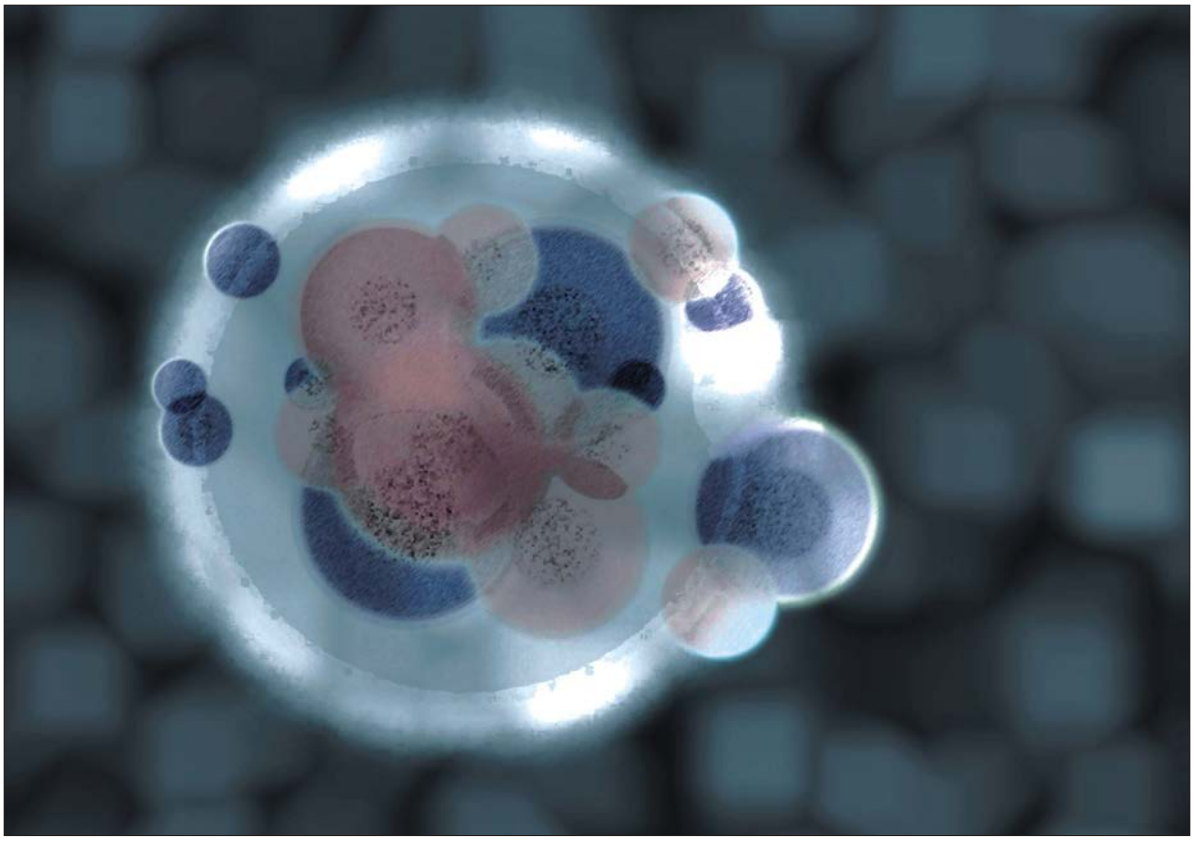

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Research Article
Identification of Low Simmondsin-High Oil Containing Accessions of *Simmondsia chinensis* (Link) Schneider (Jojoba) using HPLC

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Abstract

Background and Objective: Jojoba is an oilseed plant with cosmetics, pharmaceuticals and other industrial applications. Once the oil is extracted, the remaining cake has shown good potential as animal feed supplement but there are certain toxicity issues with the leftover feed cake due to the presence of simmondsin. This study was aimed to develop precise, faster and efficient High Performance Liquid Chromatographic (HPLC) method for the quantification of simmondsin in different accessions of jojoba and to find out best accession with low simmondsin and high oil content among them. **Materials and Methods:** Seed samples were collected from Association of Rajasthan for Jojoba Plantation and Research Project (AJORP), Rajasthan, India. Oil and simmondsin were removed by repeated extraction with water at 90°C for 1.5 h from ground jojoba seeds. Quantitative analysis of simmondsin was done by HPLC method. The separation was achieved by reverse phase C18 column with a mobile phase consisting of water-methanol (80/20; v/v), at a flow rate of 0.75 mL min^{-1}. The resultant chromatograms were analyzed by CLASS-VP V6.14 SP1 software. The correlation between concentration and peak area was calculated by Excel 2010 software. **Results:** A great diversity was observed between the simmondsin content of jojoba accessions. Oil yield were ranged from 39.0 to 45.0%. The simmondsin concentration varied from 2.6-4.2 g per 100 g seeds. On the basis of above results, the best accession under study was "48-25" which had nearly 44.7% oil content and a low content of 2.61 g simmondsin per 100 g seeds. **Conclusion:** It is concluded that planting accessions with high oil content and low simmondsin will be beneficial for both farmers and the end users of the remaining feed cake.

Key words: HPLC, jojoba oil, simmondsin, *Simmondsia chinensis*, toxicity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Jojoba [*Simmondsia chinensis* (Link) Schneider] is a perennial evergreen shrub belongs to family Simmondsiaceae which is a native to Sonoran Desert of Northern Mexico and South Western USA[1,2]. The commercial importance of the plant lies in the waxy-oil present in its seeds[3]. It is similar to the sperm whale oil in structure and uses[4]. Jojoba oil is easily extractable using simple cold press mechanism and residual cakes, which originate after seeds have been pressed for oil extraction contains about 32% protein and represent a potential amendment for animal feeds[5].

Despite this potential, there are certain toxicity issues with the cake due to the presence of simmondsin [2-(cyanomethylene)-3-hydroxy-4,5-dimethoxycyclohexyl β-D-glucoside] which cause g rowth retardation and food intake reduction[6]. A decreased appetite is observed, when monogastric animals are fed with food containing simmondsin and when they are given higher doses for a long time they die of starvation[7]. Simmondsin and simmondsin 2-ferulate have been identified as most responsible for food intake inhibition[8] and can thus serve as a dietary modifier[9], for this reason, these compounds are considered as anti-nutritional components.

It is assessed that the global marketable potential of animal feeding industry is more than 400 billion dollars annually but the major reason for problems related to animal feed and food supplement industries are the toxins present in jojoba residual cakes and are not being used in these industries. Therefore, quantity of simmondsin level is important to jojoba processors, researchers conducting animal nutrition studies, plant breeders and feed industries which requires for an efficient and a quick method for its quantitative analysis. Literature survey revealed that numerous methods have been reported for the estimation of simmondsin[7-9]. Present study involves development of HPLC method using simple mobile phase which is sensitive and rapid for the quantificationof different accessions of jojoba. However, HPLC method has been used for the quantitative analysis of simmondsin using adenosine as an internal standard[10] but the variation in jojoba accessions for oil and simmondsin content has not been reported to be used as a method for resource management.

Industries like cosmetics, pharmaceuticals, lubricants and petrochemicals have high requirement for jojoba oil. More than 300 jojoba related products are available in the market at present which include products by industries like cosmetics, pharmaceuticals and petroleum etc[11,12]. It is considered as one of the top most cosmetic lipid material because of its excellent inherent moisturizing, oxidative stability and balsamic properties. It is because of this reason that most of jojoba seeds are used by high-priced cosmetic industry. Penetration of jojoba oil into these much larger markets is expected to increase with increase in production but the shortage of high-yielding plant varieties are still an issue. Therefore, there is a serious need to develop a crop improvement program by creating a rich genetic resource base with desired traits. This will help researchers not only to identify the superior accessions but also in assessing and exploiting genetic variability of plants. This will also provide indicative guideline for collection of samples for a particular end use on the basis of predominance of particular chemical constituents.

The objective of this study was to develop a simple and fast method for the quantification of simmondsin content in different accessions of jojoba and to identify the best accession with desired traits among them.

MATERIALS AND METHODS

Plant material: Seed samples of eighteen different jojoba accessions were collected in the month of May, 2014 from AJORP (Association of Rajasthan for Jojoba Plantation and Research Project), Rajasthan, India (Table 1). These jojoba accessions having ancestral origin from Israel and USA and their seeds were collected and used to grow in different parts of India like Jaipur, Bhavnagar and Jodhpur. Further from these places they are collected and maintain at jojoba farm AJORP, Jaipur Rajasthan, India. The sample seeds were stored in zip locked polybags, weighed and milled for oil extraction.

Jojoba oil extraction: Jojoba seeds (100 g) were grounded using mortar and pestle and powder was magnetically stirred for 1.5 h in 500 mL distilled water at 90°C in a round bottom flask provided with reflux condenser[10]. After filtering the mixture, the solution was allowed to standin order to allow the oil to float above the water and oil was separated via simple pipetting and weighed. All oil samples collected were stored in sealed glass vials at 4°C till further analysis.

Simmondsin extraction: The filtrate from previous step was dried in an oven at 35°C and then residue was mixed with 30 mL methanol. In order to eliminate insoluble products, the solvent was evaporated and the obtained brown residue (simmondsin) was weighed. This was weighed and further analyzed and quantified by HPLC using standard solution of simmondsin.

Table 1: Different accessions of *S. chinensis* (Jojoba) selected for phytochemical profiling

Accession names	No. of plants selected per accession	Origin sites	Geographical coordinates
48-25	6	Israel	31.0461° N, 34.8516° E
CsMcRI	8	USA	37.0902° N, 95.7129° W
96	8	Israel	31.0461° N, 34.8516° E
32	6	Israel	31.0461° N, 34.8516° E
85	5	Israel	37.0902° N, 95.7129° W
47-21	8	Israel	37.0902° N, 95.7129° W
Q106	5	Israel	31.0461° N, 34.8516° E
CAZRI	6	USA	37.0902° N, 95.7129° W
24-8	8	USA	37.0902° N, 95.7129° W
E21-14	7	USA	37.0902° N, 95.7129° W
82-18	6	Israel	31.0461° N, 34.8516° E
Local	6	USA	37.0902° N, 95.7129° W
879-154	5	Israel	31.0461° N, 34.8516° E
Clone 64	7	Israel	31.0461° N, 34.8516° E
Q104	9	Israel	31.0461° N, 34.8516° E
58-5	5	Israel	31.0461° N, 34.8516° E
17-22	6	Israel	37.0902° N, 95.7129° W
K-11	7	Israel	31.0461° N, 34.8516° E

High Performance Liquid Chromatography (HPLC): Quantitative determination of simmondsin was done using reverse phase HPLC (Shimadzu-LC10A system-Japan) equipped with SCL10AVP analog pump, an injection valve (Hamilton syringe with 20 µL loop) and SPD10 AV detector. The conditions were optimized for separating out simmondsin from methanolic extract of different accessions of jojoba through HPLC. Briefly, the methanolic extract of simmondsin was filtered using millipore filter (0.2 µm) and HPLC was performed on reverse phase C18 column. A maximum pressure of 200 kgf cm^{-2} and a minimum of 0 kgf cm^{-2} were maintained. A mixture of water-methanol of HPLC grade (80/20; v/v) was used as a mobile phase. During the run, a flow rate of 0.75 mL min^{-1} was maintained. The eluents were monitored at 217 nm. The injection volumes of samples and standard were 20 µL.

The identification of chromatographic peaks was performed by comparison of the Retention Time (RT) to that of known simmondsin standard. Standard was accurately weighed (5 mg mL^{-1}) and dissolved in HPLC grade methanol as stock solution. Working standard solutions of 0.6, 0.8 and 1.0 mg mL^{-1} were prepared from the above stock solution. Establishment of linearity was done by triplicate injections of simmondsin standard.

Statistical analysis: The chromatograms were analyzed by Shimadzu CLASS-VP V6.14 SP1software. The peaks in chromatogram revealed the quantity of simmondsin based on their retention time and area. The correlation between concentration and peak area was calculated by Microsoft Office Excel 2010 software.

RESULTS AND DISCUSSION

In this study, high performance liquid chromatography has been applied for the simultaneous estimation of simmondsin in different accessions of jojoba. Optimization of mobile phase was performed based on asymmetric factor and peak area obtained. Different mobile phases were tried but satisfactory separation, well resolved and good symmetric peaks were obtained with the mobile phase water-methanol (80/20; v/v). Tasioula-Margari and Tsabolatidou[13] also reported methanol/water 80:20 (v/v) as an efficient extraction solvent. The retention time of simmondsin was found to be 2.85 min, which indicates a good baseline as shown in Fig. 1. The calibration curve for simmondsin was obtained by plotting the peak area versus the concentration of simmondsin over the range of 0.6-1 mg mL^{-1} and correlation coefficient (R^2) was found to be 0.99 (Fig. 2). In the related study, Medina *et al.*[14] used various ratios of isopropanol-water to extract tannins and simmondsin from defatted jojoba meal. A ratio of 7:3 was found to be optimal for extraction of simmondsin and removal of tannins. Verbiscar *et al.*[15] used isopropanol, acetone, water and dicholoromethane-methanol in the ratio of 85:15 for the extraction of simmondsin and simmondsin ferulates and reported that only water and methanol extracted simmondsin and simmondsin ferulates completely. Similarly, Abd El-Rahman *et al.*[16] have considered solvent extraction to remove more than 80% simmondsin and more than 25 and 50% of phytic acid and total phenolic content respectively, with water-isopropanol in a ratio of 3:7. For quantitative extraction of simmondsin from jojoba meal, 80:20 methanol-water was found to work best in a column extraction method,also reported by Van Boven *et al.*[17]. A number of methods including solvent extraction, chemical

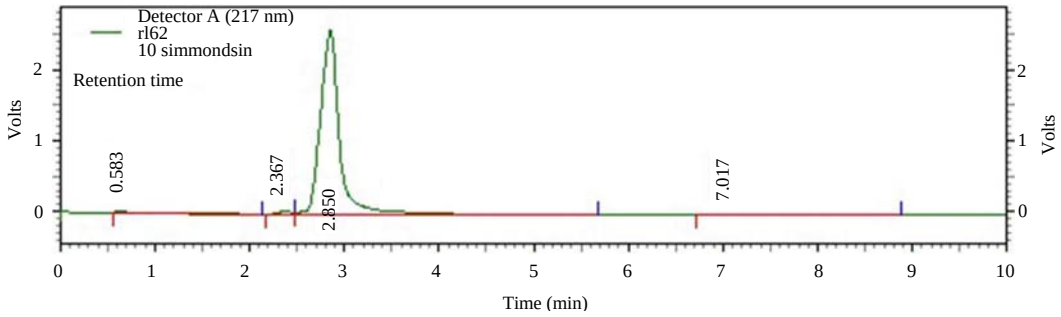

Fig. 1: HPLC chromatogram of simmondsin standard (1 mg mL^{-1})

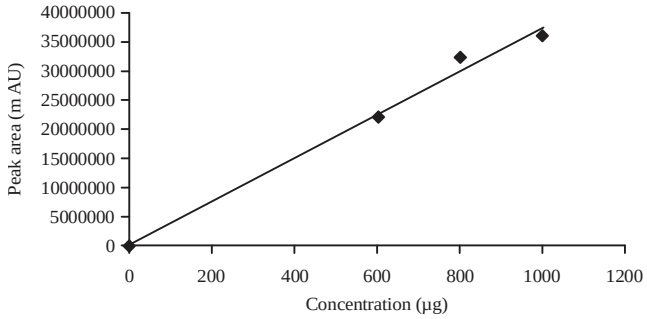

Fig. 2: Calibration curve (Linearity of simmondsin)

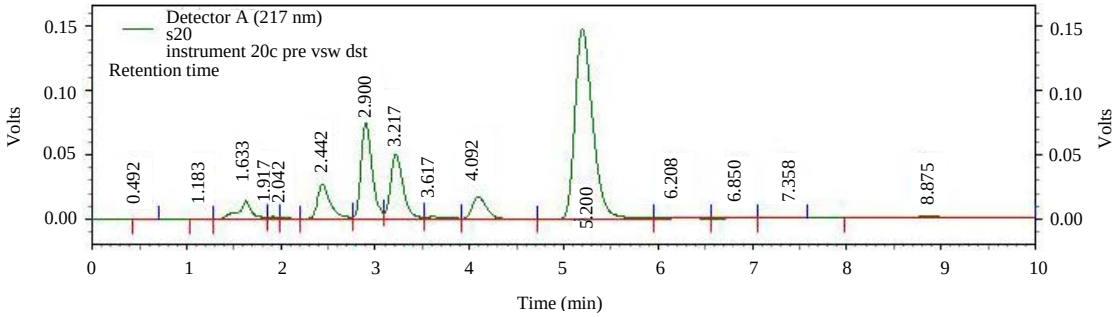

Fig.3: HPLC chromatogram of simmondsin in jojoba accession 48-25

treatment and microbial fermentation have been reported for the extraction and quantification of simmondsin[7,8,18] but the proposed HPLC method described in this study is rapid, accurate, easy to use and can be utilized in daily laboratory settings because of its short chromatographic run time of 10 min.

Seed planted populations are highly variable in their oil yield and other agronomic traits. Therefore, selection program that help us to find out the best accession are highly desirable to meet the needs of the emerging jojoba industry. Oil yield were ranged from 39.0-45.0% which was lesser if compare to the findings of Sanchez *et al.*[5] and Ranzato *et al.*[19] i.e., 50%. The simmondsin concentration varied from 2.6-4.2 g per 100 g seeds which was comparable to 3.3 and 4.5 g, reported by El-Damrawy *et al.*[20] and Bellirou *et al.*[10], respectively. After analyzing the literature and according to the results obtained in the present work, the variation in such contents among the different accessions is due to the climatic conditions, genetic antecedents of the plants and also to agro management in which they grow[8,12]. Out of the 18 accessions studied, six of them (viz. 879-154, CLONE 64, Q104, 48-25, Q106 and 58-5) produced more than 44.5% oil (Table 2). With reference to simmondsin concentration, accessions 879-154, Clone 64, Q104, Q106 and 58-5 contained more than 3 g simmondsin per 100 g seeds. The best accession was "48-25" which had nearly 44.7% oil content and a low content of 2.61 g simmondsin per 100 g seeds (Fig. 3). Planting accessions with high oil content and low simmondsin is one if the best way to

Table 2: Simmondsin and oil content in different accessions of *S. chinensis* (Jojoba)

Accessions	Retention time (min)	Peak area (mAU)	Simmondsin (g/100 g seeds)	Oil content (%)
48-25	2.90	1210110	2.610	44.7
CSMCRI	2.90	1238632	2.730	40.0
96	2.86	1232310	2.750	40.0
32	2.80	1251103	2.800	39.0
85	2.89	1268922	2.830	44.0
47-21	2.80	1312732	2.880	42.0
Q106	2.87	1327644	2.900	44.6
CAZRI	2.90	1328922	2.905	40.2
24-8	2.90	1328922	2.905	40.0
E21-14	2.82	1406343	3.110	40.2
82-18	2.80	1558279	3.520	44.2
LOCAL	2.76	1599394	3.630	40.7
879-154	2.89	1618529	3.682	44.8
CLONE 64	2.75	1690726	3.876	45.0
Q104	2.88	1802516	4.160	44.6
58-5	2.89	1796963	4.170	44.5
17-22	2.89	1838633	4.273	43.7
K-11	2.89	1838633	4.273	44.0

take advantage of feed cakes. With the availability of accession having low simmondsin content like "48-25" found in the study, planting it will be beneficial to both farmers and the end users of the remaining feed cake. These residual cakes can be used as a raw material for animal feed and also decrease the negative impact on the environment. Also, residual dry cake could be used as a fertilizer and natural soil improver as it contains 25-30% protein with nitrogen and fibres. Further, it will be useful in formulating effective breeding decisions and could be important for cosmetics, pharmaceutical and lubricant industries.

CONCLUSION

The proposed HPLC method allows the quick quantification of simmondsin in different accessions of jojoba. A great diversity was observed in the simmondsin content of jojoba accessions. The best accession under study was "48-25" with high oil and low simmondsin content and could be used as ingredient protein for animal feed and as biomass for biogas production. Further, this accession could be used for the genetic breeding program. In this respect, jojoba accessions should be selected according to its physico-chemical characteristics in order to optimize the production process and helpful in lowering down the cost of commercial jojoba products.

SIGNIFICANCE STATEMENTS

This study discovers the best accession with high oil and low simmondsin content that can be beneficial for both animal feed and food supplement industries. This study will help the researcher to uncover the critical area of detoxification and simultaneous estimation of simmondsin that many researchers were not able to explore. Therefore, the proposed methodology represents a simple and reliable approach for the estimation of simmondsin and identification of superior accessions which will be useful in formulating effective breeding decisions.

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