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Original article

The sarsaparilla market in the state of São Paulo (Brazil) and the challenges of cultivation

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ABSTRACT

This study aimed to present information about the sarsaparilla sold in establishments in the state of São Paulo, Brazil, assess the genetic diversity of *Smilax brasiliensis* Spreng., Smilacaceae, and examine the growing conditions and productivity of five species of *Smilax*. The amount of sarsaparilla sold per month at most pharmacies was 0.4 kg on average. Herbal stores and markets sold averages of 9 kg and 8 kg per month, respectively. The weight of the underground biomass of *S. fluminensis* (188.3 g) is significantly higher than those of other species (28.3-79.6 g). The study demonstrated that high genetic diversity among the *Smilax brasiliensis* plants belonging to the CPQBA germplasm bank, which was confirmed by the results of the genotyping study that used a SSR marker on *S. brasiliensis*. The high consumption of sarsaparilla and the low yield of young plants cultivated from seeds with high genetic variability reinforce the need for further studies on the production of *Smilax* species.

Introduction

According to the current Brazilian National Policy on Medicinal Plants and Herbal Medicines, investment in research and development should be made to meet the demand for raw materials for the production of herbal medicines in a safe and controlled manner. One problem faced by the Brazilian herbal medicine industry is the lack of qualified professionals to meet this demand; therefore, collaboration between pharmaceutical companies and universities is essential (Calixto, 2003; Marques and Petrovick, 2010).

Approximately 25% of therapeutic medicines are of plant origin, and 50% are synthetic medicines derived from constituents isolated from medicinal plants (Oliveira and Braga, 2003; Sousa et al., 2008). The increased use of medicinal plants in recent decades can be explained by three main factors: a) Growing consumer acceptance of plant-based medicines due to the feeling, not always justified, that natural products are safer, healthier, and more environmentally friendly than synthetic products. b) The pharmaceutical industry's renewed interest in finding natural compounds with pharmacological activity due to increased consumer acceptance and the lower research

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and development costs associated with plant-derived products. c) Validation from scientific research on commonly used plants that have been incorporated into the pharmacopoeia, creating the legal conditions necessary for them to be officially turned into medicines by pharmacists and to be prescribed by physicians (Montanari, 2002).

The Smilacaceae family is distributed in temperate and tropical regions of the world, it consists of only the *Smilax* genus (310 species) and in Brazil there are 33 species distributed in several ecosystems (Judd et al., 2008; Andreata, 2014). In Brazil, medicinal use of *Smilax* species popularly known as "sarsaparilla" dates back to the 16th century (Medeiros et al., 2007) for their anti-rheumatic, anti-syphilitic (Lorenzi, 2002), and diuretic (Schenkel et al., 2010) properties. However, there is no information on the commercialization and cultivation of these plants in Brazil.

Therefore, this study aimed to present the quantity, value, method of preparation, use, and source of the sarsaparilla sold in some establishments in the state of São Paulo, Brazil. We also aimed to characterize the genetic diversity of *Smilax brasiliensis* and to verify the yield of five species of *Smilax (S. brasiliensis* Spreng., *S. campestris* Griseb., *S. cissoids* Mart. ex Griseb., *S. fluminensis* Steud., and *S. rufescens* Griseb.) after two years of culture.

Materials and methods

Interviews in commercial establishments

From December 2010 through May 2011, commercial samples of sarsaparilla were obtained, in sealed packages or in bulk, from establishments that sell medicinal plants (including compounding pharmacies, herbal stores, supermarkets, and open markets) in nine medium-to-large sized cities throughout the state of São Paulo, Brazil: Araraquara, Campinas, Piracicaba, Presidente Prudente, Registro, Ribeirão Preto, São José dos Campos, São Paulo and Sorocaba.

The commercial samples of sarsaparilla were acquired from at least three different establishments in each city. A total of 44 establishments were visited, including 24 herbal stores, ten compounding pharmacies, and ten open markets. In eight of the 44 establishments visited, no information could be obtained on the source of the samples; therefore, 36 samples were identified as NSO (Number of Sarsaparilla Origination).

Different establishments sold sarsaparilla from the same source. In total, we identified twelve different wholesalers (Fig. 1), whose identities were kept private so as not to harm the interviewees and the establishments visited.

The person in charge of each commercial establishment was questioned about the use of plants, commercial value and source of the samples.

The packaging of the samples purchased in the pharmacies was labelled with the name, batch, production date, expiration date, plant part used, and in some cases, botanical identification, such as "*Smilax officinalis*" or simply "*Smilax* sp".

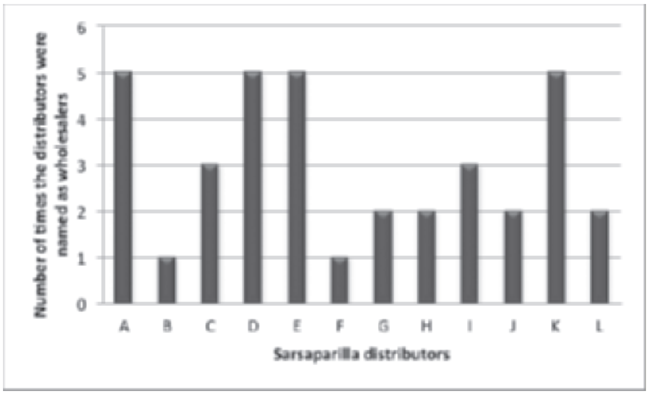

Figure 1 - Quantitative representation of the twelve sarsaparilla distributors (A-L) regarding the presence of the company's name on the packaging or the number of times the distributors were named as wholesalers by the commercial establishments visited in the state of São Paulo, Brazil.

Experiment on in-field yield

Seedlings were obtained from the seed germination of *Smilax brasiliensis* Spreng., *S. campestris* Griseb., *S. cissoids* Mart. ex Griseb., *S. fluminensis* Steud. and *S. rufescens* Griseb. in previous experiments carried out by our research group (Martins, 2009; Soares et al., 2011; Martins et al., 2012).

When the seedlings reached approximately 15 cm, they were transplanted to the experimental field of the Multidisciplinary Centre for Chemical, Biological and Agricultural Research (CPQBA), University of Campinas, in November 2009. The experiment design was of randomised blocks with six replications and five plants per plot, with a spacing of 2.5 m × 1.0 m. The plants were watered as needed, and manual weeding was performed to avoid interspecific competition or any allelopathic effect. The plants were grown on a trellis and staked as they are a climbing species. The plots were harvested in April 2012 at the end of the rainy season. The plants were subjected to just over two years of cultivation, and the dried masses of the aboveground and underground parts of the plant were measured. The underground parts and leaves were dried at 40°C in a convection gas oven to a constant weight. The results were subjected to analysis of variance (ANOVA), and treatment means were compared using Tukey's test at a 5% probability level.

DNA extraction

Genomic DNA from the fresh leaves of thirty *Smilax brasiliensis* specimens belonging to the CPQBA germplasm bank was extracted using the CTAB (cetyltrimethylammonium bromide) protocol described by Doyle and Doyle (1990) with modifications according to Martins et al. (2013). The samples were subsequently stored at -20°C.

The thirty *Smilax brasiliensis* specimens provided by the CPQBA germplasm bank were obtained at previous germination experiments (Martins et al., 2012) based on fruits, sampled randomly from individuals of different plant populations in the field, ensuring the necessary genetic variability (Martins et al., 2012). The *Smilax* genus is dioecious preventing autogamy and increasing genetic variability.

Microsatellite loci amplification conditions

For the present study, 26 microsatellite markers developed for the species *S. brasiliensis* were tested, and seventeen markers were selected for genotyping (Martins et al., 2013).

The amplification products were separated under denaturing conditions on 5% (v/v) polyacrylamide gel containing 8 M urea and 1X TBE (0.045 M Tris-borate and 0.001 M EDTA) and were analysed in an automated sequencer (NEN 4300S DNA Analyser-LI-COR Corporate) for approximately 2.5 h at 70 W. According to the sequencer operation, the signals emitted after the incidence of the laser beam on the different fluorochromes present in the primers were captured and converted into an electropherogram. Using SAGA-specific computer software, the loci were genotyped for further analyses.

Microsatellites

The genetic distance between accession pairs and the dendrogram were measured using the Rogers-W distance, and grouping was performed according to the UPGMA (unweighted pair-group method with arithmetic averaging) criterion using the TFPGA (tools for population genetic analysis) software (Miller, 1997).

Results and discussion

Interviews in commercial establishments

The samples sold in the cities of the inner region of São Paulo state, Brazil, came from twelve large herbal stores (Fig. 1), mainly located in the state capital. The managers of these herbal stores claimed that the material did not originate from cultivation and refused to disclose any further information about its origin.

Knowing the source of an herbal medicine is very important for product quality and safety assurance. The exploitation of the native flora through extractivism has led to drastic reductions in endemic populations because of predatory exploitation and a lack of knowledge of preservation mechanisms (Souza et al., 2012). Thus, the exploitation of medicinal plants can jeopardise future supplies and by mixing them with different plant species result in adulteration of the derived products (Lourenzani et al., 2004).

According to the literature, sarsaparilla is indicated for the treatment of inflammation (Xu et al., 2005; Shu et al., 2006) and rheumatism (Adams et al., 2009). Additionally, it is used as an antioxidant (Rugna et al., 2003) and a diuretic (Cáceres et al., 1987). The establishments that we visited confirmed these uses; the most frequently cited use was as a blood cleanser, followed by its use as anti-rheumatic and diuretic. In addition to these indications, the interviewees added uses as inductor of sweating, treatment for syphilis, cholesterol reductor, and as treatment for kidney stones, arthritis, acne, uric acid, gout, and poor circulation.

Medeiros et al. (2007) surveyed the indications for sarsaparilla treatments proposed by apothecaries in São Bento Monastery. They reported essentially the same therapeutic applications, as depurative, diuretic, emollient, expectorant, anti-leprosy, and sweat inducing. According to the authors, the Portuguese also used these plants for the treatment of venereal diseases, such as syphilis.

Pereira (2006) conducted another survey on the use and knowledge of medicinal plants in the Nova América district (Itápolis-SP, Brazil). Of the plants used by the local community, sarsaparilla was categorised by the researchers as a plant with therapeutic properties for diseases associated with the circulatory system. Sarsaparilla was also used as a blood cleanser by the interviewees in this community.

Infusion (9%) and decoction (73%) were the most cited methods of preparing sarsaparilla in the interviews. According to Pereira (2006), these are the most commonly used methods because they contribute to hydration, the elimination of toxins, and body temperature control.

In the apothecary shop of the São Bento Monastery, decoction was described as the most traditional method for preparing sarsaparilla tea. In some recipes for the decoction of sarsaparilla, other plants, such as shaved guaiac, shaved sassafras, liquorice (*Glycyrrhiza glabra* L.), and cut flax-leaved daphne (*Daphne gnidium* L.), were included during the decoction process (Medeiros et al., 2007).

According to Arnous et al. (2005), who interviewed 376 people from the city of Dantas (Minas Gerais-MG) on the use of medicinal plants, 75.2% of interviewees reported that the most popular method of preparation was decoction. According to the authors, this finding should be considered with caution as only roots, stems, and bark should be boiled because these parts have rigid structures. The leaves and flowers, which are less fibrous, should not be boiled.

Rodrigues & Carvalho (2001) interviewed *'raizeiros'* [herbal healers] from rural communities in southern Minas Gerais, Brazil, and found that the dosage of the medicinal plant must be precise to cure diseases. Excessive doses can cause intoxication or damage. In addition, the duration of use of medicinal plants should not be prolonged. In the present study the amount of sarsaparilla indicated by commercial establishments (Fig. 2) showed that there is no standardization of dosage. Although most ethnobotanical surveys do not report the amount of plant that should be used for tea preparation, these data should be considered for safety.

The average price for 100 g of commercial sarsaparilla was U$ 6.00 in pharmacies and U$ 3.00 in herbal stores or markets. According to the herbal healers interviewed in the study by Rodrigues & Carvalho (2001), the main factor influencing the high demand for medicinal plants is the high price of certain synthetic medicines. However, we found that the commercial cost of sarsaparilla is high and actually exceeds the cost of synthetic medicines, including widely commercialised anti-inflammatory drugs that can be purchased for approximately U$ 5.00. Other factors cited by Rodrigues & Carvalho (2001) that result in a high demand for sarsaparilla include wellness and faster healing by using both synthetic and herbal medicines and the side effects caused by synthetic medicines. The amount of sarsaparilla sold per month at most pharmacies was 0.4 kg on average. Herbal stores and markets sold averages of 9 kg and 8 kg per month, respectively. The amount of commercialized material is high considering the impact caused by extractivism, since *Smilax* species are not cultivated in Brazil and they have been used in folk medicine since the sixteenth century (Medeiros et al., 2007).

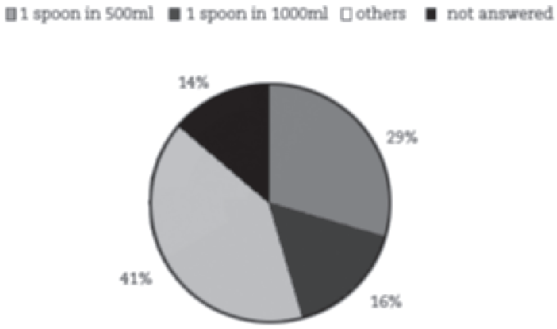

Figure 2 - Representation of the amount of sarsaparilla indicated for tea preparation.

Experiment on in-field yield

The dry masses of the aboveground and underground parts of the plant are shown in Tables 1 and 2. The analysis of variance by the F-test showed different yields for the compared species, both for the aboveground and the underground plant parts.

The mass of the underground dry matter of *Smilax fluminensis* was significantly higher than those of other species with similar masses. *S. brasiliensis* and *S. cissoides* had the highest aboveground dry masses.

The dry weight of the underground organs of sarsaparilla varied between species from 28.3 to 188.3 g (Table 2). Each commercialized package of sarsaparilla contains between 30 and 50 g of dried roots and underground stems. Therefore, it can be inferred that approximately one to two native Brazilian plants are harvested for each package suggesting that the level of extractivism is very high. This degree of extractivism may hinder the survival and conservation of these species in the Brazilian flora.

Soares et al. (2011) reported that the capacity of *Smilax fluminensis* to propagate from seeds and cuttings confirms its potential for sustainable economic use. This propagation would be an alternative to reduce the predatory extractivism of this native species. However, Martins et al. (2011; 2012) showed that, in contrast to *S. fluminensis*, the germination rates of other Brazilian species of *Smilax* are low. According to the authors, the germination of the genus *Smilax* is complex; the seeds require a long time to germinate, and seedling development is slow. They also reported that there are no studies on large-scale cultivation of this genus in Brazil and the product marketed as sarsaparilla originates from extractivism.

According to the merchants interviewed in our study, the demand for sarsaparilla increases if there is any mention of its use or importance in televised reports or advertisements. This finding was corroborated by Souza et al. (2012), who described the media as the primary vehicle for information on medicinal plants. However, this information is incomplete when it reaches the consumer, resulting in misunderstandings about the medicinal use of these plants.

In open markets and herbal stores, the packaging was frequently inadequate, without an expiration date or botanical identification. According to Bello et al. (2002), the standardisation of information on labels and information leaflets is needed, as the absence of this information may endanger the patient's health.

Although there is specific legislation for herbal medicine in Brazil, it was observed that many products on the market do not conform to the law and manufacturers consider therapeutic products of herbal origin to be "food" (Amaral et al., 2007).

After labelling verification, the sarsaparilla packages were opened and checked for the organic matter. It was observed that all samples contained large amounts of foreign organic matter, such as unidentified fragments of stems and leaves of the same plant or even parts of other plants. A similar study was conducted by Leite and Biavatti (1996) and showed that 39.47% of herbal medicines were contaminated with excessive foreign organic matter. This organic matter changed the total active ingredient content of the sample. According to the authors, this type of contamination may indicate fraud, negligence or ignorance by producers and gatherers.

Ocampo et al. (2007) reported that, under management conditions, the underground parts of *Smilax domingensis* in Guatemala can be harvested in 4 to 5 years. The average yield (fresh weight) of 8-9-year-old plants is 28 kg. These data may explain the low dry mass obtained from plants grown in the field for only 2 years.

Table 1
Summary of the analysis of variance, means, and experimental coefficients of variation of the aboveground and underground parts of five *Smilax* species. Campinas-SP, Brazil.

Variation factors	Degree of freedom	Mean square	
		Underground part	Aboveground part
		g/plant	g/plant
Block	5	2,373.2	1,062.5
Species	4	7,420.4[a]	184,564.8[b]
Residue	20	2,195.3	5,814.7
Mean		52.7	217.6
Coefficient of variation		88.81	35.0

[a] Significant at the 5% level using the F-test.
[b] Significant at the 1% level using the F-test.

Table 2
Mean dry masses (g/plant) of the aboveground and underground parts of *Smilax* species. Campinas-SP, Brazil. Means followed by different letters in the same row indicate significance at 5% using Tukey's test.

Plant part	Species				
	S. brasiliensis	*S. campestris*	*S. fluminensis*	*S. rufescens*	*S. cissoides*
Underground	59.7b	59.7b	188.3a	28.3b	79.6b
Aboveground	425.4a	122.4b	124.2b	21.0b	394.6a

Microsatellites

A higher than desirable coefficient of variation for a cultivation experiment can be attributed to high genetic diversity among plants obtained by sexual propagation and produced from seeds collected under natural conditions. Diversity was confirmed by the results of the genotyping study, which used a simple sequence repeat (SSR) marker in *Smilax brasiliensis*. The genetic variability of the germplasm bank of S. *brasiliensis* can be observed in the dendrogram obtained by the UPGMA grouping method using Roger's genetic distance matrix, modified by Wright (Fig. 3). The germplasm bank displayed wide diversity, with 1 and 2 being the closest individuals. Analysing the dendrogram, it is possible to visualise two groups at a distance of 0.90. These groups included individuals 17 and 30 (group I), the most distant from the others, and the remainder of the individuals (group II). Group II displayed a large genetic variation according to microsatellite markers. Numerous divisions were observed in group II; however, genotypes 3, 6, 15, and 27 were differentiated from the others at a distance of 0.70.

Groups showed in the dendrogram are important to characterize the genetic diversity of one of most important sarsaparilla species. These approaches will lead to a better understanding of genetic variation on this medicinal plant and to the management support of germplasm collections.

Some studies have indicated the need to establish germplasm banks of medicinal species to preserve their genetic diversity, especially those that, similar to sarsaparilla, are subject to high extractivism in Brazil. Skorupa and Assis (1998) reported that *Psychotria ipecacuanha*, Rubiaceae, has been extensively exploited throughout Brazil for decades due to the medicinal properties attributed to its roots. Biondo et al. (2007) studied the medicinal plant *Mandevilla velutina*, Apocynaceae, native of the Brazilian 'Cerrado' (tropical savannah) and has anti-ophidian properties.

Our study demonstrated the high genetic diversity among *Smilax brasiliensis* plants belonging to the CPQBA germplasm bank. Genetic diversity facilitates plant breeding, and knowledge of germplasm diversity exerts a significant impact on the cultivated plants improvement (Huang et al., 2002). According to Belaj et al. (2002), knowledge of genetic diversity among olive tree (*Olea europaea*) cultivars through random amplified polymorphic DNA (RAPD) techniques maximises the long-term use of genetic resources in breeding programs, given the genetic variability among the cultivars. In addition to RAPD techniques, microsatellite markers can also be used to detect genetic diversity, as discussed by Huang et al. (2002), who analysed wheat (*Triticum aestivum*) from germplasm banks.

In conclusion, the high volume of sarsaparilla consumption, the low yield of young plants cultivated from seeds, and the high genetic variability of plants originating from sexual propagation observed in *Smilax brasiliensis* reinforce the need for further studies on the production of *Smilax* species in Brazil.

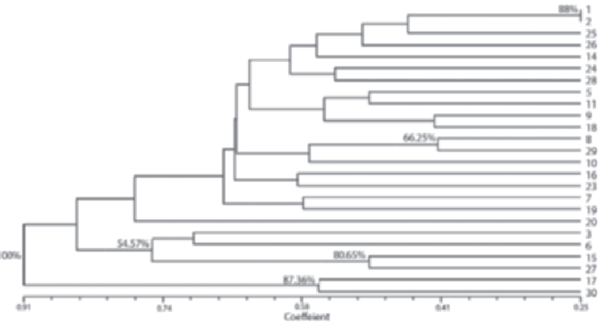

Figure 3 - Pattern of genetic divergence among the *Smilax brasiliensis* population present in the CPQBA germplasm bank in Campinas-SP, Brazil, by UPGMA clustering, based on the genetic identity obtained using Rogers-W genetic distance. The cophenetic correlation was 0.876.

Authors' contributions

MKMS (M.Sc. student) and ARM contributed in collecting commercial sample, running the laboratory work, analysis the data and writing the section related to the information about the commercialized sarsaparilla. IMJr and GMF contributed in experiment in field yield and its data analysis. ARM, MIZ and MMB contributed in genetic analysis. ARM and BAG designed the study, contributed to critical reading and final editing of the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts interest.

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