RESEARCH ARTICLE

Natural rubber identification and characterization in *Euphorbia macroclada*

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Abstract Natural rubber is one of the most important polymers produced by plants, which contains *cis*-1,4-polyisoprene, protein and fatty acids. It has unique properties compared to synthetic rubber and has many different uses in industry. Here, natural rubber of *Euphorbia macroclada* was characterized due to its abundance in arid areas. Isolation of rubber was done using both acetone and hexane solvents. FT-IR and NMR further characterized and confirmed the structure of rubber as *cis*-1,4 polyisoprene. GPC analyses showed a molecular weight of 8.180E+2 with polydispersity of 1.287. These data is useful for better understanding of latex composition in family of Euphorbiaceae.

Keywords Latex · Natural rubber · Spurge · *E. macroclada*

Introduction

Euphorbia macroclada belongs to family of Euphorbiaceae. This family includes over 2000 species distributed around the world as herbs, shrubs and trees. *E. macroclada* usually grows in different habitats such as damaged areas, arable lands, dry meadows, steppes, open oak forests, along

roads, rocks, etc. and SW Asia is one of the most important centers of its diversity (Yang et al. 2012; Dorsey et al. 2013; Peirson et al. 2013; Riina et al. 2013; Pahlevani et al. 2015). The genus *Euphorbia* is known by exudes of latex when injured (Biesboer and Koukkari 1992; Mabberley 1997; Barla et al. 2006). One of the important plants in this family is *Hevea brasiliensis* which is well known as the natural source of industrial rubber production (Dehghan and Schutzman 1994; Mooibroek and Cornish 2000). Natural rubber is a bio-polymer composed of isoprene units. The length of the biopolymers defines the importance molecular mass of rubber (Bouton 1992). Most of our knowledge of latex biochemistry and laticifers is derived from studies on *H. brasiliensis* (Arif et al. 2004; Takahashi and Koyama 2006; Wagner et al. 2007). This natural raw material is used in medical instruments, surgical gloves, and various engineering and consumable products (Mooibroek and Cornish 2000). Most developed countries are entirely dependent on the introduction of natural rubber for applications where synthetic rubber cannot be replaced. Natural rubber and latex prices continue to rise as demand grows, and global shortages are projected (Bowers 1990). As *Hevea* has a very narrow genetic base and well establishes only in tropical regions efforts have been made to develop alternative natural rubber resources (Metcalf 1967; Bowers 1990). Recently two rubber bearing plants, *Taraxacum kok-saghyz* and *Parthenium argentatum*, introduced as alternatives (Buranov and Elmuradov 2010; Cornish 2017) and many works are done to improve their processing capacity. In family of Euphorbiaceae other species like *E. characias* and *E. lactiflua* are also have been studied for rubber production (Mooibroek and Cornish 2000; Spanò et al. 2012).

To analyze tires and plastics the Fourier-transform infrared spectroscopy (FT-IR) is used which provides

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information about the chemical structure of a molecule (Amand and Tullin 1999). Nuclear magnetic resonance spectroscopy (NMR), is used for both quantitative and qualitative identification of complex organic and biological compounds (Vandersypen et al. 2001). To detect and evaluate the extracted rubber based on the molecular mass (total mass of the forming atoms) also gel permeation chromatography (GPC) is carried out (Moore 1964).

As there is no information on rubber production in *E. macroclada*, the aim of this study was to investigate *E. macroclada* latex for rubber production. Due to its broad diversity in SW Asia and growing in different habitats, this investigation adds new information on *E. macroclada* latex composition.

Materials and methods

Plant material and rubber extraction

E. macroclada wild plants were collected during autumn from Hamedan plain, located in west part of Iran and stored in Bu-Ali Sina Herbarium with a voucher specimen number EM15. Drops of latex were collected by diagonal cuts on stem using a razor into preweighed micro-centrifuge tubes. Eight samples were collected and stored at 4 °C before rubber extraction. The tubes were re-weighed to obtain the initial weight of the latex. To remove the water and dry latex completely, tubes were kept at 35 °C for 10 days. The tubes were re-weighed to obtain the total amount of water. The dried samples were extracted three times with acetone by addition of one ml acetone then vortex and centrifuge for 5 min at 6822 g, and collection of supernatant into a re-weighed clean tube. After acetone evaporation the tubes were re-weighed to measure the resin content based on the initial latex weight. In the next stage, hexane was used to extract rubber (Bell et al. 2015). Samples were centrifuged and supernatant collected into a re-weighed clean tube. Rubber was extracted three times with hexane. Hexane was dried up under the air and tubes were weighted to obtain the contained rubber. Original collection tubes containing insoluble and other material were dried and weighted to find out the percentage of other contents in initial latex (Bell et al. 2015). The extracted rubber was used for FT-IR, NMR and GPC analysis. The percentage of water, resin, rubber and other insoluble materials of latex were determined (% w/w) as the means of eight measurements.

Rubber analysis

To do the FT-IR, the Bruker Vector 22 spectrophotometer was used. Samples were dissolved in a benzene solution

and poured onto a KBr disk to form a thin film. A resolution of 2 cm^{-1} in the range of $4000\text{--}400\text{ cm}^{-1}$ was considered as described by Spanò et al. (2012) and Rolere et al. (2015). NMR spectroscopy was done by the Bruker AVANCE Aqf-300 MHz device. The rubber extracted in the chlorophorm was dissolved in CDCl_3 and measured at 25 °C and chemical shifts were reported in ppm (Mooibroek and Cornish 2000). The molecular weight was measured by GPC. Chromatogram monitor with refractive index (RI) was recorded at 30 °C. The samples were dissolved in tetrahydrofuran (THF) and stored at room temperature overnight. The clear solution was filtered from 1 micron filters and 50 μl of solution was injected by automatic sampler (GPC Agilent 1100) (Cornish et al. 2000). The flow rate was 1 ml/min for the sample. Polystyrene was used as reference material and the Agilent column, PLgel Mixed-C, 5 μm ; $300 \times 7.5\text{ mm}$ was used.

Results

Natural rubber of *E. macroclada* latex was extracted by acetone and hexane. Figure 1 shows the rubber extracted from *E. macroclada* after extraction from one milliliter of latex.

Table 1 shows the different compounds of *E. macroclada* latex. Water, resin and rubber content of 1 ml of latex were measured using eight samples.

Fourier transform-infrared spectroscopy provided bands characteristic of *cis*-1,4-polyisoprene at 886.05, 1374.84, 1458.23, 1712.07, 2867.50 and 2927.33 cm^{-1} which are due to C–H out-of-plane bending, CH_3 deformation, CH_2 deformation, C=C stretching, CH_2 stretching and CH_2 stretching respectively (Fig. 2; Table 2). The spectrum data were assigned according to the literature (Rolere et al. 2015; Ramirez-Cadavid et al. 2017). Also there were other bands not characteristic of *cis*-1,4-polyisoprene.

The ^1H NMR spectrum of *E. macroclada* extracted rubber revealed signals at 1–8. There are 8 hydrogens in polyisoprene formula (Fig. 3; Table 3) which is in accordance with the peaks in image below. The peak area of 1.68757 is related to CH_3 with three hydrogens, the peak area of 2.04889 is related to 2CH_2 with four hydrogens and peak area of 5.13508 is related to CH with one hydrogen. The other signal belongs to the solvent (Fig. 3; Table 3). In the other hand the ^1H NMR spectrum of the rubber compound showed the presence of vinyl protons (H) in the 4.5–6.5 regions. The aliphatic protons CH_2 , CH appeared in the 1–2.5 region. This information in HNMR indicates the presence of polyisoprene in *E. macroclada* (Fig. 3; Table 3).

The ^{13}C NMR spectrum of *E. macroclada* extracted rubber showed different signals in the region between 20

Fig. 1 Rubber extraction from *E. macroclada* latex.

a Dualization of samples after adding solvent (acetone), **b** the extracted resin after evaporation of acetone, **c** The extracted rubber after drying hexane extract

Table 1 The amount of water, resin and rubber determined in the latex of *E. macroclada* (%W/W)

Compounds	%
Water	25.1
Resin	9.5
Rubber	8.1
Insoluble materials	57

Fig. 2 Infrared spectroscopy of *E. macroclada* extracted rubber**Table 2** The FT-IR absorbion bands of *E. macroclada* extracted rubber

Wavenumber (cm ⁻¹)	Functional group modes
886.05	C–H out-of-plane bending
1374.84	–CH ₃ deformation
1458.23	–CH ₂ deformation
1712.07	C=C stretching
2867.50	–CH ₂ stretching
2927.33	–CH ₂ stretching

and 150 ppm (Fig. 4; Table 4). The solvent (CDCl₃) peaks revealed at 76–78 ppm. Two close peaks at 125.048 and 135.230 ppm represent the core of molecule with a double

bond (C=C). Three peaks in the beginnings 23.439, 26.415 and 32.223 are related to 2CH₂ and CH₃ in the polyisoprene formula respectively (Fig. 4; Table 4).

According to GPC analysis (Fig. 5), polydispersity index (M_w/M_n) of *E. macroclada* is 1.287 (Table 5) that is similar to this index from *H. brasiliensis* and *P. argentatum*, 1.251 and 1.240 respectively (Pearson et al. 2010).

Discussion

Natural rubber has many uses and unique properties due to proteins, lipids, carbohydrates and minerals that are present in the latex. Hevea in family of Euphorbiaceae is the only tree for rubber production in tropical regions. There is at least 60 Euphorbia species just in Europe which is needed to be explored for rubber production (Mooibroek and Cornish 2000). In addition to Hevea *E. characias* and *E. lactiflua* also have been studied for rubber production in this family (Mooibroek and Cornish 2000; Spanò et al. 2012). But there is no report on rubber content of *E. macroclada* which is growing in broad area of Asia (Pahlevani et al. 2015). The FT-IR data of *E. macroclada* rubber indicated the connection of carbon to carbon (C=C) and carbon to hydrogen (C–H). The HNMR and C¹³NMR results represented vinyl and aliphatic protons, and confirmed *cis*-1,4 polyisoprene connections in *E. macroclada* rubber. This study showed the amount of rubber and resin in the latex of this plant, 8 and 9.5 percentage, is almost comparable to *E. characias* amounts, 9.1 and 14 percentage, respectively (Spanò et al. 2012). Although *E. macroclada* has less rubber amount than *Hevea* (44%) but it has more comparing with *E. larica* and *E. tirucalli* (data are not published yet). Molecular weight of natural rubber is very important for the processing and manufacturing of rubber products. Molecular weight of *E. macroclada* rubber was $M_n = 8.180E+2$ and its polydispersity was $M_w/M_n = 1.287E+0$. In *E. characias* rubber, the molecular weight of $M_n = 93,000$ and $M_w/M_n = 2.9$ has been reported (Spanò et al. 2012). In general, longer polymer chains with narrow distribution have the best performance (Bell 2013).

Fig. 3 ^1H NMR analysis of *E. macroclada* extracted rubber (left), *cis*-1,4-polyisoprene formula (right)

Table 3 The ^1H NMR signals of *E. macroclada* extracted rubber

Peak ppm	Peak area	Related signal
1.68757	3.0000	CH_3
2.04889	4.0033	CH_2
5.13508	1.0024	CH
7.27265	—	Solvent

Table 4 The ^{13}C NMR signals of *E. macroclada* extracted rubber

Peak ppm	Related signal
23.439	CH_2
26.415	CH_2
32.223	CH_3
76.601	Solvent
77.024	Solvent
77.447	Solvent
125.048	$\text{C}=\text{C}$
135.230	$\text{C}=\text{C}$

Fig. 4 ^{13}C NMR analysis of *E. macroclada* extracted rubber

But low molecular weight rubbers can also have different uses in industry as dispersing agents or processing aids (Spanò et al. 2012). The molecular weight of the rubber is largely influenced by the concentration and characteristics of the substrate, especially with the concentration of allylic-PP initiator (Cornish and Siler 1995; Castillon and Cornish 1999; Cornish et al. 2000). *H. brasiliensis* and *P. argentatum* synthesize a high molecular weight rubber ($M_n = 10^5$ – 10^6 g/mol) with wide molecular weight distribution ($M_w/M_n = 2$ – 10) (Swanson et al. 1979; Cornish et al. 2000; Puskas et al. 2011). However, the amount of rubber and possibly its molecular characteristics depend on the culture method, plant age, climate, and plant growth conditions (Buranov and Elmuradov 2010).

Fig. 5 Molecular weight distribution of *E. macroclada* extracted rubber by GPC

Table 5 Gel permeation chromatography data for rubber extract of *E. macroclada*

Molecular weight	g/mol
M_n	8.180E+2
M_w	1.053E+3
M_z	1.884E+3
$D (M_w/M_n)$	1.287E+0
The average number of molecular weight (M_n), Weighted average molecular weight (M_w), z-average molecular weight (M_z), polydispersity (M_w/M_n)	

In conclusion, the current study concentrated on the identification and characterization of *cis*-1,4 polyisoprene in *E. macroclada*, an herbaceous and drought resistant plant, by FT-IR, NMR and GPC analysis. Further study on rubber particles and rubber structure of this plant will provide more understanding of its latex composition.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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