield

The process yield was calculated immediately after the drying experiments based on the ratio of the mass of powder (dry basis) collected in the flask (W) to the WE and its solid content (SC, % w.b.) by Eq. (1)

$$PY = \frac{W}{WE \times SC} \times 100\% \quad (1)$$

Water solubility index

The water solubility index of the spray-dried was determined using the method described by Anderson et al. (1969).[16] Spray dried powder (2.0 g) was added to distilled water (25 ml) in 100 ml centrifuge tube, mixed well, incubated in an incubator at 37^{0}C for 30 mi, and then centrifuged for 20 min at 10,000 rpm (Sigma, 13 K, Germany). The supernatant was carefully collected in a pre-weighed beaker and oven dried at 103 ± 2^0 C. The WSI (%) was calculated as the percentage of dried supernatant with respect to the dried powder.

Hygroscopicity

For hygroscopicity, 1.5 g of the powder was placed at 25^{0}C inan airtight container containing saturated solution of sodium carbonate. Sample was weighed after 1 week and hygroscopicity was expressed as gram of adsorbed moisture per 100 g ofpowder.[17]

Bulk density

Briefly, 2.0 g of dried powder was added in 10 mL of graduated measuring cylinder and vortexed for 1 min. The bulk density value (g/ml) was calculated by measuring the ratio of mass of powder to the volume occupied by the powder.[18]

Determination of ampelopsin content by HPLC

The concentration of ampelopsin of dried powder was determined by high-performance liquid chromatography (HPLC), as described above ACP (0,1 g) was added to into a 25 ml volumetric flask and filled to volume with dilution solution (22% methanol and 78% with 0.1% (wt/vol) phosphoric acid). Identification of the compounds was made by comparison of their retention's time and UV absorption spectrum with those of the standards.

Antiulcerogenic activity of the *A. cantoniensis* spray dried extract

Antiulcerogenic activity of ACP was evaluated using indomethacin-induced ulcer test**.** The experiment was carried out according to the method of Johnley *et al.*[19] After 12 h of fasting, the mice were randomly divided into five groups of ten animals per group. Groups I (normal control) and II received distilled water at a dose of 10 ml/kg. Groups IV and V were given ACP at doses 500 and 1000 mg/kg, respectively, and the group III was treated with omeprazole (30 mg/kg), as a reference drug for treatment of gastric ulcer. All mice were orally administered daily for 7 days and then deprived of food but allowed free access to water for 24 h. Gastric ulcers were induced by the administering of indomethacin (40 mg/kg) to all groups except for the control group (**Figure 1**). Then, 6 h after indomethacin administration, the animals were sacrificed by cervical dislocation, and the stomachs were removed and opened along the greater curvature. Stomach was excised and rinsed immediately in ice-cold normal saline for further tests. The number of ulcers and their severity were recorded by arbitrary scale Severity Score 0 was used for normal stomach, 0.5, 1 and 1.5 was used if there is red coloration, spot ulcers and haemorrhagic streaks respectively while 2 and 3 was used if Ulcers ≥ 3 but ≤ 5 and ≥ 5 respectively.

Ulcer index was calculated as using formula UI (Ulcer index) = UN (Average of number of ulcers) + US (average of severity score) + UP (Percentage of animals with ulcer) × 10^{-1}.

Percentage inhibition was calculated by formula % Inhibition = (UI positive control group– UI treated group) / UI positive control group

RESULTS AND DISCUSSION

Validation study

This analytical method for quantification of ampelopsin by HPLC was validated and it proved to be selective, linear, accurate, limit of detection, and limit of quantification (**Table 1**). HPLC showed that the content of ampelopsin in the plant was 18.54% (w/w) in relation to the dry basis.

Physicochemical characterization of hydroalcoholic extract

The following physicochemical parameters obtained for hydroalcoholic extract were pH 6.10, relative density 1.02. The solids content can significantly increase the yield of dry product to be obtained. Solid substance content in plant extracts is 150^o Brix. Ampelopsin level found in hydroalcoholic extract was 27.83%. Thus, it can be suggested that the extraction process led to obtaining an extract in which the concentration of that substance is about 1.4times greater than in the plant drug.

Effects of spray drying conditions on physical properties of *A.cantoniensis* extract spray dried powder

The effects of different drying temperatures on the physical properties of the ACP are shown in **Table 2**. Process yield is a key parameter

Table 1: Validation of the method for quantification of ampelopsin.

Validation parameters	Ampelopsin
Selectivity	Absence of interference
Linearity (µg/mL)	20 – 300 µg/mL
Regression equation	y = 20504x + 6849.3
Correlation coefficient (*r*)	0.9999
Limit of quantification	0.91 µg/ml
Limit of detection	0.04 µg/ml
Accuracy – percentage	98.47 - 101.92 %

when considering the feasibility of the spray-drying process as a drying methodology for the large scale manufacture. An increase in inlet temperature, from 130°C to 160°C, resulted in a general decrease in the process yield. The decrease in the process yield can be attributed to the melting of powder and the cohesion wall at higher temperature might have reduced the amount of powder production and yield. A similar trend was found while increasing drying temperature from 130°C to 160°C, resulting in significant decrease in the moisture content in ACP from 3.48 to 3.11%. Due to the increased rate of heat transfer into the particles at higher temperature, there was a greater driving force for moisture evaporation causing faster water removal.[18] Inlet temperature also influenced the hygroscopicity and bulk of the powder significantly. The highest hygroscopicity value of 55.33 g/100 g for ACP was obtained at 120°Cinlet temperature. When inlet temperature of processing was increased the hygroscopicity of ACP was decreased. The drying temperature showed a significant effect on the bulk density of ACP. A decrease in the density of the powder was observed with an increase in the inlet temperature. As explained by the high rate of drying being rapid at very high temperatures meant that there was less droplet shrinkage, giving lower powder density.[20-22] Drying temperature did not show any significant effect on WSI and ampelopsin content of the ACP. The ampelopsin content in the plant drug was 18.54% (w/w). This level in hydroalcoholic extract was 38.83%. In the extraction step, the concentration of ampelopsin was about two times higher. It was found that the spray drying conditions used caused a loss of ampelopsin when compared with the hydroalcoholic extract. This was probably due to volatilization, based on the odor of vanilla during the drying process. Nevertheless, one of the advantages of obtaining dried extracts is the possibility of getting products with a higher content of active ingredients. The results obtained in this study were adequate because the final drying process allowed for concentrations of 1.4 times greater in ampelopsin content than in the plant drug. Spray drying at the inlet temperature of 140°C was selected for the production of ACP because of the higher process yeild (68.12%) and content of ampelopsin (25.94%).

Table 2: Physicochemical properties of *Ampelopsis cantoniensis* extract spray dried powder.

Sample/Analysis	A. cantoniensis powder dried at 120°C	A. cantoniensis powder dried at 140°C	A. cantoniensis powder dried at 160°C
The process yield (%)	55.44±2.04	68.12±1.12	62.22±3.12
Moisture content (%)	3.48 ± 0.62	3.27± 0.32	3.11± 0.45
Hygroscopicity	55.33± 1.23	48.83± 1.67	46.03± 1.37
Water solubility index (%)	68.57± 1.42	72.15± 2.16	71.22±1.74
Bulk density (g/mL)	0.631± 0.007	0.572 ± 0.005	0.531± 0.006
Ampelopsin (mg/g)	252.27±3.57	259.36±2.68	256.68±4.02

Table 3: Effect of pre-treatment of ACP on ulcer index and percentage protection on gastric ulcers induced by Indomethacin.

Groups	Ulcer Index	Percentage inhibition of ulceration (%)
Group I (DW)	0	100
Group II (DW+IND)	11.45 ± 1.31	-
Group III (OMP +IND)	3.25 ± 1.10	71.62
Group IV (ACP1+IND)	6.70 ± 1.35*	41.48
Group V (ACP2+IND)	4.40 ± 1.36*	61.57

Significance at *p value < 0.05 compared to positive control (Group II). DW: distilled water (10 mg/kg b.w), IND: indomethacine (40 mg/kg b.w), OMP: omeprazole (30 mg/kg b.w), ACP1: *Ampelopsis cantoniensis* spray drided extract (500 mg/kg b.w), ACP2: *Ampelopsis cantoniensis* spray dried extract (500 mg/kg b.w).

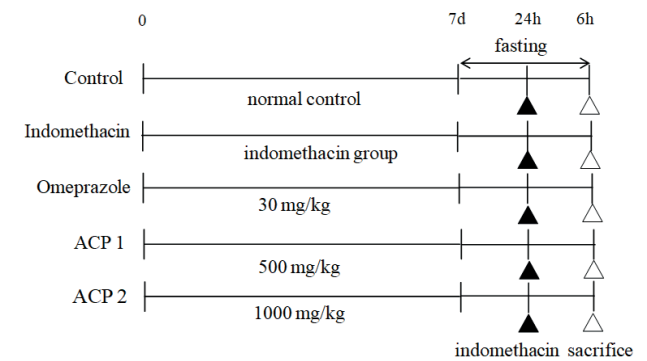

Figure 1: The schematic overview of the experimental design for ethanol-induced gastric ulcers in a rat model. The pretreatment with ACP orally administered daily for 7 days once per day. Gastric ulceration was performed by administration with ethanol and sacrificed 6 h later.

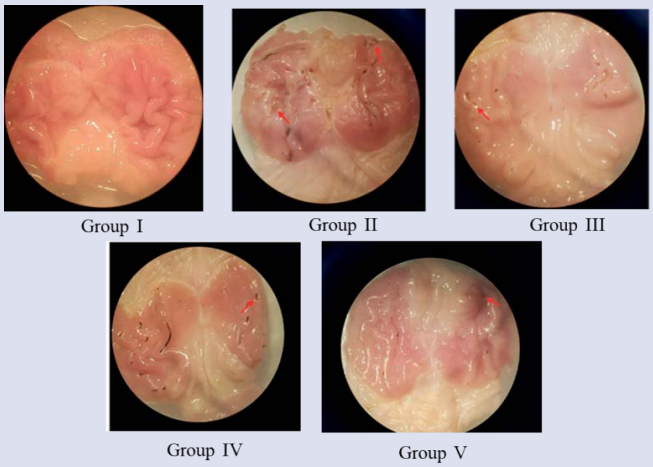

Figure 2: Showing Stomach of control rat (Group I); Indomethacin induced gastric ulcer (Group II), Effect of ACP on gastric ulcer induced by Indomethacin(Group IV and V), Effect of omeprazole on gastric ulcer induced by Indomethacin (Group III).

Antiulcerogenic activity of the A. *cantoniensis* extract spray dried powder

Effect of ACP on gastric mucosa

In positive control group (group II), there was visible gastric mucosal damage after 6 h of Indomethacin administration. In ACP (Group IV and V) and standard drug omeprazole pre-treated group (Group III), there was decrease in intensity of gastric mucosal damage but omeprazole was found better than the ACP (**Figure 2**).

Effect of A. *cantoniensis* extract spray dried powder on ulcer index and percentage protection

The effects of ACP on the ulcer index and % inhibition against ulcer in the experimental animals are shown in Table 3. It was noted that the degree of ulceration (ulcer index) by indomethacin in rats treated with ACP at doses of 500 mg/kg (n = 10) and 100 mg/kg (n = 10) was reduced by 41.46 and 61.57%, respectively, compared with the group II (indomethacin group). The degree of ulceration in animals which received omeprazole 30 mg/kg (n = 10) was reduced by 71.62%. All the doses of ACP used were significantly effective in reducing the degree of ulceration induced by indomethacin. A significant improvement in the level of inhibition against ulceration was however observed in the

ACP-treated animals. The animals treated with ACP at 1000 mg/kg b.w doses offered better protection against ulceration than the 500 mg/kg b.w. regimens and compared well with the standard drug (omeprazole) used. It was demonstrated that ACP obtained by spray drying presented gastroprotective potential.

CONCLUSION

The spray drying process of hydroalcoholic extract from *A. cantoniensis* was suitable for maintaining the chemistry profile and antiulcerogenic activity described for the medicinal plant. Pharmacological and toxicological studies should be performed in order to investigate the action mechanism of this extract and would thus contribute toward obtaining a safe and effective intermediate phytopharmaceutical product from *A. cantoniensis*.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ABBREVIATIONS

ACP: *Ampelopsis cantoniensis* spray dried extract; **DW:** distilled water; **IDN**: indomethacine; **OMP**: omeprazole.

SUMMARY

Research and development of standardized dried extract of *Ampelopsis cantoniensis* leaves obtained through spray drying and the production process was monitored by the chemical profile, physicochemical properties and potential for anti-ulcerogenic activity.

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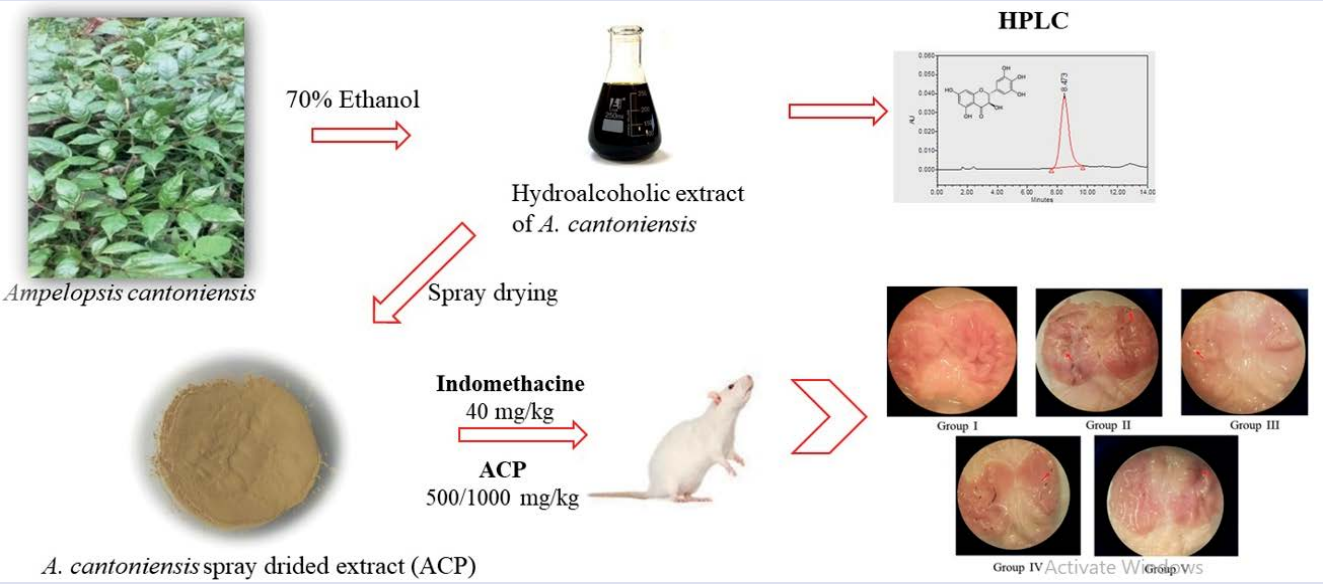
GRAPHICAL ABSTRACT



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