

# Extraction of biomedical compounds from the wood of *Pterocarpus macarocarpus* Kurz heartwood

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**Abstract:** Some wood can be used as traditional Chinese medicine. The medicinal value of wood is associated with its extractives. *Pterocarpus macarocarpus* Kurz heartwood is a kind of top valuable reddish hardwood in making furniture and handicrafts, but the research about medicine value of this wood is not enough. In order to investigate the high value biomedical compounds in *Pterocarpus macarocarpus* Kurz heartwood, the woody extractives were obtained by Soxhlet extraction and ultrasonic extraction with benzene-ethanol (1:2, v/v) solvent simultaneously and were analyzed by Gas Chromatography-Mass Spectrometer (GC-MS). Combining with the results of the two extraction methods, 44 compounds can be identified in total. Among these identified compounds, there were 5 flavonoids, 15 terpenes and 3 steroidal compounds. The representative biomedical compositions were homopterocarpin, medicarpin, (-)-pterocarpin, formononetin,  $\beta$ -eudesmol, stigmaterol, linoleic acid and so on, which indicated that the extractives from *Pterocarpus macarocarpus* Kurz heartwood have huge potential in biomedicine. This research provides scientific basis for further comprehensive utilization of *Pterocarpus macarocarpus* Kurz heartwood as Chinese medicine.

**Keywords:** *Pterocarpus macarocarpus* Kurz, biomedical compounds, woody extractives, woody biomedicine.

## INTRODUCTION

The pharmaceuticals of Chinese medicine mainly come from some plants. However, the outputs of plants are too little to meet the current market demands (Zhang *et al.*, 2015). Nowadays, exploration of new plant extractives in order to provide rich resources for woody medicine is emerging. In China, people are very fond of rosewood, which is not only made into high-grade furniture and works of art for appreciation and collection, but also is believed to have medicinal and healthy value. The medicinal value of wood is associated with its extractives existing in gum canal and parenchyma cells. Generally speaking, wood extractives contain various types of organic compounds, and the most common compounds are polyphenols, terpenes, lipids, flavonoids, lignans and water-soluble carbohydrate etc (Renugadevi and Prabu, 2010). Previous researches have reported that the some crude extractives and pure compounds from heartwood of the rosewood have various bioactivities (Ito *et al.*, 2015; Chuankhayan *et al.*, 2007; Umehara *et al.*, 2009; Innocent *et al.*, 2010). Among them, the *Dalbergia odorifera* T. Chen is the most representative wood, which heartwood is frequently used in traditional Chinese medicine, and can release a kind of mysterious faint scent (Choi *et al.*, 2009). Furthermore, the roles of anti-inflammation, inhibiting the central nervous, anticoagulation, reducing blood lipid, vasodilation and anti-cancer have been reported for the *Dalbergia odorifera* T. Chen (Guo *et al.*, 2011; Cheng *et al.*, 1998; Chan *et al.*, 1998). As the

medical functions of rosewood, a “wood health” theory has been proposed, which is based on the biochemical effects of special cheerful aromas released from wood (Roffael, 2006). Some aromas are attributable to the theory for their properties such as being bactericidal and insecticidal as well as spirit-lighting and soothing (Khyasudeen and Bakar, 2007; Wang, 2006). Meanwhile, the special cheerful aromas released from wood are also associated with woody extractives. *Pterocarpus macarocarpus* Kurz, is a kind of top valuable reddish hardwood, and there are some records and legends on the medicinal function of this wood spread in China. Unfortunately, for the extractives of *Pterocarpus macarocarpus* Kurz heartwood, published data is scarce.

In this study, intending to provide scientific basis for further comprehensive utilization of *Pterocarpus macarocarpus* Kurz heartwood as Chinese medicine, *Pterocarpus macarocarpus* Kurz heartwood was selected as the raw material. Firstly, the sample was extracted by Soxhlet extraction and ultrasonic extraction simultaneously. Then two kinds of obtained woody extractives were analyzed by Gas Chromatography-Mass Spectrometer (GC-MS). Lastly the compounds and high value biomedicines existing in this wood were identified and evaluated.

## MATERIALS AND METHODS

### Materials

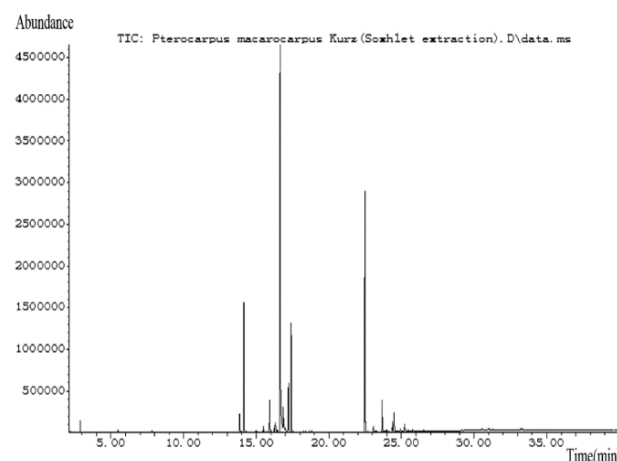
Raw material is *Pterocarpus macarocarpus* Kurz heartwood, identified and provided by Zhongshan

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Dongcheng furniture Co., Ltd (China). The initial moisture content of the material is approximately 40%. Benzene and ethanol (chromatographic grade) were prepared for the subsequent experiments. Quantitative filter paper was extracted in benzene-ethanol (1:2, v/v) solvent for 6 hours.

### Extraction

The *Pterocarpus macarocarpus* Kurz heartwood was split, ground and sieved (40 mesh). Two pieces of the wood powder weighing about 2g (accurate to 0.0001g) were wrapped in prepared quantitative filter paper, and then one was placed in a Soxhlet extractor with 90ml benzene-ethanol (1:2, v/v) solvent, another was placed in a flat bottom flask with 30ml benzene-ethanol (1:2, v/v) waiting for ultrasonic extraction. Benzene-ethanol solvent has a wide range of polarity, so almost all kinds of components in wood tissue can be dissolved, and is not easy to volatilize (Zhao *et al.*, 2002; Conde *et al.*, 2014). The Soxhlet extraction step was performed for 6h, and each extraction cycle was about 10min by adjusting temperature. The ultrasonic extraction step was performed for 2h, and this process was repeated 3 times, 90ml ultrasonic extractives solution was obtained finally. After extraction, the obtained two kinds of extractives solution were concentrated to about 1ml by rotary evaporator respectively, and then were transferred to 5ml volumetric flask, diluted with benzene-ethanol solvent to volume, waiting GC-MS analysis after filtering with micro porous membrane of 0.45 $\mu$ m.



**Fig. 1:** TIC of the extractives of *Pterocarpus macarocarpus* Kurz with Soxhlet extraction

### GC-MS analysis

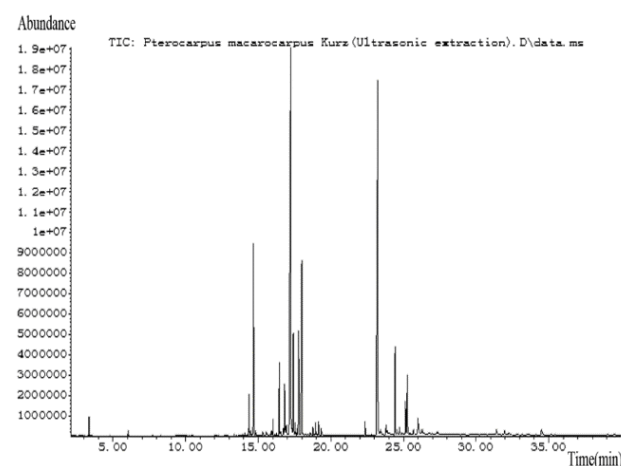
Agilent 6890N+5795C GC-MS and Agilent DB-5MS capillary quartz column (30m $\times$ 0.25mm $\times$ 0.25 $\mu$ m) were used to analyze these extractives in this study. 1 $\mu$ l of final extracted samples was injected in GC-MS using a split ratio (20:1), and the inlet temperature was 290 $^{\circ}$ C. Helium was used as carrier gas at a constant flow of 1.4 ml/min. The oven temperature program was initially at 80 $^{\circ}$ C for 5min, rose to 120 $^{\circ}$ C at a rate of 20 $^{\circ}$ C /min and then rose

to 250 $^{\circ}$ C at a rate of 10 $^{\circ}$ C /min and finally to 300 $^{\circ}$ C at a rate of 5 $^{\circ}$ C /min and held for 5min. The ionization mode of MS was electron ionization (EI), electron energy was 70eV, ion source temperature was 200 $^{\circ}$ C, quadrupole temperature was 150 $^{\circ}$ C and mass scan range was 30-500 amu (m/z). Identification of compounds was based on comparison of their spectra and relative abundance with a mass spectral data base (NIST08 MS library). Those compounds with more than 80% matching degree were qualitative in this study (Jiang *et al.*, 2016). Lastly, the relative contents of identified compounds were acquired with the method of peak area normalization (Mazimba *et al.*, 2012; Venditti *et al.*, 2014).

## RESULTS

### Analysis results after soxhlet extraction

The GC-MS had successfully separated most compounds in the extractives of *Pterocarpus macarocarpus* Kurz heartwood as shown by presence of 30 peaks and their relative quantitative contents in the total ion chromatogram (TIC) as fig. 1.



**Fig. 2:** TIC of the extractives of *Pterocarpus macarocarpus* Kurz with ultrasonic extraction

There were in total 19 compounds were identified and assigned with chemical names in table 1. These identified compounds mainly included flavonoids (25.545%), terpenes (10.625%) and so on. Among them, the flavonoids contained homopterocarpin (21.676%), medicarpin (0.568%), (-)-pterocarpin (2.748%), 6H-benzofuro[3,2-c][1]benzopyran, 3,9-dimethoxy- (0.194%) and formononetin (0.323%). These terpenes contained machilol (1.347%), (+)- $\gamma$ -gurjunene (0.112%),  $\beta$ -eudesmol (8.852%), (+)-ledene (0.089%) and 6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol (0.225%).

### Analysis results after ultrasonic extraction

After ultrasonic extraction, the obtained extractives were analysed by GC-MS and 81 peaks were successfully separated in TIC as fig. 2.

**Table 1:** Identified compounds in *Pterocarpus macarocarpus* Kurz with Soxhlet extraction

No.	Retention time/min	Compound	Formula	CAS Number	Relative content/%
1	5.474	1-Hexanol, 2-ethyl-	C <sub>8</sub> H <sub>18</sub> O	104-76-7	0.239
2	7.828	2-Butenedioic acid (Z)-, diethyl ester	C <sub>8</sub> H <sub>12</sub> O <sub>4</sub>	141-05-9	0.123
3	13.863	Machilol	C <sub>15</sub> H <sub>26</sub> O	1209-71-8	1.347
4	13.897	(+)- $\gamma$ -Gurjunene	C <sub>15</sub> H <sub>24</sub>	22567-17-5	0.112
5	14.071	Benzene, hexamethyl-	C <sub>12</sub> H <sub>18</sub>	87-85-4	0.043
6	14.149	$\beta$ -Eudesmol	C <sub>15</sub> H <sub>26</sub> O	473-15-4	8.852
7	14.227	(+)-Ledene	C <sub>15</sub> H <sub>24</sub>	21747-46-6	0.089
8	14.984	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	C <sub>10</sub> H <sub>12</sub> O <sub>3</sub>	1000297-95-5	0.326
9	16.435	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	C <sub>15</sub> H <sub>26</sub> O	1000189-10-2	0.225
10	18.414	1,2-Dimethoxy-4-(2,3-dimethoxy-1-propenyl)benzene	C <sub>13</sub> H <sub>18</sub> O <sub>4</sub>	1000313-34-6	0.144
11	18.825	9,12-Octadecadienoic acid (Z,Z)-	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	60-33-3	0.092
12	22.486	Homopterocarpin	C <sub>17</sub> H <sub>16</sub> O <sub>4</sub>	606-91-7	21.676
13	23.064	Medicarpin	C <sub>16</sub> H <sub>14</sub> O <sub>4</sub>	32383-76-9	0.568
14	23.672	(-)-Pterocarpin	C <sub>17</sub> H <sub>14</sub> O <sub>5</sub>	524-97-0	2.784
15	23.972	6H-Benzofuro[3,2-c][1]benzopyran, 3,9-dimethoxy-	C <sub>17</sub> H <sub>14</sub> O <sub>4</sub>	1433-08-5	0.194
16	24.387	9-Oxabicyclo[4.3.0]non-6-en-8-one, 7-[2-methylenebicyclo[3.3.0]octane-3,6-dione-1-yl]-	C <sub>17</sub> H <sub>18</sub> O <sub>4</sub>	1000160-28-9	1.031
17	24.487	(17aE)-D-Homo-5 $\alpha$ -pregn-17a(20)-ene	C <sub>22</sub> H <sub>36</sub>	54482-44-9	2.022
18	25.214	4-Methoxy-4',5'-methylenedioxybiphenyl-2-carboxylic acid	C <sub>15</sub> H <sub>12</sub> O <sub>5</sub>	1000242-80-7	0.986
19	25.792	Formononetin	C <sub>16</sub> H <sub>12</sub> O <sub>4</sub>	485-72-3	0.323

There were in total 39 compounds were identified and assigned with chemical names in table 2. After ultrasonic extraction, these identified compounds also mainly included flavonoids (29.536%), terpenes (10.074%) and so on. Among them, the flavonoids contained homopterocarpin (25.149%), medicarpin (0.445%) and (-)-pterocarpin (3.652%). These terpenes contained  $\beta$ -selinene (0.027%),  $\alpha$ -selinene (0.017%), elemol (0.044%), guaiol (0.024%),  $\alpha$ -eudesmol (0.091%), machilol (1.197%), (+)- $\gamma$ -gurjunene (0.077%),

(+)- $\alpha$ -elemene (0.098%), (-)- $\alpha$ -copaene (0.026%),  $\beta$ -eudesmol (8.133%), (+)-ledene (0.053%), eremophilene (0.022%), 6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol (0.058%), cycloheptane, 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl- (0.176%) and longifolenaldehyde (0.031%).

## DISCUSSION

The main compounds in two kinds of extractives were both flavonoids and terpenes. The retention time of two extractives of *Pterocarpus macarocarpus* Kurz heartwood showed a particular rule. The peaks retention time of flavonoids was between 22min and 26min, the peaks retention time of terpenes was between 12min and 17min. But those identified compounds from two kinds of

extractives were different obviously. For Soxhlet extraction, only 19 compounds were identified, including 5 flavonoids and 5 terpenes. For ultrasonic extraction, 39 compounds were identified, including 3 flavonoids and 15 terpenes. Furthermore, 3 steroids compounds (stigmasterol; 4 $\alpha$ ,14-Dimethyl-5 $\alpha$ -ergosta-8,24(28)-dien-3 $\alpha$ -ol; 9,19-Cyclolanostan-3-ol, 24-methylene-, (3 $\alpha$ -) were found in ultrasonic extractives. The difference of extractives can be explained by the mechanism and processes of two extraction methods. Soxhlet extraction make sample for continuous extraction using solvent evaporation, condensation and siphon principle. This method allows high extraction efficiency, but the high temperature for long time may cause some components to volatile or be destroyed. Ultrasonic extraction use multi effects of mechanical vibration, high acceleration, emulsifying, dispersing generated by ultrasonic to improve material molecular motion frequency and speed, increase the solvent penetration, to achieve a target that components are dissolved into the solvent. It is not necessary to heat solvent during this extraction process, so the volatilization or destruction of some components may be reduced or avoided. Soxhlet extraction and ultrasonic extraction have their own advantages. It can be more comprehensive to analyse and understand the biomedical compounds in woody extractives of *Pterocarpus macarocarpus* Kurz heartwood, combining

**Table 2:** Identified compounds in *Pterocarpus macarocarpus* Kurz with ultrasonic extraction

No.	Retention time/min	Compound	Formula	CAS Number	Relative content/%
1	3.349	Benzene, 1,3-dimethyl-	C <sub>8</sub> H <sub>10</sub>	108-38-3	0.563
2	6.041	1-Hexanol, 2-ethyl-	C <sub>8</sub> H <sub>18</sub> O	104-76-7	0.178
3	8.276	2-Butenedioic acid (Z)-, diethyl ester	C <sub>8</sub> H <sub>12</sub> O <sub>4</sub>	141-05-9	0.034
4	8.665	1H-Indene, 1-methylene-	C <sub>10</sub> H <sub>8</sub>	2471-84-3	0.006
5	10.52	Benzene, cyclohexyl-	C <sub>12</sub> H <sub>16</sub>	827-52-1	0.012
6	12.689	â-Selinene	C <sub>15</sub> H <sub>24</sub>	17066-67-0	0.027
7	12.759	α-Selinene	C <sub>15</sub> H <sub>24</sub>	473-13-2	0.017
8	13.344	Elemol	C <sub>15</sub> H <sub>26</sub> O	639-99-6	0.044
9	13.93	Guaiol	C <sub>15</sub> H <sub>26</sub> O	489-86-1	0.024
10	14.067	â-Eudesmol	C <sub>15</sub> H <sub>26</sub> O	473-16-5	0.091
11	14.366	Machilol	C <sub>15</sub> H <sub>26</sub> O	1209-71-8	1.197
12	14.407	(+)-γ-Gurjunene	C <sub>15</sub> H <sub>24</sub>	6813-21-4	0.077
13	14.465	(+)-â-Elemene	C <sub>15</sub> H <sub>24</sub>	5951-67-7	0.098
14	14.514	(-)-â-Copaene	C <sub>15</sub> H <sub>24</sub>	3856-25-5	0.026
15	14.582	Benzene, hexamethyl-	C <sub>12</sub> H <sub>18</sub>	87-85-4	0.071
16	14.675	â-Eudesmol	C <sub>15</sub> H <sub>26</sub> O	473-15-4	8.133
17	14.73	(+)-Ledene	C <sub>15</sub> H <sub>24</sub>	21747-46-6	0.053
18	14.915	Eremophilene	C <sub>15</sub> H <sub>24</sub>	10219-75-7	0.022
19	15.54	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	C <sub>10</sub> H <sub>12</sub> O <sub>3</sub>	1000297-95-5	0.284
20	15.815	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	C <sub>15</sub> H <sub>26</sub> O	1000189-10-2	0.058
21	15.911	Cycloheptane,4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-	C <sub>15</sub> H <sub>24</sub>	1000159-38-5	0.176
22	16.325	Longifolenaldehyde	C <sub>15</sub> H <sub>26</sub> O	19890-84-7	0.031
23	16.725	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	84-69-5	0.204
24	16.819	5(1H)-Azulenone,2,4,6,7,8,8a-hexahydro-3,8-dimethyl-4-(1-methylethylidene)-, (8S-cis)-	C <sub>15</sub> H <sub>22</sub> O	6754-66-1	1.891
25	16.947	Cumarin-3-carboxylic acid, 7-methoxy-	C <sub>11</sub> H <sub>8</sub> O <sub>5</sub>	20300-59-8	0.334
26	17.698	Dibutyl phthalate	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	84-74-2	0.194
27	19.154	N-â-t-Boc-L-lysine	C <sub>11</sub> H <sub>22</sub> N <sub>2</sub> O <sub>4</sub>	13734-28-6	0.916
28	19.345	Linoleic acid	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	60-33-3	0.197
29	19.599	Octadecanoic acid	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	57-11-4	0.032
30	23.234	Homopterocarpin	C <sub>17</sub> H <sub>16</sub> O <sub>4</sub>	606-91-7	25.419
31	23.413	Estra-1,3,5(10)-trien-3-ol, 16,17-epoxy-, (16â,17â)-	C <sub>18</sub> H <sub>22</sub> O <sub>2</sub>	472-56-0	0.328
32	23.792	Medicarpin	C <sub>16</sub> H <sub>14</sub> O <sub>4</sub>	32383-76-9	0.445
33	24.425	(-)-Pterocarpin	C <sub>17</sub> H <sub>14</sub> O <sub>5</sub>	524-97-0	3.652
34	25.138	2H-1-Benzopyran, 3,4-dihydro-2,2-diphenyl-	C <sub>21</sub> H <sub>18</sub> O	10419-28-0	1.234
35	25.261	3-Methylseleno-2-(1,3-dioxolan-2-yl)benzo[b]thiophene	C <sub>12</sub> H <sub>12</sub> O <sub>2</sub> S Se	71740-02-8	2.543
36	26.005	4-Methoxy-4',5'-methylenedioxybiphenyl-2-carboxylic acid	C <sub>15</sub> H <sub>12</sub> O <sub>5</sub>	1000242-80-7	1.138
37	31.403	Stigmasterol	C <sub>29</sub> H <sub>48</sub> O	83-48-7	0.356
38	31.956	4â,14-Dimethyl-5â-ergosta-8,24(28)-dien-3â-ol	C <sub>30</sub> H <sub>50</sub> O	1000105-17-1	0.309
39	34.505	9,19-Cyclolanostan-3-ol, 24-methylene-, (3â)-	C <sub>31</sub> H <sub>52</sub> O	1449-09-8	0.455

with the results of the two extraction methods.

Many studies indicated that flavonoids had strong antioxidant activity and efficiency of reducing blood sugar, anti-inflammatory, anti radiation, anti-cancer, anti-tumor. Flavonoids attract people's attention and have great development potential especially as antioxidant

drugs, because they not only have various pharmacological effects but also the side-effects is very small (Zhang, 1999; Cao *et al.* 2003). Among these identified flavonoids, homopterocarpin has reliable effects of proliferation inhibition and killing for Hep-2 cell and has therapeutic effect for liver cancer and laryngeal cancer. Medicarpin also has the effect of anti Hep-2 cell

growth, and has inhibitory effect on other tumor cell (Li *et al.* 2001; Kan *et al.* 1994; Xu *et al.*, 2009). Formononetin has the effects of regulating blood lipid metabolism, inhibition deposition of liver fat and prevention of atherosclerosis, besides anti-cancer effects (Zhao *et al.*, 2009).

Terpenes are the main source of cheerful wood aroma that could be used in aromatherapy. They are also important effective components of traditional Chinese medicine and have physiological activity, such as antivirus, anti-inflammatory and anticancer. Among these identified terpenes,  $\beta$ -endosmol is one of the main effective components of traditional Chinese medicine *Rhizoma Atractylodis*, has a certain effect on protection liver cells, treatment of diabetes and gastrointestinal disorders.  $\beta$ -Endosmol is also a drug for treatment of nervous system diseases (Kiso *et al.*, 1983; Nakai *et al.*, 2003).

The steroidal compounds have good biological activity. It is widely reported that steroidal compounds can treat cardiovascular and cerebrovascular diseases, resist cancer, reduce blood glucose, regulate immunity. And steroidal compounds can be used to synthesise hormone drugs. For example, stigmasterol with strong anti-tumor and antioxidant activity, can inhibit liver cancer cells effectively (Zhang *et al.*, 2007; Li *et al.*, 2012). And furthermore, many steroidal drugs are synthesised from stigmasterol as raw material.

In addition to these above bioactive components, linoleic acid has the effects of decreasing the blood lipid level and blood pressure, softening the human blood vessels and facilitating microcirculation, which functions can prevent or reduce the incidence of cardiovascular disease and especially is useful for prevention of high blood pressure, high cholesterol, angina, coronary heart disease and atherosclerosis. Linoleic acid is also regarded as scavenger for blood vessel for its effects on prevention of cardiovascular disease and atherosclerosis (Rodrigues *et al.*, 2010). At the same time, 2-butenedioic acid(Z)-, diethyl ester; octadecanoic acid and other components are often used as pharmaceutical intermediates. From these compounds we can see *Pterocarpus macarocarpus* Kurz heartwood has high medical value and health care potential to human

## CONCLUSION

Soxhlet extraction and ultrasonic extraction both have their own advantages. Soxhlet extraction has higher extraction efficiency, ultrasonic extraction can avoid volatilization and destruction of some components in woody extractives. Therefore, it is more comprehensive to analyse and understand the biomedical compounds in woody extractives of *Pterocarpus macarocarpus* Kurz heartwood, combining with the results of the two

extraction methods. The obtained woody extractives were analyzed by GC-MS, 44 compounds existing in *Pterocarpus macarocarpus* Kurz heartwood were identified totally. These identified compounds mainly included 5 flavonoids, 15 terpenes and 3 steroidal compounds. The functional analytical results suggest that the identified compounds of *Pterocarpus macarocarpus* Kurz heartwood have a huge potential in biomedicine, especially including homopterocarpin, medicarpin, (-)-pterocarpin, formononetin,  $\beta$ -eudesmol, stigmasterol, linoleic acid and so on.

## ACKNOWLEDGEMENTS

This work was financially supported by the Project on the Integration of Industry, Education and Research of Zhongshan City, China (No. 2013C2FC0038), the National Natural Science Foundation of China (Grant Nos. 31570558 and 31500479) and China Postdoctoral Science Foundation funded project (2015M581805).

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