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Isolation and Characterisation of L-quebrachitol from Rubber Factory Wastewater

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In the production process of natural rubber (NR), large quantities of wastewater containing substantial amounts of L-quebrachitol are discharged. To date, published methods of extracting L-quebrachitol from wastewater are still at the laboratory stage. In this study, membrane separation technology was applied to the extraction of L-quebrachitol from wastewater and the experimental parameters were studied in order to determine optimal experimental conditions. Results show that the optimum source material for L-quebrachitol extraction was the serum collected by squeezing the latex coagulum. When this serum was passed through a microfiltration (MF) membrane and two ultrafiltration (UF) membranes, majority of the organic impurities were removed. The resulting ultrafiltrate was concentrated by a nanofiltration (NF) membrane and washed twice. Finally, a new and convenient approach for large scale extraction of L-quebrachitol is proposed combining membrane separation and crystallisation. The output achieved with this approach would be 10 kg per year under laboratory conditions with 99.20% purity. The structure of L-quebrachitol was confirmed by elementary analysis, infrared (IR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, mass spectroscopy (MS) and single-crystal X-ray diffraction.

Keywords: L-quebrachitol; wastewater; membrane separation technology

L-quebrachitol (1-L-(-)-2-O-methyl-chiro-inositol, *Figure 1*) is a naturally occurring optically active inositol. It exists in many kinds of plants and has gained increasing attention as a chiral source in organic synthesis[1–4]. In metabolism, where it is mainly found in the form of a phosphate ester or phosphate, it serves an important role as a second messenger that controls cellular processes by generating internal calcium signals[4–9]. Extensive research on the synthesis of chiral inositol derivatives from L-quebrachitol has been carried out. These derivatives are significant because they can be used in antibiotics, antioxidants, enzyme inhibitors, inhibition of angiogenesis and treatment of diabetes[10].

L-quebrachitol was first isolated from *Aspidosperma quebracho* from South America and then found in several other plants, including *Elaeagnus formosana*, *Acer saccharum*, *Allophylus edulis*, *Mitrephora*

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vulpina, *Cannabis sativa*, and *Hippophae rhamnoides*[1,11–15]. Though L-quebrachitol exists in many kinds of plants, the amount contained is generally low. Rubber tree, *Hevea brasiliensis,* contains relatively large quantities of L-quebrachitol that can be conveniently isolated from natural rubber (NR) factory wastewater[2,10,16–17].

Rubber trees are widely planted in tropical and subtropical regions of the world. After several decades of development, the yield of NR tends to be stable. In order to increase the value of the products generated by the NR industry, L-quebrachitol is extracted as a by-product of the NR industry and a number of companies already hold patents on methods for extracting L-quebrachitol[2,17–19]. However, these patented methods are currently not suitable for large scale applications because the quantities that can be extracted are limited to the laboratory scale and the extraction costs are high. Therefore, it was considered to be a challenging problem to solve[20].

Membrane separation technology has been developed recently and widely used in various industries, including food, medicine, environmental protection, biological and chemical fields. In many applications, the technology has been shown to be economically viable and technically efficient[21]. In the isolation and purification of natural products, microfiltration (MF) membranes, which serve to remove suspended or colloidal solids, are usually used in pretreatment steps to obtain clarified sample solutions. Ultrafiltration (UF) membranes can then be used to concentrate and collect the macromolecular solutes, while small solutes easily pass through these membranes. Nanofiltration (NF) membranes are used to carry out partial demineralisation of the ultrafiltrate[22].

In this work, L-quebrachitol was isolated from NR factory wastewater using membrane separation technology instead of the more traditional approach involving concentration by evaporation followed by column chromatography. With this novel approach, the results show that 10 kg of L-quebrachitol can be obtained from 10 tonnes of wastewater per year under laboratory conditions. The structure of L-quebrachitol was determined by elementary analysis, infrared (IR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, mass spectroscopy (MS) and single-crystal X-ray diffraction. The described method of L-quebrachitol extraction has been applied for a patent in China and claimed priority under the Patent Cooperation Treaty (PCT)[23–24].

EXPERIMENTAL

Materials

Wastewater was collected from three different sources from the Jinghong NR factory. The sources were the coagulation tank (referred to as A-wastewater), the effluent outlet (B-wastewater) and the reuse pond (C-wastewater). A-wastewater was collected by squeezing latex coagulum in the coagulation tank. It contained a small amount of uncoagulated latex and various non-rubber components, such as proteins, lipids, sugars as well as inorganic and organic salts, which

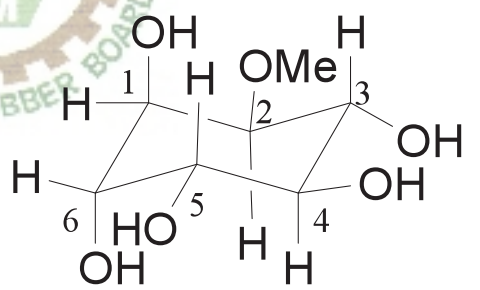

Figure 1. Structure of L-quebrachitol

originate from field latex[25]. B-wastewater was untreated wastewater discharged from the NR processing plant. It contained latex serum and large quantities of water, which were used to dilute the field latex and clean the materials and machinery. C-wastewater was derived from B-wastewater after the latter was treated successively by hydrolysis-acidification, anaerobic digestion, aerobic digestion and sedimentation. C-wastewater is generally reused to clean the latex coagulum and machinery in the NR processing factory. L-quebrachitol standards purity ≥98% were purchased from Carbosynth Ltd. (UK).

Membrane Separation System

A spiral-wound membrane separation device was designed by Hangzhou Donan Memtec Co., Ltd. Four spiral-wound membranes, each with an effective area of 0.32 m^2, were purchased from General Electric Company (GE). They are EW1812C-34D (pore diameter 0.04 μm), PT1812C-34D (molecular weight cut-off (MWCO) 5 kDa), GE1812C-34D (MWCO 1 kDa) and DL1812C-34D (rejection $MgSO_4$ of 96%).

Isolation and Purification

First, the wastewater from the NR factory was heated till it boiled and the floccules were skimmed off. Then, the wastewater was filtered through a 300 mesh screen to remove solid impurities, resulting in a clear liquid. Soluble macromolecular matter such as proteins in the clear liquid was removed by an MF membrane with a pore size of 0.04 μm. In order to get rid of additional soluble impurities, two UF membranes with MWCOs of 5 and 1 kDa were applied successively. Then the ultrafiltrate was concentrated by an NF membrane with a MWCO of 150 – 300 Da and the concentrate was washed twice with distilled water. A thick paste was obtained by vacuum distillation after the concentrate was decolourised using activated carbon and then kept at 4ºC for 12 h to promote crystallisation. The resulting crude extract thus obtained was recrystallised three times from distilled water by cooling. The pure crystals obtained were re-dissolved in hot water and the final product was precipitated by the addition of three volumes of ethanol[23–24].

General Experimental Procedures

Samples prepared as above were subjected to selected analysis. The melting point was determined with a WRS-2 micro melting point apparatus and was uncorrected. The optical rotation was measured using a JASCO P-1030 automatic digital polarimeter. Elemental analysis was determined on an Elementar Vario Micro cube. The IR spectrum was measured on a PerkinElmer Spectrum 65 infrared spectrometer with a KBr pellet. The electrospray ionisation mass spectrometry (ESI-MS) spectrum was recorded on a LCQ classic mass spectrometer. NMR spectra were recorded on a Bruker AVIII600 spectrometer with tetramethylsilane (TMS) as the internal standard and the chemical shifts were expressed in δ values (p.p.m.). The single-crystal X-ray diffraction data were collected using Mo Kα radiation (λ = 0.71073 Å) on a Bruker Smart 1000 diffractometer.

RESULTS AND DISCUSSION

Wastewater Source Selection

L-quebrachitol extraction was carried out on the three different types of wastewater A, B and C-wastewater. Experimental results show that the yields of L-quebrachitol from A and B-wastewater were 45.80 g (0.1145% w/w) and 5.52 g (0.0138% w/w), respectively. The significant decrease in the

yield of L-quebrachitol from B-wastewater in comparison to that of A-wastewater is due to the large amounts of water used for diluting the latex and washing the latex coagulum. No L-quebrachitol was extracted from C-wastewater. As previously reported, the reason is that L-quebrachitol was consumed by microorganisms in the wastewater[26]. In summary, the findings show that A-wastewater is the main source material for L-quebrachitol extraction.

Effect of Membrane Pore Size on Crystallisation

The chemical composition of the wastewater from the NR factory is complex. It contains a large amount of proteins, lipids, pigments and about 0.1% of L-quebrachitol. The high impurity content makes it difficult to crystallise L-quebrachitol. In this work, experiments were performed to obtain products of higher purity. First, 120 kg of A-wastewater was dispensed into three equal volume containers (labelled Samples A, B and C) and each of them were pretreated by passing through an MF membrane (pore diameter 0.04 μm). Sample B was subsequently filtered with a UF membrane of MWCO 5 kDa. Sample C was passed through two UF membranes, first through one with a MWCO of 5 kDa and then through a second one with a MWCO of 1 kDa. Finally, these solutions were concentrated using an NF membrane (rejection $MgSO_4$ of 96%). The effect of membrane pore size on crystallisation is shown in *Table 1*.

The results show that Sample C had the fastest crystallisation rate and that Sample A did not crystallise, which was due to the high concentration of impurities. The yield of L-quebrachitol from Sample A was 44.72 g (0.1118% w/w), which was obtained by column chromatography. The yields of L-quebrachitol from Samples B and C were 41.85 g (0.1046% w/w) and 41.57 g (0.1039% w/w), respectively. There is an obvious decrease in the yields of L-quebrachitol from Sample B and C with respect to Sample A, as addition of the UF step resulted in the loss of solution, causing the yield of L-quebrachitol to decrease. Compared with the yield of L-quebrachitol from Sample B, the yield of L-quebrachitol from Sample C had little decrease. This is

TABLE 1. EFFECT OF MEMBRANE PORE SIZE ON CRYSTALLISATION

Wastewater	Type and order of membranes used	Crystallisation time (h)	Yield (g), % (w/w)
Sample A	1. MF (pore diameter 0.04 μm) 2. NF (rejection $MgSO_4$ of 96%).	No crystallisation occurred	44.72*, 0.1118
Sample B	1. MF (pore diameter 0.04 μm) 2. UF (MWCO 5 kDa) 3. NF (rejection $MgSO_4$ of 96%).	12	41.85, 0.1046
Sample C	1. MF (pore diameter 0.04 μm) 2. UF (MWCO 5 kDa) 3. UF (MWCO 1 kDa) 4. NF(rejection $MgSO_4$ of 96%).	< 1	41.57, 0.1039

* The yield of L-quebrachitol from Sample A was determined by column chromatography.

because although more crude extract was obtained from Sample B, it also contained more impurities. In order to obtain a purer final product, additional recrystallisation steps are necessary, which may reduce the yield of L-quebrachitol. In summary, while a purer ultrafiltrate is obtained with the smaller pore size of UF membrane, though beneficial for crystallisation process, its use has little effect on the yield of L-quebrachitol.

Concentration and Purification of the Ultrafiltrate

NF membranes are adopted to separate the small neutral and charged solutes from aqueous solutions[27]. In this study, the ultrafiltrate was passed through an NF membrane and a portion of the small inorganic salt impurities was removed with the filtrate. However, after this step majority of the small inorganic salt impurities still remained in the concentrate, affecting both the rate of crystallisation and purity of the final product. The concentration of the impurities can be reduced effectively by washing the concentrate with water. First, 40 kg of A-wastewater was filtered with one MF membrane and two UF membranes. Next, the ultrafiltrate was concentrated with the use of an NF membrane to a volume of about two litres, which took approximately 1.5 hours. Two litres of distilled water was then added for washing; this step was repeated nine times. Electrical conductivity (EC) values were measured prior to each washing.

Pure water is a very poor conductor of electrical current, whereas water containing dissolved salts conducts current approximately in proportion to the amount of salt present[28]. Based on this information, the EC of the sample was used to monitor impurities in the form of small inorganic salts. The effect of filtration time on EC is shown in *Figure 2*. As expected, EC values of the concentrate and the filtrate rapidly increased over time due to the increase in concentration

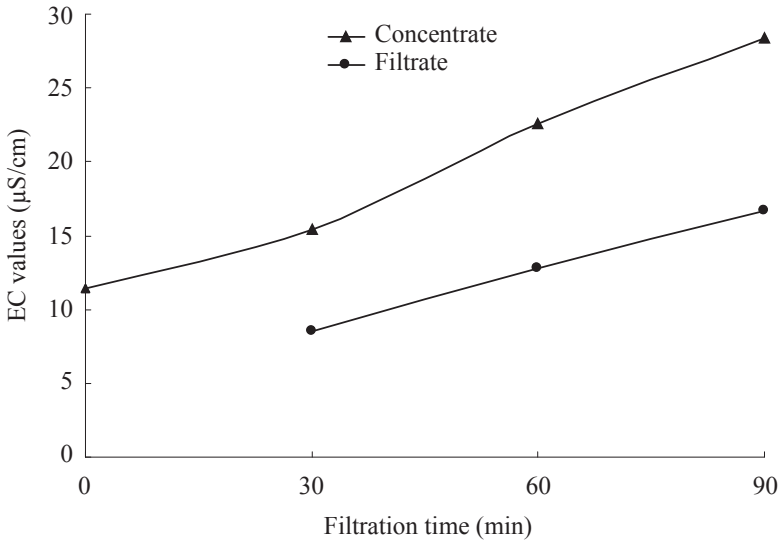

Figure 2. Effect of nanofiltration time on EC of wastewater

of inorganic salts in the concentrate and the highest EC values were attained prior to washing.

Figure 3 shows the effect of washing times performed on a sample on the EC. The results indicate that the EC values of the concentrate and filtrate decreased rapidly the first three times a sample was washed. After being washed five times, EC values for a sample decreased slowly, illustrating that majority of the impurities had already been removed.

Additional impurities can be removed by adding more washing steps, but this may cause a decrease in the yield of L-quebrachitol. The effect of washing times on crystallisation is shown in *Table 2*. The results show that the concentration of the impurities decreased when the washing times increased, the first wash had a major effect on the yield of L-quebrachitol. Crystallisation occurred in 1 h after two washes. With additional washes, there was almost no improvement on the crystallisation rate, but the yield decreased significantly. The reason is that majority of the impurities were removed after the first two washes. After this number of washes, the low remaining impurity level had little effect on the crystallisation rate. In summary, washing two or three times was found to be the a good approach for improving the yield of L-quebrachitol.

IR Spectrum

The infrared spectrogram of the sample is shown in the frequency range of 400 – 4000 cm^{-1} in *Figure 4*. A wide, strong absorption peak was observed at 3338 cm^{-1} which corresponds to the overlap of the stretching vibration absorption peaks of five hydroxyl groups and the effect of intramolecular or intermolecular hydrogen bondings. The multiple absorption peaks at 2939, 2928, 2901, 2882, 2835 cm^{-1} were assigned to the six C-H bonds and one methyl group. The

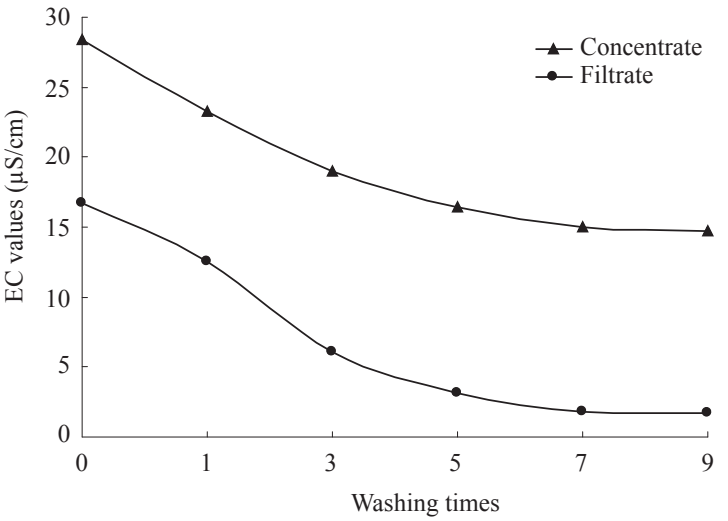

Figure 3. Effect of washing times on EC of nanofiltrate during the nanofiltration stage

TABLE 2. EFFECT OF WASHING TIMES ON CRYSTALLISATION

Washing times	Crystallisation time (h)	Yield (g) , %(w/w)
0	12	45.21, 0.1130
1	6	41.77, 0.1044
2	< 1	40.52, 0.1013
5	< 1	36.76, 0.0919

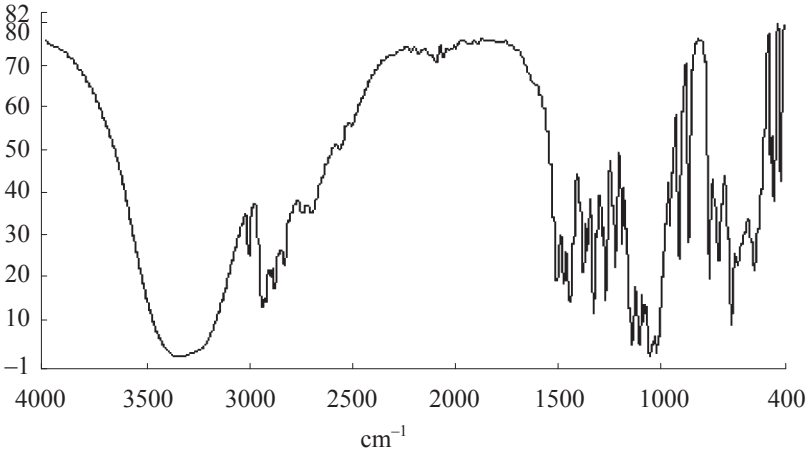

Figure 4. IR spectrum of L-quebrachitol

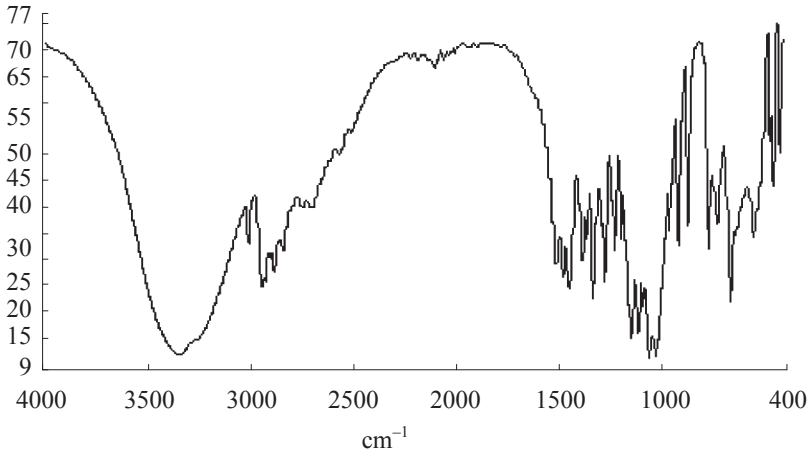

Figure 5. IR spectrum of L-quebrachitol standards

strong stretching vibration absorption peaks of five C-OH, C-OCH_3 and C-O bonds appeared at 1139, 1102, 1086, 1078, 1063, 1048, 1015 cm^{-1} respectively. Compared with the IR spectrum of the corresponding standards (*Figure 5*), results show that the characteristics and patterns found in both spectra are very similar.

X-ray Crystal Structure Analysis of L-quebrachitol

The crystal structure of L-quebrachitol was determined from the X-ray diffraction data, solved by direct methods and refined by full-matrix least-squares optimisation of F^2 over all unique reflections (SHELXS-97). The ORTEP diagram of L-quebrachitol is shown in *Figure 6*. The C-C bond lengths vary from 1.5210(19) Å to 1.5366(19) Å; the C-C bond lengths vary from 1.4268(15) Å to 1.4405(16) Å; minimum and maximum bond angles observed for non-hydrogen bonds were 107.61(11)º and 112.55(11)º, respectively. The crystal data for the structure determinations is given in *Table 3*. L-quebrachitol crystallises in a monoclinic system, space group $P2_1$, with cell parameters of, a = 6.624(2) Å, b = 7.193(3) Å, c = 8.687(3) Å, β = 90.634(5)º and Z = 2. The ring is in chair conformations[1,16].

Other Analysis

The final product was also characterised by melting point, specific rotation, elemental analysis, MS and NMR. The values are presented in *Table 4*.

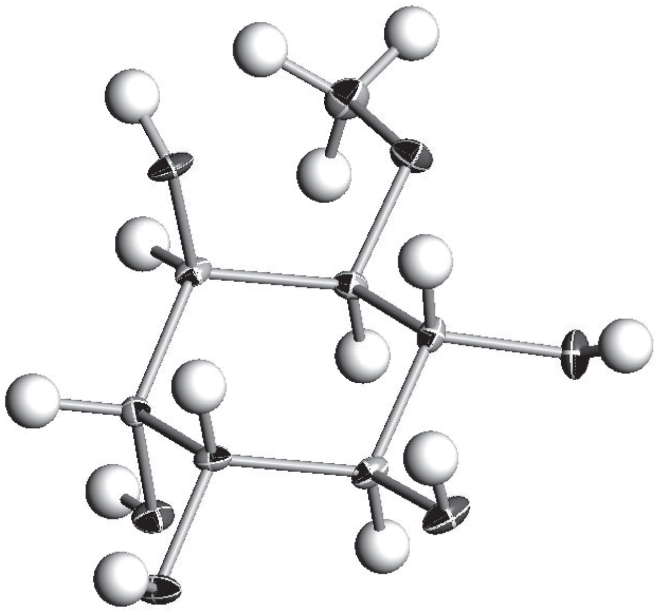

Figure 6. ORTEP diagram of L-quebrachitol

TABLE 3. CRYSTAL DATA FOR L-QUEBRACHITOL

Empirical formula	$C_7 H_{14} O_6$
Formula weight	194.18
Colour	colourless
Crystal size, mm	0.20 × 0.18 × 0.12
Crystal system	Monoclinic
Space group	$P2_1$
Symmetry	x, y, z; -x, y + 1/2, -z
Unit cell dimensions, Å	a = 6.624(2), b = 7.193(3), c = 8.687(3)
Unit cell angles, º	$\alpha = \gamma = 90$, $\beta = 90.634(5)$
Volume, $Å^3$	413.9(2)
Z	2
Calculated density, g/cm^3	1.558
Absorption coefficient, mm^{-1}	0.137
F(000)	208

TABLE 4. CHARACTERISATION DATA OF L-QUEBRACHITOL

Melting point	194.1 – 194.3ºC
Specific rotation	$[\alpha]_D^{20}$ = -81.55 (*c*, 5.00, H_2O)
Negative ESIMS	193.00 [M-H]- (Calculated for $C_7H_{13}O_6^-$)
Elemental analysis	C 43.28, H 7.19; found C 43.30, H 7.27.
^{1}H NMR (600 MHz, D_2O)	δ: 4.28 (t, 1H, J = 6.6 HZ), 4.07 (t, 1H, J = 6.6 HZ), 3.75 (dd, 1H, J = 3.6 HZ, 3.0 HZ), 3.62 (m, 2H, J = 34.2 HZ), 3.46 (s, 1H), 3.41(dd, 1H, J = 2.4HZ, 1.2HZ).
^{13}C NMR (150 MHz, DMSO)	δ: 81.58, 73.78, 72.70, 72.53, 70.96, 68.52, 57.5.

CONCLUSIONS

The work reported in this paper suggests that serum collected by squeezing the coagulated rubber particles is the best source for L-quebrachitol extraction, which was then concentrated and purified using membrane separation technology. The results indicate that small amounts of impurities had a strong influence on crystallisation of L-quebrachitol which shall be removed as much as possible prior to crystallisation. Optimising the membrane pore size and washing times allowed an effective removal of impurities. Using a UF membrane with an MWCO of 1 kDa and washing the ultrafiltrate two to three times generated a relatively pure extract that crystallised effectively and did not substantially decrease the yield of L-quebrachitol.

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REFERENCES

1. HUANG, K.F. AND LUO, W.L. (1994) Crystal Structure of L-Quebrachitol. *Chin. Chem. Soc*., **41(1)**, 115–117.

2. KIDDLE, J.J. (1995) Quebrachitol: A Versatile Building Block in the Construction of Naturally Occurring Bioactive Materials. *Chem. Rev*., **95(6)**, 2189–2196.

3. DE ALMEIDA, M.V., DE ASSIS, J.V., COURL, M.R.C., ANCONI, C.P.A., GUERREIRO, M.C., DOS SANTOS, H.F. AND DE ALMEIDA, W.B. (2010) Experimental and Theoretical Investigation of Epoxide Quebrachitol Derivatives Through Spectroscopic Analysis. *Org. Lett*., **12(23)**, 5458–5461.

4. QIAO, L.X., HU, Y.H., NAN, F.J., POWIS, G. AND KOZIKOWSKI, A.P. (2000) A Versatile Approach to PI(3,4)P2,PI(4,5)P2, and PI(3,4,5)P3 from L-(-)-Quebrachitol. *Org. Lett*., **2(2)**, 115–117.

5. DING, Y.X., ZHOU, S.F. AND SHEN, J. (1995) The Synthesis of Myo-inositol Phospates by S-L Phase Transfer Catalytic Method. *Acta Chim. Sinica*, **53**, 305–309. (in Chinese)

6. BERRIDGE, M.J. AND IRVINE, R.F. (1989) Inositol Phosphates and Cell Signaling. *Nature*, **341**, 197–205.

7. BERRIDGE, M.J. AND IRVINE, R.F. (1993) Inositol Trisphosphate and Calcium Signaling. *Nature*, **361**, 315–325.

8. KOZIKOWSKI, A.P., OGNYANOV, V.I., FAUQ, A.H., NAHORSKI, S.R. AND WILCOX, R.A. (1993) Synthesis of 1D-3-deoxy-,1D-2,3-dideoxy-, and 1D-2,3,6-trideoxy-myo-inositol 1,4,5-trisphosphate from Quebrachitol, Their Binding Affinities, and Calcium Release Activity. *J. Am. Chem. Soc*., **115**, 4429–4434.

9. CHEN, P.R. AND XIANG, P. (2011) Recent Advances in the Ferrier-Type Rearrangement Reactions and Their Applications in the Complex Bio-active Molecules. *Chin. J. Org. Chem*., **31(8)**, 1195–1201. (in Chinese)

10. AO, N.J. (2005) The Development and Use of the Quebrachitol as Valuable Botanical Drug Resource in Latex from *Hevea brasiliensis*. *Journal of Yunnan Agricultural University*, **20(4)**, 467–473. (in Chinese)

11. STINSON, E.E., DOOLEY, C.J., PURCELL, J.M. AND ARD, J.S. (1967) Quebrachitol - A New Component of Maple Sap and Sirup. *J. Agric. Food Chem*., **15(3)**, 394–397.

12. DIAZ, M., GONZALEZ, A., CASTRO-GAMBOA, I., GONZALEZ, D. AND ROSSINI, C. (2008) First Record of L-Quebrachitol in Allophylus Edulis (Sapindaceae). *Carbohydr. Res*., **343(15)**, 2699–2700.

13. MOHARAM, B.A., JANTAN, I., JALIL, J. AND SHAARI, K. (2010) Inhibitory Effects of Phylligenin and Quebrachitol Isolated from Mitrephora vulpina on Platelet Activating Factor Receptor Binding and Platelet Aggregation. *Molecules*, **15(11)**, 7840–7848.

14. ADAMS, R., PEASE, D.C. AND CLARK, J.H. (1940) Isolation of Cannabinol, Cannabidiol and Quebrachitol from Red Oil of Minnesota Wild Hemp. *J. Am. Chem. Soc*., **62(8)**, 2194–2196.

15. YANG, B.R., KALLIO, H., JUDIN, V.P. AND KORTENIEMI, V.M. (2009) Extract with High Amount of Quebrachitol and Its Use. *PCT Patent Application No. PCT/FI2009/050273*.

16. DOWD, M.K. AND STEVENS, E.D. (2002) The Crystal Structures of D-Pinitol and L-Quebrachitol by Low-Temperature X-RAY Differaction *J. Carbohydr. Chem*., **21(5)**, 373–383.

17. UDAGAWA, Y., MACHIDA, M. AND OGAWA, S. (1991) Method of Recovering L-Quebrachitol from Rubber Latex Serums. *US Patent No. 5041689*.

18. HART, W.J. (1945) Method for Recovering Quebrachitol from Rubber Latex Serum. *US Patent No. 2378141*.

19. MCGACACK, J. AND BINMORE, G.B. (1930) Method for Recovering

Quebrachitol from Rubber Latex Serum. *US Patent No. 1758616*.

20. GOPALAKRISHNAN, J., THOMAS, M. AND JACOB, J. (2010) A Process for Obtaining Pure L-Quebrachitol from Rubber Latex Serum. *Indian Patent No. 238511*.

21. TABE-MOHAMMADI, A. (1999) A Review of the Applications of Membrane Separation Technology in Natural Gas Treatment. *Sep. Sci. Technol*., **34(10)**, 2095–2111.

22. SUÁREZ, E., LOBO, A., ALVAREZ, S., RIERA, F. A. AND ÁLVAREZ, R. (2009) Demineralization of Whey and Milk Ultrafiltration Permeate by Means of Nanofiltration. *Desalination*, **241(1)**, 272–280.

23. JIANG, S.K., WU,Y. AND ZHANG, G.M.(2013) Method of Extracting L-Quebrachitol from Rubber Factory Wastewater. *CN Patent No. 201310007459*. (in Chinese)

24. WU, Y. JIANG, S.K., LI, G.H., ZHANG, G.M., ZOU, J.Y. AND XU, R. (2013) Method of Large-scale Extracting L-Quebrachitol from Natural Rubber Factory Wastewater. *PCT Patent Application No. PCT/CN2013/075778*. (in Chinese)

25. HE, Y.P. (2007) Natural Rubber Processing. Haikou: Hainan press. (in Chinese)

26. DENG, Y.J. AND DENG, D.Y. (2000) Study on Extraction of Quebrachitol from Natural Rubber. *Natural Product Research and Development*, **12(6)**, 61–65. (in Chinese)

27. WANG, X.L., YING, A.L. AND WANG, W.N.(2002). Nanofiltration of L-phenylalanine and L-aspartic acid aqueous solutions. *J. Membrane Sci*., **196(1)**, 59–67.

28. PAGE, A.L. (1982) Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. American Society of Agronomy, Soil Science Society of America.