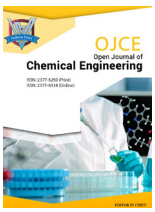
Open Journal of Chemical Engineering (OJCE)

DOI: http://doi.org/10.7508/ojce.01.2020.06.10

ISSN: 2377-6250 (Print)
ISSN: 2377-8318 (Online)
CODEN : OJCEB4



ARTICLE

MOLECULES AND FUNCTIONS OF ROSEWOOD: *MILLETTIA LAURENTII*

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ARTICLE DETAILS

Article History:

Received 24 May 2020
Accepted 21 June 2020
Available online 31 July 2020

ABSTRACT

In this study, we examined the constituents of Millettia laurentii that positively affect human health using pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS), thermal desorption system-gas chromatography-mass spectrometry (TDS-GC-MS), and gas chromatography-mass spectrometry (GC-MS). The composition of the plant's chemicals with known benefits to human health functions was studied by reviewing the literature. Cedrane, 8-propoxy- can alleviate cough, jaundice, and can prevent flu. Hexadecanoic acid, methyl ester has a certain anti-leukemia effect. 9-Octadecenamide, (Z)- can act as an expectorant, stomachic, diuretic, and adjust blood pressure. 2-Methoxy-4-vinylphenol has the effect of clearing heat, and detoxifying and moistening the intestines.

KEYWORDS

Millettia laurentii; Py-GC-MS; GC-MS; TDS-GC-MS; health care ingredients.

1. INTRODUCTION

Millettia laurentii (family Leguminosae) is a legume tree that is mainly distributed in the Congo, Cameroon, and other Central African regions. Millettia laurentii is a loose material, its growth round is not obvious. The dark colored wood that is the product of this tree is called wenge, African rosewood, and faux ebony. The heartwood of Millettia laurentii is dark brown with straight black stripes. The wood is rough and uneven, but it is oily with a high gloss. Because this wood possesses high strength, hardness, and strong decay resistance, it is commonly used to produce luxury furniture, musical instruments, sports equipment, and is used for carving works of art. Traditionally, the wood from Millettia laurentii is considered to have benefits to human health and is often used to make tea sets. Therefore, Millettia laurentii powder was analyzed by pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS), thermal desorption system-gas chromatography-mass spectrometry (TDS-GC-MS), thermogravimetric analysis (TGA), and Fourier transform infrared (FT-IR) spectroscopy. Ethanol, ethanol/benzene, and ethanol/methanol extractives of Millettia laurentii were analyzed by gas chromatography-mass spectrometry (GC-MS) and FT-IR to determine the active molecules of Millettia laurentii and their possible effects on human bodily functions.

2. MATERIALS AND METHODS

2.1 Materials

The Millettia laurentii used in the experiments was harvested in Congo, Africa. The wood was first pulverized, and then the obtained wood powder was tested. Chromatography was performed with ethanol, benzene, and methanol used in the experiments. Quantitative filter paper was extracted with ethanol for 5 h. The three solvents used in the experiments were ethanol, ethanol/benzene (volume ratio of 1:1), and ethanol/methanol (volume ratio of 1:1).

2.2 Experimental methods

2.2.1 Extraction method

The crushed and processed powder of Millettia laurentii wood was weighed, divided into 3 parts, and the mass determined to be 16 g ± 1.0 mg. The well-weighed powder and 300 ml of ethanol, ethanol/benzene (1:1 by volume), or ethanol/methanol (1:1 by volume) were each added to three round-bottom flasks and then refluxed at 83°C, 81°C, and 80°C, respectively, for 5 hours. The obtained extractives were subjected to suction filtration with a circulating water-type vacuum pump (Yuhua SHZ-D (III)) using a quantitative filter paper subjected to ethanol extraction treatment for 5 hours. Finally, the obtained extract was steamed and concentrated with a rotary evaporator (Yuhua RE-2000A) [1,2].

2.2.2 FT-IR analysis

The untreated powder of Millettia laurentii and the concentrated extractives refluxed with three types of extractants were subjected to FT-IR detection (Thermo Fisher Nicolet, 670 FT-IR). The scan of each powder was collected at a spectral resolution of 4 cm^{-1}, and the spectral range was 400 cm^{-1}-4000 cm^{-1} [3-6].

2.2.3 TG analysis

Thermogravimetric analysis (TGA) was conducted on the powder of Millettia laurentii (TA Instruments Q50, V20.8 Build 34). The carrier gas used in the experiment was high purity nitrogen, and the nitrogen release rate was 60 ml/min. The TGA temperature program began at

30°C and increased to 250°C at a rate of 5°C/min [7-10].

2.2.4 GC-MS analysis

The three extracts were analyzed using a gas chromatograph-mass spectrometer (Agilent GC-MS 7890B 5977A) with a HP-5MS non-polar column (30 m×250 μm×0.25 μm). For the elastic quartz capillary column, the carrier gas used was high-purity helium with a flow rate of 1 mL/min; the split ratio was 20:1. The temperature program for the GC began at 50°C, increased to 250°C at a rate of 8°C/min, and then increased to 300°C at a rate of 5°C/min. The MS program set the scanning range of the mass at 30 amu-600 amu with an ionization voltage of 70 eV and an ionization current of 150 μA for electron ionization (EI). The ion source and the quadrupole temperature were set at 230°C and 150°C, respectively.

2.2.5 TDS-GC-MS analysis

The powder of Millettia laurentii was analyzed with thermal desorption system-gas chromatography-mass spectrometry. The starting temperature for thermal desorption (TD) was 30°C, which was applied for 1 min at a rate of 10°C/min, increased to 100°C, held for 5 min, and then increased to 200°C at a rate of 10°C/min, with a transmission line temperature of 230°C. The cooled injection system (CIS) starting temperature was -50°C, which was held for 0.1 min, and then increased to 230°C at a rate of 10°C/s and maintained for 1 min. The temperature program for the gas chromatograph-mass spectrometer (Agilent GC-MS 7890B 5977A) began at 50°C, increased to 250°C at a rate of 8°C/min, and then increased to 300°C at a rate of 5°C/min. The MS program set the scanning range of the mass at 30 amu-600 amu with an ionization voltage of 70 eV, and ionization current of 150 μA for electron ionization (EI). The ion source and the quadrupole temperature were set at 230°C and 150°C, respectively. The analytical standard library was analyzed with the NIST14.L program.

2.2.6 Py-GC-MS analysis

Powdered Millettia laurentii was analyzed by thermal cracking-gas chromatography-mass spectrometry (CDS 5200-TRACE™ 1310 ISQ™). High purity helium was used as the carrier gas, the pyrolysis temperature was 500°C, the heating rate was 20°C/ms, and the pyrolysis time was 15 s. The pyrolysis product transfer line and the injection valve temperature were set to 300°C; the column used was a TRACE™ TR-5MS capillary column (30 m×0.25 mm×0.25 μm); shunt mode was used with a split ratio of 1:60 and a shunt rate of 50 mL/min. The GC program began with the temperature at 40°C for 2 min, increased to 120°C at a rate of 5°C/min, and then increased to 200°C at a rate of 10°C/min for 15 min. An ion source temperature of 280°C was used for electron ionization (EI) with a scanning range of 28 amu-500 amu.

3. RESULTS

3.1 FT-IR analysis

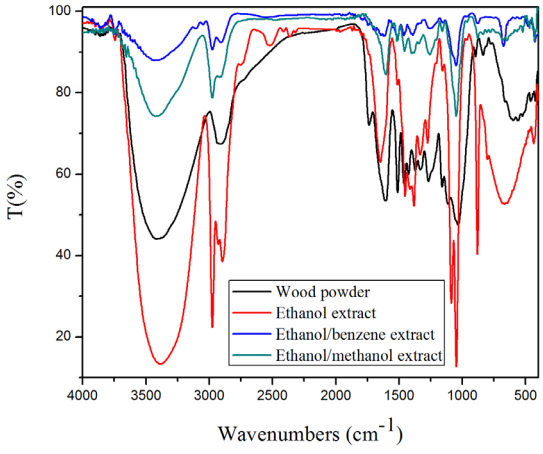

Figure 1: FT-IR spectra of Millettia laurentii powder and three extracts.

Figure 1 shows the infrared comparison spectra of the powder and the three extractives for Millettia laurentii. The infrared spectrum of 3360 cm^{-1} denotes the O-H stretching vibration in the cellulose, phenol, alcohol, and carboxylic acid compounds [11]. The infrared spectrum of 2900 cm^{-1} denotes the C-H stretching vibration and C-H bending vibration in cellulose and hemicellulose [12]. The lignin aromatic carbon skeleton vibration appears at 1600 cm^{-1} [13]. At 1425 cm^{-1} of the infrared spectrum is the CH_2 bending vibration in lignin and the CH_2 shear vibration in cellulose [14]. At 1266 cm^{-1} of the infrared spectrum is the G ring and acyloxy CO-O stretching vibration. The infrared spectrum at 1033 cm^{-1} is the C-H aromatic in-plane bending vibration [15]. The infrared spectrum at 817 cm^{-1} is the C-H of the G-ring outside the bending vibration.

3.2 TG analysis

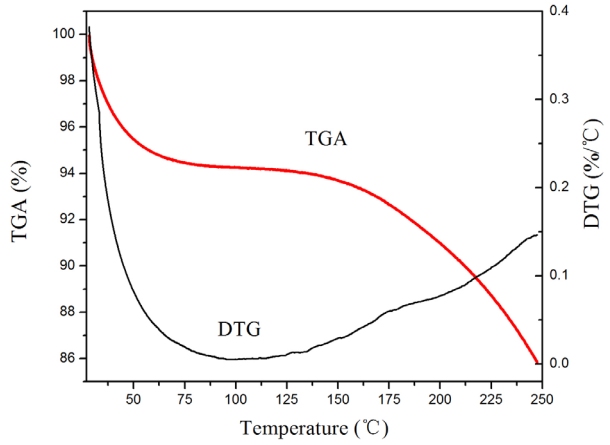

Figure 2: The TG curve for Millettia laurentii.

Figure 2 shows the TG curve for Millettia laurentii. At the 30°C-75°C temperature section in the figure, the quality of Millettia laurentii changed faster, mainly due to evaporation of water and a small amount of oil. The 75°C-150°C temperature section in the figure illustrates the continuous endothermic process of wood powder. In the 160°C-250°C temperature range, the Millettia laurentii powder underwent a more violent pyrolysis reaction, resulting in a faster decrease in the quality of the wood powder.

3.3 GC-MS analysis

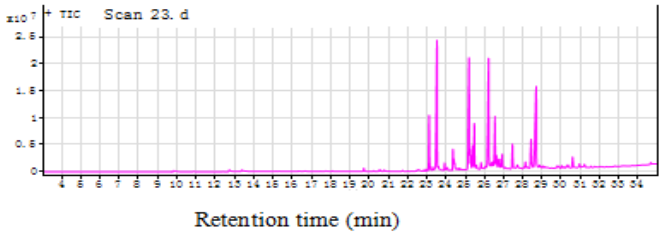

Figure 3: Total ion chromatogram of ethanol extractives of Millettia laurentii.

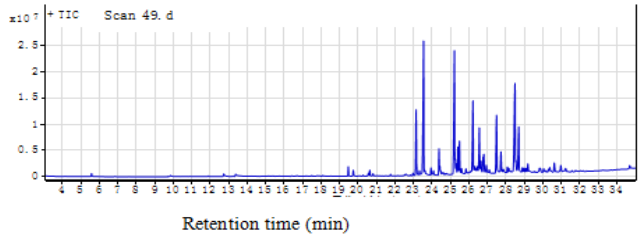

Figure 4: Total ion chromatogram of ethanol/benzene extractives of Millettia laurentii.

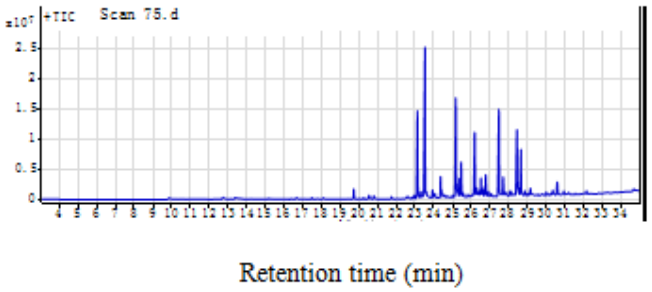

Figure 5: Total ion chromatogram of ethanol/methanol extractives of Millettia laurentii.

Table 1: GC-MS analysis results for ethanol extractives.

No.	Retention time (min)	Peak area (%)	Compounds
1	9.776	0.281	m-Guaiacol
2	12.732	0.386	1,4-Benzenediol, 2-methoxy-
3	13.379	0.540	4-Methoxybenzene-1,2-diol
4	19.731	0.272	(-)-Spathulenol
5	22.966	0.152	cis-Trismethoxyresveratrol
6	25.482	4.025	3,3',4,4'-Tetramethoxystilbene
7	26.937	1.625	10,11-Dihydro-10-hydroxy-2,3-dimethoxydibenz(b,f)oxepin
8	28.438	3.670	10,11-Dihydro-10-hydroxy-2,3,6-trimethoxydibenz(b,f)oxepin
9	28.703	12.309	2H-1-Benzopyran-2-one, 7-hydroxy-3-(4-methoxyphenyl)-

Table 2: GC-MS analysis results for ethanol/benzene extractives.

No.	Retention time (min)	Peak area (%)	Compounds
1	9.866	0.383	m-Guaiacol
2	12.745	0.521	1,4-Benzenediol, 2-methoxy-
3	13.392	0.664	4-Methoxybenzene-1,2-diol
4	16.671	0.210	Cryptomeridiol
5	17.493	0.133	Ledene oxide-(II)
6	19.731	0.851	Tricyclo[4.4.0.0(2,7)]dec-8-ene-3-methanol, .alpha.,.alpha.,6,8-tetramethyl-, stereoisomer
7	21.749	0.252	Phenol, p-1-indanyl-
8	22.965	0.277	cis-Trismethoxyresveratrol
9	25.475	2.820	3,3',4,4'-Tetramethoxystilbene
10	27.714	1.666	-Indacene-1,7-dione, 2,3,5,6-tetrahydro-3,3,4,5,5,8-hexamethyl-
11	28.451	7.573	10,11-Dihydro-10-hydroxy-2,3,6-trimethoxydibenz(b,f)oxepin
12	28.503	0.811	10,11-Dihydro-10-hydroxy-2,3,6-trimethoxydibenz(b,f)oxepin

Figure 3 to Figure 5 show the total ion chromatograms of the extractives of ethanol, ethanol/benzene, and ethanol/methanol, respective. Tables 1-3 are the results of the GC-MS analysis of extractives of ethanol, ethanol/benzene, and ethanol/methanol of Millettia laurentii.

The chemical constituents of the three extracts of Millettia laurentii were determined by a GC-MS qualitative analysis technique [16]. A total of 75 peaks were isolated by GC-MS gas chromatographic analysis of the ethanol extract of Millettia laurentii, and 9 compounds were identified. The results show that the components are: 2H-1-benzopyran-2-one, 7-hydroxy-3-(4-methoxyphenyl)- (12.309%), 3,3',4,4'-tetramethoxystilbene (4.025%), 10,11-dihydro-10-hydroxy-2,3,6-trimethoxydibenz(b,f)oxepin (3.67%), 10,11-dihydro-10-hydroxy-2,3-dimethoxydibenz(b,f)oxepin (1.625%), 4-methoxybenzene-1,2-diol (0.54%), 1,4-benzenediol, 2-methoxy- (0.386%), m-guaiacol (0.281%), (-)-dpathulenol (0.272%), and cis-trismethoxyresveratrol (0.152%).

A total of 87 peaks were isolated by GC-MS gas chromatographic analysis of the ethanol/benzene extract, and 12 compounds were identified. The results show that the components are: 10,11-dihydro-10-hydroxy-2,3,6-trimethoxydibenz(b,f)oxepin (11.769%), 3,3',4,4'-tetramethoxystilbene (2.634%), S-indacene-1,7-dione, 2,3,5,6-tetrahydro-3,3,4,5,5,8-hexamethyl- (2.095%), 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester (0.599%), 4-methoxybenzene-1,2-diol (0.515%), (-)-spathulenol (0.483%), 1,4-benzenediol, 2-methoxy- (0.459%), 1,2-benzenedicarboxylic acid, butyl 2-methylpropyl ester (0.382%), .alpha.-methylstyrene (0.276%), m-guaiacol (0.199%), cis-trismethoxyresveratrol (0.18%), abd ledene oxide-(II) (0.068%).

A total of 79 peaks were isolated by GC-MS gas chromatographic analysis of the ethanol/methanol extract, and 11 compounds were identified. The results show that the components are: 10,11-dihydro-10-hydroxy-2,3,6-trimethoxydibenz(b,f)oxepin (8.384%), 3,3',4,4'-tetramethoxystilbene (2.82%), S-indacene-1,7-dione, 2,3,5,6-tetrahydro-3,3,4,5,5,8-hexamethyl- (1.666%), tricyclo[4.4.0.0(2,7)]dec-8-ene-3-methanol, .alpha.,.alpha.,6,8-tetramethyl-, stereoisomer (0.851%), 4-methoxybenzene-1,2-diol (0.664%), 1,4-benzenediol, 2-methoxy- (0.521%), m-guaiacol (0.383%), cis-trismethoxyresveratrol (0.277%), phenol, p-1-indanyl- (0.252%), cryptomeridiol (0.21%), and ledene oxide-(II) (0.133%).

3.4 TDS-GC-MS analysis

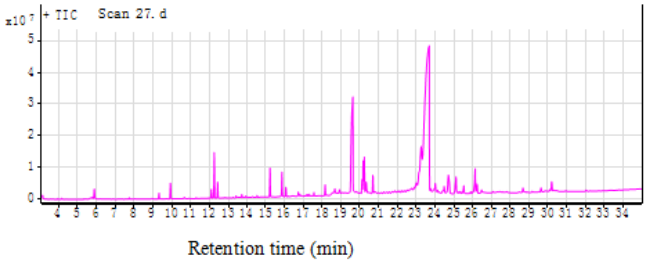

Figure 6: Total ion chromatogram of the Millettia laurentii powder.

The total ion chromatogram of the Millettia laurentii powder is shown in Figure 6. The chemical constituents of Millettia laurentii powder were determined by a TDS-GC-MS qualitative analysis technique [17]. A total of 38 peaks were isolated by TDS-GC-MS gas chromatographic analysis of Millettia laurentii powder, and 13 compounds were identified.

Table 4: TDS-GC-MS analysis results for the Millettia laurentii powder.

No.	Retention time (min)	Peak area (%)	Compounds
1	5.797	0.431	2-Cyanoethyl acrylate
2	5.885	0.667	2-Pyrimidinamine
3	9.326	0.258	Ethanol, (2-butoxyethoxy)-
4	9.931	0.759	m-Guaiacol
5	12.111	0.434	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate
6	12.262	2.136	Ethanol, -(2-butoxyethoxy)-, acetate
7	12.439	0.745	Propanoic acid, 2-methyl-, 3-hydroxy-2,2,4-trimethylpentyl ester

8	15.224	1.297	Benzene, ,2,3-trimethoxy-5-(2-propenyl)-
9	15.867	1.134	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate
10	16.068	0.450	Cedrane, 8-propoxy-
11	19.635	12.848	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
12	20.177	1.449	Hexadecanoic acid, methyl ester
13	20.24	1.918	piro(4,5)deca-6,9-diene-2,8-dione
14	25.118	1.464	9-Octadecenamide, (Z)-

Table 4 shows the results of TDS-GC-MS analysis of Millettia laurentii powder. The results show that the components are: 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester (12.848%), ethanol, 2-(2-butoxyethoxy)-, acetate (2.136%), 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione (1.918%), 2,2,4-trimethyl-1,3-pentanediol diisobutyrate (1.568%), 9-octadecenamide, (Z)- (1.464%), hexadecanoic acid, methyl ester (1.449%), benzene, 1,2,3-trimethoxy-5-(2-propenyl)- (1.297%), m-guaiacol (0.789%), propanoic acid, 2-methyl-, 3-hydroxy-2,2,4-trimethylpentyl ester (0.745%), 2-pyrimidinamine (0.667%), cedrane, 8-propoxy- (0.45%), 2-cyanoethyl acrylate (0.431%), ethanol, 2-(2-butoxyethoxy)- (0.258%).

3.5 Py-GC-MS analysis

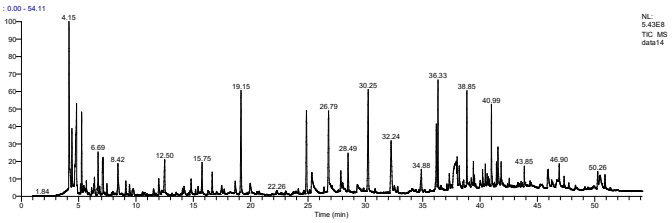

Figure 7: Relative abundance curve for the Millettia laurentii powder.

Table 5: Py-GC-MS analysis results for the Millettia laurentii powder.

No.	Retention time (min)	Peak area (%)	Compounds
1	5.26	14.595	2-Propanone, 1-hydroxy-
2	7.13	1.817	Propanoic acid, 2-oxo-, methyl ester
3	9.11	0.512	2-Furanmethanol
4	16.64	0.652	2-Cyclopenten-1-one, 2-hydroxy-3-methyl-
5	19.15	3.867	Phenol, 2-methoxy-
6	25.33	2.191	Catechol
7	26.79	4.372	m-Guaiacol
8	27.86	1.649	1,2-Benzenediol, 3-methoxy-
9	28.49	1.257	Phenol, 4-ethyl-2-methoxy-
10	30.25	4.723	2-Methoxy-4-vinylphenol
11	32.24	4.114	Phenol, 2,6-dimethoxy-
12	39.42	0.413	Phenol, 2,6-dimethoxy-4-(2-propenyl)-
13	40.22	0.385	Phenol, 2,6-dimethoxy-4-(2-propenyl)-
14	40.46	0.370	Benzaldehyde, 4-hydroxy-3,5-dimethoxy-
15	40.61	0.249	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol
16	40.99	1.707	Phenol, 2,6-dimethoxy-4-(2-propenyl)-
17	41.56	2.800	2-Propenal, 3-(4-hydroxy 3-3-methoxyphenyl)-

Figure 7 shows the relative abundance curve of the Millettia laurentii powder. The chemical constituents of the Millettia laurentii powder were determined by the Py-GC-MS qualitative analysis technique [18]. A total of 50 peaks were isolated by Py-GC-MS gas chromatographic analysis of Millettia laurentii powder, and 15 compounds were identified.

Table 5 shows the results of Py-GC-MS analysis of Millettia laurentii powder. The results show that the components are: 2-propanone, 1-hydroxy- (14.595%), 2-methoxy-4-vinylphenol (4.723%), m-guaiacol (4.372%), phenol, 2,6-dimethoxy- (4.114%), phenol, 2-methoxy- (3.867%), 2-propenal, 3-(4-hydroxy-3-methoxyphenyl)- (2.8%), phenol, 2,6-dimethoxy-4-(2-propenyl)- (2.505%), catechol (2.191%), propanoic acid, 2-oxo-, methyl ester (1.817%), 1,2-benzenediol, 3-methoxy- (1.649%), phenol, 4-ethyl-2-methoxy- (1.257%), 2-cyclopenten-1-one, 2-hydroxy-3-methyl- (0.652%), 2-furanmethanol (0.512%), benzaldehyde, 4-hydroxy-3,5-dimethoxy- (0.37%), and 4-((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol (0.249%).

4. DISCUSSION

The Py-GC-MS, TDS-GC-MS and GC-MS techniques were used to qualitatively analyze the chemical components of Millettia laurentii for determination of which ones contribute to human health, and the related compounds were obtained. Ledene oxide-(II) has the ability to detoxify the body and can be used to treat cold headaches and sore throat [19]. 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester has medical value and can be synthesized with anticancer drugs [20]. 1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester benefits the stomach, liver, and kidney, and alleviates anemia and lower back pain [21]. Propanoic acid, 2-methyl-, 3-hydroxy-2,2,4-trimethylpentyl ester can contribute heat to wet conditions with a purging fire detoxification effect, as well as antibacterial effects [22]. Cedrane, 8-propoxy- can relieve cough, jaundice, and can prevent flu [23]. Hexadecanoic acid, methyl ester exhibits a certain anti-leukemia effect [24]. 9-Octadecenamide, (Z)- acts as an expectorant, stomachic, diuretic, and can adjust blood pressure [25]. 2-Methoxy-4-vinylphenol can effectively clear heat, and detoxify and moisten the intestines [26].

5. CONCLUSION

A total of 75 peaks were isolated by GC-MS gas chromatographic analysis of the ethanol extractives of Millettia laurentii, and 9 compounds were identified; a total of 87 peaks were isolated by GC-MS gas chromatographic analysis of ethanol/benzene extractives, and 12 compounds were identified; a total of 79 peaks were isolated by GC-MS gas chromatographic analysis of ethanol/methanol extractives, and 11 compounds were identified.

For the TDS-GC-MS analysis, a total of 38 peaks were isolated by TDS-GC-MS gas chromatographic analysis of Millettia laurentii powder, and 13 compounds were identified.

For the Py-GC-MS analysis, a total of 50 peaks were isolated by Py-GC-MS gas chromatographic analysis of Millettia laurentii powder, and 15 compounds were identified.

Through access to the literature and related reports, we have determined that Millettia laurentii contains components that are beneficial to human health and function. Cedrane, 8-propoxy- can alleviate cough and jaundice, and can prevent flu. Hexadecanoic acid, methyl ester exerts an anti-leukemia effect. 9-Octadecenamide, (Z)- acts as an expectorant, stomachic, diuretic, and adjusts blood pressure. 2-Methoxy-4-vinylphenol has the ability to clear heat, and detoxify and moisten the intestines.

ACKNOWLEDGMENTS

This research was supported by the Planned Science and Technology Project of Hunan Province, China (No. 2016SK2089; No. 2016RS2011), Major scientific and technological achievements transformation projects

of strategic emerging industries in Hunan Province (2016GK4045), Academician reserve personnel training plan of lift engineering technical personnel of Hunan Science and Technology Association (2017TJ-Y10).

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