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ORIGINAL ARTICLE

Gas Chromatography Coupled with Mass Spectrometry for the Rapid Characterization and Screening of Volatile Oil of *Euphorbia fischeriana* Steud

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ABSTRACT

Gas chromatography (GC)-mass spectrometry is a powerful tool which is used in the analysis of volatile components in food and herbal medicine. *Euphorbia fischeriana* Steud., a traditional Chinese herb, has been used for thousands of years in China. Recently, scientists are exploring more on the pharmacological action of *E. fischeriana*. However, so far, there is no information regarding the volatile constituents of *E. fischeriana*. Therefore, in this study, we aimed to investigate and characterize the global chemical ingredients of volatile oil of *E. fischeriana* under *in vitro* and *in vivo* conditions. In this study, an accurate and rapid GC combined with the mass spectrometric method and MassHunter tool were employed to identify the comprehensive constituents and explored the absorbed components in rat serum after oral administration of *E. fischeriana*. According to our results, a total of 28 compounds were identified via *in vitro* analysis by matching with the NIST database. Of them, the following 14 compounds were tentatively characterized in the serum samples: (−)-alpha-cedrene; (+/-)-sesquithuriferone; cedrol; acorenone; neocembrene; geranylgeraniol; abietatriene; abieta-7,13-diene; cembrenol; sclareol; ethyl linoleate; isopimara-7,15-dien-3-one; abietadienal; and abietinol. Most of the compounds identified were diterpenes and sesquiterpenes. These results might be useful in various pharmacological and pharmacodynamic research, and the established method could offer valuable chemical information for bioactive volatile components characterization.

Key words: Characterization, constituent, mass spectrometry, volatile oil

SUMMARY

- The established Gas Chromatography Coupled with Mass Spectrometry analysis combined with data processing approach was suitable for the rapid identification of constituents from Volatile Oil of *Euphorbia fischeriana* Steud *in vitro* and *in vivo*

Abbreviations used:
EFS: *Euphorbia fischeriana* Steud; VOEFS: Volatile Oil of *Euphorbia fischeriana* Steud; TCM: Traditional Chinese medicine; NIST: the National Institute of Standards and Technology; GC-MS: Gas Chromatography Coupled with Mass Spectrometry

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INTRODUCTION

Since many decades, Traditional Chinese Medicine (TCM), including various kinds of Chinese herbs, has played a critical role in drug discovery and human health.[1,2] *Euphorbia fischeriana* Steud.(EFS), a perennial herbaceous plant, locally known as *Lang-Du*, is a remarkable toxic TCM in northern China [Figure 1]. In recent years, there has been an increase in the number of pharmacological studies conducted on EFS extract which demonstrate its potential as anticancer, antivirus, anti-edema, and anti-inflammatory agent.[3,4] This encouraged us to further explore the bioactive constituents of EFS. Recent studies have shown that diterpenoids, triterpenes, sesquiterpenes, acetophenones, steroids, and tannins are the primary active components of EFS.[5-7]

Volatile oils contain a mixture of various chemicals including aldehydes, aliphatic and aromatic compounds, and terpenes. These compounds are an indispensable part of Chinese herbs which play an important role in the therapeutic application of TCM. Owing to the favorable pharmacological activities of EFS, it is important to further study the volatile oil of *E. fischeriana* Steud. (VOEFS) for the sake of broadening the application of EFS. However, to the best of our knowledge, so far, the composition of VOEFS remains unknown. Hence, it is important to identify the constituents of VOEFS under *in vitro* and *in vivo* conditions. Literature shows great development in the field of analytical methods for the rapid and accurate determination of various chemical components. For example, gas chromatography-mass spectrometry (GC-MS) is a powerful tool used in the analysis of volatile ingredients in TCM. Furthermore, with the advent of serum pharmacochemistry, various volatile bioactive constituents have been identified via GC-MS analysis.[8-10] In this study, GC-MS combined with an automatic data processing software was employed to identify and characterize the bioactive components of VOEFS.

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Figure 1: *Euphorbia fischeriana* Steud. and its dried root

MATERIALS AND METHODS

Chemicals and reagents

Deionized water was purified by a Millipore Milli-Q water purification system (Millipore Co., Ltd, China); ethyl acetate (High-performance liquid chromatography grade) was purchased from Dikma Technologies Inc.(China); Pentobarbital sodium and tween-80 were purchased from Sigma-Aldrich (Sigma, USA). The root of EFS was collected from Inner Mongolia in China and identified by doctor Qi Liu of Qiqihar Medical University.

Extraction and preparation of volatile oil of *Euphorbia fischeriana* Steud.

Deionized water (five times) was added to the dried root of EFS (6200 g) and was steeped overnight. Then, the mixture was hydro-distilled for 8 h to obtain a yellow oil. The oil was further extracted by ethyl acetate, and the extract was further dried by anhydrous sodium sulfate and evaporation of ethyl acetate. Finally, VOEFS was diluted in 2% Tween 80 (6 mg/mL VOEFS solution) and analyzed by GC-MS.

Animal experiments

A total of 16 male Wistar rats (weighing 220 ± 20 g) were purchased from Harbin Medical University (Harbin, China) and optionally fed in a standard animal house for 7 days. Then, the rats were randomly divided into experimental group and control group. Rats belonging to the experimental group and control group were administered orally with 6 mg/mL VOEFS solution and 2% Tween 80 solution at a dose of 10 mL/kg body weight. After 30 min, blood was collected from the hepatic portal vein and the animals were sacrificed. All the animal experiments were performed according to the protocols approved by the Review Committee of Animal Care and Use of Qiqihar Medical University.

Sample preparation for gas chromatography-mass spectrometry

The blood sample was centrifuged at 3500 rpm for 15 min at 4°C and serum was separated. Briefly, 200 μL of phosphoric acid was added to 5 mL of serum and vortexed for 1 min. Next, the mixture was extracted thrice with 5 mL of ethyl acetate. Then, 15 mL of the ethyl acetate extract was collected and dried under a stream of nitrogen gas at 40°C. The residue was redissolved in 100 μL of ethyl acetate and vortexed for 1 min. Serum samples from both groups were obtained via centrifugation of blood at 13,000 rpm for 10 min at 4°C. Meantime, the preceding VOEFS sample was processed as the same procedures. After filtration through a 0.22 μm membrane, 1 μL of dosed serum sample, blank serum sample, and VOEFS sample were injected into the GC-MS system consecutively.

Gas chromatography-mass spectrometry analyzing

GC-MS analysis was conducted using an Agilent 7890B-5977A system on an HP-5MS capillary column (30 m × 0.25 mm id, 0.25 μm). Helium was used as the carrier gas, and the flow rate was set at 1 mL/min during the acquisition period of 66 min. The oven temperature of GC was initially maintained at 50°C for 4 min and then increased to 160°C at a rate of 30°C/min and held for 8 min, and then increased to 170°C at a rate of 2°C/min and held for 27 min. Then, the oven temperature was rapidly increased to 190°C at a rate of 5°C/min and held for 4 min. Finally, the oven temperature was increased to 290°C at a rate of 15°C/min and maintained for 4 min. The temperatures of the injection port and interface temperatures were both 280°C, and the ion source temperature and quadrupole temperature were set at 230°C and 150°C, respectively. The optimal split ratio was 10:1. The electron energy was set at 70 eV and the MS was acquired in a full-scan mode at a range of 50–550 amu and the scan rate was set at 0.5 s.

Data processing

The data of dosed serum, blank serum, and VOEFS sample were processed using MassHunter software. Then, the peaks with an intensity higher than 10-fold of signal-to-noise ratio were filtered, and then the available MS/MS information including peak retention time, MS fragmentation, and intensity of every peak were extracted and automatically compared with the National Institute of Standards and Technology 14 (NIST14). Then, the compounds from VOEFS sample with a score higher than 80 points were listed and the information of the corresponding fragments were imported to establish a new library. Compared with the newly established constituents' library, components only found in dosed serum after oral administration of VOEFS were deemed as the constituents migrating to blood.

RESULTS AND DISCUSSION

Figure 2 shows the experimental procedures, and Figure 3 shows all the total ion chromatographs. The data were analyzed by MassHunter software, and the fragments were further screened via NIST 14. Finally, the results provided by NIST 14 were artificially confirmed again in sequence. According to the results, 28 peaks in VOEFS were exactly identified. Among them, 16 compounds were diterpenoids, 5 were sesquiterpenes, 2 were long-chain fatty acid ethyl esters, and 2 were long-chain alkenes. For example, take the identification of cedrol; the retention time was 13.339 min and its precise molecular weight was 222.198. The degree of unsaturation was calculated as 3, which means that it may be a ring compound. The calculated molecular formula was speculated to be $C_{15}H_{26}O$ based on the elemental analysis and fractional isotope abundance. By taking the fragmentation pattern and empirical knowledge into consideration, the main typical MS fragments were analyzed to be *m/z* 222$[C_{15}H_{26}O]^+$, 207$[C_{14}H_{23}O]^+$, 189$[C_{14}H_{21}]^+$, 177$[C_{13}H_{21}]^+$, 164$[C_{12}H_{20}]^+$, 150$[C_{11}H_{18}]^+$, 135$[C_{10}H_{15}]^+$, 121$[C_9H_{13}]^+$, 107$[C_8H_{11}]^+$, 95$[C_7H_{11}]^+$, 81$[C_6H_9]^+$, 69$[C_5H_9]^+$, and 55$[C_4H_7]^+$, which might match with cedrol in NIST 14 library. Figure 4 shows the relative mass spectrum and proposed fragmentation pathway.

Using the aforementioned conditions, the database included 28 identified chemical constituents from the VOEFS sample *in vitro* was created and all the corresponding information about the constituents including their retention time, molecular weight, and fragments' information were exported to a new library. Based on the new library, after deducting the background, chemical profiles of the dosed serum sample were compared with the blank serum sample. Next, 14 compounds including (−)-α-cedrene; (+/-)-sesquithuriferone; cedrol; acorenone; neocembrene; geranylgeraniol; abietatriene; abieta-7,13-diene; cembrenol; sclareol; ethyl linoleate; isopimara-7,15-dien-3-one; abietadienal; and abietinol were simultaneously screened in the dosed serum from the new library. Among the 14 constituents migrating to

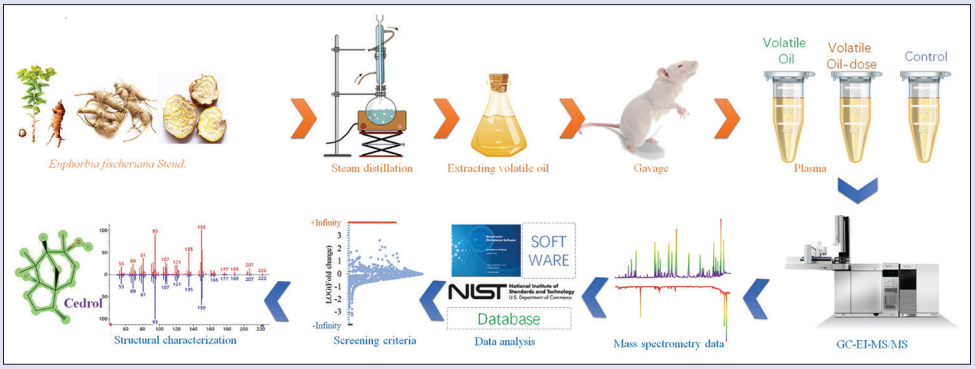

Figure 2: Experimental procedures for rapid discovery and global characterization of multiple components in volatile oil of *Euphorbia fischeriana* Steud.

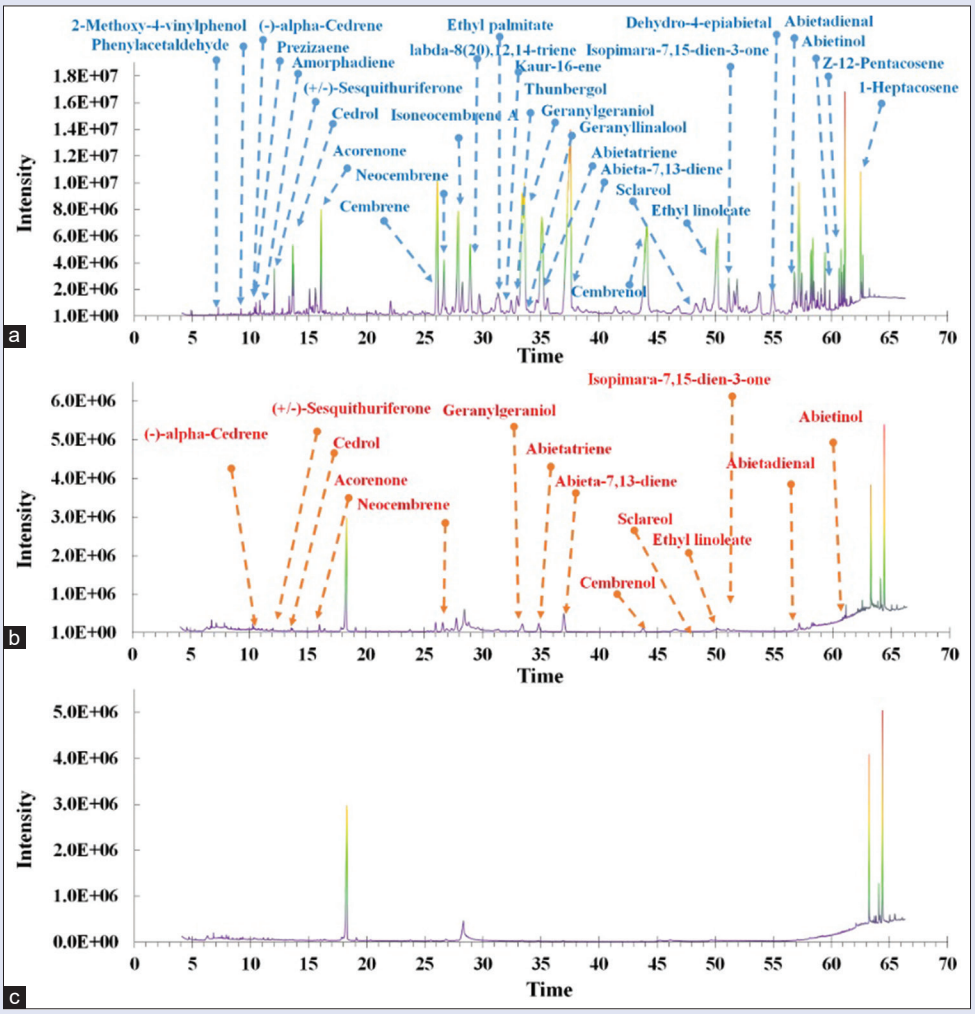

Figure 3: The total ion chromatograms of volatile oil of *Euphorbia fischeriana* Steud. sample (a), dosed serum sample (b) and control sample (c)

the blood, 9 were diterpenoids, 3 were sesquiterpenes, and there were still some other ingredients. It is worth mentioning that bioactive diterpenoids and sesquiterpenes which demonstrate activities especially anticancer activity accounted for 64% and 22%, respectively. Figure 5 shows the detailed results and related activities.

Based on network pharmacology method (http://lsp.nwu.edu.cn/index.php), the constituents migrating to the blood were closely related to multiple targets such as prostaglandin G/H synthase 2, thrombin, muscarinic acetylcholine receptor M2, acetylcholinesterase, dipeptidyl peptidase IV, peroxisome proliferator-activated receptor-γ, estrogen receptor, β2 adrenergic receptor, muscarinic acetylcholine receptor M1, nitric oxide synthase, endothelial, muscarinic acetylcholine receptor M3, sodium channel protein type 5 subunit α, neuronal acetylcholine receptor protein, alpha-7 chain, γ-aminobutyric acid

receptor subunit alpha-1, prostaglandin G/H synthase 1, androgen receptor, γ-aminobutyric-acid receptor alpha-2 subunit, retinoic acid receptor RXR-alpha, γ-aminobutyric-acid receptor alpha-3 subunit, neuronal acetylcholine receptor subunit alpha-2, alpha-1B adrenergic receptor, α-1A adrenergic receptor, β-lactamase, γ-aminobutyric-acid receptor subunit alpha-6, sodium-dependent noradrenaline transporter, α-1D adrenergic receptor and nuclear receptor coactivator 2 [Figure 5]. Moreover, four constituents such as cedrol, geranylgeraniol, sclareol, and ethyl linoleate were shown to have definite pharmacological activities. Cedrol, a kind of sesquiterpene alcohols, expresses various activities such as anticancer,[11,12] anti-inflammatory,[13] sedative,[14] and antifungal.[15-17] Zhang[12] investigated that cedrol can inhibit cell proliferation, induce apoptosis in A549 cells via mitochondrial and PI3K/Akt signaling pathways and pro-death autophagy by increasing intracellular production of reactive oxygen species. Moreover, Jeong *et al.*[13] found that cedrolcan inhibited the activity of cytochrome P450 enzyme in human liver microsomes. Geranylgeraniol, an important biosynthetic precursor of multiple biochemical products including terpenes, carotenoids, steroids, cholesterol, and paclitaxel, is a straight chain diterpene possessing extensive physiological activities such as anticancer, antivirus, and so on.[18-20] According to a previous study,[19] geranylgeraniol can significantly improve the steroidogenesis of testis-derived cells by adjusting cAMP/PKA signaling, which shows that geranylgeraniol may be a novel therapeutic agent. Moreover, it has been demonstrated that geranylgeraniol had toxic effect on human DU145 prostate carcinoma cells.[21] Furthermore, geranylgeraniol improved wound healing and tissue proliferation markedly, which means that it may be a promising option for the prevention and treatment of bisphosphonate-related osteonecrosis of the jaws.[22,23] Sclareol is a fragrant raw material which shows diverse activities such as anticancer, anti-inflammation, antiphotoaging, and antifungal.[24-27] Park *et al.*[24] found that sclareol inhibited the ultraviolet-induced mRNA expression of matrix metalloproteinases by adjusting the protein expression of AP-1 constituents, and it may be an effective candidate ingredient alleviating the facial wrinkle formation. Zhang *et al.*[28] declared that sclareol inhibited the growth of tumor cells via upregulation of tumor suppressor gene of caveolin-1 in cervical cancer cells, which offer a potential therapeutic target for human cancer in a clinical setting. Hsieh *et al.*[29] found that sclareol effectively ameliorated the lipopolysaccharide-induced acute lung edema via inhibition of phosphorylation of mitogen-activated protein kinases and induction of heme oxygenase-1 signaling. Furthermore, ethyl linoleate is used as an antiaging, antibacterial, anti-inflammatory, anti-atherosclerotic agent.[30,31] According to a previous study,[30] ethyl linoleate decreased the process of melanogenesis by blocking the phosphorylation of Akt and glycogen synthase kinase 3 β and by decreasing the level of β-catenin, suggesting that ethyl linoleate can be used as a whitening agent in cosmetics aimed at reducing hyperpigmentation. Park *et al.*[31] found that ethyl linoleate can attenuate the formation of lipopolysaccharide-induced pro-inflammatory cytokines via induction of heme oxygenase-1 in RAW264.7 cells. Finally, abietatriene inhibited melanogenesis by regulating the expression of melanogenic factors, suggesting its application in hyperpigmentation and skin-whitening creams.[32] The various constituents of VOEFS enter to circulation and exhibit their pharmacological activities, suggesting that it plays a vital role in healing many diseases.

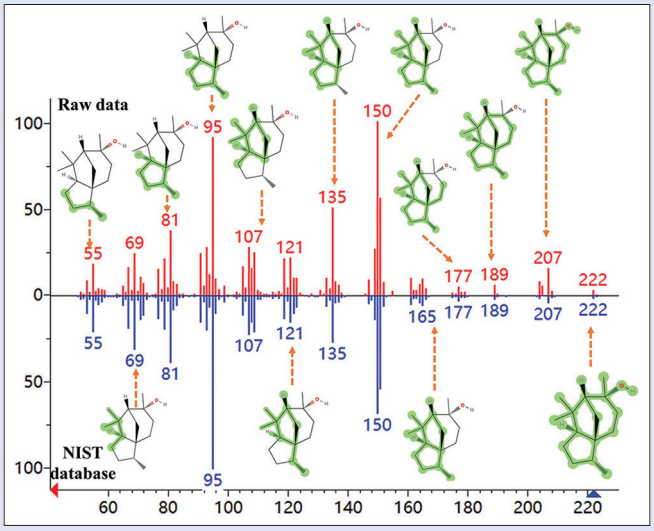

Figure 4: The schematic diagram for fragmentation pathway of cedrol

CONCLUSION

In this study, a rapid and efficient GC-MS technique combined with serum pharmacochemistry was employed to screen and track the multiple bioactive components entering the blood after the oral administration of VOEFS. Our results demonstrate that a total of 28 interesting ions of VOEFS were identified tentatively under *in vitro* conditions, and among them, 50% of the constituents were absorbed in the bloodstream. Among

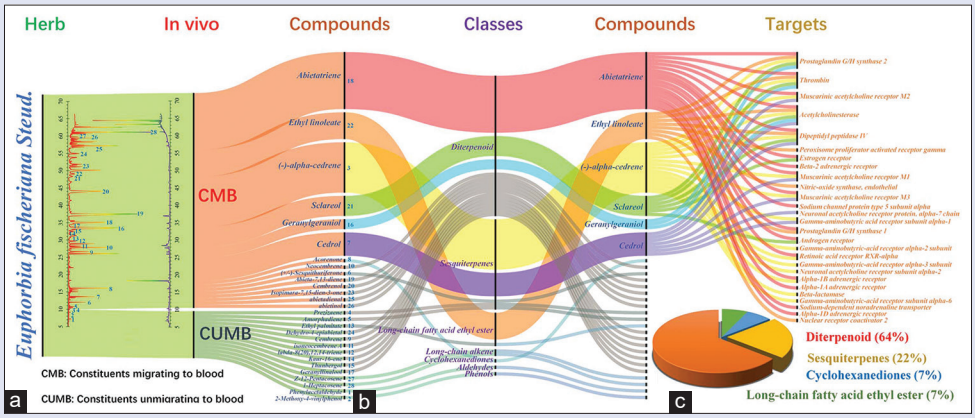

Figure 5: The composite diagram for the compounds information of *Euphorbia fischeriana* Steud. The total ion chromatograms of volatile oil of *Euphorbia fischeriana* Steud. sample and dosed serum sample (a), the classification and targets of constituents migrating to blood (b), and the proportion of these compounds (c)

the constituents absorbed into the blood, the bioactive diterpenoids and sesquiterpenes accounted for 64% and 22% respectively. This is a novel systematic study which explored the bioactive constituents of VOEFS. The characterization of VOEFS not only enriched the knowledge about its volatile constituents but also indicated their possible role in healing.

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Conflicts of interest

There are no conflicts of interest.

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