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Molecular characteristics of immune activities in *Cercis chinensis* stem extractives for physical anthropology

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As one of dominant species of medicinal plants in North China, *Cercis chinensis* was also considered as the important immune active drug resources for physical anthropology, however, the molecule constituents of *Cercis chinensis* stems was not known. Therefore, the molecular characteristics of extractives from *Cercis chinensis* stems were studied to further utilize the drug resources. The GC/MS analysis results showed that 6, 13, 3, and 8 components were identified by GC/MS in the SY extractives, syJC extractives, syjcBY extractives, jcsyBY extractives from *Cercis chinensis* stems, respectively. The multi-steps extraction method was fit to extraction and separation for the particular extractives from *Cercis chinensis* stems. What's more, the extractives of *Cercis chinensis* stems contained biomedical constituents, such as phytol, 4,6-di-O- methyl- .alpha.-d-galactose, 2-C-methyl-myo-Inositol, and so on. The analytical result suggested that the extractives of *Cercis chinensis* stems were used to prepare the rich and rare immune activities for the better physical anthropology.

Keywords: Immune activity, Physical anthropology, *Cercis chinensis* stem, Extractives molecule, GC/MS

INTRODUCTION

Cercis chinensis, which easily growing in average, medium and well-drained soil, should be planted when young and left undisturbed. *Cercis chinensis* was native in central and southern China, commonly called Chinese redbud, and usually grown much smaller as an open, densely branched, multi-stemmed shrub or small tree to 8-15' tall [1]. Its leaves would turn a respectable yellow in fall [2]. Especially, Chinese redbud was less affected by the common diseases and pests of our native redbud. *Cercis chinensis* was also an important or namental shrubs for large beautiful flowers with bright color, and then was widely welcomed for good adaptability, high survival rate, low price, and good greening effect. *Cercis chinensis* tree should be transplanted in the bud before or after the leaves, roots could be cultivated, but could not be the first root transplanting and colonization [3, 4]. *Cercis chinensis* had the well-developed root system, and was not easy to cut so as to avoid tearing the root bark. When transplanting the proper pruning, part old branches should be updated each year before germination. It need be fertilized for 2-3 times during the growth period.

Cercis chinensis was a tree which had economic value. In the past, *Cercis chinensis* stems were mainly used in furniture and building. However, *Cercis chinensis* stems were always abandoned. *Cercis chinensis* biomass had the functions of clearing heat and cooling blood, detoxification, activating blood flow, swelling pain [5, 6, 7, 8]. It could cure rheumatism, traumatic injury, wind cold dampness arthralgia, amenorrhea, snake bite, bloodfeud, rabies and other diseases [2]. Its bark was bitter and flat to activate blood flow and swell detoxification for wind cold dampness arthralgia, amenorrhea, blood gas pain, sore throat, stranguria, carbuncle swollen, tinea cabies, traumatic injury, insect and snake bite. Its wood was bitter and flat to promote blood circulation, stranguria for blood stasis dysmenorrhea, abdominal pain and stranguria; Its flowers had heat-cleared detoxification for rheumatism muscles pain and nasal abscess; Its fruit was used for coughs and pregnant women [1, 5, 6]. *Cercis chinensis* biomass was also used as traditional Chinese medicine for thousands of years. However, the medicinal components of *Cercis chinensis* were less recorded. Yanyan *et al.* [9] found N naphthyl 2 aniline, biocyclomahanimbine, mahanimbine and girinimbine in Bauhinia variegate. Wanxi *et al.* [10, 11, 12, 13, 14, 15, 16, 17]; Lansheng *et al.* [18] had studied many woody extractives and their pyrolysis products [11, 19]. Zhu-Ping *et al.* [20] had studied the synthetic bio-drugs [21, 22, 23, 24, 25, 26, 27]. Some researchers had explored a variety of immune biological active ingredients [5, 6, 7, 8, 28, 19, 29, 30, 18, 31, 32, 33]. And *Cercis chinensis* was also

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considered as the important immune active drug resources to maintain and enhance the fine physical anthropology. However, the molecule constituents of immune activities from *Cercis chinensis* stems were not known. Therefore, the molecular characteristics of the stems extractives from *Cercis chinensis* were investigated and analyzed by the optimized extracting techniques in order to better utilize immune activities from *Cercis chinensis* stems as bio-drug resources for the better physical anthropology.

EXPERIMENTAL

Materials

In winter, fresh *Cercis chinensis* stems were collected from the Tongbai Mountain, Henan Province, China. The fresh stems were directly pound to pieces and kept in the dryer. Acetic ether, methanol, benzene, petroleum ether and ethanol, which were prepared for the subsequent experiments, were chromatographic grade. Cotton thread and cotton bag were both extracted in benzene/ethanol solution ($V_{ethanol} / V_{benzene} = 2$) for the treatment time of 12 h.

Experimental methods

Weighed 27 pieces of stems, each was 20 g (1.0 mg accuracy), and finally parceled by cotton bag tied by cotton thread. Method of three-step extraction was carried out by large-caliber Soxhlet according to the solution's different orders combined by JC-SY-BY (methanol → petroleum ether/acetic ether ($V_{petroleum\ ether} / V_{acetic\ ether} = 2$) → benzene/ethanol), SY-BY-JC (petroleum ether/acetic ether → benzene/ethanol → methanol), BY-JC-SY (benzene/ethanol → methanol → petroleum ether/acetic ether), respectively. After each step extraction, the extractives solutions were removed and evaporate in 60°C-70°C air, and then kinds of extractives of *Cercis chinensis* stems were obtained. The extraction times were both 2.5 hours.

GC/MS condition

Among the above extractives, the SY extractives in SY-JC-BY method (Number LD-120), syJC extractives in SY-JC-BY method (Number LD-123), syjcBY extractives in SY-JC-BY method (Number LD-125), and jcsyBY extractives in JC-SY-BY method (Number LD-127) were analyzed, respectively. Each 0.5 mg extractives was analyzed by online linked GC/MS (gas chromatograph / mass spectrometer), respectively. The GC/MS analysis was done as the same as the documents [18, 12, 13, 14, 15].

EXPERIMENTAL RESULTS

Results of stem extracts from Cercis chinensis

The SY extractives, syJC extractives, jcsyBY extractives, and syjcBY extractives were obtained respectively. The total ion chromatograms of four extractives by GC/MS were shown in Figure 1. Relative content of each component was counted by area normalization. The components and their contents were identified by analyzing the MS data, the NIST standard MS map, open-published books and papers. And the results were listed in table 1, table 2, table 3 and table 4, respectively.

RESULTS' ANALYSES

Molecular properties of stem extracts from Cercis chinensis

According to GC/MS result, 6 components were identified from SY extractives of *Cercis chinensis* stems. The result showed that the main components were 2-butanone, 4,4-dimethoxy- (14.602%), 4,6-di-O- methyl-.alpha.-d-galactose (50.990%), n-hexadecanoic acid (1.811%), 9,12-octadecadienoic acid, methyl ester (4.059%), tetrasiloxane, decamethyl- (7.041%), 1H-indole, 1-methyl-2-phenyl- (21.497%).

The 13 components were identified from syJC extractives of *Cercis chinensis* stems. The result showed that the main components were 1,3-hexadien-5-yne (2.619%), benzene, 1-pentynyl- (0.392%), 5,6-epoxy-6- methyl-2-heptanone (1.114%), diazene, butyl[1-(2,2-dimethylhydrazino)ethyl]-(87.380%), myo-Inositol, 2-C-methyl- (0.278%), n-hexadecanoic acid (0.543%), dibutyl phthalate (0.897%), (R)-(-)-14-methyl- 8-hexadecyn-1-ol (0.140%), phytol (0.377%), 9,12-octadecadienoic acid (Z,Z)- (0.914%), 2-bromo- 4,5-dimethoxycinnamic acid (2.730%), 2,4,6-cycloheptatrien-1-one, 3,5-bis-trimethylsilyl- (0.550%), 1H-indole, 1-methyl-2-phenyl- (2.068%).

The 3 components were identified from syjcBY extractives of *Cercis chinensis* stems. The result showed that the main components were 2,4-hexadiyne (92.924%), dibutyl phthalate (4.520%), 1,2-benzenedicarboxylic acid, diisooctyl ester (2.555%).

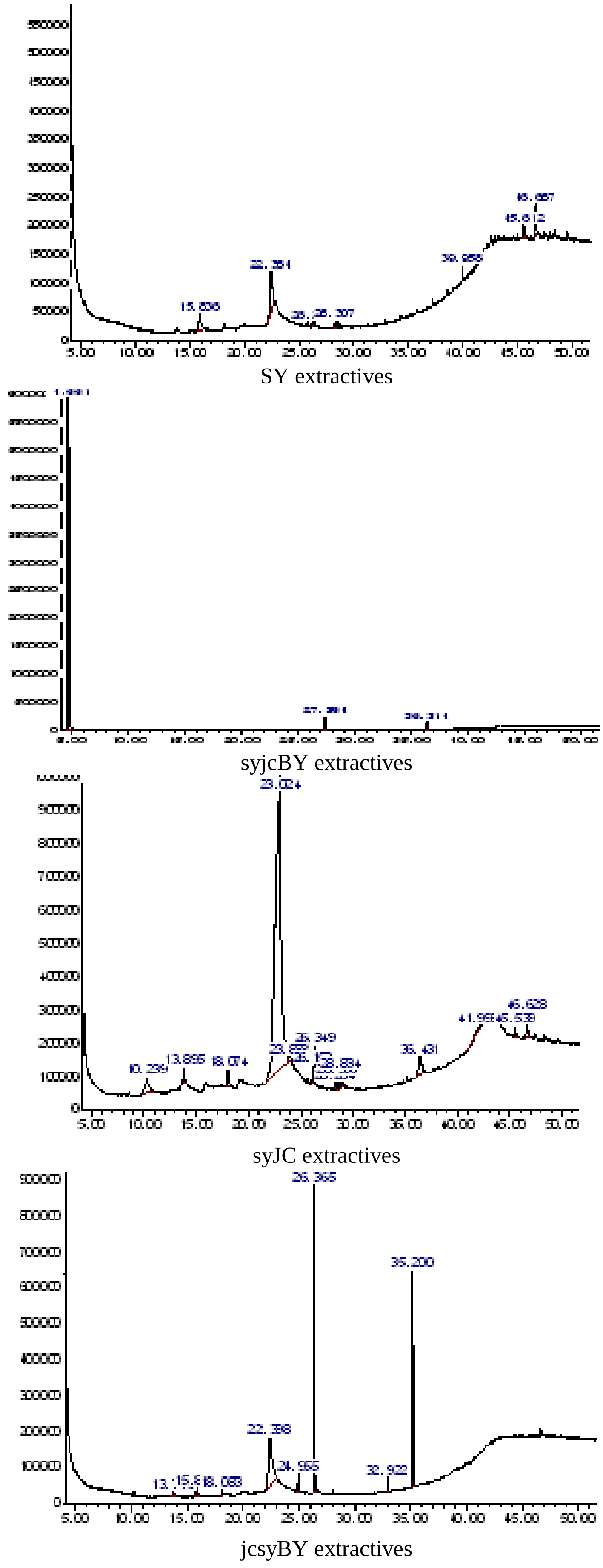

Fig. 1. Total ion chromatograms of four stem extractives of *Cercis chinensis* by GC/MS.

Table 1. The compounds of SY extractives of *Cercis chinensis* stems by GC/MS.

No.	Retention time [min]	Peak area [%]	Chemical compound
1	15.836	14.602	2-Butanone, 4,4-dimethoxy-
2	22.384	50.990	4,6-Di-O-methyl-.alpha.-d-galactose
3	26.223	1.811	n-Hexadecanoic acid
4	28.307	4.059	9,12-Octadecadienoic acid, methyl ester
5	39.958	3.154	1H-Indole, 1-methyl-2-phenyl-
6	45.612	7.041	Tetrasiloxane, decamethyl-
7	46.687	18.343	1H-Indole, 1-methyl-2-phenyl-

Table 2. The compounds of syJC extractives of *Cercis chinensis* stems by GC/MS.

No.	Retention time [min]	Peak area [%]	Chemical compound
1	10.239	2.619	1,3-Hexadien-5-yne
2	13.895	0.392	Benzene, 1-pentynyl-
3	18.074	1.114	5,6-Epoxy-6-methyl-2-heptanone
4	23.024	87.380	Diazene, butyl[1-(2,2-dimethylhydrazino)ethyl]-
5	23.888	0.278	Myo-Inositol, 2-C-methyl-
6	26.152	0.543	n-Hexadecanoic acid
7	26.349	0.897	Dibutyl phthalate
8	28.264	0.140	(R)-(-)-14-Methyl-8-hexadecyn-1-ol
9	28.556	0.377	Phytol
10	28.834	0.914	9,12-Octadecadienoic acid (Z,Z)-
11	36.431	2.730	2-Bromo-4,5-dimethoxycinnamic acid
12	41.998	0.370	1H-Indole, 1-methyl-2-phenyl-
13	45.539	0.550	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-
14	46.628	1.698	1H-Indole, 1-methyl-2-phenyl-

Table 3. The compounds of syjcBY extractives of *Cercis chinensis* stems by GC/MS.

No.	Retention time [min]	Peak area [%]	Chemical compound
1	4.661	92.924	2,4-Hexadiyne
2	27.364	4.520	Dibutyl phthalate
3	36.314	2.555	1,2-Benzenedicarboxylic acid, diisooctyl ester

Table 4. The compounds of jcsyBY extractives of *Cercis chinensis* stems by GC/MS.

No.	Retention time [min]	Peak area [%]	Chemical compound
1	13.716	1.425	Ethanone, 1-(2,4,5-trimethylphenyl)-
2	15.817	2.909	2-Butanone, 4,4-dimethoxy-
3	18.083	0.980	3,3'-Thiodipropanol
4	22.398	34.002	4,6-Di-O-methyl-.alpha.-d-galactose
5	24.955	1.854	Phthalic acid, isoporpyl propyl ester
6	26.365	35.106	Dibutyl phthalate
7	32.922	1.380	Hexanedioic acid, bis(2-ethylhexyl) ester
8	35.2	22.344	Phthalic acid, 2-ethylhexyl hexyl ester

The 8 components were identified from jcsyBY extractives of *Cercis chinensis* stems. The result showed that the main components were ethanone, 1-(2,4,5-trimethylphenyl)- (1.425%), 2-butanone, 4,4-dimethoxy-(2.909%), 3,3'-thiodipropanol (0.980%), 4,6-di-O-methyl-.alpha.-d-galactose (34.002%), phthalic acid, isoporpyl propyl ester (1.854%), dibutyl phthalate (35.106%), hexanedioic acid, bis(2-ethylhexyl) ester (1.380%), phthalic acid, 2-ethylhexyl hexyl ester (22.344%).

Based on the above molecular properties, kinds of extractives *Cercis chinensis* stems were significantly different in several solvents and extraction order. There were the vast differences in the different three-step extraction method. Therefore, the multi-step extraction method was fit to extraction and separation of the particular *Cercis chinensis* extractives.

Resource properties of stem extractives from Cercis chinensis

There were many biomedicine and drug components in stem extractives of *Cercis chinensis*. Because of its bio-drug value, dibutyl phthalate was a pesticide to keep internal environment homeostasis [16]. Phthalic acid, isobutyl nonyl ester, which had the potential efficacy on curing chronic cardiovascular and cerebrovascular diseases, had anti-tumor, anti-inflammatory, antibacterial functions [34]. Phytol, which was an acyclic diterpene alcohol that could be used as a precursor for the manufacture of synthetic forms of vitamin E and vitamin K1, was used not only as food which converted to phytanic acid and stored in fats of body, but also in the fragrance industry, cosmetics, shampoos, toilet soaps, household cleaners, and detergents [35, 36, 37, 38]. 4, 6-di-O-methyl-.alpha.-d-galactose was a form of sugar that could provide a great deal of energy in a very small amount of product, enhance nutrient properties, and be used as a nutritive sweetener. The n-hexadecanoic acid was an anti-inflammatory agents whose binding energy was calculated by in silico method and compared with known inhibitors [39]. Myo-Inositol, 2-C-methyl, which are found in many foods, in particular fruit, especially cantaloupe and oranges, played an important role as the structural basis for a number of secondary messengers in eukaryotic cells, the various inositol phosphates, and had a taste which has been assayed at half the sweetness of table sugar. 2, 4, 6-cycloheptatrien-1-one, 3,5-bis-trimethylsilyl- was one of the bioactive components of *Microcosmus exasperatus* which might heal some diseases [41]. 9,12-octadecadienoic acid, methyl ester, and 9,12-octadecadienoic acid (Z,Z)- had been identified as the main medical component of dried worms, and has diuretic, swelling and detoxification properties [41, 15]. Hexanedioic acid was also rare medicinal herds. The analytical result suggested that there were many bio-drug activities in kinds of extractives from *Cercis chinensis* stems..

CONCLUSION

The analytical analysis result of different *Cercis chinensis* stem extractives was obtained. 6, 13, 3, and 8 components were identified by GC/MS in SY extractives, syJC extractives, syjcBY extractives, jcsyBY extractives, respectively. There were the vast differences in the different three-step extraction methods. The multi-step extraction method was fit to extraction and separation of the particular extractives from *Cercis chinensis* stem. What's more, the kinds of extractives from *Cercis chinensis* stem were rich in bio-drug activities, such as Phytol was used not only as food which converted to phytanic acid and stored in fats of body, but also in the fragrance industry, cosmetics, shampoos, toilet soaps, household cleaners, and detergents; 4,6-di-O-methyl-.alpha.-d-galactose was used as a nutritive sweetener; myo-Inositol, 2-C-methyl played an important role as the structural basis for a number of secondary messengers in eukaryotic cells, and so on. And the stem extractives of *Cercis chinensis* were rich in rare bioimmune-drug activities for the better physical anthropology.

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REFERENCES

1. L. H. Mu, Study on chemical constituents and biological activity of Cercis chinensis redbud part., Peking Union Medical College, 2007.
2. L. Yuan, Study on chemical constituents and biological activity of Cao Shanhu, Bauhinia and Lobular Shi Nan, Doctoral Dissertation of Peking Union Medical College, 2006.
3. W. Tuanrong, *J. Hebei Forest Sci Technol.*, **4**, 10 (1998).
4. X. Boshu, D. Chunlei, L. Hongrong, *Land Gree.*, **2**, 43 (2006).
5. J. C. Xie, B. G. Sun, F. P. Zheng, Y. U. Min, *Food Sci.*, **26**, 281 (2005a).
6. J. C. Xie, B. G. Sun, Y. U. Min, *Food and Fermentation Ind.*, **7**, 100 (2005b).
7. X. B. Liu, H. Cao, *Amino Acids Biotic Resour.*, **1**, 75 (2010)
8. F. S. Wang, S. X. Ren, D. P. Yang, C. S. Zhu, H. N., *J Chin Mass Spectr Soc.*, **1**, 1 (2004)..
9. Z. Yanyan, C. Chengbin, C. Bin, H. Bing, S. Qishi, *Chin J Med Chem.*, **14**, 169 (2004).
10. Q. Z. Ma, W. X. Peng, Y. Q. Wu, X. Zhang, H. P. Deng, *Applied Electromagnetics And Mechanics (II)*, **13**, 373 (2009).
11. W. X. Peng, F. J. Wang, L. S. Wang, X, Z Qiu. *Kristallogr. NCS*, **227**, 61 (2012a).

12. W. X. Peng, L. S. Wang, Z. Lin, M. L. Zhang, *J. Pure Appl. Microbio.*, **7(**SE), 67 (2013a).
13. W. X. Peng, Z. Lin, J. B. Cao, F. L. Gao, X. W. Zhao, Z. F. Zhang, *J. Pure Appl. Microbio.,* **7**(2), 1237 (2013b).
14. W. X. Peng, Z. F. Zhang, Z. Lin, M. Ohkoshi, J. B. Cao, F. L. Gao, X. W. Zhao, *J. Pure Appl. Microbio.*, **7**, 2017 (2013c).
15. W. X. Peng, Z. Lin, J. B. Cao, F. L. Gao, X. W. Zhao, *Biotechnol. Biotec. Eq.,* **27**, 4311 (2013d).
16. W. X. Peng, Wang, Z. Lin, M. L. Zhang, Z. Lin, *Pak. J. Pharm. Sci.,* **27**(3S3), 723 (2014a).
17. W. X. Peng, X. Qiu, O. Makoto, *Pak. J. Pharm. Sci.,* **27**(4S), 991 (2014b).
18. L. S. Wang, W. X. Peng, M. L. Zhang, Z. Lin. *J. Pure Appl. Microbio.*, **7**(SE), 59 (2013a).
19. H. C. Qiu, W. X. Peng,, Y. Q. Wang, S. B. Wang, G. J. Xu, *J. Comput. Theor. Nanos.,* **9**, 1525 (2012).
20. Z. P. Xu, Z. Y Peng, Jing-Jun D, Rui-Cheng D, Xu-Dong W, Hui O, Pan Y, Juan H, Yuan-Feng W, Man Z, Xiao-Chun P, Wan-Xi P, Hai-Liang Z, *Eur. J. Med. Chem.,* **68**, 212 (2013).
21. R. Chaiyarat, C. Sookjam, K. Eiam-Ampai, P. Damrongphol, *Sci Asia***,** **40**, 198 (2014).
22. S. Ruangsanka, *Sci Asia,* **40**, 204 (2014).
23. C. H. Lu, W. L. Liao, T. Y. Wang, C. C. Chen, Y. H. Chen, S. S. Tang, Y. C. Han, F. J, *Sci Asia,* **40**, 212 (2014).
24. W. X. Peng, L. S. Wang, F. J. Wang, Q. Xu. *Acta Crystallog. E,* **67**, O2329 (2011).
25. W. X. Peng, L. Cao, *Z. Kristallogr. NCS,* **227**, 267 (2012).
26. W. X. Peng, L. S. Wang, Q. Xu, Q. D. Wu, S. L. Xiang, *J. Comput. Theor. Nanos.,* **9**, 1431 (2012c).
27. I. Gunes, K. Aslantaş, S. Ulker, S. Taktak, *Journal of the Balkan Tribological Association,* **38**, 173 (2015).
28. Y. C. Sun, Z. Lin, W. X. Peng, T. Q. Yuan, F. Xu, Y. Q. Wang, J. Yang, Y. S. Wang, R. C. Song, *BioResources,* **9**, 1055 (2014).
29. Q. Xue, W. X. Peng, M. Ohkoshi, *Pak. J. Pharm. Sci.,* **27**, 975 (2014).
30. C. Le, P. Wanxi, S. Zhengjun, S. Lili, C. Guoning, *Pak. J. Pharm. Sci.,* **27**(4S), 1083 (2014).
31. L. S. Wang, W. X. Peng, Z. Lin, M. L. Zhao, *J. Pure Appl. Microbiol .,* **7**, 1345 (2013b).
32. R. S. Clements Jr, B. Darnell, *Am J Clin Nutr.,* **33**(9), 1954 (1980).
33. V. L. Boyadzhieva, P. Georeva, T. Kuneva, N. Stoilov, *Oxidation Commun.,* **38**, 271 (2015).u
34. H. Huang, X. Zhao, B. Jiang, *Chinese Tradit. & Herb. Drugs,* **37**, 307 (2006).
35. T. Netscher, *Synthesis of Vitamin E. Vita Horm.,* **76**, 155 (2007).
36. M. D. Alison, J. P. Richard, E. H. Mark, D. A. Andrew, *Curr Org Chem.,* **7,** 1625 (2003).
37. D. M. V. D. Brink, R. J. A. Wanders, *Cell Mol Life Sci.,* **63**, 1752 (2006).
38. D. Mcginty, C. S. Letizia, A. M. Api McGinty, *Food Chem Toxicol,* **48**, S59 (2010).6
39. V. Aparna, K. V. Dileep, P. K.Mandal, P. Karthe, C. Sadasivan, M. Haridas, *Chem. Biol. Drug Des.,* **80**, 434 (2012).
40. V. K. Meenakshi, S. Gomathy, *J, Curr, Chem, Pharm, Sc*, **2**, 271 (2012).
41. C. Guo, D. Wei, *Chin. J. Anal. Chem.,* **34**, 15 (2006).