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Journal of Chemical and Pharmaceutical Research, 2018, 10(1):30-37

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Pharmacognostic Characterization of *Laurus nobilis* L. Leaves

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ABSTRACT

Laurus nobilis L. (Lauraceae) leaves are used on popular medicine as well as food flavoring and in the perfumery. This paper aimed to realize a pharmacognostic characterization L. nobilis leaves from different brands to evaluate authenticity, integrity and purity parameters. Morphoanatomic studies of the vegetable has been done, in the same way as tests for starch and tannin histochemical reactions, impurities determination not inherent in the drug, foam index and intumescence, nitrogen determination, proteins, calcium and phytochemical profile evaluation of its hydroalcoholic extracts. External morphology variations were observed. The base, apex, margin and petiole were predominantly of cuneate, acuminate, entire and curved type, respectively. All the leaves were intact, penninervate, smooth and glabrous, with length of 6.2 to 9.8 cm and width of 2.8 to 3.7 cm. The epidermis showed isodiametrical cells, annular collenchyma, dorsiventral mesophyll composed by one or two layers of palisade parenchyma and three to four rows of spongy parenchyma. It was characteristic in all samples the presence of multiple secretory cells, tannins, starch, nitrogen, calcium and protein. The analyzed samples were in the accepted limits of impurities not inherent to drug. The determined intumescence index showed similar results. But in the determination of the foam index, the results of the analyzed samples were different. Phytochemical screening showed the presence of phenols, coumarins, steroids, triterpenes, flavonoids and saponins. The dried leaves standardization of L. nobilis, which are reported for the first time, can be used to establish the quality assurance of this plant in future investigations and applications.

Keywords: *Laurus nobilis* leaves; Morphoanatomy; Phytochemical; Quality control

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INTRODUCTION

The use of plants and its derivative products for medical purposes represents a practice in increasing expansion at the present time [1]. This practice is based primarily on the popular knowledge, passed from generation to generation, but has been incorporating scientific advances in the search to guarantee security by the use of plant species with proven therapeutic efficacy [2-4]. Although there is growth in the use of medicinal plants there is still a significant lack of research data in this area [5]. Condiments are used worldwide to increase and/or add flavor to food and, secondarily, for conservation purposes, due to its antimicrobial and antioxidant properties. The condiments of the Lamiaceae family, for example, have been widely used in food products such as laurel (*Laurus nobilis* L.) [6].

Laurus nobilis L., commonly known as louro-comum (Portuguese), loureiro-de-presunto (Portuguese), loureiro-de-apolo (Portuguese), sweet-bay (English), yuegui (Chinese), and others, is a tree that can reach 14 meters in height, perennial, smooth-stemmed, with many branches and leaves, yellow inflorescences and dark globular fruits [7-10]. Its leaves are clearly odoriferous, traditionally used as food condiment and essential oil source in the perfumery [11,12]. In popular medicine is used against digestive disorders and flatulence [13], ulcer treatment [14], menstrual cramps and oligomenorrhea [15].

The correct evaluation of the quality of medicinal plants and their compliance with pharmacopeial criteria allows greater safety to the drug identification, quality of their extracts and also provides the exact amount to be incorporated into formulations, according to the concentration of the active substances present. Standardization is considered an essential stage to guarantee the quality of the herbal medicine and, through it, is possible to establish a plant drug standard and reproducibility in several production batches. In this way, this paper aims to perform a pharmacognostic evaluation of *Laurus nobilis* L. leaves from different brands available in the market, in order to evaluate and determine parameters of authenticity, integrity and purity.

MATERIALS AND METHODS

Dried leaves of the species *Laurus nobilis* L. marketed in São Luís-Maranhão (Brazil) were obtained and they were purchased at random from the commercial brands Cheiro Verde (no lot number, no manufacturing data and maturing in August, September and October 2012) and Ricco (no lot number, manufactured on September 1, 2011, with an expiration date of 12 months). There were also obtained single samples commercialized in the Central Market of the city of São Luís-Maranhão (without specifications).

Initially the samples were evaluated for size (length and width), color and smell (odor) from the parameters described by the Brazilian Pharmacopoeia 5th Edition [16]. For the external morphological study of the leaves was observed the phyllotaxis, composition, color, consistency, leaf blade surface, size, shape, apex, base, margin and venation system [17] venation pattern. The microscopic determination was performed by usual techniques of vegetal anatomy, in which the transverse cuts in the dried leaves of the analyzed species were realized in the middle third of the leaf blade for identification and classification of plant tissues, epidermal attachments and cellular inclusions, using fucsin and safranin solutions as coloring agents [16-18]. The material was observed by microscopy (Bio3 Technical, Bel, Monza, Italy) and photographed with a digital camera (Eurekan 5.0, Bel, Monza, Italy).

Histochemical reactions for the determination of starch and tannins, strange material, foam indices and swelling were realized following a methodology adopted by the Brazilian Pharmacopoeia 5th Edition [16]. The total nitrogen determination was performed according to the Kjeldahl method [19] with consequent determination of crude protein by the conversion calculation of nitrogen into protein, in which the percentage of proteins is estimated multiplying the percentage of nitrogen by 100/16 or multiplying the percentage of nitrogen by 6.25. Calcium was determined by titrimetric method of EDTA (complexometry) [20].

In the preparation of the hydroalcoholic extracts of the three samples of *L. nobilis*, the dried leaves were pulverized and subjected to the extraction process by dynamic maceration (under constant agitation), during 24 hours, with ethanol 70%, in the ratio of 1: 6 weight/volume. This procedure was repeated three times and the filtrate from the three extractions was collected, concentrated in a rotavaporator and denominated hydroalcoholic extract (HE). Phytochemical prospecting of HE was realized according to Matos [21] modified, in order to verify the presence of the following secondary metabolites classes: phenols, tannins, resins, coumarins, anthocyanins, anthocyanidins, chalcones, flavones, flavonols, xanthones, flavononones, steroids, triterpenes, saponins and alkaloids.

Results were expressed as mean ± standard error of mean (s.e.m.). The differences were detected by One way analysis of variance (One-way ANOVA) followed by Newman-Keuls post-test using GraphPad Prism Software Inc, version 5.00 (GraphPad Software Inc., San Diego, CA, USA). Differences were considered statistically significant when $p<0.05$.

RESULTS AND DISCUSSION

Regarding the size, samples from Mercado Central and the Cheiro Verde brand presented statistically different lengths of the mark Ricco. And as for width, the Cheiro Verde sample was statistically different from the Ricco brand and the Central Market (Figures 1 and 2).

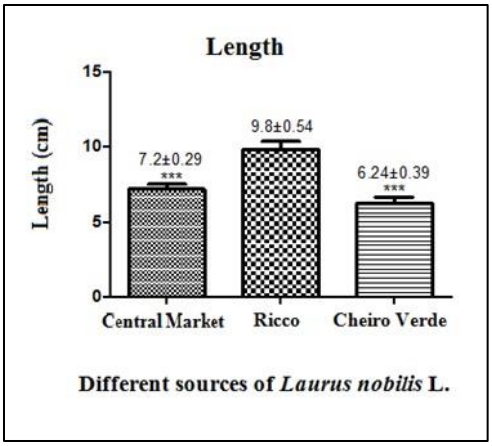

Figure 1: Determination of leaf length of *Laurus nobilis* L. from different brands marketed in São Luís-Maranhão (Brazil). * *P*<0.0002 comparing "Cheiro Verde *vs* Ricco" and "Mercado Central *vs* Ricco", respectively**

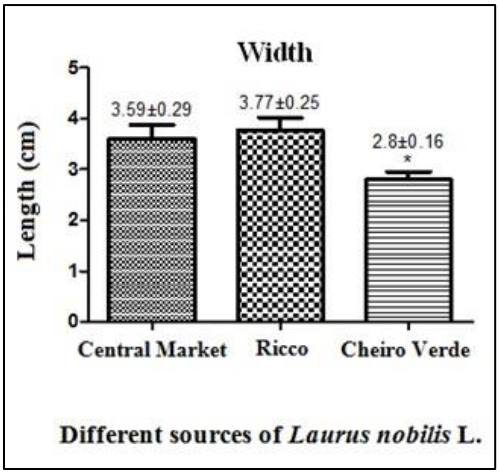

Figure 2: Determination of leaf width of *Laurus nobilis* L. from different brands marketed in São Luís-Maranhão (Brazil). *P< 0,03 comparing "Cheiro Verde *vs* Ricco" and "Cheiro Verde *vs* Mercado Central", respectively

All samples were presented in green-gray colors and coriaceous. It was confirmed that most showed intense and characteristic odor. In the analysis of external morphology, the contour varied from elliptic-lanceolate to obovate-elliptical, oval-lanceolate and obovate-lanceolate. In relation to apex, the acuminate predominated. It also had variation as to the base, being common of the type cuneate. As for the margin, the entire margin prevailed. All samples were intact, penninervate, smooth and glabrous. The petiole was straight and curved to biconvex and concave-convex (Figure 3).

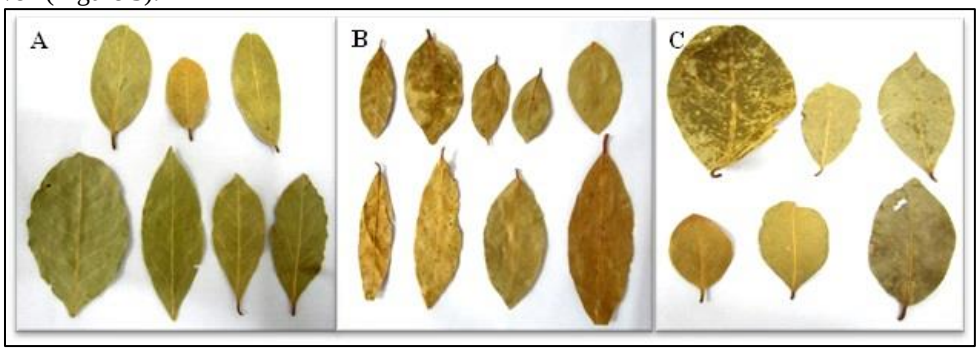

Figure 3: External morphology of *Laurus nobilis* L. leaves from different brands marketed in São Luís-Maranhão (Brazil). A – Cheiro Verde. B - Ricco. C – Mercado Central

There is no record in the literature of a leaf pattern for *L. nobilis* L. ANVISA itself [22] mentions as general characteristics only dry-looking leaves, color green-brown, fragrance and own taste. By the results can be confirmed a difficulty in establishing morphological characteristics due to the observed variability. According to Kaurinovic and collaborators [23], benzene compounds present in percentages between 1% and 12% are responsible for spicy aroma of laurel leaves and are factors of extreme importance in determination of the sensorial quality of the vegetable. The subdivision of the limbus and contour showed similarities to the characteristics reported by Lorenzi and Matos [9] and Di Stasi and Hiruma-Lima [24] who mention being common to the species: simple and lanceolate leaves. The bases of the leaves of Ricco and Cheiro Verde brands were equally consistent with the literature [25] that reported an acute basis for *L. nobilis*. However, the samples presented divergent apices from the literature [25], where they mention obtuse apex.

The epidermis presented isodiametric cells on both sides. The collenchyma is an annular type with three to four layers of cells. It was possible to observe the endoderm and pericyclic fibers forming a sheath around the collateral vascular bundle. And it was confirmed, in cross-section, a biconvex foliar base. In the mesophyll region was verified the presence of one to two layers of palisade parenchyma facing to the adaxial epidermis and three to four rows of spongy parenchyma tissue on the abaxial side. The sample of the Cheiro Verde brand showed moderately thickened cuticle on the abaxial face when compared with the other samples. It was characteristic of all the samples the presence of several secretory cells, large and relatively rounded, close to the epidermis, dispersed in the fundamental parenchyma and in the mesophyll (Figure 4).

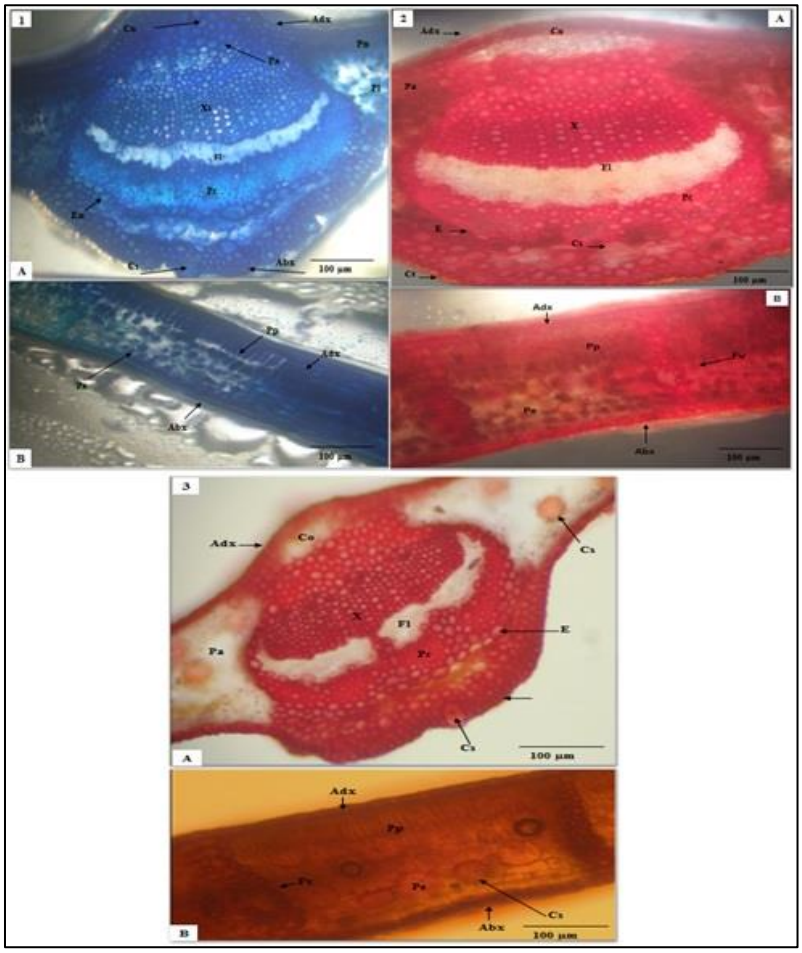

Figure 4: Transverse section of the leaf blade of *Laurus nobilis* L. from the Mercado Central (1), Cheiro Verde (2) e Ricco (3). A – Central rib. B – Mesophyll. Adx = adaxial epidermis. Abx = abaxial epidermis. Co = collenchyma. Pa = parenchyma. Pc = pericyclic fibers. En = endoderm. Xi = xylem. Fl = phloem. Pp = palisade parenchyma. Pe = spongy parenchyma. Cs = secretory cell. Fv = vascular bundle

Representatives from Lauraceae family usually have in common dorsiventral mesophyll, small and medium vascular bundles with sclerenchyma sheath extension, secretory cells containing mucilage or essential oil, sclerenchyma sheath in the central vein, stone cells with U-thickening and small calcium oxalate raphides [26]. In the present

study, many of these aspects have been confirmed, except for the absence of stone and crystal cells in leaves. However, according to Nakata [27] the formation of calcium oxalate crystals is in dependence of the synthesis of oxalate by the vegetable and the absorption of calcium from the external environment. The deficiency of this ion in the environment can lead to disappearance of crystals in plant tissues. In this way, non-observance of these formations in the leaf of the species in question can be attributed to an environmental variation.

Secretory cells in in *L. nobilis* contain essential oil (oil cells). They are generally spherical, with suberized walls and yellowish content [26] and the central vein commonly presents annular collenchyma [28] similar to that observed in this investigation. It was observed the presence of tannins and starch in the three analyzed samples, in greater proportion in the Cheiro Verde and Ricco brands than that of the Mercado Central (Figure 5).

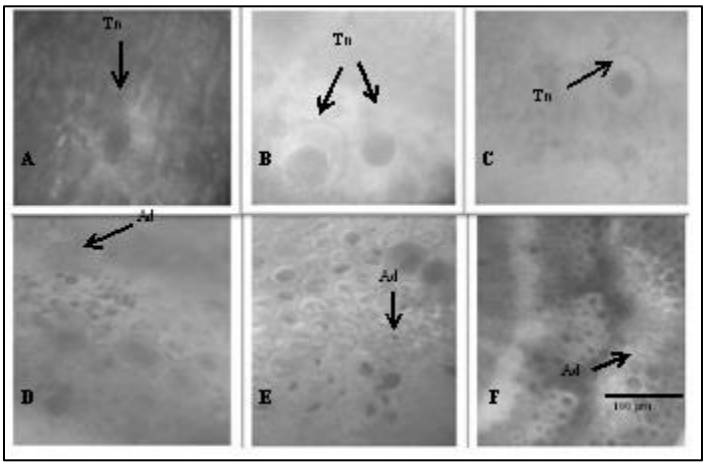

Figure 5: Histochemical test for tannins and starch in *Laurus nobilis* L. leaves from different brands marketed in São Luís-Maranhão. A and D – Mercado Central. B and E – Cheiro Verde. C and F – Ricco. Ad = starch. Tn = tannin

The results of the histochemical reaction for tannins showed a correlation with previous research, which detected the presence of tannins in *Laurus nobilis* L. [29-31]. Vegetables rich in tannins are used on the popular medicine to the combat of bleeding, diarrhea, wounds, burns, stomach problems, kidney problems and other diseases involving inflammatory processes [32]. Related to the presence of starches, no data were found in the literature that indicate the presence of such compounds in the *L. nobilis* leaves. The sample of Ricco brand presented 1.49% of impurities of vegetal nature, mineral or organic, not inherent to the drug, while the Cheiro Verde brand presented 0.26% and the Mercado Central 1.04%. However, the presence of fungi was observed in most of the analyzed samples (Figure 3). The analyzed samples were within the accepted limits of impurities not inherent to the drug, due to the percentage of strange elements not exceeding 2% as recommended by the Brazilian Pharmacopoeia 5th Edition [18].

In relation to the nitrogen, proteins and calcium determinations of the analyzed samples, the sample from Mercado Central presented higher percentage of nitrogen (0.14%) and protein (0.875%), followed by the Ricco brand with percentage of 0.098% and protein of 0.612% and the Cheiro Verde with the lowest values (0.084% and 0.525% for nitrogen and protein, respectively). This, in turn, gained focus in relation to others as to the percentage of calcium with the value of 0.94%, followed by the Mercado Central with 0.92% and Ricco with 0.46% (Table 1).

Table 1: Percentage of nitrogen, proteins and calcium of the *Laurus nobilis* L. leaves from different brands marketed in São Luís-Maranhão

Parameters (%)	Leaves of *Laurus nobilis* L.		
	Ricco	Cheiro verde	Mercado Central
Nitrogen	0,098	0,084	0,14
Proteins	0,612	0,525	0,875
Calcium	0,46	0,94	0,92

Mineral nutrients are commonly known as macronutrients and micronutrients depending on the quantity required by the plant. So, nitrogen and calcium are considered macronutrients, the first being of the structural type and the second of the ionic type. Proteins are basic and vital compounds of vegetables, and others living organisms. They are present in the formation of cell walls, besides participate and catalyze almost all chemical reactions [33].

Vegetables rich in protein and calcium are widely used as functional foods. Although *Laurus nobilis* L. is used in alimentation, no data were found in the literature with relation to the determination of nitrogen concentration, proteins and calcium in this plant species.

Foam index (the rate of foam) determination in the analyzed samples, only the Cheiro Verde brand presented unsatisfactory results. It was verified in the Ricco sample the foam reaching 0.4 cm. In the sample from the Mercado Central, foam of 0.2 was observed (Table 2). The determined intumescence index showed similar results, because the Mercado Central sample presented 0.5 cm of intumescence, the Ricco brand presented 0.5 cm and the Cheiro Verde brand presented 0.6 cm of intumescence (Table 2).

Determination of foam and intumescence indices is useful, respectively, to estimate the amount of saponins in the sample and the extra volume of solvent that must be added in the production of an extract, being important in the technological process of the vegetal drug [34]. However, no data were found in the literature reporting the foam index and intumescence on *L. nobilis* leaves. The hydroalcoholic extract phytochemical prospecting of marketed *L. nobilis* leaves presented poorly positive results for phenols, coumarins, steroids and triterpenes, with the exception for Ricco brand that had moderately positive for steroids and triterpenes. The extracts did not present hydrolyzed and condensed tannins, with the exception of the Mercado Central sample that presented suspect of condensed tannins. Also were not found in the samples: resins, alkaloids, anthocyanins, anthocyanidins, flavones, flavonols, xanthones and chalcones. The Mercado Central and Ricco samples showed only suspect of flavonols, while the Cheiro Verde presented a moderately positive result for this metabolite. No saponins were found in samples from Mercado Central and Ricco, while was observed the weakly positive presence of such metabolite in the Cheiro Verde brand (Table 2).

Table 2: Intumescence index, foam and phytochemical prospecting of the hydroalcoholic extract of *Laurus nobilis* L. leaves from different brands marketed in São Luís-Maranhão. (-) negative; (S) suspicious; (+) weakly positive; (++) moderately positive e (+++) strongly positive [21]

Tests	Leaves of *Laurus nobilis* L.		
	Ricco	Cheiro verde	Mercado Central
Intumescence (cm)	0.5	0.6	0.5
Foam	0.4	0	0.2
(cm)			
Phenols	+	+	+
Hydrolyzed tannins	-	-	-
Condensed tannins	S	-	-
Resins	-	-	-
Coumarins	+	+	+
Alkaloids	-	-	-
Steroids	+	+	++
Triterpenes	+	+	++
Anthocyanins and anthocyanidins	-	-	-
Flavones, flavonols and xanthones	-	-	-
Chalcones	-	-	-
Flavonols	S	++	S
Saponins	-	+	-

The presence of tannins in the three samples analyzed was not observed, except for the suspicion of condensed tannins in the Mercado Central, diverging from that found in other studies [29-31].

The presence or absence of certain groups of secondary metabolites may justify the use of certain herbal drugs in popular medicine, because in general these compounds are associated with certain biological activities. And the compounds classes may serve as standard of identification and quality of the plant species [35].

The alkaloids were divergent in relation to the results obtained and those reported in the literature, in which relatively low concentrations of alkaloids were detected in previous studies [29-31].

Methanolic extracts of leaves, bark and fruit of *L. nobilis* contain flavonoids, sesquiterpenes and phenolic acids [36], which were also observed in this study through phytochemical tests. From the infusion of its leaves it was also possible to isolate glycosylated flavonoids [37]. According to Kim and collaborators [38], *Laurus nobilis* is source of a series of polyphenolic compounds, as well as monoterpenes and sesquiterpenes that are directly linked by their activities against allergic reactions and anti-inflammatory action. No reports were found in the literature about the presence of resins, coumarins, saponins, steroids, anthocyanins and anthocyanidins in *L. nobilis*.

CONCLUSION

The results obtained by the all analyzed parametersof the pharmacognostic features of *Laurus nobilis leaves* are presented here for the first time may help to evaluate the usefulness of these characters in establishing the botanical

identity of the plant can be used as vegetable identification standard, determining the authenticity, integrity and purity of the pharmacogen. The standardization of dried leaves commercialized of *Laurus nobilis* L. also can be used to establish the quality control of this specie and the reproducibility in several production batches where adulterants and substituents can also be detected.

ACKNOWLEDGMENTS

The authors thank to Fundação de Amparo à Pesquisa e ao Desenvolvimento Científico e Tecnológico do Estado de Maranhão (FAPEMA) and Universidade Federal do Maranhão (UFMA) for providing financial support.

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