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RECOVERY OF L- QUEBRACHITOL FROM DIFFERENT LATEX SERUM SOURCES OF *HEVEA BRASILIENSIS*

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Hevea latex contains several non-rubber components in addition to rubber particles. Among them L-quebrachitol (methyl inositol), a commercially important compound, is prominently present. Hence attempts were made for its isolation from different sources of latex serum. In the present study, latex serum of four clones obtained by four different extraction methods, *viz*. coagulation of latex with acetic acid, coagulation of latex with alcohol, centrifugation of latex and low temperature extraction, as well as the effluent of latex centrifuging factory were used for quebrachitol separation. The results showed that yield/ extraction rate of quebrachitol from natural rubber latex varied from clone to clone and with different methods of serum preparation. The highest recovery (1.4%) was obtained from the serum obtained by the latex centrifugation method in the clone RRII 430. Of the four serum sources used, serum from latex centrifugation gave the best results in all the clones. Among the four clones, RRII 430 gave the highest yield of quebrachitol followed by RRII 414, RRII 105 and RRIM 600 which had similar levels of extraction rates irrespective of the source of the serum. Recovery was the lowest (0.005%) in factory effluent which may be due to prolonged preservation of latex.

Keywords: Latex serum, Quebrachitol, Secondary metabolite

INTRODUCTION

Hevea brasiliensis is mainly cultivated as the major source of natural rubber. Apart from rubber, latex also contains inositols as secondary byproducts. Clonal differences were reported in total inositol content in the latex (Gopalakrishnan *et al*., 2008). In *Hevea* latex, L-quebrachitol (methyl inositol) is the predominant inositol with small amounts of L-and myo-inositols (Bealing, 1969, 1981 Anderson, 1972; d' Auzac and Jacob, 1989).

Inositols are cyclohexane hexols having six hydroxyl groups bonded on a cyclohexane nucleus. L-quebrachitol (1L-2-O-methyl-chiro-inositol) is a naturally occurring optically active inositol. The chemical structure of quebrachitol is shown in Figure 1. It is a high-value compound with several commercial applications and is mainly used in the pharmaceutical industry and in medical research (Lau, 1993; 1996; Deng and Deng, 1999). It has gained much attention because of its optical properties and also due to its various derivatives which

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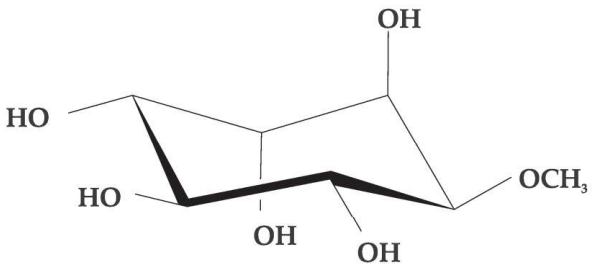

Fig. 1. Chemical structure of L- quebrachitol

are involved in cell signaling mechanisms (Lau, 1993; Ningjian, 2005). Moreover, by suitable chemical modifications, some inositol derivatives are reported to function as anticancer drugs, antibiotics or enzyme inhibitors (Lau, 1993). The usefulness of this compound to mankind triggered the development of methods for the extraction of L-quebrachitol from natural rubber latex serum (Lau, 1993; Deng and Deng, 1999; Gopalakrishnan *et al*., 2010).

Plant secondary metabolites are currently the subject of much research interest. Development of a quick, reliable and efficient extraction protocol for a particular class of compounds is highly in demand. The present study was aimed at finding out the rate of recovery of quebrachitol from different *Hevea* clones and varying sources of latex serum.

MATERIALS AND METHODS

Latex samples were collected from four different *Hevea* clones, *viz*. RRII 105, RRIM 600, RRII 430 and RRII 414. Three replications were made. Fresh latex without preservatives was used in this study. Serum obtained from the latex samples through four different latex coagulation methods and from the effluent of latex processing factory was used as the source material for quebrachitol.

(1) Coagulation of latex with acetic acid (A- serum)

Fresh latex samples were diluted with an equal quantity of water and 3% acetic acid was added. After coagulation, the serum was collected by removing the coagulated rubber.

(2) Coagulation of latex with alcohol

Fresh latex samples were mixed with 80% alcohol, heated at 80 ^{0}C for 30 min, cooled and filtered to collect the serum. This step was repeated thrice with the remaining coagulam. The serum samples were pooled and used as the source material for quebrachitol extraction.

(3) Centrifugation of latex (C-serum)

Latex samples were centrifuged using an ultracentrifuge (Sorvall OTD 55B) at 23,000 rpm for 45 min at 4 ^{0}C. The latex was separated into three layers and the middle layer containing the C-serum was collected for extraction of quebrachitol.

(4) Low temperature extraction

Fresh latex samples were frozen immediately and kept for 3 to 4 days. Coagulated samples were squeezed and the serum was collected for isolation of quebrachitol.

(5) Serum from the effluent of latex processing factory

In addition to the sera of the four clones obtained by different extraction methods, effluent from a latex centrifuging factory

was also collected and tested as a source material for quebrachitol extraction.

Serum samples obtained by the five different methods were processed as per the protocol developed for the extraction of quebrachitol from *H. brasiliensis* latex (Gopalakrishnan *et al*., 2010). The serum samples collected were concentrated and then deproteinised using acetone and kept overnight in a refrigerator. After 24 h, the samples were filtered and the filtrate was collected. It was then evaporated and the residue was dissolved in acetone and passed through a silica column (silica gel) for removing lipids and related impurities. After concentrating and resuspending in distilled water, the resultant solution was passed through mixed bed ion exchange resin column (Amberlite MB 150) for further purification. The eluate thus obtained was concentrated to obtain L-quebrachitol crystals.

HPLC analysis

The crystals (quebrachitol) obtained from different serum samples were analyzed by HPLC (Waters, Austria). A known quantity of quebrachitol was dissolved in water (milli Q) and the solutions were filtered through a 0.45 μm syringe filter. The filtrates were analyzed using a Ca loaded cation exchange column of Shodex Sugar SC1011 (8 X 300 mm, Showa Denko, Tokyo) with a refractive index detector. The column and RI detector were kept at 30 °C and elution was carried out using milli Q water. The isolates were identified based on the retention time of standard L-quebrachitol (Sigma Chemicals, USA).

RESULTS AND DISCUSSION

Methods of latex coagulation were found to have direct effect on the yield of quebrachitol from the latex sera. Analyses indicated that a higher extraction rate of quebrachitol was obtained from the C-serum, while the remaining three methods gave similar extraction rates (Table 1). The average rate of extraction of quebrachitol was 1.29% (w/w) using serum obtained by centrifugation, while the remaining methods yielded 0.22 to 0.27% of the inositol. The clone RRII 430 was superior to other clones and gave the highest recovery rate (1.41%) by centrifugation method.

In general, RRII 400 series clones had a relatively higher per cent of quebrachitol than the other two popular *H. brasiliensis* clones, RRII 105 and RRIM 600. The clone RRII 105 is cultivated in more than 80% of the traditional rubber growing region whereas RRIM 600 is mainly cultivated in the non-traditional region. Therefore, a

Table 1. **Recovery of L-quebrachitol from different clones and mode of serum extraction**

Clone	Extraction rate (%)				
	Acid coagulation (A-serum)	Alcoholic extraction	Centrifugation (C-serum)	Low temperature extraction	Factory effluent
RRII 105	0.14	0.15	1.25	0.12	
RRIM 600	0.12	0.13	1.26	0.12	
RRII 414	0.27	0.28	1.25	0.22	
RRII 430	0.49	0.50	1.41	0.43	
Average	0.26	0.27	1.29	0.22	0.005

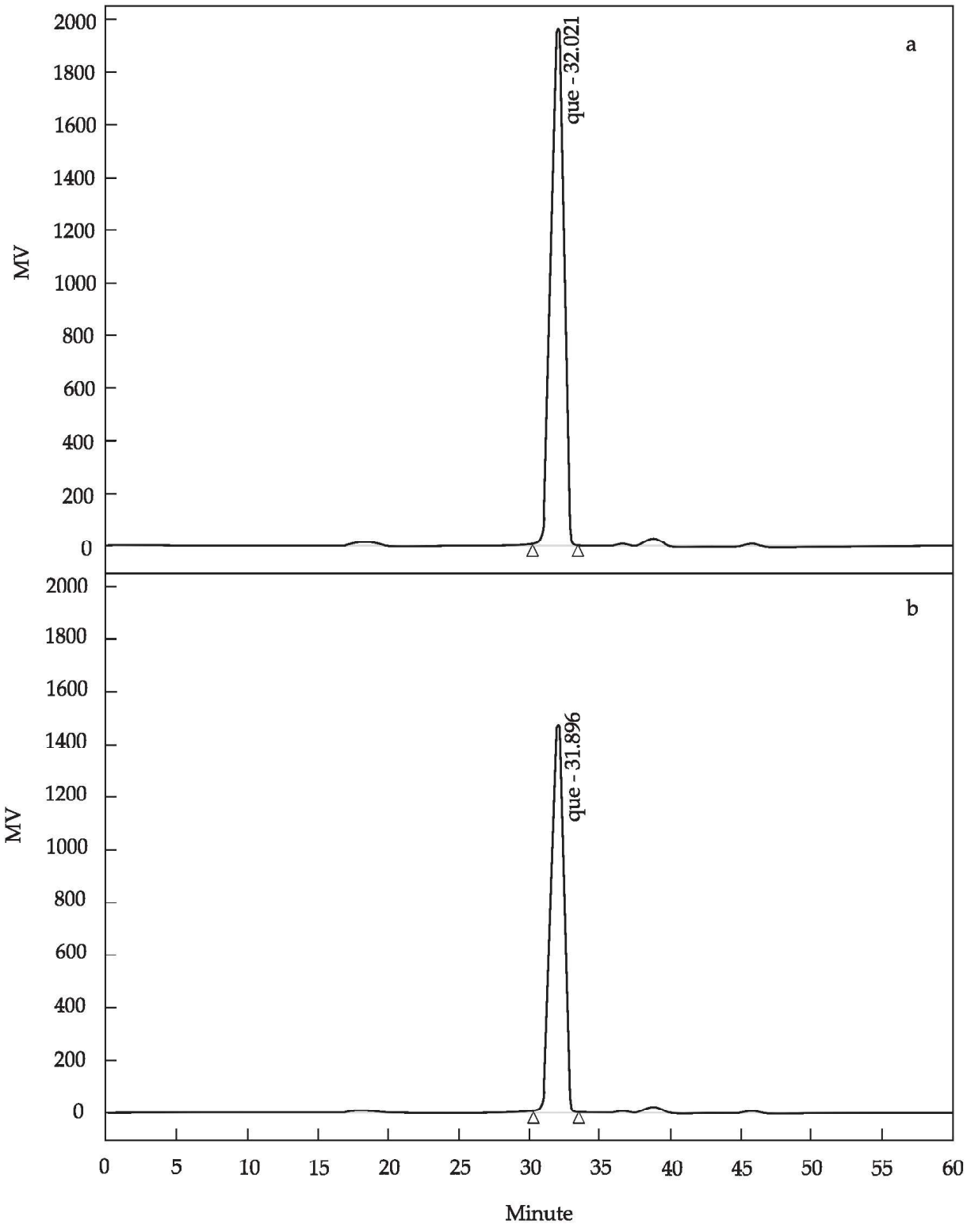

Fig. 2. The chromatographic profile of (a) quebrachitol standard and (b) quebrachitol isolated from *Hevea* latex by alcohol extraction

comparative account of recovery rate of quebrachitol in these clones was worked out. The recovery rate of quebrachitol was almost similar in these clones and the highest rate was obtained for centrifugation (Table 1).

The recovery of quebrachitol was very low (0.005%) in the factory effluent sample. Preservatives such as ammonia change the non–rubber constituents in the latex, affecting the colloidal stability and thereby hindering the release of some non-rubber constituents such as quebrachitol. Thus the very low rate of quebrachitol recovery from factory effluent may be due to high amount of preservatives and prolonged storage of latex. The addition of chemical preservatives and duration of preservation were reported to have a profound effect on the extractable amount of quebrachitol from latex. The ammonia content of the preservatives is the key factor determining the preserving effect on latex. Latex can be preserved effectively only if the ammonia content of the latex is maintained around 1% (Deng *et al*., 2004).

The chromatographic profiles obtained by HPLC analysis of standard L-quebrachitol and quebrachitol isolated from *Hevea* latex by alcohol extraction are given in Figure 2. Similar chromatograms are also obtained for quebrachitol isolated from other serum sources. The results revealed that the protocol developed was suitable for the isolation of quebrachtiol from the sera obtained from various latex coagulation methods.

CONCLUSION

The present study revealed that the amount of quebrachitol extracted from natural rubber latex varied with respect to clone and the source of the serum. The highest extraction rate/recovery was observed in C-serum, while the lowest was in factory effluent. An efficient and quick extraction method and identification of suitable serum source would give opportunities to use natural rubber latex as a source of quebrachitol which would further fetch revenue to the growers.

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