HOẠT TÍNH GIẢM ĐAU VÀ TIÊU CHUẨN HÓA BỘT SẤY PHUN ĐƯỢC CHIẾT XUẤT TỪ CÁC BỘ PHẬN CỦA CÂY NGẢI CỨU ARTEMISIA VULGARIS L

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TÓM TẮT

Giới thiệu: Cây Ngải cứu có tên khoa học là Artemisia vulgaris L., là một loài có tầm quan trọng rất lớn trong lịch sử y học. **Phương pháp nghiên cứu**: Nghiên cứu này được thực hiện với mục tiêu là chuẩn hóa bột phun sấy của A. vulgaris (SDA) bằng cách xác định các thông số hóa lý, sự hiện diện hay có mặt của kim loại nặng và một số loài vi khuẩn; sàng lọc hóa thực vật; xây dựng phương pháp sắc ký lỏng áp suất cao (HPLC) để xác định hàm lượng eupatilin; đánh giá tác dụng giảm đau trên mô hình gây đau quặn bằng acid acetic. **Kết quả**: Tỷ lệ độ ẩm, tro toàn phần, tro không tan trong aacid của SDA lần lượt là 3,95% - 4,57%, 6,59% - 7,02%, 0,004%-0,006%. Nồng độ của tất cả các kim loại nặng được thử nghiệm đều nằm dưới giới hạn chấp nhận được của WHO và các loài vi khuẩn như Escherichia coli, Salmonella spp, Staphylococcus aureus và Pseudomonas aeroginosa không có trong SDA. Phân tích hóa thực vật cho thấy SDA có chứa flavonoid, tannin và phenolics. Hàm lượng eupatilin là 0,89 – 0,90 mg/g. Bột phun sấy có tác dụng giảm đau. **Kết luận**: kết quả thu được từ nghiên cứu này có thể được sử dụng để chuẩn hóa bột phun sấy A. vulgaris.

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SUMMARY

ANALGESIC ACTIVITY AND STANDARDIZATION OF SPRAY-DRIED POWDERS PREPARED FROM AERIAL PARTS EXTRACTS OF ARTEMISIA VULGARIS L

Introduction: Artemisia vulgaris L., commonly known as mugwort, is a species with great importance in the history of medicine. **Methods:** The present investigation was carried out to standardize the spray-dried powders of A. vulgaris (SDA) by determination of physicochemical parameters, presence or absence of heavy metals, and microbial contamination; screening for phytochemicals; development of High Pressure Liquid Chromatography (HPLC) for the determination of eupatilin; and acetic acid induced writhing assays were employed to as certain analgesic effect. **Results:** The percentages of moisture content, total ash, acid insoluble ash of SDA were 3.95% - 4.57%, 6.59% - 7.02%, 0.004%-0.006%, respectively. The concentrations of all the tested heavy metals were below the WHO acceptable limits and bacterial species, such as Escherichia coli, Salmonella spp, Staphylococcus aureus, and Pseudomonas aeroginosa were not present in SDA. The phytochemical analysis showed that SDA contain flavonoids, tannins, and phenolics. The content of eupatilin was 0.89 – 0.90 mg/g.

The spray-dried powders possessed analgesic effect. **Conclusion:** the results obtained from this study can be used to standardize the spray-dried powder of A. vulgaris.

Keywords: Artemisia vulgaris, Asteraceae, Analgesic.

I. INTRODUCTION

Pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage.[1] According to the National Institutes of Health, pain is one of our most important national public health problems, a silent epidemic. For many people, pain is more or less a permanent feature of their lives and has a profound impact on their quality of life. Currently, opioids and non-steroidal anti-inflammatory drugs (NSAIDs) are used in the treatment of pain.[2] Although these drugs have excellent analgesic effects, however, they are they are said to have numerous side effects: opioids cause physical dependency, tolerance, and addiction while NSAIDs usually cause gastrointestinal disorders.As such, research to discover other alternatives to treat pain is crucial. Plants are being used in the traditional systems of medicine in many parts of the world for the control, management and/or treatment of a variety of human and animal ailments. Many of these herbs with analgesic activity had been used without any adverse effects.

The plant Artemisia vulgaris L., a perennial weed, commonly known as mugwort, and locally known in the Vietnam as "Ngai cuu", is distributed widely in Asia, Europe, Northern Africa and North America.[1-2] The plant is traditionally used for treatment of gynecological ailments, gastrointestinal diseases, cholera, leprosy nervous system disorders such as insomnia, epilepsy, depression, and excessive stress exposure. Furthermore, it is recommended for relieving hypertension and inducing labor or miscarriage. Scientific study on the plant shows a wide range of activities, including antioxidant, antitumor, antispasmodic, anti-steroidogenic, anti-fertility, antihypertensive, muscle relaxant, antiviral, antibacterial, cholinergic, diuretic etc. Previous studies dealing with A. vulgaris phytochemistry point out the presence of different chemical groups, such as essential oil, polysaccharide, flavonoids, hydroxycinnamic acids, and quinic acid derivatives.[3,4] Thus, this study aimed to investigate the potential analgesic activity of a standardised spray dried powder extract of A. vulgaris in animal model.

II. MATERIALS AND METHODS

Plant material

The aerial parts of A. vulgaris were collected from Phu Tho 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. A voucher specimen (LHT-QY001) was deposited in the Institute of Pharmaceutical Education, Vietnam Military Medical University, 160 Phung Hung, Ha Dong District, Hanoi, Viet Nam.

Preparation of spray-dried powder from hydroalcoholic extract of Artemisia vulgaris

The extraction of A. vulgaris aerial parts was done using ultrasonic bath (Model 2510, Branson Ultrasonics Corporation, Connecticut, USA) designed with a fixed frequency of 40 kHz and power intensity 160 W. The sample was mixed with 95 % ethanol

in the flask in the ratio of 1:15 (g/mL). The ultrasound-assisted extraction was done at temperature 40°C for 30 min. The extract was then filtered through Whatman no. 1, dried using a rotary evaporator (Buchi R220, Germany) under vacuum at 40°C until reaching the solid content of 12%. Drying carriers (maltodextrin:aerosil, 7:1) were added to the concentrated extract (8%, wet base) was further subjected to a Mini Spray Dryer B-290 (Buchi, Flawil, Switzerland) to obtain the powder form the extract. The operational conditions were: inlet temperature 140°C, outlet temperature 102°C; 4 mL/min feed rate and 2 bar spraying pressure.

Determination of physicochemical parameters of Artemisia vulgaris spray-dried powder

Physicochemical parameters such as moisture content, total ash content, acid insoluble ash content were determined for A. vulgaris spray-dried powder according to the methods described in guidelines of WHO.[5]

Determination of moisture content

The powdered material (1 g) was placed in a moisture dish and dried to a constant weight in an oven at 105°C. The loss of weight in mg/g of air-dried material was calculated.[6]

Determination of total ash content

The powdered material (1 g) was accurately weighed and placed in a crucible. The material was ignited to a constant weight by gradually increasing the heat to 600°C ± 25°C until it was white. The residual ash was allowed to cool in a desicator. The content of total ash in mg/g of air dried material was calculated.[7]

Determination of acid insoluble ash content

Hydrochloride acid (2 N; 25 mL) was added to the crucible containing the total ash, covered with a watch glass and boiled gently for 5 min. The watch glass was rinsed with 5 mL of hot water and the rinsed contents were added to the crucible. The acid insoluble matter was collected on an ashless filter paper and washed with hot water until the filtrate was neutral. The filter paper containing acid insoluble matter was transferred to the original crucible, dried and ignited to a constant weight. The residue was allowed to cool in a desicator and weighed. The content of the acid insoluble ash in mg/g of air dried material was calculated.[8]

Determination of heavy metals

The contents of lead (Pb), cadmium (Cd), arsenic (As) and mercury (Hg) have been detected in spray dried powder using AAS (Atomic Absorption Spectroscopy) according to AOAC.[9]

Determination of microbial contamination in the spray dried powder

Presence or absence of yeasts, moulds, Escherichia coli, Salmonella spp, Staphylococcus aureus and Pseudomonas aeroginosa were determined according to the standards of TCVN.[10]

Preliminary phytochemical screening of Artemisia vulgaris spray-dried powder

The qualitative chemical tests were performed for the A. vulgaris spray-dried powder according to the methods described by Farnsworth[11] with some modifications.

Test for phenolics

Two to three drops of 1% $FeCl_3$ solution was added into 2 mL portions (1%) of the spray-dried powder. Phenolic compounds produce a deep violet color with ferric ions.

Test for tannins

The spray-dried powder was dissolved in methanol (1-2 mL) by heating. A few drops of 5% $FeCl_3$ solution was added. Tannins give a blackish blue or green blackish color in the presence of $FeCl_3$.

Test for flavonoids

The spray-dried powder was dissolved in methanol (1-2 mL) by heating. Then metal magnesium and 5-6 drops of conc. HCl were added. The solution turns red when flavonoids are present.

Determination of eupatilin in spray-dried powders of Artemisia vulgaris

A 500 mg portion of the extracts of SDA was dissolved in 20 ml methanol and subjected to ultrasonication for 20 min. Then the volume of the content was made up to 25 ml using a volumetric flask. Finally, all the samples were passed through a 0.45 µm PTFE filter before HPLC injection. Similarly, the reference standards (eupatilin, 5 mg) were dissolved in 5 ml methanol and then filtered. The stock solutions were used to prepare further dilutions. Eupatilin concentration was determined by an Eclipse XDB-C18, 5µm, 4.6 × 150 mm (Agilent, USA) in a Waters high-performance liquid chromatography equipped with a photodiode array detector, quaternary pump, online degasser, auto sampler, automatic injector, column heater (Milford, MA, USA). The content of eupatilin was determined by the HPLC method described by Kim et al.,.[12] The calibration curve was set up at 350 nm by subjecting the eupatilin standard solution to the isocratic mobile phase, which consisted of acetonitrile/water/acetic acid (38:62:0.5, v/v/v), at a flow rate of 1 ml/min at 30°C with an injection volume of 20 µl. Identification and calibration of the samples were performed by comparing absorbance spectra and retention times with those of the standard reference. The concentrations of eupatilin in the samples were estimated based on the regression line of eupatilin in the range of 6.25 - 100 µg/ml, which was Y = 29927X + 84570, where Y is the peak area of the analyte and X is the concentration of the analyte (µg/ml).

Analgesic activity of A. vulgaris spray-dried powders

To evaluate whether SDA has peripheral analgesic activity, we performed acetic acid writhing test. Acetic acid-induced writhing test was performed as reported previously.[12] The animals of either sex were weighed and divided into four groups of ten animals in each. Control group (CG), which received gavage with normal saline (0.1 ml/10 g); positive group (PG), which received diclofenac sodium (20 mg/kg, i.p.), a standard analgesic drug[13] ; low dosage group (LG), which received gavage with SDA (170 mg/kg) and high dosage group (HG), which received gavage with SDA (340 mg/kg). The experiment lasted for 7 days. Two hours after gavage on the seventh day, mice were injected intraperitoneally with 0.2 ml of 0.6% acetic acid, and the number of writhes of each mouse was counted starting from 5 min up to 20 min and expressed as percentage protection.

III. RESULTS AND DISCUSSION

Physicochemical parameters

Results are listed in Table 1. The moisture content at 105ºC of spray dried powders from 3.95% to 4.57% was obtained, which can be considered as adequate assuming the maximum moisture content recommended by the U.S. Pharmacopoeia[13]

for dry extracts of medicinal plants (≤5%). Moisture content of drug should be at minimum level to discourage the growth of bacteria, yeast or fungi during storage. Low moisture content indicates the appropriate standard, quality and stability of plant material and can be considered in future study or application. If the drying process is not efficient, it may lead to the degradation of phytoconstituents of the drug during storage.[14] The total ash value of spray-dried powders was found range from 6.59% to 7.02%, while acid insoluble ash ranged from 0.004% to 0.006%, respectively. The ash values of the drug are also a significant parameter for the detection of nature of material, adulteration, impurities, authenticity of drug, quality and purity of the test

sample. The total ash value indicates the impurities like carbonate, oxalate and silicate. The acid-insoluble ash is used to estimate the amount of silica present, especially sand which is the indication of contamination with earthy material.[14] Relatively less amount of these two parameters indicate low inorganic matter and silica were detected in spray-dried powders.

Heavy metal contamination testing aims to determine the levels of metal content Hg, Pb, Cd and As contained in spray-dried powders, which is dangerous and toxic to the body. The concentrations of all the tested heavy metals were below the WHO acceptable limits. Microbial contamination reveals the impurity in medicinal plants, which come from the preparation or final products. Based on microbial contamination test, the results of total yeast and mold number test spray-dried powders did not contain yeast and mold. The identification test of pathogenic bacteria is carried out to detect the presence of pathogenic bacteria including Escherichia coli, Salmonella sp, Staphylococcus aureus, Pseudomonas aeruginosa. Based on the test results, in all samples there were no pathogenic bacteria tested.

Preliminary phytochemical analysis

Phytochemical screening was carried out to identify the phytoconstituents present in the spray-dried powders. Based on the screening (Table 2), the compounds contained in the spray-dried powders of A. vulgaris are flavonoid, tannin and phenolic. The eupatilin content (Table 2) ranged from 0,89 mg/g to 0,90 mg/g.

Analgesic activity of A. vulgaris spray-dried powders

To evaluate whether or not analgesic activities of SDA are through acting on peripheral tissues, we performed acetic acid writhing test. As shown in Table 3, SDA (low and high dosage) and diclofenac sodium significantly reduced the writhing number as compared to control mice treated with CG group ($p < 0.05$). The percentage of inhibition of writhing was 63.57%, 35.71% and 37.86% in PG, LG and HG groups, respectively. Expectedly, positive drug diclofenac sodium showed significant analgesic activity with conspicuous decrease in number of writhes and increased inhibition ratio. These data suggest that SDA may have certain analgesic activity.

IV. CONCLUSION

In conclusion, the results obtained from physicochemical parameters, phytochemical screening studies and development of HPLC for the determination of eupatilin can be used to standardize spray-dried powders of A. vulgaris. Analgesic activity of A. vulgaris spray-dried powders by writhing induced method showed certain analgesic effect. Hence it is recommended that further advance and high level work should be done

on A. vulgaris spray-dried powders to use it as a natural, economic and safe drug.

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VI. CONFLICT OF INTEREST

The authors declare no conflict of interest.

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