Analysis of the Components in Moxa Smoke by GC-MS and Preliminary Discussion on Its Toxicity and Side Effects

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Moxibustion plays an important role in the prevention and treatment of diseases and the promotion of human health. In this study, the components in moxa smoke from Jiangxi Poai Biotechnology Co., Ltd., namely, Qingmoxa sticks, were absorbed by five solvents (cyclohexane, ethyl acetate, n-butanol, anhydrous ethanol, and water) and identified by gas chromatography-mass spectrometry. The identification results of the smoke from the Qingmoxa sticks that was absorbed in liquid are as follows: a total of 294 compounds were identified, including 139 in cyclohexane, 145 in ethyl acetate, 60 in n-butanol, 89 in anhydrous ethanol, and 77 in water, and of those, 112 toxic compounds were identified. Furthermore, Ingenuity Pathway Analysis software and the PubChem database were successfully applied to analyze the toxic compounds. There were 812 target proteins related to the toxic components, 25 molecular networks, and 54 biological pathways. The results showed that the toxic compounds of moxa smoke may have some side effects on the heart, liver, and kidney of humans. This study revealed that the components of moxa smoke are complex and diverse. Due to the findings of toxic compounds in moxa smoke, we recommend that moxibustion rooms should be equipped with ventilation equipment or enough artificial ventilation to ensure the health of patients and practitioners.

1. Introduction

Moxibustion is an important part of clinical treatment in traditional Chinese medicine. In moxibustion, wormwood or other drugs are used to place acupoints or pain points on the body surface for warming meridians and stimulating acupuncture points [1]. As people pay more attention to health, the use of moxibustion to treat diseases in China and other Asian countries is growing [2]. Heat and moxa smoke are produced during moxibustion. The heat of moxibustion has the function of assisting Yang Qi, lifting subsidence, and solidifying. Recent studies have shown that moxa smoke also has antibacterial, antitumor, antiviral, anti-inflammatory, and air purification functions [3–7]. Ancient books on Chinese Medicine contain records of the use of moxa smoke in the treatment of irritable bowel syndrome [8], inflammatory bowel disease [9], and neurological symptoms [10]. Additionally, the antioxidants in moxa smoke play an antiaging role through the penetration of heat [11].

However, some patients feel uncomfortable during moxibustion and can even have noticeable adverse reactions, such as watery eyes and coughing, which has caused people to question the safety of moxa smoke [12, 13]. Some studies have shown that there were harmful components such as monoaromatic hydrocarbons and formaldehyde in moxa smoke [14–16]. The inhalation of these substances induced eustachian tube irritation, throat itching, eye pain, tonsil swelling, and other toxic effects [12]. Therefore, it is very important to determine the toxic compounds in moxa smoke.

The aim of the present study was to analyze the components in Qingmoxa smoke based on enrichment with five solvents. A set of smoke absorption devices were designed with cyclohexane, ethyl acetate, n-butanol, anhydrous...
ethanol, and water as absorbents with the help of an extraction pump to concentrate the moxa smoke in the solvents. The benefits of this device for enrichment of moxa smoke include: (1) moxa sticks can burn completely in the air to avoid incomplete combustion; (2) the devices can detect as many compounds as possible by increasing the concentration of moxa smoke; and (3) the use of different polar solvents can provide reference for the absorption and treatment of moxa smoke. Then, the toxic compounds were queried by the Comparative Toxicogenomics Database (CTD) [17, 18]. In addition, we aimed to estimate the toxic compounds in moxa smoke that would have an impact on the human body by applying Ingenuity Pathway Analysis (IPA) software and the PubChem database to provide an experimental basis for the safety evaluation of moxa smoke [19, 20].

2. Materials and Methods

2.1. Materials. We followed the steps outlined in our patent, “A method of using Terahertz Wave to detect the quality of moxa column,” patent number: ZL 2020 1 0000161.6, which are as follows: (1) sample placement; (2) determination of the background value; (3) measurement of the terahertz wave energy at different bands of the combustion column; (4) data processing; and (5) column quality judgment. If the terahertz wave intensity of each band is stronger than others and the waveform slightly changes, the quality is better. The results revealed which Qing moxa stick had the best quality, and that one was selected for smoke enrichment analysis [21]. Qing moxa sticks (18 × 27 ± 1 mm, Jiangxi Poai Biotechnology Co., Ltd., Poyang, China), which are widely used by the Chinese population, were used in this study. Moxa sticks were encased in Artemisia argyi (Chinese mugwort) floss, which was made of dried A. argyi leaves. The Qing moxa sticks were produced with a 10:1 ratio, which means that 10 kg of dried A. argyi leaves were processed into 1 kg of moxa floss. Analytical grade cyclohexane, ethyl acetate, n-butanol, and ethanol were all purchased from Guangdong Xilong Science Co., Ltd., and used as received.

2.2. Sample Preparation. A set of smoke absorption devices was designed as shown in Figure 1. With 1000 mL cyclohexane, ethyl acetate, n-butanol, anhydrous ethanol, or water as the solvent, 50 moxa sticks were burned in the air until combustion was complete. During the combustion process, the air pump control combustion speed was adjusted such that the blank flask did not fill with white smoke, so that the solvent fully absorbed the moxa smoke. The glass ball in the absorption flask had holes in it to reduce the production of bubbles and prevent the solvent from escaping. The absorption solution was emptied from the absorption filter, with a 0.22 μm microporous membrane, and 2 mL of each solution was added into the sample bottle for gas chromatography-mass spectroscopy (GC-MS) analysis.

2.3. GC-MS Analysis. An Agilent Technologies 7890 GC system (Agilent Technologies Inc., Palo Alto, CA, USA) coupled with an Agilent Technologies 5975 mass spectrometer (Agilent Technologies Inc.) was used for moxa smoke analysis. A HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm) was used to separate compounds. High-purity helium was applied as the carrier gas. The following conditions were used: column flow rate: 1.0 mL/min; split injection, split ratio: 100:1; injection volume: 1 μL; and injection port temperature: 250°C. The temperature procedure was as follows: 0–3 min, 40–40°C; 3–39 min, 40–220°C; 39–43 min, 220–220°C; 43–49 min, 220–280°C; and 49–50 min, 280–280°C.

The MS working conditions were as follows: the electron ionization energy was 70 eV, the full-scan acquisition was used in the range of 50–650 m/z, the ion source temperature was 230°C, the transmission ion temperature was 280°C, and the four-stage pole temperature was 150°C. The identification of each peak in the total ion flow chromatogram was automatically retrieved from the National Institute of Standards and Technology (NIST) 11.L as the standard mass spectrometry database and verified with standard mass spectrometry. Some components were confirmed with the retention value of a standard sample. The identified components were semiquantified by comparing the peak area of each component with the total peak area, and the relative percentage of components was calculated by the peak area normalization method.

2.4. Network Toxicological Analysis. The compounds identified by the NIST 11.L were then queried for related toxicity through the CTD database (https://ctdbase.org/about/). Then, the molecular information corresponding to the toxic compounds of moxa smoke was obtained from the PubChem database (http://pubchem.ncbi.nlm.nih.gov/) [22]. In addition, the Swiss Target Prediction database (http://www.swisstargetprediction.ch/) was used to predict toxic compounds relevant targets, and exporting Uniprot ID. Next, the molecular networks of toxic compound target proteins and its biological pathways were constructed by IPA software (Qiagen, Redwood City, CA, USA).

3. Results

3.1. Total Ion Chromatogram. The total ion chromatograms (TIC) of moxa smoke from solvents by GC-MS are shown in Figure 2 [23, 24]. As shown in Figure 2, the compounds in moxa smoke were detected within 40 min.

3.2. GC-MS Analysis Results. A total of 294 compounds, including 139 in cyclohexane, 145 in ethyl acetate, 60 in n-butanol, 89 in anhydrous ethanol, and 77 in water were found and identified in Qing moxa smoke. As shown in Tables 1–5, only 52 unique compounds were detected in cyclohexane smoke absorption liquid, 57 in ethyl acetate, 10 in n-butanol, 17 in anhydrous ethanol, and 47 in water, and other components were identified in more than one solvent. Toluene, pyridine, 2-methylpyridine, 2-methyl-2-cyclopenten-1-one,
2-furanmethanol, 2-acetylfuran, phenol, eucalyptol, o-cresol, indole, and biphenyl were detected in all five solvents and are shown in Figure 3, but the same components had different concentrations in different solvents. This shows that the components of moxa smoke were absorbed differently by different polar solvents.

As shown in Figure 3, the common components from moxa smoke in the five solvents included toluene (0.650%–3.872%), pyridine (0.137%–2.847%), 2-methylpyridine (0.267%–1.878%), 2-methyl-2-cyclopenten-1-one (0.412%–1.649%), 2-furanmethanol (0.526%–1.320%), 2-acetylfuran (0.266%–1.092%), phenol (2.686%–5.405%), eucalyptol (1.037%–1.605%), o-cresol (0.661%–1.419%), indole (0.780%–1.257%), and biphenyl (0.179%–0.338%). Among the above common components, the relative contents of phenol were more than 2% in all solvents. Phenol is a corrosive compound that is a strong irritant, which can lead to acute poisoning, skin ulcers, and tissue burns and can even be life-threatening [25, 26]. However, the amount of harmful substances produced by moxibustion will dictate thenegative impact on the human body, and the duration of exposure to a moxa fume environment will determine if damage is caused to the body. There is no unified answer to these questions, which requires a large amount of case analysis and clinical trials.

3.3. Toxic Compounds of Moxa Smoke. The toxicity of compounds was determined based on the CTD database (https://ctdbase.org/about/), which provided abundant toxicological information for researchers. Among the 294 compounds detected in the moxa smoke absorption liquid, 112 compounds were confirmed to be toxic. Further study is needed to explore the toxicity of the 112 compounds. Table 6 provides details of the 112 toxic compounds.

3.4. Targets of Toxic Compounds. Through the PubChem database (http://pubchem.ncbi.nlm.nih.gov/), molecular information for the 112 toxic compounds in moxa smoke was identified, and the corresponding number of "Canonical SMILES" was obtained. Then, using the Swiss Target Prediction database (http://www.swisstargetprediction.ch/) to predict the 112 relevant targets of the toxic compounds, the UniProt ID was exported. In addition, the UniProt ID was analyzed with IPA software to obtain the targets of toxic compounds. There were 812 targets for the toxic compounds in moxa smoke, compared to 810 identified with the IPA database.

3.5. Molecular Networks of Toxic Compounds. The UniProt IDs of the 810 target proteins of the 112 toxic compounds were imported into the IPA bioanalysis software. Under the "tox analysis" module, IPA was used to construct the molecular networks of target proteins. A total of 25 molecular networks were constructed for 112 toxic compounds, with a maximum score of 43, as shown in Figure 4. The results showed that these target proteins were related to cell signal transduction, nucleic acid metabolism, inflammatory response, organ damage, and cell apoptosis. Therefore, this can be used to frame a correlation study on moxa smoke.

3.6. Biological Pathways of Toxic Compounds. Using the "tox analysis" module in the IPA software, a total of 54 biological pathways were found for the 112 toxic compounds. The main biological pathways of the toxic compounds from moxa smoke included cardiotoxicity, hepatotoxicity, and nephrotoxicity. Consequently, the toxic compounds of moxa smoke may have some side effects on the human heart, liver, and kidneys. A heat map of the biological pathway of toxic compounds is shown in Figure 5. According to it, the pathway with the highest −log (p value) was cardiac arriphathy, which was classified as cardiotoxicity, with a value of 79.429. Drug target molecules acting on this pathway include ABCB1, ABCCC8, ACE, ADORA1, ADORA2A, ADORA2B, ADORA3, ADRA2A, ADRA2B, ADRA2C, ADRB1, ADRB2, ADRB3, ALDH5A1, ALOX5AP, AR, ASIC3, CA1, CA12, CA13, CA14, CA2, CA3, CA4, CA5A, CA5B, CA6, CA7, CA9, CACNA2D1, CETP, CNR1, CYP2C19, CYP2C9, DPP4, ESRI, ESRI2, FIT1, F2, F2R, FADS1, FKB1A, FLT1, FLT4, GAA, GABRA1*, GABRA2*, GABRA3*, GABRA5*, GABRB2*, GABRB3*, GABRG2*, GLP1R, GLRA1, GRIA4, HCAR2, HMGCR, HRH2, HTT, ICAM1, INSR, ITGAL, ITGB2, KCNA5, KCNJ11, KDM1A, KDR, MTNR1A, MTNR1B, MTOR, NOS3, NPC1L1, NR3C1, NR3C2, OPRD1, OPRK1, OPRM1, PDE10A, PDE11A, PDE3A, PDE3B, PDE4A, PDE4B, PDE4C, PDE4D, PDE5A, PDE7A, PDE7B, PGR, PLA2G2A, PLA2G7, PLG, PPARG, PRKCH, PTGER1, PTGER2, PTGER3, PTGER4, PTGER, PTGS1, PTGS2, RHOA, S1PR1, SCARB1, SCN10A, SCNSA,
SCN9A, SELE, SERPINE1, SLC6A4, SOAT1, TBXA2R, TERT, TLR4, TNF, TNNT2*, TSPO, TUBB1, TUBB3, VDR, VEGFA, and XDH. This also guides the development of follow-up toxicology experiments and research on the effects of moxa smoke on the organs of Sprague Dawley rats.

4. Discussion

GC-MS was applied to study the compounds in moxa smoke absorbed in five different polar solvents from Qing moxa sticks. This study found that a total of 294 compounds were
identified, including 139 in cyclohexane, 145 in ethyl acetate, 60 in n-butanol, 89 in anhydrous ethanol, 77 in water, and 11 in all five polar solvents. Among the 294 compounds detected in the moxa smoke absorption liquid, 112 compounds were confirmed to be toxic. With the “tox analysis” module, IPA was used to construct molecular networks of target proteins. The results showed that these target proteins were related to cell signal transduction, nucleic acid metabolism, inflammatory response, organ damage, and cell apoptosis. At the same time, the main biological pathways of the toxic compounds from moxa smoke included cardiotoxicity, hepatotoxicity, and nephrotoxicity. The safety of smoke has

| No. | Rt (min) | Compound | Retention index | Relative content (%) |
|-----|----------|----------|-----------------|----------------------|
| 1   | 4.038    | Bicyclo[4.1.0]hept-2-ene | 706  | 0.033     |
| 2   | 4.602    | 4-Methyl-1,4-hexadiene | 737  | 0.175     |
| 3   | 4.978    | 1-Methylcyclohexene | 757  | 0.078     |
| 4   | 5.093    | 1,3,5-Heptatriene | 763  | 0.115     |
| 5   | 5.290    | 1,7-Octadiene | 774  | 0.048     |
| 6   | 5.400    | 2-Methyl-1-heptene | 780  | 0.105     |
| 7   | 5.514    | 1-Octene | 786  | 0.693     |
| 8   | 5.947    | (E)-2-Octene | 806  | 0.086     |
| 9   | 6.458    | 1,3-Dimethyl cyclohexene | 822  | 0.222     |
| 10  | 6.647    | (E,E,E)-2,4,6-Octatriene | 828  | 0.027     |
| 11  | 7.111    | 5,6-Dimethyl-1,3-cyclohexadiene | 843  | 0.040     |
| 12  | 7.749    | 3-Methylenecycloheptene | 863  | 0.146     |
| 13  | 8.060    | (1Z,2Z)-1,2-Di(ethylidene)cyclobutane | 873  | 0.094     |
| 14  | 8.334    | Cyclohexanol | 882  | 0.703     |
| 15  | 9.025    | 2-Ethylpyridine | 904  | 0.095     |
| 16  | 10.064   | 2-Methyl-1-octen-3-yne | 935  | 0.105     |
| 17  | 10.166   | 1-Methylcycloheptene | 938  | 0.141     |
| 18  | 11.164   | Mesitylene | 968  | 0.307     |
| 19  | 11.629   | Alpha-methyl styrene | 981  | 0.176     |
| 20  | 12.372   | Gamma-terpinene | 1004 | 0.227     |
| 21  | 12.960   | 1,2,4-Trimethylbenzene | 1022 | 0.866     |
| 22  | 13.395   | Trans-beta-methyl styrene | 1035 | 0.371     |
| 23  | 14.973   | 1-Phenyl-2-butene | 1083 | 0.228     |
| 24  | 15.181   | 1-Methyl-4-(prop-1-en-2-yl)benzene | 1090 | 1.054     |
| 25  | 15.901   | Cosme | 1112 | 0.163     |
| 26  | 16.210   | 2,4-Dimethylstyrene | 1122 | 0.156     |
| 27  | 16.305   | 1-Phenyl-1-butene | 1125 | 0.336     |
| 28  | 16.448   | 1-Allyl-2-methylbenzene | 1130 | 0.223     |
| 29  | 16.749   | Phenyl acetonitrile | 1140 | 0.610     |
| 30  | 17.051   | 2,3-Dimethylphenol | 1149 | 0.687     |
| 31  | 17.155   | 1,2,3,4-Tetramethylbenzene | 1153 | 0.203     |
| 32  | 17.510   | 1,1a,6,6a-Tetrahydrocyclopenta| 1164 | 0.258     |
| 33  | 18.241   | 1,2-Dimethylindan | 1188 | 0.200     |
| 34  | 19.970   | 4-Methylindole | 1247 | 0.200     |
| 35  | 20.792   | 7H-Benzocycloheptene | 1275 | 0.200     |
| 36  | 21.045   | 2-(2-Hydroxyphenyl)buta-1,3-diene | 1284 | 0.168     |
| 37  | 22.396   | 1H-Indene,2,3-dihydro-1,3-trimethyl | 1332 | 0.226     |
| 38  | 23.401   | 5-Methylindole | 1369 | 0.099     |
| 39  | 23.527   | 1,8-Cyclostatradecadiyne | 1374 | 0.108     |
| 40  | 24.816   | 1,4-Dimethylnaphthalene | 1422 | 0.227     |
| 41  | 25.073   | 1,4,5-Trimethylnaphthalene | 1432 | 0.142     |
| 42  | 26.434   | 2,4,6-Trimethylbenzonitrile | 1485 | 0.137     |
| 43  | 27.199   | 1-Phenylpyridin-2-one | 1516 | 0.186     |
| 44  | 28.473   | 2,4-Dimethoxyacetophenone | 1568 | 0.234     |
| 45  | 28.910   | Spathulenol | 1586 | 0.163     |
| 46  | 29.355   | 2-Methylbiphenyl | 1605 | 0.114     |
| 47  | 30.735   | (+)-y-Gurjunene | 1665 | 0.217     |
| 48  | 33.434   | 9-Methylene-9H-fluorene | 1786 | 0.103     |
| 49  | 34.460   | 3,7,11,15-Tetramethyl-2-hexadecene | 1835 | 0.062     |
| 50  | 34.710   | 2,6,10,14-Tetramethyl-2-hexadecene | 1847 | 0.135     |
| 51  | 35.671   | 1-Nonadecene | 1893 | 0.163     |
| 52  | 37.669   | E-15-Heptadecenal | 1993 | 0.123     |
Table 2: Relative content (%) of unique compounds in ethyl acetate.

| No. | Rt (min) | Compound                                           | Retention index | Relative content (%) |
|-----|----------|----------------------------------------------------|-----------------|----------------------|
| 1   | 4.106    | 3-Methyl-butanenitrile                             | 710             | 0.159                |
| 2   | 4.535    | Dimethyl aminocacetanitrile                        | 733             | 0.191                |
| 3   | 4.603    | 2,4-Dimethyl-1,3-pentadiene                        | 737             | 0.152                |
| 4   | 5.236    | 3-Methylenecyclohexene                             | 771             | 0.095                |
| 5   | 5.300    | Cyclooctene                                        | 775             | 0.030                |
| 6   | 5.522    | 2-Octene                                           | 787             | 0.557                |
| 7   | 5.837    | 2,3-Dimethyl-1,4-hexadiene                         | 802             | 0.120                |
| 8   | 5.957    | (Z)-2-Octene                                       | 806             | 0.044                |
| 9   | 6.462    | Pyrazine, methyl                                   | 822             | 0.208                |
| 10  | 6.869    | 2,5-Dimethylpyrrole                                | 835             | 0.088                |
| 11  | 8.073    | 1,4-Dimethylenecyclohexane                         | 874             | 0.051                |
| 12  | 8.411    | 2,3-Dimethylpyridine                               | 885             | 0.166                |
| 13  | 9.820    | 3-Ethyl-1H-pyrrole                                 | 928             | 0.295                |
| 14  | 9.893    | 3,4-Dimethylpyridine                               | 930             | 0.509                |
| 15  | 10.078   | 2-Ethyl-5,5-dimethyl-1,3-cyclopentadien            | 935             | 0.082                |
| 16  | 10.480   | 2-Methylborazine                                   | 947             | 0.250                |
| 17  | 11.291   | 2,5-Cyclooctadien-1-one                            | 971             | 0.115                |
| 18  | 11.406   | Benzene                                            | 975             | 0.060                |
| 19  | 11.503   | Aniline                                            | 978             | 0.183                |
| 20  | 12.166   | 3-Methylstyrene                                    | 997             | 0.337                |
| 21  | 12.385   | Alpha-phellandrene                                 | 1004            | 0.151                |
| 22  | 12.591   | 2-Ethyl-4-methyl-1H-pyrrole                        | 1010            | 0.295                |
| 23  | 13.065   | o-Cymene                                           | 1025            | 1.118                |
| 24  | 13.402   | Allylbenezene                                      | 1035            | 0.309                |
| 25  | 15.039   | 3-Ethyl-o-xylene                                   | 1085            | 0.593                |
| 26  | 15.620   | 7-Methylbenzofuran                                 | 1103            | 0.383                |
| 27  | 15.921   | Azulene                                            | 1113            | 0.406                |
| 28  | 16.454   | 4-Allyltoluene                                     | 1130            | 0.171                |
| 29  | 16.762   | 3-Ethyltylaniline                                  | 1140            | 0.367                |
| 30  | 17.065   | 3-Methyl-1H-indene                                 | 1150            | 0.557                |
| 31  | 17.163   | 1,2,3,4-Tetramethylylufen                          | 1153            | 0.138                |
| 32  | 17.523   | 1,4-Dihydonaphthalene                              | 1165            | 0.214                |
| 33  | 18.254   | 1-Methyl-3-(1-methyl-2-propenyl)benzene            | 1188            | 0.188                |
| 34  | 18.501   | Dihydrocarveol                                     | 1196            | 0.140                |
| 35  | 18.696   | Catechol                                           | 1203            | 0.223                |
| 36  | 19.034   | 2,6-Dimethylundecane                               | 1215            | 0.195                |
| 37  | 19.250   | (E)-Cinnamaldehyde                                 | 1222            | 0.391                |
| 38  | 19.355   | Cyclododecene                                      | 1226            | 0.166                |
| 39  | 19.758   | Isoquinoline                                       | 1239            | 0.210                |
| 40  | 19.983   | 3-Methylindololizine                               | 1247            | 0.172                |
| 41  | 21.057   | 1,11-Dodecadiene                                   | 1284            | 0.162                |
| 42  | 22.733   | 2-Methylhydroquinone                               | 1345            | 0.174                |
| 43  | 22.816   | Naphthalene, 1,2,3,4-tetrahydro-1, 1-dimethyl      | 1348            | 0.125                |
| 44  | 23.825   | 2-Methyl-5-(1-mylethenthyl)-cylohexanone           | 1385            | 0.131                |
| 45  | 23.955   | 3-Methylindole                                     | 1389            | 0.257                |
| 46  | 24.827   | 1,3-Dimethylnaphthalene                            | 1423            | 0.205                |
| 47  | 24.930   | 1,6-Dimethylnaphthalene                            | 1427            | 0.460                |
| 48  | 25.761   | 2-Phenyl-1,3-cyclohexadien                         | 1459            | 0.104                |
| 49  | 28.833   | Phenylephrine                                      | 1583            | 0.020                |
| 50  | 31.836   | Thiazolo[5,4-f]quinolin                            | 1713            | 0.158                |
| 51  | 32.239   | 1,1,2-Trimethylcycloundecane                       | 1732            | 0.250                |
| 52  | 33.442   | Phenanthrene                                       | 1787            | 0.116                |
| 53  | 34.719   | 3,7,11,15-Tetramethyl-2-hexadecene                 | 1847            | 0.146                |
| 54  | 35.480   | (S)-6,6-Dimethyl-2-azaspir[4,4]non-1-ene            | 1884            | 0.158                |
| 55  | 37.681   | 3-Icosene                                          | 1994            | 0.179                |
| 56  | 39.610   | 10-Heneicosene (c,t)                               | 2093            | 0.140                |
| 57  | 39.729   | Heneicosane                                        | 2100            | 0.110                |
Table 3: Relative content (%) of unique compounds in n-butanol.

| No. | Rt (min) | Compound                        | Retention index | Relative content (%) |
|-----|----------|---------------------------------|-----------------|----------------------|
| 1   | 6.843    | 3-Furaldehyde                   | 834             | 1.167                |
| 2   | 9.850    | 3-Methylheptan-4-one            | 928             | 0.377                |
| 3   | 9.998    | 2,4-Dimethylpyridine            | 933             | 0.457                |
| 4   | 10.819   | Limonene                        | 957             | 0.442                |
| 5   | 12.169   | Butyl butyrate                  | 997             | 6.894                |
| 6   | 16.330   | (E)-1-Phenyl-1-butene           | 1126            | 0.273                |
| 7   | 16.771   | Benzyl(methylidyne)azanium      | 1140            | 0.559                |
| 8   | 18.371   | cis-2-dodecene                  | 1192            | 1.064                |
| 9   | 19.262   | Tricyclo[3.3.1.0(2,8)]nona-3,6-dien-9-one | 1222  | 0.600                |
| 10  | 23.958   | 1-Methylindolizine              | 1390            | 0.361                |

Table 4: Relative content (%) of unique compounds in anhydrous ethanol.

| No. | Rt (min) | Compound                        | Retention index | Relative content (%) |
|-----|----------|---------------------------------|-----------------|----------------------|
| 1   | 5.142    | Thiophene                       | 766             | 0.064                |
| 2   | 6.488    | 2-Methylpyrazine                | 823             | 0.137                |
| 3   | 9.811    | 2,3-Dimethyl-1H-pyrrrole        | 927             | 0.155                |
| 4   | 11.170   | 6-Methyl-6-ethylfulvene         | 968             | 0.191                |
| 5   | 12.724   | Acrylamide                      | 1014            | 0.098                |
| 6   | 14.877   | (−)-Camphor                     | 1080            | 0.477                |
| 7   | 14.974   | 2-Methyl-1-phenylpropene        | 1083            | 0.137                |
| 8   | 15.691   | 4-Pyrindol                      | 1105            | 0.157                |
| 9   | 17.059   | 3-Phenyl-1,2-butadiene          | 1150            | 0.649                |
| 10  | 17.518   | Benzo[2,3]bicyclo[3.1.0]hexane  | 1165            | 0.188                |
| 11  | 20.713   | Citral                          | 1272            | 0.283                |
| 12  | 21.053   | 4-Methyl-2H-benzopyranne        | 1284            | 0.160                |
| 13  | 22.331   | 1,7-Dimethylnaphthalene         | 1330            | 0.224                |
| 14  | 25.053   | 2,3,6-Trimethylnaphthalene      | 1432            | 0.123                |
| 15  | 29.065   | (Z)-8-Hexadecene                | 1593            | 0.393                |
| 16  | 35.803   | Nonadecane                      | 1900            | 0.118                |
| 17  | 37.154   | Dibutyl phthalate               | 1968            | 0.425                |

Table 5: Relative content (%) of unique compounds in water.

| No. | Rt (min) | Compound                        | Retention index | Relative content (%) |
|-----|----------|---------------------------------|-----------------|----------------------|
| 1   | 4.688    | Methallylcyanide                | 741             | 0.065                |
| 2   | 5.083    | Cyclopentanone                  | 763             | 0.730                |
| 3   | 5.558    | Tetrachloroethylene             | 789             | 0.076                |
| 4   | 6.010    | 4-Aminopyridine                 | 808             | 0.470                |
| 5   | 6.479    | 2-Methycyclopentane             | 823             | 0.375                |
| 6   | 6.549    | 4-Methylpentanenitrile          | 825             | 0.314                |
| 7   | 6.679    | (R)-(−)-3-Methylcyclopentanone  | 829             | 0.150                |
| 8   | 7.870    | 2,6-Dimethylpyridine            | 867             | 0.596                |
| 9   | 8.091    | Cyclohexanone                   | 874             | 0.378                |
| 10  | 8.420    | 5,5-Dimethyl-1,3-hexadiene      | 885             | 0.079                |
| 11  | 8.816    | 2-Ethylpyrazin                  | 898             | 0.426                |
| 12  | 8.975    | 2,3-Dimethylypyrazine           | 903             | 0.126                |
| 13  | 10.000   | 2,5-Dimethylpyridine            | 933             | 0.497                |
| 14  | 10.378   | 5-Methylfurfural                | 944             | 1.092                |
| 15  | 11.178   | Phenetole                       | 968             | 0.185                |
| 16  | 11.346   | 1-Isopropylcyclopentene         | 973             | 0.312                |
| 17  | 12.121   | 2-Ethyl-6-methylpyridine        | 996             | 0.438                |
| 18  | 12.400   | 2-Ethyl-5-methylpyridine        | 1004            | 0.282                |
| 19  | 12.687   | 5-Ethyl-2-methylpyridine        | 1013            | 0.207                |
| 20  | 12.743   | 2-Acetyl-5-methylfuran          | 1015            | 0.095                |
| 21  | 13.570   | 1-Acetyl-2-methyl-1-cyclopentene| 1040            | 0.378                |
| 22  | 14.229   | 2-Methyl-6-methylene-2,7-octadien-4-ol | 1060  | 0.540                |
| 23  | 14.663   | Sabinene hydrate                | 1074            | 0.750                |
| 24  | 14.803   | p-Tolunitrile                   | 1078            | 0.511                |
become greater concern. The question of whether moxa smoke is harmless or not has become key to restricting the use of moxibustion.

At present, most studies on moxa smoke have shown that it has many pharmacological effects. A study [27] showed that the superoxide anion scavenging activity of moxa smoke was superoxide dismutase 24.4 U/mg, which was slightly higher than that of partially purified moxa extract and alkali-lignin, but lower than that of sodium ascorbate, gallic acid, and catechin, which further confirmed the antioxidant and pro-oxidative effects of moxa smoke. The methanol extract of moxa smoke has the functions of antioxidant and eliminates free radicals [28]. Another study demonstrated that moxa smoke can improve sperm concentration and promote sperm movement in rats [29].

Although it was suggested that the toxic compounds in moxa smoke were harmful to the human heart, liver, and kidneys, low and middle concentrations had no effects. Moxa smoke at higher concentrations might destroy heart, liver, and kidney function. In fact, it has been reported that moxa smoke can cause related symptoms, such as eustachian tube and throat itching, eye pain, tonsil enlargement, and other symptoms [30–33]. Tar contains two-tenths of a million of a kind of thick cyclic aromatic hydrocarbon called benzo(a) pyrene, which is a strong carcinogen [34]. In a few cases, patients undergoing moxibustion treatment or after treatment had erythema, blisters, and other hypersensitive symptoms, and these conditions disappeared after leaving the moxa smoke environment [35–37]. Research results show that moxibustion may have a greater impact on some

| No. | Rt (min) | Compound | Retention index | Relative content (%) |
|-----|---------|----------|----------------|----------------------|
| 25  | 15.168  | 2-Methylbenzoazole | 1089           | 0.568                |
| 26  | 15.419  | 2,6-Dimethylphenol  | 1097           | 0.820                |
| 27  | 15.601  | Phenylacetone       | 1103           | 0.116                |
| 28  | 15.698  | 1-Isopropyl-1-cyclohexene | 1106 | 0.226                |
| 29  | 16.045  | 4-Ethylphenol       | 1117           | 0.681                |
| 30  | 16.490  | Decamethylcyclopentasiloxane | 1131 | 0.372                |
| 31  | 16.721  | Endo-borneol        | 1139           | 2.029                |
| 32  | 16.918  | 2-Acetyltoluene     | 1145           | 0.314                |
| 33  | 17.065  | (−)-Terpinen−4-ol   | 1150           | 2.220                |
| 34  | 17.262  | 1-(3-Methylphenyl)ethanone | 1156 | 0.818                |
| 35  | 17.460  | (−)-Alpha-terpineol | 1163           | 1.438                |
| 36  | 17.581  | (+)-Dihydrocarvone  | 1167           | 0.338                |
| 37  | 17.960  | (+/-)-cis-piperitol | 1179           | 0.350                |
| 38  | 18.027  | Verbenone           | 1181           | 0.190                |
| 39  | 18.279  | (−)-cis-carveol     | 1189           | 0.357                |
| 40  | 18.610  | 2,4-Dimethylanisole | 1201           | 0.248                |
| 41  | 19.293  | Piperitone          | 1223           | 0.187                |
| 42  | 20.994  | 1-Methylindan-2-one | 1282           | 0.261                |
| 43  | 21.356  | Dodecamethylcyclohexasiloxane | 1294 | 0.193                |
| 44  | 22.485  | 3,3-Dimethyl-1-indanone | 1336 | 0.233                |
| 45  | 23.314  | Methyl eugenol      | 1366           | 0.229                |
| 46  | 23.669  | 2,3-Dimethylnaphthalene | 1379 | 0.146                |
| 47  | 26.001  | 2,4-Di-tert-butylphenol | 1468           | 0.644                |

Figure 3: Relative contents (%) of common compounds in the five solvents.
### Table 6: Toxic compounds in moxa smoke.

| No. | Compound               | CAS RN   | Chemical ID | PubChem CID |
|-----|------------------------|----------|-------------|-------------|
| 1   | Toluene                | 108-88-3 | D014050     | 1140        |
| 2   | Pyrimidine             | 108-88-3 | C030986     | 9260        |
| 3   | 1-Methylpyrrole        | 96-54-8  | C096654     | 7304        |
| 4   | Pyridine               | 110-86-1 | C023666     | 1049        |
| 5   | Cyclopentanone         | 120-92-3 | C007201     | 8452        |
| 6   | 1-Octene               | 111-66-0 | C037690     | 8125        |
| 7   | Tetrachloroethylene    | 127-18-4 | D013750     | 31373       |
| 8   | Octane                 | 111-65-9 | C026728     | 356         |
| 9   | 4-Aminopyridine        | 504-24-5 | D015761     | 1727        |
| 10  | Ethylbenzene           | 100-41-4 | C004912     | 7500        |
| 11  | Styrene                | 100-42-5 | D020058     | 7501        |
| 12  | p-Xylene               | 106-42-3 | C031286     | 7809        |
| 13  | Furfural               | 98-01-1  | D000562     | 7362        |
| 14  | 2,5-Dimethylpyrrole    | 625-84-3 | C067286     | 12265       |
| 15  | 2-Furanmethanol        | 98-00-0  | C012986     | 7361        |
| 16  | 2-Acetylfuran          | 1192-62-7 | C039669    | 14505       |
| 17  | 3-Methylpyridine       | 108-99-6 | C053603     | 7970        |
| 18  | 2,6-Dimethylpyridine   | 108-48-5 | C013093     | 7937        |
| 19  | o-Xylene               | 95-47-6  | C026114     | 7237        |
| 20  | Butyro lactone         | 96-48-0  | D015107     | 7302        |
| 21  | Cyclohexanone          | 108-94-1 | C036468     | 7967        |
| 22  | Phenyl ethyne          | 536-74-3 | C044736     | 10821       |
| 23  | m-Xylene               | 108-38-3 | C031285     | 7929        |
| 24  | Propyl benzene         | 103-65-1 | C024288     | 7668        |
| 25  | Nonane                 | 111-84-2 | C017573     | 8141        |
| 26  | 2-Ethylpyridine        | 100-71-0 | C005167     | 7523        |
| 27  | Benzaldehyde           | 100-52-7 | C032175     | 240         |
| 28  | Cumene                 | 98-82-8  | C015763     | 7486        |
| 29  | 2,4-Dimethylpyridine   | 108-47-4 | C078448     | 7936        |
| 30  | Benzo furan            | 271-89-6 | C105430     | 9223        |
| 31  | 5-Methyl furfural      | 620-02-0 | C048065     | 12097       |
| 32  | Phenol                 | 108-95-2 | D019800     | 996         |
| 33  | Limonene               | 138-86-3 | D000077222  | 22311       |
| 34  | 3-Ethyltoluene         | 620-14-4 | C029719     | 12100       |
| 35  | 1,2,3-Trimethylbenzene  | 526-73-8 | C010179     | 10866       |
| 36  | Mesitylene             | 108-67-8 | C010219     | 7947        |
| 37  | Phenetole              | 103-73-1 | C079413     | 7674        |
| 38  | Eucalyptol             | 470-82-6 | D000077591  | 2758        |
| 39  | Benzene                | 71-43-2  | D001554     | 241         |
| 40  | Aniline                | 62-53-3  | C023650     | 6115        |
| 41  | Alpha-methyl styrene   | 98-83-9  | C017915     | 7407        |
| 42  | Butyl butyrate         | 109-21-7 | C022793     | 7983        |
| 43  | Decane                 | 124-18-5 | C012867     | 15600       |
| 44  | Gamma-terpinene        | 99-85-4  | C018669     | 7461        |
| 45  | Alpha-phellandrene     | 99-83-2  | C005403     | 7460        |
| 46  | 5-Ethyl-2-methylpyridine | 104-90-5 | C019196     | 7728        |
| 47  | o-Cresol               | 95-48-7  | C034047     | 335         |
| 48  | Acrylamide             | 79-06-1  | D020106     | 6579        |
| 49  | 2-Acetyl-3-methyl furan | 1193-79-9 | C057528    | 14514       |
| 50  | p-Cresol               | 106-44-5 | C032538     | 2879        |
| 51  | 1,2,4-trimethyl benzene | 95-63-6  | C010313     | 7247        |
| 52  | o-Cymene               | 527-84-4 | C046257     | 10703       |
| 53  | p-Cymene               | 99-87-6  | C007210     | 7643        |
| 54  | Guaiacol               | 90-05-1  | D001639     | 460         |
| 55  | m-Cresol               | 108-39-4 | C042041     | 342         |
| 56  | Allyl benzene          | 300-57-2 | C102347     | 9309        |
| 57  | Indene                 | 95-13-6  | C093581     | 7219        |
| 58  | Acetophenone           | 98-86-2  | C038699     | 7410        |
| 59  | Methyl benzoate        | 93-58-3  | C044605     | 7150        |
| 60  | 2,6-Dimethyl phenol    | 576-26-1 | C036531     | 11335       |
people with chronic pharyngitis, leading to coughing due to moxa smoke allergy, but these symptoms gradually improved after ventilation [38]. Some scholars have placed rats in a dynamic exposure cabinet and observed the content of Ox-LDL in their serum. The results showed that the content of Ox-LDL decreased gradually with the increase of moxa smoke concentration, suggesting that moxa smoke can reduce the degree of platelet aggregation. Therefore, it may

| No. | Compound                | CAS RN  | Chemical ID | PubChem CID |
|-----|-------------------------|---------|-------------|-------------|
| 61  | Undecane                | 1120-21-4 | C022884    | 14257       |
| 62  | Phenylacetone           | 103-79-7 | C008863    | 7678        |
| 63  | 4-Pyridinol             | 626-64-2 | C534143    | 12290       |
| 64  | Naphthalene             | 91-20-3  | C031721    | 931         |
| 65  | Azulene                 | 275-51-4 | C005525    | 9231        |
| 66  | 4-Ethylphenol           | 123-07-9 | C042291    | 31242       |
| 67  | 4-Allyltoluene          | 3333-13-9 | C092903    | 76851       |
| 68  | Indolizine              | 274-40-8 | C035094    | 9230        |
| 69  | Phenyl acetonitrile     | 140-29-4 | C006725    | 8794        |
| 70  | 2,3-Dimethylphenol      | 526-75-0 | C054067    | 10687       |
| 71  | 1,2,3,4-Tetramethylbenzene | 488-23-3 | C021246    | 10263       |
| 72  | 4-Caffeoylquinic acid   | 10482-56-1 | C016775   | 443162      |
| 73  | 3,5-Dimethylphenol      | 108-68-9 | C016834    | 7948        |
| 74  | Terpenin-4-ol           | 562-74-3 | C034019    | 11230       |
| 75  | Dodecane                | 112-40-3 | C007548    | 8182        |
| 76  | Catechol                | 120-80-9 | C034221    | 289         |
| 77  | 5,6-Dimethylbenimidazole| 582-60-5 | C015158    | 675         |
| 78  | Tridecane               | 629-50-5 | C094074    | 12388       |
| 79  | (E)-Cinnamaldehyde      | 104-35-2 | C012843    | 637511      |
| 80  | Indole                  | 120-72-9 | C030374    | 798         |
| 81  | Isoquinoline            | 119-65-3 | C039109    | 8405        |
| 82  | Citral                  | 5392-40-5 | C007076   | 638011      |
| 83  | Hydroquinone            | 123-31-9 | C031927    | 785         |
| 84  | Biphenyl                | 92-52-4  | C010574    | 7095        |
| 85  | 1-Tridecene             | 2437-56-1 | C028691   | 17095       |
| 86  | Tetradecane             | 629-59-4 | C024713    | 12389       |
| 87  | 2-Methylnaphthalene     | 91-57-6  | C027384    | 7055        |
| 88  | 1-Methylnaphthalene     | 90-12-0  | C025968    | 7002        |
| 89  | 2-Methoxy-4-vinylphenol| 7786-61-0 | C014245   | 332         |
| 90  | 2,6-Dimethylnaphthalene| 581-42-0 | C028519    | 11387       |
| 91  | 2-Methylhydroquinone    | 95-71-6  | C062397    | 7253        |
| 92  | Methyl eugenol          | 93-15-2  | C005223    | 7127        |
| 93  | 5-Methylindole          | 614-96-0 | C093726    | 11978       |
| 94  | 2,3-Dimethylnaphthalene| 581-40-8 | C091753    | 11386       |
| 95  | 3-Methylindole          | 83-34-1  | D012862    | 6736        |
| 96  | 1,4-Dimethylnaphthalene| 571-58-4 | C031969    | 11304       |
| 97  | 2,4-Di-tert-butylphenol| 96-76-4  | C056559    | 7311        |
| 98  | Dibenzo[b,d]furan       | 132-64-9 | C023614    | 568         |
| 99  | Phenylenedine           | 59-42-7  | D010656    | 6041        |
| 100 | Heptadecane             | 629-78-7 | C016486    | 12398       |
| 101 | Spathulenol             | 6750-60-3 | C013258  | 92231       |
| 102 | 1-Octadecene            | 112-88-9 | C109760    | 8217        |
| 103 | Chamazulene             | 529-05-5 | C013872    | 10719       |
| 104 | Phenanthrene            | 85-01-8  | C031181    | 995         |
| 105 | Octadecane              | 593-45-3 | C022883    | 11635       |
| 106 | Pinane                  | 473-55-2 | C030216    | 10129       |
| 107 | Nonadecane              | 629-92-5 | C061580    | 12401       |
| 108 | Hentriacontane          | 630-04-6 | C049203    | 12410       |
| 109 | Methyl palmitate        | 112-39-0 | C019012    | 8181        |
| 110 | Ambrettolide            | 123-69-3 | C008563    | 5365703     |
| 111 | Dibutyl phthalate       | 84-74-2  | D003993    | 3026        |
| 112 | Icosane                 | 112-95-8 | C050821    | 8222        |
improve microcirculation and promote metabolism of the body. Low concentrations of moxa smoke have no noticeable damage to vascular endothelium, while medium concentrations can cause a certain degree of vascular endothelium damage [39, 40].

The moxa sticks were encased in *A. argyi* floss, which is made of dried *A. argyi* leaves. There have been many experimental studies on the toxicity of *A. argyi*, which were not limited to conventional acute toxicity, subacute toxicity, or chronic toxicity. Domestic scholars have conducted in-depth studies on the hepatorenal toxicity, embryonic toxicity, and genetic toxicity caused by *A. argyi*. The research objects were not limited to the whole animal, but also extended to the cellular level, and the intrinsic mechanism of some toxicity of *A. argyi* was also discussed. The relationship between quantity, time and toxicity, and a safe time span for use were also discussed. However, some of the results showed that *A. argyi* had hepatotoxicity, especially the essential oil of *A. argyi* [41, 42]. The dosage of *A. argyi* or moxa sticks used in toxic experiments was more than 10 to 200 times the clinical dosage. According to the results of this paper, we carried out toxicological experiment of moxa smoke in rats. We followed the steps outlined in our patent, “A device for enriching moxa smoke and its analytical method,” patent
number: CN202010327163.6 [43]. Rats exposed to 756650 mg/m³ concentration of moxa smoke (concentration of moxa smoke in 50 moxa sticks) were compared with the control group, and the structure of myocardial cell, hepatic cell, and the renal tubules showed changes (Supplementary Figure S1) such as cardiac hypertrophy, degeneration and necrosis, and dilatation of renal tubules, respectively.

In a word, we should not discuss the toxicity in terms of toxicity in isolation but should comprehensively consider the clinical use characteristics of traditional Chinese medicine. However, in clinical application, we should pay attention to its “toxicity” to human body and try to avoid overdose. Therefore, moxibustion rooms should have installed ventilation equipment or the room should have adequate artificial ventilation so that the health of patients and practitioners can be guaranteed. The safety of compounds in moxa smoke needs to be further studied. The results of this study provide a basis for a safety evaluation of moxa smoke in the future.

Data Availability

The data used to support the findings of this study are included within the article and in the supplementary figure. The prior studies (and datasets) are cited at relevant places within the text as references [21, 43].

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors’ Contributions

L.H.N. conceptualized the study. S.S. and X.X.Y. contributed to methodology and formal analysis, validated the study, and wrote, reviewed, and edited the manuscript. W.W.L. provided software. X.X.Y. wrote and prepared the original draft. L.H.N. was responsible for project administration. All authors have read and agreed to the published version of the manuscript.

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Supplementary Materials

Supplementary Figure S1: microscopic observations of heart, liver, and kidney pathology. (Supplementary Materials)

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