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PHYSICAL-CHEMICAL CHARACTERIZATION, ANATOMICAL AND SEASONAL EVALUATION OF *THUJA OCCIDENTALIS* L. (CUPRESSACEAE)

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ABSTRACT: *Thuja occidentalis* L. is a plant widely used therapeutically. Having seen the necessity to standardize the vegetal drug and to evaluate the interference of certain environmental and seasonal factors on the synthesis of metabolites, notably flavonoids, this work considered anatomical and physical-chemical characterization assays for the aforesaid vegetal drug, in addition to phytochemical prospecting and evaluation of the flavonoid content for one year. Characterization demonstrated that a thick powder (484 µm), with loss by desiccation (6.9%), water determination (5.3%), ash content (4.5%) and volatile oils (0.7%) to be acceptable. The average content of total polysaccharides obtained for analysis was 233.2 mg/g. The anatomical evaluation pointed to structures such as spongy parenchyma, secretor channels, heteromorphic cells and ciclocitic stomata. Phytochemical prospecting showed the presence of the majority of the metabolites investigated in all the months of study. Alkaloids and coumarins were absent. The verified results for flavonoid content indicate that *T. occidentalis* presents a dynamic metabolism with maximum attainment of flavonoids in march/2012, with a suggestive relationship with the verified low pluviometric index. The characterization data correspond to the first parameters for quality that allow for standardization of the vegetal drug and future phytomedicines obtained from this plant.

INTRODUCTION: *T. occidentalis*, called white cedar or tree of life, possesses medicinal use in diverse homeopathic and phytotherapeutical formulations, such as in the example of the immunostimulant Esberitox®N [1, 2].

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The studies that cite the phytochemical composition of *T. occidentalis* clearly show a series of compounds [1, 3], notably flavonoids, lignans, polysaccharides [1] and essential oils [4]. Among these, flavonoids were identified in an ethanolic portion of *T. occidentalis* aerial parts in numerous pharmacological activity assays related to hepatoprotective [5], antidiabetic [6], antitumor [7], antiulcer [8], antioxidant [9] and hypolipid activity [10].

So, given the chemical and pharmacological importance of this specie's group of metabolites, the determination of the vegetal drug's contents in a study is vital.

Similarly, when faced with the interference that secondary metabolites can undergo in the presence of environmental factors (climatic, time of collection, place of cultivation, etc); it also becomes necessary to evaluate the influence of certain environmental conditions to maximize the active constituent production [11] and to obtain greater quality of the vegetal raw material [12].

Associated with these factors, in the face of the National Policy for Medicinal Plants and Phytotherapies in Brazil and the required regulations, which establishes the adherence to rigorous botanical, microbiological and chemical quality criteria, the importance of quality control and standardization of vegetal products becomes clear. Faced with these aspects, this work intends to perform physical-chemical and anatomical assays of the vegetal drug *T. occidentalis*, as well as evaluate the influence of seasonality and certain environmental factors on the specie's main metabolites, including the flavonoids.

MATERIALS AND METHODS:

Plant material: The plant material was cultivated in Cabo de Santo Agostinho city, Pernambuco-Brazil (8º29' 86, 07"' 45, 29" S and 35º06 W), near Barra de Pirapama. Identification was carried out by researcher and curator Dr. Rita de Cássia Araújo Pereira and the exsiccata deposited in the Agronomic Institute of Pernambuco (IPA) Herbarium, under nº 87.752. Of the material collected, aerial parts (leaves and branches), at equal stages of development and in the absence of depredation, were selected. The fresh vegetal material was washed with purified water, stabilized with 70% ethanol (v/v) and exposed for drying in a circulating air cabinet dryer for 120 h at 40 ºC. After drying, the material was pulverized in a cutting mill with 20 mesh sieves (0.84 mm). For the physical-chemical and anatomical characterization of the vegetal drug, the material was collected during the month of October/ 2011 and for the seasonal evaluation of the phytochemical profile and total flavonoid content (TFC), the harvests were performed during the course of one year (May/2011 to April/2012).

Seasonal evaluation of the phytochemical profile: Phytochemical prospecting, with monthly collected samples, obtained from the pulverization and methanolic decoction of about 6 g of aerial parts *in natura* and 100 mL of methanol, was performed. Later, the solution was filtered and concentrated, then reserved for analyses. For the analysis of alkaloids, an acid extraction with a heated solution of HCl 5%, followed by dilution with methanol and filtration, was done. After that, it was followed by a Thin Layer Chromatography (TLC) analysis, applying aliquots of 10 µL of these extracts on chromatography silica gel plates (Alugram® SIL G/UV, Ref: 818133). Developers and mobile phase systems were used consistent with the group of metabolites to be investigated (**Table 1**).

TABLE 1: REAGENTS AND STANDARDS USED FOR EVALUATION OF THE CLASSES OF SECONDARY METABOLITES

Secondary metabolites	Mobile phase	Developer	Standard	References
Alkaloids	E-F-G-W	Dragendorff	Pilocarpine	Wagner and Bladt[13]
Monoterpenes, sesquiterpenes	T-E*	Vanillin-sulphuric	Thymol	Wagner and Bladt[13]
Triterpenes, steroids	T-A	Lieberman-Buchard	β-sitosterol and ursolic acid	Harbone[14]
Flavonoids, Phenylpropane glycosides, cinnamic derivatives	E-F-G-W	NEU-UV	Quercetin	Wagner and Bladt[13]
Coumarins	T-E	KOH	Umbelliferone	Wagner and Bladt[13]
Proanthocyanidins, Leucoantocianidins	E-F-G-W	Vanillin-hydrochloric	(-) epicatequin	Robertson et al.[15]

Legend: E-F-G-W – Ethyl acetate - Formic acid – Glacial acetic acid - Water (100:3:3:3 v/v); T-A – Toluene - Ethyl acetate (90:10 v/v); T-A* – Toluene - Ethyl acetate (97:3 v/v); NEU reagent - diphenylboric acid-β-ethilamino ester

Evaluation of the Total Flavonoid Content (TFC): In addition to qualitative tests for secondary metabolites, TFC of seasonal evaluation samples was obtained, expressed as the total percentage (% m/m) of flavonoid content calculated as quercetina [16], according to the equation below:

$$TFC = \frac{A \times DF}{m \times E^{1}_{1cm}} \quad (1)$$

Where: TFC = Total Flavonoid Content; = absorbency measured; DF = Dilution Factor; m = mass of the dry drug (g); $E^{1}_{1\,cm}$ = 500 (specific absorption of the quercetin complex with aluminum chloride).

The TFC obtained was converted to mg of quercetin per 100 g of dry drug. To attain the vegetal dry drug mass, the result, 8.97%, from the loss by desiccation, was used. The absorbency, from which TFC was calculated, was obtained by the spectrophotometry flavonoid dosage method using a UV/Vis-mini 1240 Shimadzu® spectrophotometer, previously validated by the vegetal drug. For this analysis, a reflux extraction for each analyzed month was carried out; the sample readings were done in triplicate from the extractive solution obtained. The difference between TFCs throughout the months was evaluated by One Way Analysis of Variance (ANOVA) with a reliable level of 95%.

On the basis of average monthly TFC and in the surveys of climatic precipitation data [17], average temperature and relative humidity [18], the monthly average temperature from the average of the daily maximum temperatures registered in each month was obtained. In this context, interference of these environmental factors on the content of flavonoids and on the qualitative evaluation of remaining metabolites, derived from phytochemical prospecting was evaluated.

Anatomical evaluation: Leaves from *T. occidentalis* were collected and, later, preserved in FAA50 (formaldehyde - ascetic acid - ethyl alcohol 50%). Sections were done free-hand, colored with safranin and astra blue, mounted with glycerin 50%, according to usual vegetal anatomy procedure [19]. Under optical (light) microscopy (Olympus) with an attached camera (Sony W5), digital images were obtained for later anatomical structure analysis.

Physical-chemical characterization: The vegetal drug was submitted to assays for "determination of powder granulometry", "determination of loss by desiccation" (gravimetric method) and "determination of total ash" and "water determination" (Karl Fischer volumetric method) [20].

For determination of granulometric distribution, about 25 g of the vegetal drug were weighed and submitted to vibration for 30 min in sieves with the following meshes: 20, 30, 40, 60, 100 and 170 mesh, corresponding to mesh openings of 850, 500, 425, 250, 150 and 90 µm, respectively. By means of passage and retention curves, the average particle size was calculated. All the assays were conducted in triplicate.

Volatile oil determination: For the quantification of volatile oils present in the sample, extraction was preceded by hydrodistillation, using a Clevenger apparatus. About 30 g of the dry vegetal drug was weighed which was transferred to a flat-bottomed flask with approximately 250 mL of distilled water, enough to cover the material. The hydrodistillation process lasted about 3h. This determination was carried out in triplicate based on the described procedure of Santos *et al* [21].

Polysaccharide dosage: Polysaccharide dosage was determined by antrona colorimetric method in a UV/Vis-mini 1240 Shimadzu® Spectrophotometer. The samples were obtained in accordance with Tang et al [22], modifying the following parameter: one aliquot of 2.0 mL of the aqueous extract was removed, adding one aliquot of 0.24 mL of trichloroacetic acid 10% (v/v).

The antrona reagent was added to samples in test tubes in an aliquot of 5.0 mL containing 1.0 mL of aquaeous extract, obtained at the drug/solvent ratio of 1:24; in standard sample test tubes of glucose (Sigma®) used to attain the standard curve, and in test tubes with distilled water to obtain the compensation solution [23]. Analysis was carried out in triplicate at the wavelength of 620 nm.

RESULTS AND DISCUSSION:

Seasonal evaluation of the phytochemical profile and TFC: Observations performed during the monthly collections of *T. occidentalis* allowed for important confirmations. During the period evaluated, the branches remained in vertical and erectile planes, with dark green coloration and small in size, characteristic of young branches. The bark of the stalks exhibited a brown-orange color, also characteristic of its stage of development. The plants produced a characteristic aroma and were found in a bushy state during the period of study. Herbivory was not visible and the ground was found to be humid and with a pH varying from 5.7 to 6.7.

According to Stangerlin *et al* [24], *T. occidentalis* has a preference for deep soil, with a pH between 5.2 and 7.0; adequate conditions for cultivation. In relation to the phytochemical prospecting performed, the results are summarized in **Table 2**.

TABLE 2: RESULT OF PHYTOCHEMICAL PROSPECTING OF METHANOLIC EXTRACTS OF *T. OCCIDENTALIS* L. FOR SEASONAL EVALUATION

Secondary metabolites	May 2011	Jun 2011	Jul 2011	Aug 2011	Sep 2011	Oct 2011	Nov 2011	Dec 2011	Jan 2012	Feb 2012	Mar 2012	Apr 2012
Alkaloids	-	-	-	-	-	-	-	-	-	-	-	-
Monoterpenes and sesquiterpenes	+	+	+	+	+	+	+	+	+	+	+	+
Triterpenes and steroids	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids, Phenylpropane glycosides and cinnamic derivatives	+	+	+	+	+	+	+	+	+	+	+	+
Coumarins	-	-	-	-	-	-	-	-	-	-	-	-
Proanthocyanidins and leucoantocianidins	+	+	+	+	+	+	+	+	+	+	+	+

Legend: (+) presence of the chemical group, (-) absence of the chemical group.

Violet or blue color spots when seen in visible light (VIS), after nebulization with vanillin-sulfuric acid, confirm the presence of monoterpenes and sesquiterpenes, present in every month of the study and, therefore without seasonal variation during the year.

Additionally, the presence of violet-colored spots with an Rf of 0.6, in these conditions of elution and development, also mentioned in the work described by Wagner and Bladt [13] for *Salvia officinalis* L. leaves, suggests the confirmation of the monoterpene thujone in *T. occidentalis*, also observed in every month of the study.

The findings of these analyses corroborate the study described by Castellón *et al* [3], which highlight the constituents of the essential oil as a characteristic metabolic group of the genus Thuja. Most of the phytochemical surveys for the species highlight, inclusively, the thujone monoterpene as the major constituent [4, 25, 26]. Naser *et al* also highlighted the following terpenes in the species: borneol, thujone, camphene, fenchone, limonene, miricene, α-terpineol, terpinolene, thuja alcohol, carvotan acetone, origanol, origanese, myrcene [1].

As to the presence of triterpenes and steroids, analysis by TLC indicated the presence of the component β-sitosterol in *T. occidentalis* in every month evaluated, as per **Table 2**. The presence of these metabolites in the species was previously described in the work of Castellón *et al* [3] with nine mother tinctures commercialized in Cuba.

However, in this case, the presence of β-sitosterol was not described, specifically. In the same manner, for concentrated tannins, by TLC analysis, evidence of proanthocyanidins and leucoanthocyanidines every month, specifically (-) epicatechin was observed.

In dealing with the evaluation of polyphenols by TLC, the presence of fluorescent spots under UV light (at about 254 nm) indicates the presence of flavonoids.

At 365 nm, depending on the structure of the flavonoids, these polyphenols display a dark yellow, green or blue fluorescent color, that intensifies or changes in the presence of various developers [13]. Therefore, the fluorescent yellow and green spots, present in every month's collection, are indicative of this group of metabolites.

The flavonoids in glycosylated forms should be emphasized, since the representative spots of quercetin with less Rf than the standard substance (agyicone), suggests the presence of quercitin glycosides. This result corroborates the finding of this flavonol in *T. occidentalis* in the survey carried out by Naser *et al* [1]. This group of metabolites, for this species, was also mentioned in the studies of Meenu *et al* [27], involving methanolic extracts and hydroalcoholic extracts and Castellón et al [3], with homeopathic mother tinctures.

Among the chemical groups researched concerning *T. occidentalis*, alkaloids were absent, which agreed with the findings of Meenu *et al* [27]. Coumarins were also absent, disagreeing, in this case, with what Naser *et al* [1] described, who identifies the presence of p-cumaric acid and umbelliferone in the vegetal drug.

Thus, it was observed that, as to the groups of metabolites investigated, the species is not so sensitive to seasonal variations, as there was no qualitative variation of these constituents throughout the period of study. However, in fact, the temporal and spacial variations in the content of the secondary metabolites in plants can occur at a climatic, seasonal and daily level and, in spite of the existence of genetic control, the expression can suffer resultant modifications of the interaction of biochemical, physiological, ecological and evolutionary processes; or even as a result of foliar development and/or the sprouting of new organs [28].

The flavonoids, a group of metabolites of chemical and pharmacological importance for *T. occidentalis*, were investigated quantitatively in our experiments during the same period as the phytochemical prospecting, described in the evaluation assay with the objective of establishing a relationship to the main registered climatic conditions at the site of cultivation (**Table 3**).

The relationship between precipitation, relative humidity (RH), monthly average temperature and TFC is represented in **Figure 1**.

TABLE 3: ANALYSIS OF THE CLIMATIC CONDITIONS FOR SEASONAL EVALUATION OF THE SECONDARY METABOLITES AND TFC

Months	TFC (%) ± sp	Precipitation (mm) ± sp*	Average temperature (°C) ± sp**	Relative Umidity (%) ± sp**
May/ 2011	7.86 ± 0.09	16.60 ± 27.53	28.94 ± 0.96	85.22 ± 9.18
Jun/2011	8.44 ± 0.16	4.,2 ± 7.13	28,.40 ± 0.76	82.63 ± 9.05
Jul/2011	6.43 ± 0.04	11.51 ± 16.28	27.44 ± 0.79	84.86 ± 8.93
Aug/ 2011	8.57 ± 0.25	3.58 ± 6.36	28.05 ± 0.63	77.40 ± 9.99
Sep/ 2011	8.37 ± 0.13	1.51 ± 4.27	28.44 ± 0.56	72.97 ± 8.91
Oct/ 2011	7.99 ± 0.10	1.32 ± 0.81	29.,71 ± 0.68	69.87 ± 8.18
Nov/2011	7.97 ± 0.12	2.69 ± 7.00	30.13 ± 0.66	69.16 ± 8.37
Dec/ 2011	6.89 ± 0.14	0.33 ± 0.81	30.94 ± 0.76	67.32 ± 8.67
Jan/ 2012	7.53 ± 0.07	6.00 ± 14.54	30.23 ± 1.46	73.11 ± 10.79
Feb/ 2012	7.53 ± 0.08	2.93 ± 9.20	29.91 ± 0.77	71.87 ± 9.16
Mar/2012	9.51 ± 0.09	1.94 ± 5.29	30.65 ± 0.55	71.62 ± 7.86
Apr/2012	7.81 ± 0.06	1.13 ± 3.78	30.49 ± 0.49	72.12 ± 9.06

Legend: ± sd = standard deviation. Source: *Pernambuco Water Agency and Climate [17]; ** National Institute of Meteorology [18].

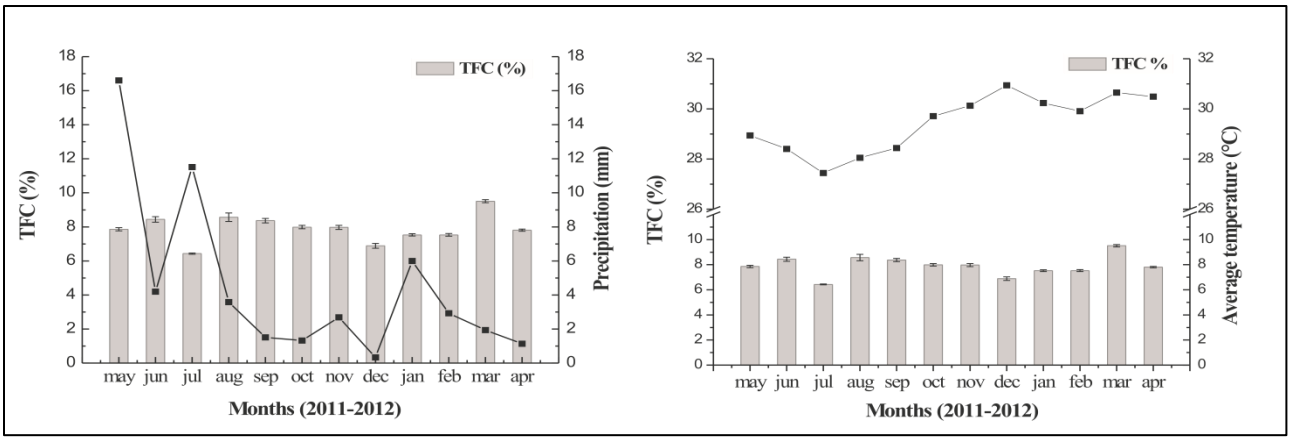

FIG. 1: SEASONAL ANALYSIS OF TFC IN RELATION TO THE PLUVIOMETRIC INDEX AND THE MONTHLY AVERAGE TEMPERATURES

On the basis of the data presented it was observed that there was a statistical difference for TFC during the months ($F_{calculated}$ = 127.28; $F_{tabulated}$ = 2.21), that registered an average value of 7.91% ± 0.80. Minimum TFC was found in the month of July/2011 (6.10%), a period of an elevated pluviometric index (11.51 mm), elevated UR (84.86%) and warmer temperatures (27.44ºC). Even when there was an elevated pluviometric index in May/2011, there was considerable variation in the precipitation throughout the days of this month (sd ± 27.53). Additionally, it must be emphasized that between the months of January and July/2011, a pluviometric average of 18.6% was registered, above the expected for the entire State of Pernambuco, mainly in the months of May and July for the Metropolitan Region of Recife, where Cabo de Santo Agostinho is located [17].

In December/2011, the second lowest value for TFC was found (6.89%), related, in contrast, to the lowest level of precipitation for the year (0.33 mm), low UR (67.32%) and high temperatures (30.74ºC). Similarly, the maximum value for TFC was observed in the month of March/2012, a period corresponding to the end of summer, with a low pluviometric index (1.94 mm), low UR (71.62%) and an elevated average temperature (30.65ºC). The differences in the pluviometric index and UR in this month were 35.8% and 15.40%, respectively, in relation to what was observed in the month of July/2011. Thus, in accordance with the data presented, they suggest that a low pluviometric index favors a greater attainment of flavonoids in *T. occidentalis* with factors such as high temperature and low UR favoring these findings; however extreme minimum precipitation conditions negatively influence the biosynthesis of flavonoids, as seen in December/2011.

Studies, by other authors cited, affirm that such environmental conditions described as favorable for maximum TFC were also found for other species, as described by Alves *et al* [29] in a study carried out with 20 medicinal plants from the savannah, which presented a greater yield of flavonoids during the dry season. On the other hand, Borella *et al* [30] observed that *Baccharis trimera* yielded many more flavonoids during the summer harvest. In the case of *T. occidentalis*, Brzezińska and Kozłowska [31] reported that the phenolic composite level is related to insolation intensity, as part of an adaptation mechanism.

Likewise, the hydric factor, that exerts influence on physiological processes, such as the opening and closing of stomata, photosynthesis, growth and foliar expansion, can also generate changes in the secondary metabolism of the plant. When in a condition of hydric stress, however, there is a physiological adaptive response of increasing productivity of some terpenoids and flavonoids [11], as seen in our study of *T. occidentalis with* regard to maximum TFC in March, the dry season. This result agrees with the findings of Edwards and Dixon [32], who affirm that this species exhibit a great tolerance for water availability, and can be found in diverse habitats, varying from dry to marshy surfaces. In this in case, evidence indicates that *T. occidentalis* exhibits osmotic adjustments in reply to the hydric deficit and this response is common in some herbaceous and woody species.

Moreover, it is known that new tissues generally possess greater biosynthetic rates of metabolites, as verified in the contents of rutin, vitexin and total flavonoids from *Passiflora edulis Sims f. flavicarpa* due to the presence of younger leaves [33]. A recent study shows still a significant increase in the levels of flavonoids, including, in conditions where grains of pollen from *T. occidentalis* were exposed to air pollutants. This increase was justified as a physiological mechanism of characteristic defense of polyphenols [34].

So, it can be concluded that there was a fluctuation in TFC, which shows that this species possess a dynamic metabolism and, even in drier periods, *T. occidentalis* continues synthecizing flavonoids. In other plants, to the contrary, there can be stabilization in the synthesis of some metabolites[35]. However, other factors must also be evaluated so that the role of flavonoids can be understood in the adaptation of this plant in its surroundings.

Moreover, differences in TFC over the months can provide parameters for the selection of the best months for harvesting this medicinal plant, and thus provide greater quality for the vegetal drug and the products commercialized from it, reducing extractivist pressure [36]. Therefore seasonal evaluation can be considered a starting point for the elaboration of new studies, aiming at enriching knowledge concerning the species, as well as adding criteria for the production and development of future phytomedicines.

Anatomical evaluation: Considering the importance of microscopic identification to guarantee the authenticity of the vegetal material and the normative requirement of this evaluation, plus the scarcity of such information on *T. occidentalis*, the main anatomical characteristics of the vegetal species under study were traced.

T. occidentalis leaf exihibts a thick cuticle covering over a unistratified epidermis, consisting of cells with straight or slightly sinuous anticlinal walls, followed by a layer of hypodermis (**Figure 2A**), inerrupted only in regions of stomata; these characteristics were also found by Ivănescu et al [37]. In some regions of the leaves two layers of palisade parenchymata are found, in the external and internal sides (**Figure 2B, 2D**), with its interior presenting voluminous cells, forming the spongy parenchymata (**Figure 2A**).

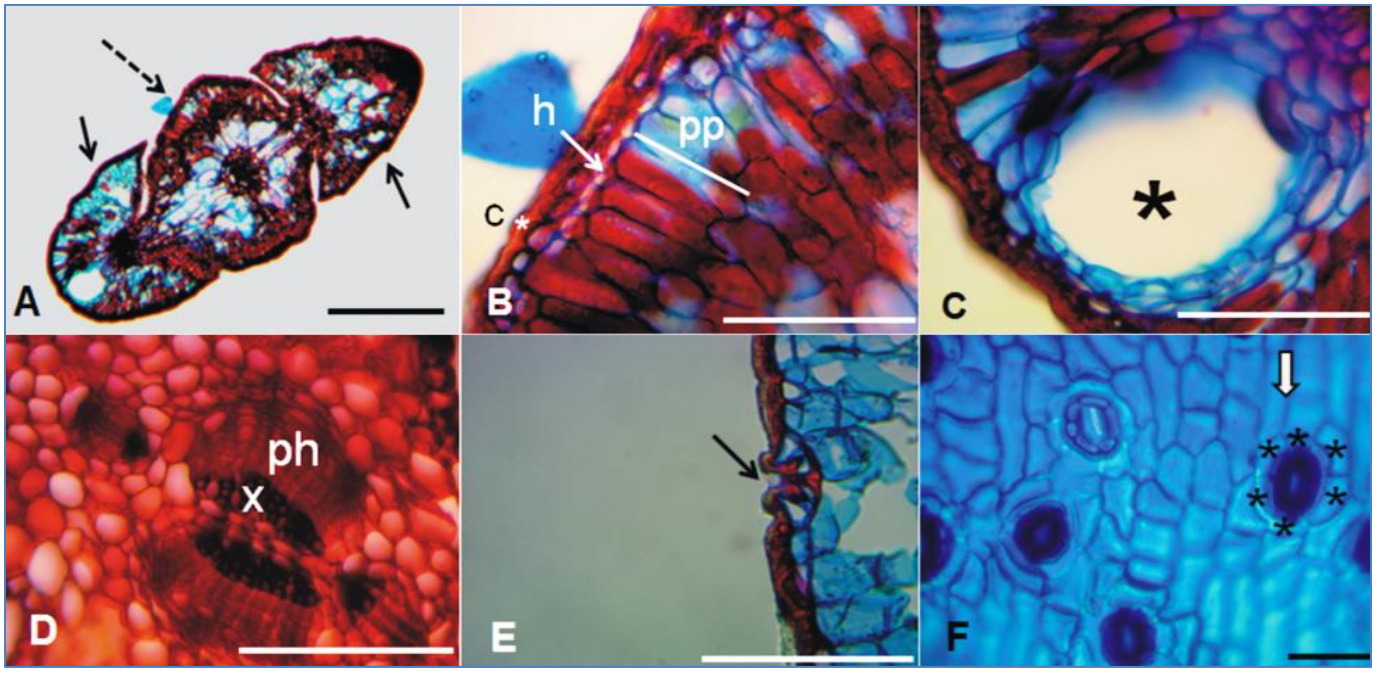

FIG. 2: ANATOMY OF *THUJA OCCIDENTALIS* LINN. (CUPRESSACEAE)

Legend: A) Transversal view of branch (dashed arrow) with opposing leaves (arrow), showing leaves with external convex side and internal straight side. B) palisade parenchymata (pp) just below the external side of the epidermis. C) Secretor Channel (*). D Vascular bundle with xilem (x) and phloem (ph) in a side by side arrangement. E) Stoma (arrow) in transversal view. F) Ciclocitic stoma (hollow arrow) frontal view surrounding subsidiary cells (*). Bars A = the 200 µm; B-E = 50 µm; F = 20 µm. Source: our own crop.

On the abaxial side secretor channels are found immediately below the hypodermis (**Figure 2C**), agreeing with what was described in the studies by Ivănescu *et al* [37]. According to these authors, cells of *T. occidentalis* lateral leaves are classified as heteromorphic in the main nervure region, where there is a central collateral vascular bundle, enclosed in a layer of endoderm, flanked by two lesser bundles, one with the phloem toward the adaxial side and the other phloem toward the abaxial side (**Figure 2D**).

Since the leaves are convex, the adaxial side is convex and the abaxial face is plane. The leaf is hypostomatic, with ciclocitic stomata, showing 5-6 surrounding subsidiary cells (**Figure 2E-2F**); a fact corroborated by Ivănescu et al [34]. In accordance with Metcalfe and Chalk [38], the type of stoma is an important sign in the taxonomic identification of many species.

The mesophyle is dorsiventral, with the palisade parenchymata situated immediately below the adaxial side of the epidermis, serving for the greatest incidence of exposure to luminosity in this area, with a cylindrical cell shape and greater amount of chloroplasts in its interior. This is the most important tissue in the production of photo-assimilated compounds.

Physical- chemical characterization: ***Granulometric distribution and particle size in sieving:*** The results obtained for the granulometric distribution histogram of the aerial parts of *T. occidentalis* (**Figure 3**) demonstrate that particles of the vegetal material are predominantly retained in a 500 and 425 µm sieve, representing a percentage of 27.59% and 25.59% respectively of retained material.

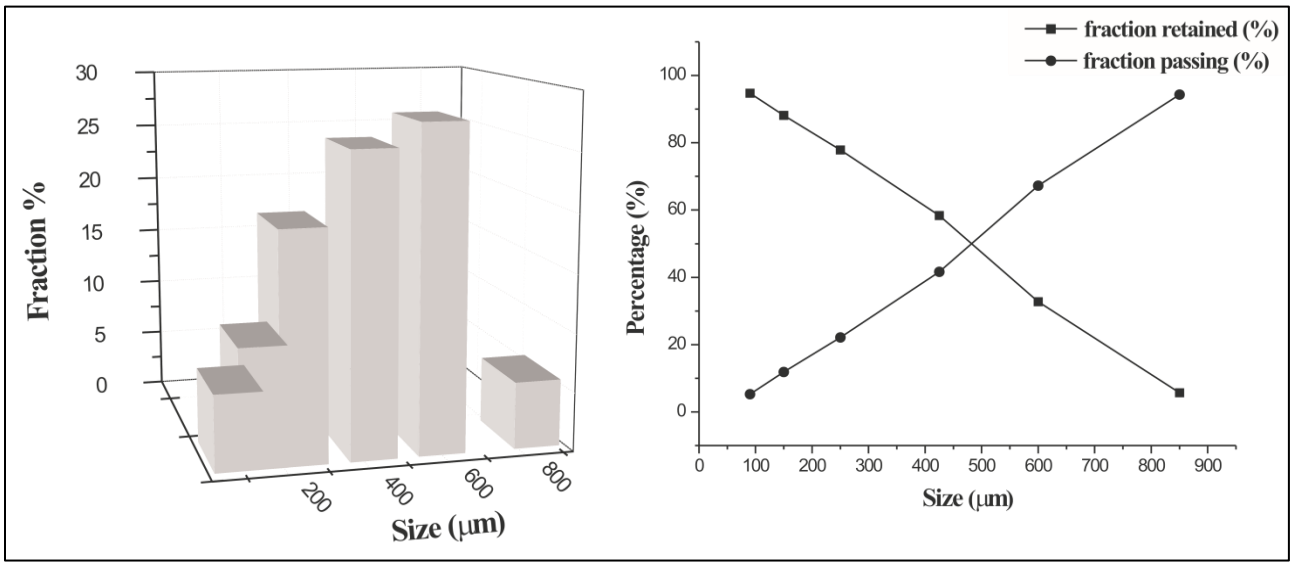

FIG. 3: HISTOGRAM OF GRANULOMETRIC DISTRIBUTION AND RETENTION AND PASSAGE CURVES OF *T. OCCIDENTALIS* AERIAL PARTS

The average size of particles, determined from the point of intersection of the retention and passage curves, was 484 µm (± 4.24) (**Figure 3**), characterizing it as a thick powder, according to the pharmacopeia criteria Brazilian Pharmacopeia [20]. This result constitutes an important factor for vegetal material conservation, since reduced granulometry with a consequent increase in the powder's contact surface makes eventual stability problems possible, due to adsorption of humidity [39]. Granulometry is also directly related to the available contact surface for interaction with the solvent used in extraction, thus it is an important preliminary parameter, because it has a direct relation to the efficiency of the extractive process [40], besides constituting a determining factor in the homogeneity and reproducibility of extractive processes. Particles with a very small diameter (less than 0.2 mm) compromise filtration and, consequently, the content of the active substances, since there is a formation of preferential channels that can make processes difficult such as percolating and maceration, that requires a process of filtering under pressure [41].

Moreover, very fine particles can adhere to larger ones, increasing the viscosity of the medium and creating a barrier that hinders the penetration of solvents. Thus, thick powder is the most indicated for extraction from leaves, flowers and herbs, as determined for *T. occidentalis* However, particles with a very large average diameter (greater than 0.8 mm) also compromise extraction, by not allowing the penetration of the extracting liquid in every cell[41].

Loss by desiccation, water determination, total ash content and determination of volatile oils: The loss by desiccation is a parameter that allows for the evaluation of the vegetal drug, because it supplies, at a measurable value, residual humidity of volatile composites, including water, that favor hydrolysis and enzymatic activity reactions, with consequent deterioration of the chemical constituents, besides affording the growth of fungi and bacteria. Low humidity content indicates efficiency during the drying process and that the material is stable. So, determination of humidity is an important parameter, since it conveys information regarding the storage of a vegetal drug [42].

The results, following this test with the above-mentioned vegetal drug, demonstrated a value that corresponds to 6.92% (±0.04) and, therefore, below the maximum limits established by the Brazilian Pharmacopeia [20] in the monographs of vegetal drugs (8 to 14%), thus it is possible to infer the chemical and microbiological stability of the vegetal drug *T. occidentalis.*

Additionally, water determination by the Karl Fischer method revealed an average value of 5.30% water (±0.17). Thus, in accordance with the value obtained in the loss by desiccation (6.92%), one can conclude that about 1.62% could be attributed to other volatile constituents, the majority being essential oils. Notwithstanding, the results for the determination of essential oils by Clevenger showed a value of 0.7% and, therefore, inferior to that foreseen (1.62%). This value, nevertheless, is in accordance with the content of essential oils shown in other studies conducted with *T. occidentalis* that relate them to certain pharmacological activities [26].

Ash content is comprised of mineral salts (physiological ash) or impurities (non-physiological ash) present as vegetal drug contaminants, mainly sand and soil silicon [42]. In this analysis, a value of 4.53% (± 0.05) was found and therefore below the index of 14% presented by the monographs on vegetal drugs in the Brazilian Pharmacopeia [20], indicating that the cited vegetal drug does not possess an excess of soil and/or sand.

Polysaccharide dosage: Polysaccharides are indicated as important constituents for antiviral and immunostimulant activities of *T. occidentalis* leaves [1], however, until now; a series of pharmacological studies has identified the presence of these composites in *T. occidentalis* only by means of phytochemical investigations [8-10]. Therefore, the dosage for polysaccharides in *T. occidentalis* leaves became necessary, not only because of the absence of data in the literature, but also because of the fact that immunostimulant activity evaluated by Sunila et al [7] was similar for unprocessed methanolic extracts and isolated polysaccharide fractions of *T. occidentalis* leaves, indicating that the extract can be used as a vegetal raw material, without harm to its pharmacological activity.

For the polysaccharide dosage, the antrona method was used one of the most used colorimetric methods for the determination of polysaccharides [43]. The antrona reaction is based on the hydrolytic and dehydrating action of the concentrated sulfuric acid on the polysaccharides. The dehydrated simple sugars for furfural or hydroxymethylfurfural are condensed with antrona (9, 10-dihidro-9-oxoanthracene) resulting in a teal colored product that can be analyzed by spectrophotometry [44]. The absorbency values obtained for the vegetal drug samples under study were converted into total polysaccharide content using the glucose calibration curve equation that presented a correlation coefficient of 0.9935. Glucose can be used to estimate polysaccharide content, due to reports that the reaction of antrona with equivalent amounts of glucose, starch and glycogen develops similar absorbencies [45]. Therefore, the average content of total polysaccharides obtained for analysis was 233.2±2.0µg/g, a parameter that could be used for quality control of the vegetal drug.

The values presented in this work represent initial parameters that can be used in the quality control of the vegetal drug.

CONCLUSION: The results obtained with physical-chemistry tests, anatomy and seasonal evaluation of the major metabolites represent parameters for quality control of the aerial parts of *T. occidentalis* and enable the realization of the months with the highest relevance of metabolites. These data will be useful for quality control of plant drug and phytomedicines obtained from this species.

Pharmacological activities previously described and attributed to the flavonoid fraction of *T. occidentalis* encourage further testing for the standardization of extracts obtained from the plant drug characterized this group of metabolites.

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