Composition of the Essential Oil of Aristolochia Manshurientsis Kom

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Abstract. This study demonstrated the chemical constituents of the essential oil of Aristolochia manshurientsis Kom and improved the essential oil efficiency by the enzyme-assisted extraction followed by hydrodistillation. The essential oils of Aristolochia manshurientsis Kom acquired by hydrodistillation after the solvent extraction with and without the assistance of cellulase have been investigated by gas chromatography/Mass spectrometry (GC-MS). The predominant constituents of both types of essential oils are camphene, 1,7,7-trimethyl-bicyclo [2.2.1] hept-2-yl acetate, 1,6-dimethyl-4-(1-methylethyl) naphthalene, caryophyllene oxide, borneol, and (-)-Spathulenol. The enzyme-assisted extraction not only increased extracting efficiency of the essential oil from 4.93% to 9.36%, but also facilitated the extraction of additional eight compounds such as 2-methano(-6,6-dimethyl) bicycle [3.1.1] hept-2-ene, (+)--terpineol and 1-propyl-3-(propen-1-yl) adamantane, which were not identified from the non-enzyme extraction sample.

1. Introduction
Aristolochia manshurientsis Kom, commonly known as Guanmutong in Chinese, is a kind of ligneous liane [1]. It is widely distributed in Liaoning, Jilin, Heilongjiang, Shanxi, Gansu, Sichuan, and Hubei provinces of China, north of Korea, and Russia [2]. This plant usually grows in the shady and wet mountainous region. It is often used to treat nephropathy and edema in Chinese Traditional Medicine [3]. The chemical composition of the ligneous stem of Aristolochia manshurientsis Kom has been already investigated. It has been reported that the predominant non-volatile chemical constituents from the ligneous stem of this plant are aristolochic acids, 6-methoxyaristolochic acid, aristoloside, magnoflorine, hederagenin, oleanolic acid, β-sitosterol, (+)-isocy clopermacrenal, manshurolide, (+)-(6S,7R)-6, debilic acid, and 11-bicycloergomacrenal (10) E,4E-dien-14-al [4]. Some of these compounds such as aristolochic acids were found to be responsible for the toxicity for the human body. It was claimed that an excess intake of Aristolochia manshurientsis Kom could lead to the acute renal failure (3). This became, to some extent, an obstacle to the practical use of Aristolochia manshurientsis Kom.

In contrast, the extraction of volatile component is an alternative way to the development of Aristolochia manshurientsis Kom-based products. Many essential oils from plants have shown to be applicable in many fields such as flavors, fragrances and antioxidants [5] [6] [7]. It is known that
essential oils from plants, e.g. traditional medicine, clove leaf and lemon, are usually recovered by hydrodistillation [8] [9] [10]. In addition, it has been reported that the enzyme treatment prior to the extraction will facilitate the improvement of the recovery efficiency of essential oils [11], natural pigments [12], moniliformin [13], antioxidative phenols [14], and capsaicinoids and carotenoids [15], and so forth.

To the best our knowledge, up to the present there has been no report yet with regard to chemical constituents of the essential oil from the ligneous stem of Aristolochia manshurientsis Kom. This paper report the constituents analysis of the essential oil obtained from Aristolochia manshurientsis Kom collected from northeast of China by GC-MS method. The chemical constituents and the extracting efficiency of the essential oils by using the hydrodistillation with and without the enzyme-assisted solvent extraction were compared. We found that the enzyme-assisted extraction not only gave rise to the increased extracting efficiency of the essential oil, but also facilitated the extraction of additional eight compounds.

2. Materials and Methods
The materials in this work, Aristolochia manshurientsis Kom, was obtained from ligneous stem of the corresponding plant from Northeast of China, and was hand-chopped to 2-3 cm pieces after being naturally dried at room temperature. Ferrous sulphate and anhydrous sodium sulfate were commercially available from ACROS, respectively. Anhydrous ethyl ether was purchased from Aldrich and freshly distilled after the treatment with ferrous sulphate. The enzyme used was Cellucalast 1.5 L (Novo Nordisk, Switzerland). The stated activity of the enzyme was 1500 NCU (Novo cellulose units). Ten units were added per gram of the dried plants materials.

Essential oils were obtained by using the hydrodistillation according to references [5] [6] [7] [8] [9] [10]. The raw materials were pretreated by immersion in water with and without cellulase for 24 hours at 45°C, respectively, and underwent the subsequent ultrasonic pretreatment for 30 minutes. Then the mixture was distilled for 4 hours. The essential oil was cooled and separated from the water. In order for drying the extract and removing the high-molecular-weight substances, the extract was passed through a small column comprising of anhydrous sodium sulfate and silica gel. Samples were kept in amber vials at 4 °C until chromatographic-mass spectroscopic analysis. The yields of the essential oil were expressed in percentage (w/w) of dry weight of the plant dry matter.

The yellowish sample consisting of 2 µL of distilled essential oils in ethyl ether were analyzed on GC-MS. The content of each kind of chemical composition was calculated and represented as a peak area percentage provided by the GC system automatically. Every oil sample was analyzed three times and the average was obtained. For this portion of the work, a Hewlett-Packard 6890 series gas chromatograph, equipped with a HP-5 phenylmethyl polysiloxane 30 m x 0.25 mm fused-silica capillary column with 0.25 µm film thickness, was used. The GC was linked to a Hewlett-Packard model 5973A mass spectrometry detector. The parameters were set as follows: The injector temperature 230 °C, the split injection mode with split ratio 20:1, and the oven temperature from 60 °C (maintain 5 min) to 200 °C (maintain 10 min) at a ramp of 5 °C/min. Helium was used as carrier gas with a flow of 1 mL/min. The mass spectrometer was operated in the 70 eV EI mode with scanning from 20 to 500 aum. The EI ion source is at 230°C, with the quadripole temperature at 150 °C and emission current 34.6 µA. Identification of the chemical constituents of the essential oil was according to computer matching with commercial mass spectra (NIST 98/EPA/NIH), the Wageningen Collection of Mass Spectra of Natural Products, and the mass spectroscopy literature data [16].

3. Results and Discussion
Figure 1 showed the TIC chromatogram chart of the essential oil of Aristolochia manshurientsis Kom without the addition of cellulases. Table 1 made a detailed list about the constituents of the essential oil, in which 35 chemical compounds were identified and accounted for 73.41%. The extraction efficiency of the essential oil was 4.93% based on the calculation on the dried plant materials. The predominant constituents of the essential oil are borneol (10.81%), camphene (2.91%), caryophyllene oxide (6.08%), 1,7,7-trimethyl-bicyclo [2.2.1] hept-2-yl acetate (10.12%), and
1,4-dimethyl-7-(1-methylethyl) -azulene (4.05%), and (-)-spathulenol (3.72%). Other important constituents are 1, 2, 3, 5, 6, 8-hexahydro -4,7-dimethyl-1-(1-methylethyl) -naphthalene (2.59%), 2-methyl-5-(1-methylethenyl)-trans-2-cyclohexen-1-ol (1.04%), hexanoic acid (1.97%), and 4-(1-methylethyl)-1-cyclohexene-1-carboxaldehyde (1.87%).

Figure 2 gives the TIC chromatogram of the essential oil of Aristolochia manshuriensis Kom with the addition of cellulases. The chemical constituents of the essential oil were listed in Table 2, in which 53 compounds were identified, accounting for 70.66% of the whole essential oil. The present study was carried out with the hope that the enzyme-assisted process would increase the efficiency of extraction of essential oil from Aristolochia manshuriensis Kom. In effect, we found that the efficiency increased up to 9.36%, calculated on the dried plant materials.

The predominant constituents of this type of essential oils are 1,7,7-trimethyl-bicyclo [2.2.1] hept-2-yl acetate (9.90%), 1,4-dimethyl-7-(1-methylethyl)-azulene (1.77%), camphene (3.89%), caryophyllene oxide (4.90%), borneol (10.40%), and (-)-spathulenol (3.12%). There was also some increase in percentage of these compounds when the enzyme was added compared to that of the non-enzyme-assisted extraction. It is interesting to note that the most conspicuous changes were the appearance of (+)-α-terpineol (1.90%), and 2-methano (-6,6-dimethyl) bicycle [3.1.1] hept-2-ene (0.81%), which were not identified in the sample with the non-enzyme extraction.

**Table 1.** Identification Data of Volatile Compounds in Aristolochia manshuriensis Kom without the addition of cellulases.

| No | Constituents | R. T. | Formula | M.W. | %   |
|----|--------------|-------|---------|------|-----|
| 1  | α-pinene     | 4.16  | C_{10}H_{16} | 136.13 | 1.66 |
| 2  | camphene     | 4.43  | C_{10}H_{16} | 136.13 | 2.91 |
| 3  | β-pinene     | 4.94  | C_{10}H_{16} | 136.13 | 0.62 |
| 4  | hexanoic acid| 5.25  | C_{6}H_{12}O_{2} | 116.08 | 1.97 |
| 5  | 1-methyl-3-(1-methylethyl)-benzene | 5.89 | C_{10}H_{14} | 134.11 | 0.72 |
| 6  | D-limonene   | 5.98  | C_{10}H_{16} | 136.13 | 0.72 |
| 7  | trans-Mentha-2,8-dienol | 8.13 | C_{10}H_{16}O | 152.12 | 0.42 |
| 8  | 6,6-dimethyl-2-methylene-bicyclo[3.1.1]heptan-3-ol | 8.60 | C_{10}H_{16}O | 152.12 | 0.79 |
| 9  | borneol      | 9.30  | C_{10}H_{16}O | 154.14 | 10.81 |
Table 1 Cont.

|   | Formula                  | Retention Time | Molecular Weight | Molecular Formula |
|---|--------------------------|----------------|------------------|-------------------|
| 10| 4-(1-methylethyl)-2-cyclohexen-1-one | 9.80           | C₈H₁₄O            | 138.10            |
| 11| 4-trimethyl-3-cyclohexene-1-methanol | 9.91           | C₁₀H₁₆O            | 154.14            |
| 12| 6,6-dimethyl-bicyclohept-2-ene-2-methanol [3.1.1] | 10.05 | C₁₀H₁₆O            | 152.12            |
| 13| 4,6,6-trimethyl-bicyclohept-3-en-2-one [3.1.1] | 10.38 | C₁₀H₁₆O            | 150.10            |
| 14| trans-2-methyl-5-(1-methylethenyl)-2-cyclohexen-1-ol | 10.63 | C₁₀H₁₆O            | 152.12            |
| 15| 1,7,7-trimethyl-formate,endo-bicyclo[2.2.1]heptan-2-ol, | 10.86 | C₁₁H₁₆O₂           | 182.13            |
| 16| cis-2-methyl-5-(1-methylethenyl)-2-cyclohexen-1-ol | 10.94 | C₁₀H₁₆O            | 152.12            |
| 17| 2-methyl-5-(1-methylethenyl)-2-cyclohexen-1-one | 11.28 | C₁₀H₁₆O            | 150.10            |
| 18| 4-(1-methylethyl)-1-cyclohexene-1-carboxaldehyde | 12.08 | C₁₀H₁₆O            | 152.12            |
| 19| 1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl acetate | 12.35 | C₁₂H₂₀O₂           | 196.15            |
| 20| Caryophyllene | 15.76 | C₁₅H₂₄            | 204.19            |
| 21| 1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethenyl)-azulene | 16.71 | C₁₅H₂₄            | 204.19            |
| 22| decahydro-1,1,7-trimethyl-4-methylene-1H-cycloprop[e]azulene | 16.77 | C₁₅H₂₄            | 204.19            |
| 23| butylated hydroxytoluene | 17.99 | C₁₅H₂₂O            | 220.18            |
| 24| 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylthyl)-1(1S-cis)-naphthalene | 18.26 | C₁₅H₂₄            | 204.19            |
| 25| (-)-spathulenol | 19.54 | C₁₅H₂₂O            | 220.18            |
| 26| Caryophyllene oxide | 19.67 | C₁₅H₂₄O           | 220.18            |
| 27| (4aR-trans)-decahydro-4a-methyl-1-methylene-7-(1-methylethylidene)-naphthalene | 19.87 | C₁₅H₂₆O           | 222.20            |
| 28| 2-acetyl-6-methoxynaphthalene | 20.56 | C₁₃H₁₅O₂           | 200.08            |
| 29| 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylthyl)-naphthalene | 20.68 | C₁₅H₂₄            | 204.19            |
| 30| cadinol | 21.00 | C₁₅H₂₆O           | 222.20            |
| 31| Copaene | 21.08 | C₁₅H₂₄            | 204.19            |
| 32| Selina-6-en-4-ol | 21.27 | C₁₅H₂₆O           | 222.20            |
| 33| 1,4-dimethyl-7-(1-methylethyl-azulene) | 21.73 | C₁₅H₁₈            | 198.14            |
| 34| 1-ethyl-1-methyl-2-(1-methylethenyl)-4-(1-methylethylidene)-cyclohexane, | 25.77 | C₁₅H₂₄            | 204.19            |
| 35| Nonadecane | 26.32 | C₁₉H₄₀            | 268.31            |
Percentages obtained by peak area normalization.
Identification by gas chromatography-mass spectrometry and the mass spectroscopy literature data.

![Abundance](image)

**Figure 2.** TIC chromatogram of the essential oil of *Aristolochia manshuriensis* Kom with the addition of cellulases.

**Table 2.** Identification Data$^a$ of Volatile Compounds in *Aristolochia manshuriensis* Kom with the addition of cellulases.

| No | Constituents$^b$ | R. T. | Formula | M.W. | %  |
|----|-----------------|------|---------|------|----|
| 1  | α-pinene        | 4.16 | C$_{10}$H$_{16}$ | 136.13 | 2.09 |
| 2  | camphene        | 4.43 | C$_{10}$H$_{16}$ | 136.13 | 3.89 |
| 3  | 3-thujen-2-ol   | 4.52 | C$_{10}$H$_{16}$O | 152.12 | 0.44 |
| 4  | β-pinene        | 4.94 | C$_{10}$H$_{16}$ | 136.13 | 0.64 |
| 5  | hexanoic acid   | 5.25 | C$_{6}$H$_{12}$O$_2$ | 116.08 | 1.48 |
| 6  | 1-methyl-3-(1-methylethyl)-benzene | 5.90 | C$_{10}$H$_{14}$ | 134.11 | 0.43 |
| 7  | D-limonene      | 5.98 | C$_{10}$H$_{16}$ | 136.13 | 1.26 |
| 8  | eucalyptol      | 6.04 | C$_{10}$H$_{16}$O | 154.14 | 0.30 |
| 9  | 3,7-dimethyl-1,6-octadien-3-ol | 7.60 | C$_{10}$H$_{16}$O | 154.14 | 0.19 |
| 10 | trans-p-mentha-2,8-dienol | 8.12 | C$_{10}$H$_{16}$O | 152.12 | 0.42 |
| 11 | 2,2,3-trimethyl-3-cyclopentene-1-acetaldehyde | 8.27 | C$_{10}$H$_{16}$O | 152.12 | 0.12 |
| 12 | 6,6-dimethyl-2-methylene-bicyclo [3.1.1] heptan-3-ol | 8.60 | C$_{10}$H$_{16}$O | 152.12 | 0.81 |
| 13 | 4-isopropylcyclohexanone | 9.08 | C$_{8}$H$_{16}$O | 140.12 | 0.14 |
| 14 | 6,6-dimethyl-2-methylene-bicyclo [2.2.1] heptan-3-one | 9.20 | C$_{10}$H$_{16}$O | 150.10 | 0.32 |
| 15 | borneol         | 9.30 | C$_{10}$H$_{16}$O | 154.14 | 10.40 |
| 16 | 4-(1-methylethyl)-2-cyclohexen-1-one | 9.80 | C$_{8}$H$_{12}$O | 138.10 | 1.34 |
| 17 | 4-trimethyl-3-cyclohexene-1-methanol | 9.92 | C$_{10}$H$_{16}$O | 154.14 | 1.90 |
| No. | Chemical Structure | Formula | Molecular Weight | Molar Refractivity |
|-----|--------------------|---------|------------------|-------------------|
| 18  | 6,6-dimethyl-bicyclo [3.1.1] hept-2-ene-2-methanol | C_{10}H_{16}O | 152.12 | 1.17 |
| 19  | 4,6,6-trimethyl-bicyclo [3.1.1] hept-3-ene-2-one | C_{10}H_{16}O | 150.10 | 2.14 |
| 20  | trans-2-methyl-5-(1-methylethenyl)-2-cyclohexene-1-ol | C_{10}H_{16}O | 152.12 | 2.02 |
| 21  | 1,7,7-trimethyl-1-formylendo-bicyclo[2.2.1]heptan-2-ol | C_{10}H_{16}O | 182.13 | 2.30 |
| 22  | cis-2-methyl-5-(1-methylethenyl)-2-cyclohexen-1-ol | C_{10}H_{16}O | 152.12 | 0.52 |
| 23  | 2-methyl-3-phenyl-propanal | C_{10}H_{16}O | 148.09 | 0.27 |
| 24  | 2-methyl-5-(1-methylethenyl)-2-cyclohexen-1-one | C_{10}H_{16}O | 150.10 | 1.15 |
| 25  | 4-(1-methylethyl)-1-cyclohexene-1-carboxaldehyde | C_{10}H_{16}O | 152.12 | 1.03 |
| 26  | 1,7,7-trimethyl-bicyclo [2.2.1] hept-2-yl acetate | C_{12}H_{20}O_2 | 196.15 | 9.90 |
| 27  | 4-(1-methylethyl)-benzenemethanol | C_{10}H_{16}O | 150.10 | 0.41 |
| 28  | 2-methoxy-4-viylphenol | C_{8}H_{10}O | 150.07 | 0.98 |
| 29  | 2-(1,1-dimethylethyl)-phenol | C_{8}H_{10}O | 150.07 | 0.11 |
| 30  | Caryophyllene | C_{15}H_{24} | 204.19 | 0.48 |
| 31  | Cyclohexene,6-ethenyl-6-methyl-1-(1-methylethyl)-3-(1-methylethyl idene)-(s)- | C_{15}H_{24} | 204.19 | 0.19 |
| 32  | 1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethylidene)-azulene | C_{15}H_{24} | 204.19 | 0.34 |
| 33  | decahydro-1,1,7-trimethyl-4-methylene-1H-cyclpropene|azulene | C_{15}H_{24} | 204.19 | 0.36 |
| 34  | (+)-epi-bicyclosesquiphellandrene | C_{15}H_{24} | 204.19 | 0.26 |
| 35  | Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-7-methyly-4-methylene-1-(1-methylethyl)-(1.alpha.,4a.al pha.,8a.alpha.) | C_{15}H_{24} | 204.19 | 0.19 |
| 36  | isoledene | C_{15}H_{24} | 204.19 | 0.20 |
| 37  | octahydro-1,1,4,7-tetramethyl-1H-cyclopropene|azulene | C_{15}H_{24} | 204.19 | 0.40 |
| 38  | α-Muurolene | C_{15}H_{24} | 204.19 | 0.20 |
| 39  | 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-cyclohexene | C_{15}H_{24} | 204.19 | 0.17 |
| 40  | 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methyl ethyl)-(1S-cis)-naphthalene | C_{15}H_{24} | 204.19 | 1.43 |
| 41  | 6,10-dimethyl-3-(1-methylethylidene)-1-cyclodene | C_{15}H_{24} | 206.20 | 0.43 |
Table 2. Cont.

| No | compound | C_{19}H_{30}O | mass spectrum data |
|----|----------|----------------|-------------------|
| 42 | (-)-spathulenol | 19.55 | 220.18 | 3.12 |
| 43 | caryophyllene oxide | 19.68 | 220.18 | 4.90 |
| 44 | (4αR-trans)-decahydroy-4a-methyl-1-methylene -7-(1-methylethylidene)-napthalene | 19.88 | 204.19 | 2.60 |
| 45 | viridiflorol | 20.12 | 222.20 | 1.32 |
| 46 | 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylene)napthalene | 20.68 | 204.19 | 0.81 |
| 47 | 1α,2,3,3a,4,5,6,7b-octahydro-1,1,3a,7-tetramethylene 1H-cyclopropa[a]napthalene | 20.78 | 204.19 | 0.25 |
| 48 | cadinol | 21.00 | 222.20 | 0.77 |
| 49 | copaene | 21.08 | 204.19 | 0.94 |
| 50 | selina-6-en-4-ol | 21.27 | 222.20 | 0.81 |
| 51 | 1,4-dimethyl-7-(1-methylethyl)-azulene | 21.73 | 198.14 | 1.77 |
| 52 | 1,6-dimethyl-4-(1-methylethyl)napthalene | 21.79 | 198.14 | 0.29 |
| 53 | (1α,2β,5α)-2-methyl-5-(1-methylethenyl)-cyclohexanol | 23.89 | 154.14 | 0.26 |

*Percentages obtained by peak area normalization.

Identification by gas chromatography-mass spectrometry and the mass spectroscopy literature data.

Totally speaking, alcohols, ketones, terpenoids, esters, etc. were identified in the two kinds of essential oils. Among these compounds, camphene is one of the most odorous constituents of camphor. The identification of a relatively large amount of camphene in the essential oil of the studied species confirms the particular odor [1], similar to camphor, of the living plant. Terpenoids in the essential oils including α-pinene, β-pinene, limonene, camphene have received extensive attentions for the antiflammatory, analgesic, antimicrobial and insecticidal properties [17]. The borneol in two kinds of essential oils showed the highest in quantity (10.40% and 10.81%). It has been reported that borneol is a colorless monoterpen, which have many effects such as antibacterial, antifungal, antispasmodic, chloeretic and tranquilizing [18]. It is also interesting to note that the enzyme-assisted extraction facilitated the existence of a large amount of 1-propyl-3-(propen-1-yl) adamantane (5.69%) in the essential oil. It has been reported that the adamantanes derivatives can be used to modulate the reactive oxygen species [19] [20]. This means that the essential oil of Aristolochia manshuriensis Kom obtained by enzyme-assisted extraction may also potentially be used as the antioxidant. Therefore, in term of this function, enzyme-assisted extraction has an advantage over the regular extraction of the essential oil of Aristolochia manshuriensis Kom. The aforementioned activities of compounds existed in the essential oil indicate that it will be useful in antiflammatory, analgesic, tranquilizing, antioxidant, antimicrobial and insecticidal areas.

As for extraction of valuable materials from plants, it is important to study not only efficiency, but also changes that might be caused in the target substances by the addition of enzyme. In this study, the enzyme-assisted extraction not only gave rise to the increased extraction efficiency of the essential oil from 4.93% to 9.36%, but also facilitated the extraction of additional eight compounds. It is believed that the introduction of cell-wall hydrolyzing enzyme in the pretreatment process usually has two functions. On the one hand, partial hydrolysis of the cell wall increases its permeability and facilitates higher recovery yields of the target products. On the other hand, softening of cell wall gives rise to the mild extraction conditions. The essential oil in Aristolochia manshuriensis Kom is usually confined within specific cells in certain tissues in the ligneous stems, and will be released from the cells by the solvent when the tissues are hydrolysed or/and softened by the enzymes [11] [12] [13] [14] [15].
This study demonstrated the chemical constituents of the essential oil of *Aristolochia manshuriensis* Kom, and the increased essential oil efficiency and the changes in its composition by the enzyme-assisted extraction followed by hydrodistillation. It would be worthwhile for guidance of the practical use of the Aristolochia manshuriensis Kom in a new form of essential oil, instead of the form of toxic-containing whole ligneous stem. It would be also beneficial for the quality control of the essential oil in viewpoint of its application in such as fragrances, anti-inflammatory, analgesic, tranquilizing agents and antioxidants.

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