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Antidepressant-like effect and phytochemical profile of supercritical CO_2 extract from *Citri reticulatae pericarpium*

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Citri reticulatae pericarpium is a condiment, adding much flavor in Chinese food. Also it can be used to treat depression as a Traditional Chinese Medicine (TCM). The study here aimed to evaluate the antidepressant effect between the supercritical CO_2 extract (**SC-E**) from *Citri reticulatae pericarpium* and the essential oil extracted by steam distillation (**SD-E**). And chemical compositions of **SC-E** were qualitatively analyzed by ultra-performance liquid chromatography quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF/MS) and gas chromatography-mass spectrometry (GC-MS). Compared with **SD-E**, **SC-E** showed a stronger antidepressant-like effect in FST and TST mice. And it also decreased the content of monoamine oxidase (MAO) in the cerebral cortex of stressed mice. A total of 60 compounds were identified in **SC-E**. Among them, 28 compounds were characterized in UPLC-Q-TOF/MS analysis and all are polymethoxyflavones (PMFs). Three main compounds, 3,5,6,7,8,3',4'-heptamethoxyflavone, nobiletin and tangeretin, together account for 66.09% of the total relative peak area. 33 terpenes were identified by GC-MS analysis, such as D-limonene (12.34%), β-elemene (8.86%), germacrene D (5.59%) and (Z, E)-α-farnesene (5.44%). Polymethoflavones and terpenes are the main constituents of **SC-E** responsible for its antidepressant-like effect. The study could stimulate further investigations into the antidepressant effects and mechanism of *Citri reticulatae pericarpium*.

1. Introduction

Citri reticulatae pericarpium (Chen-Pi in Chinese) is the dry mature peel of *Citrus reticulata* Blanco. (Rutaceae), rich in vitamins, dietary fiber, polysaccharides and potassium, calcium various trace elements (Yu et al. 2018). It is often used as condiment and herbal diet, such as tea, beverage, and cake (Yu et al. 2018; Yi et al. 2015). Most importantly, *Citri reticulatae pericarpium* has been used for treatment of gastrointestinal disorders due to its spleen-strengthening and liver-qi regulating functions (Ma 2018; Shi et al. 2009). It contains essential oil, flavonoids and alkaloids (Yu et al. 2018; Yi et al. 2015), which contribute to protecting nerve cells from oxidative stress, promoting gastrointestinal motility, antioxidant, and anti-inflammatory effects (Lu et al. 2010). It has become a high-frequency herbal medicine in current clinical prescription for post-stroke depression (Li et al. 2011) and is a key herb in Chaihu-Shu-Gan-San (CSGS), a Traditional Chinese Medicine (TCM) formula effectively used in the treatment of depression (Chang et al. 2015). The essential oil of *Citri reticulatae pericarpium* and its main component D-limonene have been considered as the constituents leading to the depression-like effect (Zhang et al. 2019).

Supercritical fluid extraction is a quick and simple way to selectively extract and analyse natural active ingredients from food, plants and herbal medicines (Wei et al. 2019; Pourmortazavi and Hajimirsadeghi 2007). Carbon dioxide (CO_2) is the most commonly used supercritical fluid. It provides a range of attractive advantages, such as selectivity, rapidity, harmlessness (Oba et al. 2017), particularly for the extraction of essential oils. Supercritical CO_2 extraction has already been used to extract the essential oil and PMFs from citrus fruits (Long et al. 2019; Chen and Huang 2016). However, there are no reports about the antidepressant effects and chemical profile of supercritical CO_2 extract from *Citri reticulatae pericarpium*.

In this study, we firstly compared the antidepressant-like effects of the supercritical CO_2 extract (**SC-E**) from *Citri reticulatae pericarpium* with the essential oil obtained by conventional steam distillation (**SD-E**) in forced swim test (FST) and tail suspension test (TST). Additionally, the chemical profile of **SC-E** was qualitatively determined by UPLC-Q-TOF/MS and GC-MS, respectively. We hope that this work could provide evidence for further studies of the antidepressant effects and the mechanism behind it.

2. Investigations, results and discussion

2.1. Effect of SC-E in the open-field test

The open-field test mainly evaluate animals' autonomous activities. The increase or decrease of the activities can reflect the excitement or inhibition on the central nervous system caused by a drug (Duan et al. 2015). Compared with the control, both **SC-E** and **SD-E** treatment showed no obvious changes in the total distance of movement, residence time in central area and resting time. The results suggested that both had no central nervous system excitatory effect.

2.2. Effects of SC-E and SD-E on immobility time in TST and FST

The tail suspension test (TST) and the forced swim test (FST) are commonly used tests of depression-related behavior in

Table 1: Autonomous activities of mice after SC-E and SD-E treatment (*x ± s, n=10*)

Groups	Total distance (m)	Residence time in the central area (s)	Resting time (s)
Control group	664.80±84.58	16.78±7.76	37.65±6.43
Positive conotrol group	680.64±97.65	18.08±4.44	37.10±7.64
SD-E group	676.84±87.41	18.18±5.79	37.59±8.38
SC-E group	762.62±82.47	19.47±6.01	36.80±9.23

rodents, and the immobility time of mice reflects their hopeless and unresponsive state to stress in experiments (Li et al. 2007). Fig. 1 shows that the positive control group with amitriptyline treatment significantly shortened the immobility times in TST and FST compared with the control group. Both **SC-E** and **SD-E** shortened the immobility time of the two tests mentioned. **SD-E** only significantly shortened the immobility time in FST. The results suggested that **SC-E** had a stronger antidepressant effect than **SD-E**.

2.3. Effect of SC-E on the activity of monoamine oxidase

Monoamine oxidase, located in the outer mitochondrial membrane of neuronal and non neuronal cells, catalyses the oxidative domi-

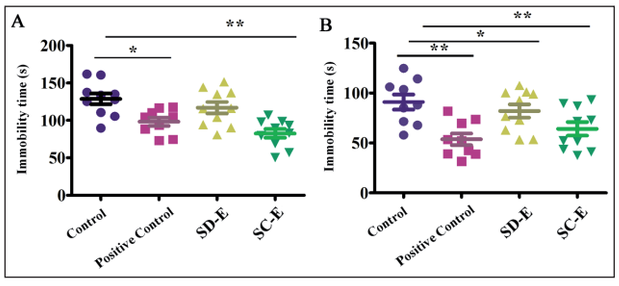

Fig. 1: Immobility times of TST and FST after SC-E and SD-E treatment A: TST; B: FST (x ± s, n=10, blue – control group; purple – positive control group; yellow- SD-E group; green- SC-E group).

nation of primary, secondary, and tertiary amines (Shulman et al. 2013). The regulation of MAO seems vital in some psychiatric and neurological disorders because low level of MAO could alleviate depression. Thus, monoamine oxidase inhibitors are commonly used as clinical antidepressants (Shulman et al. 2013). Fig. 2 shows that the supercritical CO_2 extract significantly reduced the activity of monoamine oxidase in the cerebral cortex of mice ($p < 0.001$),

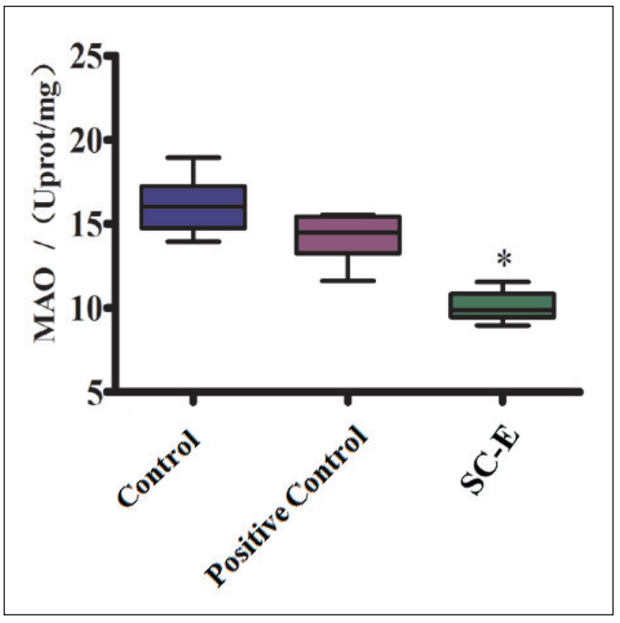

Fig. 2: Activity of MAO in the cerebral cortex of mice after SC-E treatment (x ± s, n=10, blue - control group; purple - positive control group; green- SC-E group).

with an inhibition rate of 37.69 % comparing with the control set. The outcome indicated that monoamine oxidase inhibition might one of the ways how **SC-E** exerts antidepressant activity.

2.4. Chemical components of SC-E by UPLC-Q-TOF/MS

The chemical profile of **SC-E** was analysed by the optimized UPLC-Q-TOF/MS detection condition, and the base peak chromatogram is shown in Fig. 3. The chemical components of **SC-E** were identified by the retention time, MS data, and UV spectrum. As a result, 28 polymethoxyflavonoids (PMFs) were identified (Table 2). Among them, isosinensetin, 5-demethylnobiletin, 5,7,8,4'-tetramethoxyflavone, nobiletin, 3,5,6,7,8,3', 4'-heptamethoxyflavone and tangeretin were confirmed by comparing with the reference standards. In addition, PMFs have a common structural skeleton, and usually have hydroxyl/methoxyl groups in different positions. But the substitution positions of hydroxyl or methoxy were difficult to determined by mass spectra. For example, compound **3** was inferred as monohydroxy-pentamethoxyflavone, but the specific connection position of the hydroxyl on 3' or 4' cannot be determined. Further identification of the compounds is required to be completed in the future.

Table 2: UPLC-Q-TOF/MS data of the major constituents of SC-E

Peak number	Retention time (min)	Identification	Molecular formula	$[M + H]^+$ (*m/z*)	Error (*ppm*)	Fragment ions in the positive ion mode (*m/z*)
1	4.26	7-hydroxy-5, 6, 8, 3', 4'-pentamethoxyflavone	$C_{20}H_{20}O_8$	389.1227	2.31	359.0698$[M+H-2CH_3]^+$
2*	4.34	isosinensetin	$C_{20}H_{20}O_7$	373.1290	-0.80	343.0821$[M+H-2CH_3]^+$ 328.0569$[M+H-3CH_3]^+$ 315.0881$[M+H-2CH_3\text{-}CO]^+$ 300.0646$[M+H-3CH_3\text{-}CO]^+$
3	4.45	3' -hydroxy-5, 6, 7, 8,4'-pentamethoxyflavone /4' -hydroxy-5, 6, 7, 8, 3' -pentamethoxyflavone	$C_{20}H_{20}O_8$	389.1227	2.31	373.1290$[M+H-CH_4]^+$ 359.0698$[M+H-2CH_3]^+$ 313.0679$[M+H-H_2O\text{-}2CH_3\text{-}CO]^+$
4	4.49	monohydroxy -hexamethoxyflavone	$C_{21}H_{22}O_9$	419.1368	-6.20	389.1227$[M+H-2CH_3]^+$

Peak number	Retention time (min)	Identification	Molecular formula	$[M + H]^+$ (*m/z*)	Error (*ppm*)	Fragment ions in the positive ion mode (*m/z*)
5*	4.60	5, 7, 8, 3', 4', 5' -hexamethoxy-flavone	$C_{21}H_{22}O_8$	403.1376	4.22	827.2534$[2M+Na]^+$ 425.1165$[M+Na]^+$ 373.0868$[M+H_2\text{-}CH_3]^+$ 358.0670$[M +H\text{-}3CH_3]^+$ 345.1017$[M+H\text{-}2CH_3\text{-}CO]^+$
6	4.69	monohydroxy hexamethoxyfla-vone	$C_{21}H_{22}O_9$	419.1368	-6.20	403.1376$[M+H\text{-}CH_4]^+$ 389.0882$[M+H\text{-}2CH_3]^+$
7	4.74	sinensetin	$C_{20}H_{20}O_7$	373.1290	-0.80	357.0987$[M+H\text{-}CH_4]^+$ 343.0802$[M+H\text{-}2CH_3]^+$ 327.0510$[M+H\ \text{-}2CH_3\text{-}CH_4]^+$ 315.0845$[M+H\text{-}2CH_3\text{-}CO]^+$ 313.0756$[M+H\text{-}2CH_4\text{-}CO]^+$ 300.0652$[M+H\text{-}3CH_3\text{-}CO]^+$
8*	4.78	5, 7, 8, 4' -tetramethoxyflavone	$C_{19}H_{18}O_6$	343.1207	-7.29	313.0679$[M+H\text{-}2CH_3]^+$, 285.0716$[M+H\text{-}2CH_3\text{-}CO]^+$
9	4.85	pentamethoxyflavone	$C_{20}H_{20}O_7$	373.1290	-0.80	343.1126$[M+H\text{-}2CH_3]^+$ 328.0586$[M+H\text{-}3CH_3]^+$ 315.0881$[M+H\text{-}2CH_3\text{-}CO]^+$ 300.0652$[M+H\text{-}3CH_3\text{-}CO]^+$
10	4.97	dihydroxy -tetramethoxyflavone / pentamethoxyflavanone	$C_{20}H_{22}O_7$	375.1438	0.00	397.1438$[M+Na]^+$ 211.0677$[M+H\text{-}C_{10}H_{10}O_2]^+$
11	5.07	5, 6, 7, 3', 4', 5' -hexamethoxy-flavone	$C_{21}H_{22}O_8$	403.1376	4.22	827.2534$[2M+Na]^+$ 425.1165$[M+Na]^+$ 387.0952$[M+H\text{-}CH_4]^+$ 373.0952$[M+H\text{-}2CH_3]^+$
12	5.16	pentamethoxyflavone	$C_{20}H_{20}O_7$	373.1290	-0.80	357.0987$[M+H\text{-}CH_4]^+$ 343.0802$[M+H\text{-}2CH_3]^+$ 300.2921$[M+H\text{-}3CH_3\text{-}CO]^+$
13*	5.22	nobiletin	$C_{21}H_{22}O_8$	403.1376	4.22	827.2534$[2M+Na]^+$ 425.1165$[M+Na]^+$ 388.1161$[M+H\text{-}CH_3]^+$ 373.0868$[M+H\text{-}2CH_3]^+$ 358.0670$[M+H\text{-}3CH_3]^+$ 330.0734$[M+H\text{-}3CH_3\text{-}CO]^+$
14	5.34	hexamethoxyflavone	$C_{21}H_{22}O_8$	403.1376	4.22	425.1165$[M+Na]^+$ 373.0868$[M+H\text{-}2CH_3]^+$ 343.1126$[M+H\text{-}4CH_3]^+$
15	5.34	monohydroxy -pentmethoxyfla-vones	$C_{20}H_{20}O_8$	389.1227	2.31	373.0868$[M+H\text{-}CH_4]^+$ 313.0756$[M+H\text{-}H_2O\text{-}2CH_3\text{-}CO]^+$
16	5.38	hexamethoxyflavone	$C_{21}H_{22}O_8$	403.1376	4.22	373.0868$[M+H\text{-}2CH_3]^+$
17	5.49	hexamethoxyflavone	$C_{21}H_{22}O_8$	403.1376	4.22	373.0952$[M+H\text{-}2CH_3]^+$ 343.1207$[M+H\text{-}4CH_3]^+$
18*	5.57	3, 5, 6, 7, 8, 3', 4' -heptame-thoxyflavone	$C_{22}H_{24}O_9$	433.1490	2.08	403.1024$[M+H\text{-}2CH_3]^+$ 345.0610$[M+H\text{-}4CH_3\text{-}CO]^+$
19	5.67	heptemethoxyflavone	$C_{22}H_{24}O_9$	433.1490	2.08	455.1267$[M+Na]^+$ 403.1024$[M+H\text{-}2CH_3]^+$ 345.0610$[M+H\text{-}4CH_3\text{-}CO]^+$
20	5.73	pentamethoxyflavone	$C_{20}H_{20}O_7$	373.1290	-0.80	315.0845$[M+H\text{-}2CH_3\text{-}CO]^+$
21	5.78	5-hydroxy-3, 6, 7, 8, 3', 4' -pen-tamethoxyflavanone	$C_{21}H_{22}O_9$	419.1342	0.00	389.0882$[M+H\text{-}2CH_3]^+$ 361.0879$[M+H\text{-}2CH_3\text{-}CO]^+$
22*	5.80	tangeretin	$C_{20}H_{20}O_7$	373.1290	-0.80	343.0802$[M+H\text{-}2CH_3]^+$ 328.0569$[M+H\text{-}3CH_3]^+$ 315.0845$[M+H\text{-}2CH_3\text{-}CO]^+$ 300.0646$[M+H\text{-}3CH_3\text{-}CO]^+$
23	6.24	monohydroxy -pentmethoxyfla-vones	$C_{20}H_{20}O_8$	389.1234	0.51	373.0868$[M+H\text{-}CH_4]^+$ 359.0781$[M+H\text{-}2CH_3]^+$
24	6.28	hexamethoxyflavone	$C_{21}H_{22}O_8$	403.1376	4.22	827.2534$[2M+Na]^+$ 425.1165$[M+Na]^+$ 373.0952$[M+H\text{-}2CH_3]^+$ 330.0734$[M+H\text{-}3CH_3\text{-}CO]^+$

Peak number	Retention time (min)	Identification	Molecular formula	$[M + H]^+$ (*m/z*)	Error (*ppm*)	Fragment ions in the positive ion mode (*m/z*)
25*	6.40	5 - hydroxy-6, 7, 8, 3', 4' -penta-methoxyflavone	$C_{20}H_{20}O_8$	389.1227	2.31	373.0868$[M+H-CH_4]^+$ 359.0781$[M+H-2CH_3]^+$ 313.0602$[M+H-H_2O-2CH_3-CO]^+$
26	6.54	monohydroxy -pentmethoxyfla-vones	$C_{20}H_{20}O_8$	389.1227	2.31	411.1027$[M+Na]^+$ 359.0781$[M+H-2CH_3]^+$
27	6.65	monohydroxy -hexamethoxy-flavone	$C_{21}H_{22}O_9$	419.1368	-6.20	411.1097$[M+Na]^+$ 389.0882$[M+H-2CH_3]^+$ 371.0691$[M+H-2CH_3-H_2O]^+$ 346.0698$[M+H-3CH_3-CO]^+$
28	6.87	monohydroxy -tetramethoxy-flavone	$C_{19}H_{18}O_7$	359.1113	5.01	344.0942$[M+H-CH_3]^+$ 329.0644$[M+H-2CH_3]^+$ 301.0738$[M+H-2CH_3-CO]^+$

* Chemical components were identified by the reference standards.

The UPLC-Q-TOF/MS analysis indicated that polymethoxyflavones (PMFs) were the main components in **SC-E**. Moreover, as shown in Fig. 3, three main constituents, 3,5,6,7,8,3',4'-heptamethoxyflavone, nobiletin and tangeretin with the highest relative peak areas were observed in the UPLC-Q-TOF/MS chromatogram, and together account for 66.09% of the total relative peak area. The PMFs were usually used for pharmacology and dietary supplemental resources for their broad spectrum of biological effects, including anti-inflammatory, anti-carcinogenic, and anti-atherogenic properties (Li et al. 2007). Nobiletin exerts antidepressant effect through affecting the monoaminergic system and regulating the BDNF-Trk B pathway. (Yi et al. 2011; Li et al. 2013). 3,5,6,7,8,3',4'-Heptamethoxylflavone ameliorates depressive-like behavior in chronic unpredictable mild stressed mice by activating ERK (Sawamoto et al. 2017). However, no report has been found on the antidepressant activity of other PMFs, so further studies are required to confirm the antidepressant effects of the identified PMFs from *Citri reticulatae pericarpium*.

2.5. Chemical components of SC-E by GC-MS

Optimized GC-MS detection conditions were used to analyze the supercritical CO_2 extract (**SC-E**) of *Citri reticulatae pericarpium*, and the total ion chromatogram is shown in Fig. 4. By

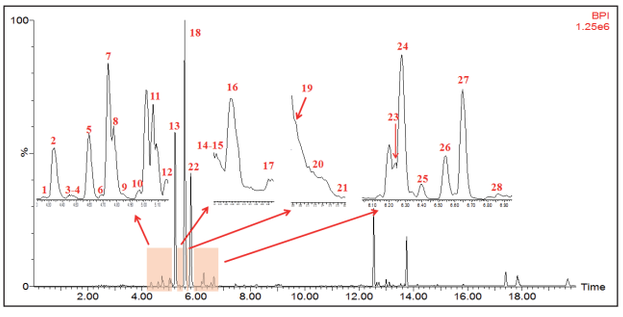

Fig. 3: Bass peak intensity chromatogram of SC-E detected by UPLC-Q-TOF/MS.

comparing the ion fragments with the NIST MS search 2.0 library, 33 compounds were figured out (Table 3 and Fig. 5), in which 6 components including D-limonene, *γ*-terpinene, *β*-elemene, linalool, carvacrol, neryl acetate were verified comparing with the reference standards.

Furthermore, the area normalization method determined the relative percentage of every component in the volatile oil. 33 components accounted for 62.03% in the total volatile oil. Among them, the content of D-limonene was the highest at 12.34%, and the other components with relative content greater than 5% were *β*-elemene (8.86%), germacrene D (5.59%) and (Z, E) -*α*-farnesene (5.44%). D-limonene can significantly shorten the forced immobility time and increase spontaneous activity in mice and plays an antidepressant role by reactivating 5-HT, dopamine, and total cholesterol levels in mice brain, reducing the content of corticotropin, and activating AMPK pathway to prevent neuronal death (Piccinelli et al. 2015).

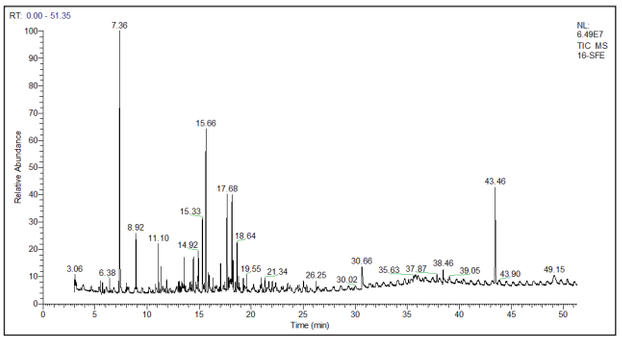

Fig. 4: Total ion chromatogram of SC-E detected by GC-MS.

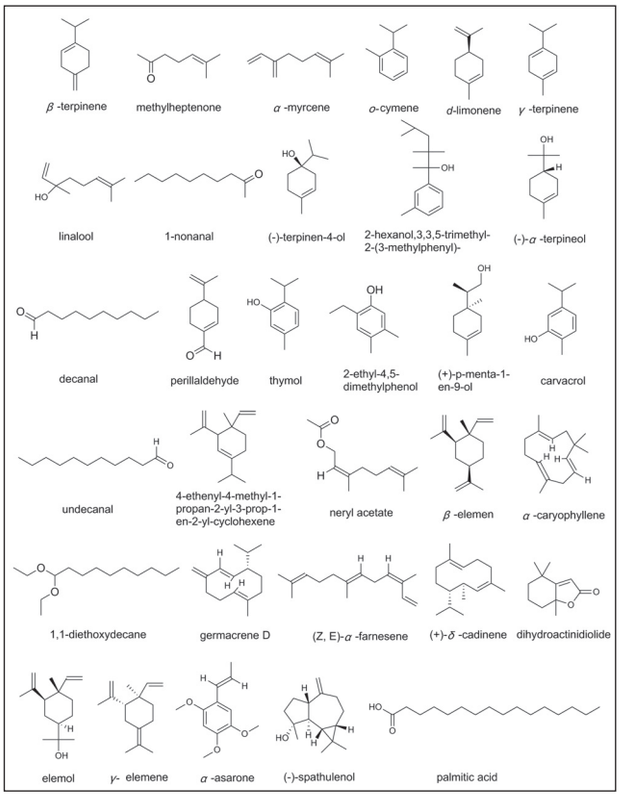

Fig. 5: Structures of compounds in SC-E identified by GC-MS

Table 3: GC-MS data of the major constituents of SC-E

Peak number	Retention time (min)	Identification	Molecular formula	Molecular weight	Relative percentage (%)
1	5.39	*β*-terpinene	$C_{10}H_{16}$	136	0.09
2	6.38	methylheptenone	$C_8H_{14}O$	126	0.50
3	6.49	*α*-myrcene	$C_{10}H_{16}$	136	0.01
4	7.27	*o*-cymene	$C_{10}H_{14}$	134	0.35
5*	7.36	*d*-limonene	$C_{10}H_{16}$	136	12.34
6*	8.04	*γ*-terpinene	$C_{10}H_{16}$	136	0.33
7*	8.92	linalool	$C_{10}H_{18}O$	154	2.68
8	9.02	1-nonanal	$C_9H_{18}O$	142	0.04
9	10.80	(-)-terpinen-4-ol	$C_{10}H_{18}O$	154	0.41
10	10.93	2-hexanol,3,3,5-trimethyl-2-(3-methylphenyl)-	$C_{10}H_{14}O$	150	0.17
11	11.09	(-)-*α*-terpineol	$C_{10}H_{18}O$	154	2.40
12	11.37	decanal	$C_{10}H_{20}O$	156	1.12
13	13.02	perillaldehyde	$C_{10}H_{14}O$	150	0.40
14	13.19	thymol	$C_{10}H_{14}O$	150	0.18
15	13.41	2-ethyl-4,5-dimethylphenol	$C_{10}H_{14}O$	150	0.24
16	13.48	(+)-p-menta-1-en-9-ol	$C_{10}H_{18}O$	154	0.03
17*	13.55	carvacrol	$C_{10}H_{14}O$	150	1.63
18	13.67	undecanal	$C_{13}H_{26}O$	198	0.16
19	14.46	4-ethenyl-4-methyl-1-propan-2-yl-3-prop-1-en-2-yl-cyclohexene	$C_{15}H_{24}$	204	2.24
20*	14.92	neryl acetate	$C_{12}H_{20}O_2$	196	2.61
21*	15.66	*β*-elemene	$C_{15}H_{24}$	204	8.86
22	17.06	*α*-caryophyllene	$C_{15}H_{24}$	204	1.59
23	17.48	1,1-diethoxydecane	$C_{14}H_{30}O_2$	230	0.31
24	17.68	germacrene D	$C_{15}H_{24}$	204	5.59
25	18.16	(Z, E)-*α*-farnesene	$C_{15}H_{24}$	204	5.44
26	18.64	(+)-*δ*-cadinene	$C_{15}H_{24}$	204	3.07
27	18.80	dihydroactinidiolide	$C_{11}H_{16}O_2$	180	0.72
28	19.26	elemol	$C_{15}H_{26}O$	222	0.69
29	19.55	*γ*-elemene	$C_{15}H_{24}$	204	0.98
30	20.99	*α*-asarone	$C_{12}H_{16}O_3$	208	0.81
31	21.34	(-)-spathulenol	$C_{15}H_{24}O$	220	0.88
32	30.66	palmitic acid	$C_{16}H_{32}O_2$	256	1.87
33	49.10	sinensetin	$C_{20}H_{20}O_7$	372	0.15

* Chemical components were identified by the reference standards.

However, the other components have not been reported any activities related to antidepressant-like effects. In summary, **SC-E** showed stronger antidepressant-like effects in FST and TST than the essential oil extracted by steam distillation. Polymethoflavones and terpenes are the main constituents of **SC-E** responsible for its antidepressant-like effect.

2.6. Conclusion

The supercritical CO_2 extract from *Citri reticulatae pericarpium* (**SC-E**) exhibited stronger antidepressant-like effects than its conventional essential oil extracted by steam distillation. **SC-E** significantly shortened the immobilization time of TST and FST through inhibition of monoamine oxidase (MAO) in the cerebral cortex of mice. Furthermore, UPLC-Q-TOF/MS and GC-MS were combined to describe the phytochemical profile of **SC-E**. A total of 60 compounds were identified. Among them, 28 PMFs were identified by UPLC-Q-TOF/MS, in which 3,5,6,7,8,3',4'-heptamethoxyflavone, nobiletin and tangeretin account for 66.09% of the total relative peak. The GC-MS analysis identified 33 constituents and D-limonene (12.34%) had the highest relative content, followed by *β*-elemene (8.86%), germacrene D (5.59%) and (Z, E)-*α*-farnesene (5.44%). This study highlights the key role of PMFs in the antidepressant effect of *Citri reticulatae pericarpium*. The findings may offer an incentive in expanding its use during relief and prevention of symptoms of depression or anxiety.

4. Experimental

4.1. Materials and chemicals

The raw herb was purchased from Beijing Tongren Tang Pharmaceutical Co., Ltd. (Beijing, China). It was identified as the dry mature peel of *Citrus reticulata* Blanco. by ZhongMei Zou of the Institute of Medicinal Plant Development (IMPLAD), Chinese Academy of Medical Sciences & Peking Union Medical College. The voucher specimen (No. SCI-NCL003168-1) was deposited in the National Compound Library of Traditional Chinese Medicine (NCL-TCM) at IMPLAD.

Amitriptyline hydrochloride was bought from Sigma-Aldrich (St Louis, USA). The reference standards of nobiletin, 3,5,6,7,8,3',4'-heptamethoxyflavone, isosinensetin, 5-demethylnobiletin, *γ*-terpinene, *β*-elemene, D-limonene, neryl acetate, 5,7,8,4'-tetramethoxyflavone, tangeretin, linalool, and carvacrol were provided by NCL-TCM at IMPLAD. The purity of all compounds was higher than 98%.

Carbon dioxide (purity 99.5%) was received from the Beijing Chengweixin Industrial Gas Sales Co., Ltd. (Beijing, China). Monoamine oxidase (MAO) detection kit and BCA protein quantitative kit were bought from the Jiancheng Institute of Biotechnology (Nanjing, China). HPLC-grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). The water used for HPLC analysis was purified by Milli-Q system from Millipore (Billerica, USA). Formic acid (HPLC grade) was obtained from Tedia (Fairfield, USA). Leucine-enkephalin was bought from Sigma Aldrich (St Louis, USA). Tween-80 was purchased from Tianjin kwangfu Fine Chemical Industry Research Institute (Tianjin, China). N-hexane was purchased from Oceanpak Alexative Chemical. Co., Ltd. (Beijing, China). All other chemicals used were of analytical grade.

4.2. Supercritical CO_2 extraction

The dried *herb* (400 g) were crushed and sieved through a 60 mesh sieve. A supercritical fluid extraction apparatus (HA120-50-01, Jiangsu Hua'an Scientific Research Instrument Co., Ltd.) was used to extract under the following conditions: pressure 30 MPa, temperature 45 °C, CO_2 flow rate 35 L/h, extraction time 2 h. As a result, 12.00 g extract (**SC-E**) was obtained, and the extraction rate was 3.00 %.

4.3. Steam distillation

The dried *herb* (400 g) were crushed and sieves through a 60 mesh sieve and soaked in distilled water at a ratio of 1:10 (w/v) overnight. The essential oil (**SD-E**) was extracted with volatile oil extractor for 10 h, and the yield was 1.05%.

4.4. Animals and treatments

Forty male ICR mice (20–25 g), purchased from the Institute of Beijing Viton Lihua Experimental Animal Technology Co., Ltd. (Beijing, China), were habituated in cages with five mice one cage and kept (23–25 °C and 40–60% humidity) under a standard 12 h light/dark cycle with free access to water and diet. The animals were raised for 7 days before the experiment. The open-field experiment was carried out to eliminate mice with abnormal autonomic activity. All the experimental procedures were approved by the Ethics Committee of the Institute of Medicinal Plant Development, CAMS & PUMC.

We separated the mice into four groups in random: control group (1% Tween-80 -normal saline, 0.9% NaCl), positive control group (amitriptyline hydrochloride, 1-7 d: 20 mg/kg, 8-14 d: 30 mg/kg), **SC-E** treatment group (0.156 g/kg, equal to 5.2 g crude drug/kg), **SD-E** treatment group (0.0546 g/kg, equivalent to 5.2 g crude drug/kg) for 14 days without stop.

4.5. Open-field test

Based on the method described previously, the voluntary activities of mice were observed before and after consecutive administration of drug (Duan et al. 2015). The size of open field arena is 50 × 50 × 40 cm and divided into 16 equal squares. The mouse was placed in the center of the open field arena, and the spontaneous activities were automatically recorded within 5 min. The records included total distance, central region residence time and rest time. Test one mouse at a time, and the arena floor was cleaned between the tests avoiding anxiety behavior. Silence of the experimental environment was ensured during the test.

4.6. Tail suspension test

Based on the conventional method of Porsolt (Porsolt et al. 1977), the tail suspension test was performed. One hour after the last drug administration, each mouse was individually suspended by the tail with a clamp in a box (40 × 40 × 60 cm). And the head of mouse needs to be at least 10 cm off the bottom. Video camera was used to record the immobility duration of 6 min. The last 4 min of the 6 min observation data was analysis by SuperTst tail suspension and forced swimming analysis software (Shanghai XinRuan Soft Information Technology Co., Ltd). The experimental environment was ensured to be independent and quiet during the experiment.

4.7. Forced swim test

Based on the conventional method of Porsolt (Porsolt et al. 1977), the forced swim test was performed. One hour after the last drug administration, mouse was forced swimming for 6 min in a glass cylinder (diameter: 11 cm) containing fresh water up to a height of 12 cm at 25 ± 1 °C. Video camera was used to record the immobility duration of 6 min. The last 4 min of the 6 min observation data was analysis by SuperTst tail suspension and forced swimming analysis software (Shanghai XinRuan Soft Information Technology Co., Ltd). The experimental environment was ensured to be independent and quiet during the experiment.

4.8. Monoamine oxidase (MAO) activity assay

The cerebral cortex was rapidly separated on an ice bath, frozen in liquid nitrogen, and then transferred to a -80 °C refrigerator for preservation until detection. Samples were then taken out and thawed at room temperature. The MAO activity assay was carried out according to the instructions of the MAO detection kit. Specific steps were as follows: The cortex was precisely weighed, proportioned with ice saline (1:9, m/v), and then homogenated. The homogenization buffer was centrifuged at 3000 rpm/min for 10 min and the final 500 μL supernatant was gathered. Then, 300 μL reagent 1 (substrate) and 3 mL reagent 2 (buffer solution) were successively added in the above supernatant. The mixture reacts in a water bath at 37°C for 3 h. 300 μL reagent 3 (enzyme inactivating agent) was added in the above mixture for terminating the reaction. The MAO was then extracted by 3 mL reagent 4 through vortexing (2 min) and centrifugating (3500 rpm/min, 10 min). Supernatant was transferred to quartz cuvette (10 mm optical diameter), and then measured the absorbance at 242 nm.

4.9. UPLC-Q-TOF/MS analysis

4.9.1. Sample preparation

All reference standards were accurately weighed, dissolved in methanol, and stored at 4°C before use. Then, **SC-E** was accurately weighed and dissolved in methanol. After vortexing (2 min) and ultrasonic dissolving (20 min), the extracted solution was then filtered through a 0.22 μm microporous filter for analysis.

4.9.2. UPLC-Q-TOF/MS conditions

A Waters Acquity™ Ultra Performance LC system (Waters Corp., Milford, USA) with PDA and Q-TOF/MS detectors were used for chromatographic analysis. We gained the sample separation on an ACQUITY CORTECS UPLC C18 column (2.1×100 mm, 1.6 μm, Waters Corp, Milford, USA). Flow rate: 0.45 mL/min, injection volume: 5 μl, column incubator: 40 °C. The mobile phase consisted of water (0.2% FA, A) and acetonitrile (0.2% FA, B) with the linear gradient programs of 1 - 40% B at 0-3 min, 40 - 100% B at 3-13 min, 100% B at 13 - 20 min. PDA detector wavelength was set at 330 nm.

Mass spectral data were measured by a Waters SYNAPT G2 HD MS Q-TOF mass spectrometer (Waters Corp, Manchester, UK) with an electrospray ionization (ESI) source under positive ion scan mode. The parameters were: capillary voltage 3.0 kV, extraction cone voltage 3.0V, sample cone voltage 40 V, desolvation gas temperature 350 °C, desolvation gas rate 600 L/h, source temperature 100 °C, cone gas rate 50 L/h, interscan delay time 0.02 s, interscan scan time 0.5 s. The lock mass *m/z* 556.2771in all analyses was at a concentration of 0.5 μg/mL. Centroid mode data were obtained and the mass range was set at *m/z* 50-1200.

4.10. GC-MS analysis

4.10.1. Sample preparation

All reference standards were accurately weighed and dissolved in n-hexane. Then, **SC-E** was dissolved in n-hexane. After vortexing (2 min) and ultrasonic dissolving (20 min), the extracted solution was then filtered through a 0.22 μm microporous filter for analysis.

4.10.2. GC-MS conditions

The samples gained separation on an elastic quartz capillary column (TG-5MS 30 m× 0.32 mm i.d., 0.25 μm film thickness, Thermo, America). ISQ GC-MS (Thermo, America) was used to acquire the MS data. Oven temperature program was initiated at 60 °C, ramped at 6 °C/min to 150 °C, and then ramped at 3 °C/min to 250 °C and held for 2 min. The carrier gas was helium (99.999 %) at a constant flow of 1 mL/min, and a sample volume of 1 μL was injected at a split ratio of 30. EI source: 70 eV, solvent delayed for 3 min, scan from m/z 50-450 amu. NIST MS search 2.0 mass spectrometry retrieval standard library was used to structural identification.

4.11. Statistical analysis

One-way analysis of variance (ANOVA) was carried out for analyzed the results using the Statistical Package for Social Science program (SPSS 16.0, SPSS, Chicago, IL, USA). All data are represented as means ± SEM, $*p < 0.05$, $**p < 0.01$.

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