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THUJA OCCIDENTALIS L. (CUPRESSACEAE): REVIEW OF BOTANICAL, PHYTO-CHEMICAL, PHARMACOLOGICAL AND TOXICOLOGICAL ASPECTS

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ABSTRACT: *Thuja occidentalis* called the tree of life is an endemic plant in eastern North America and cultivated in northern Europe and in Brazil as ornamental tree. According to the popular use of this plant has been used in herbal and homeopathic preparations for acute and chronic infections of the upper respiratory tract, warts, as adjunctive antibiotic, immunostimulant. However, these and other data are old or unavailable, and in view of the therapeutic potential of the species, it was necessary to upgrade the recently published studies and not included in previous reviews about the botanical, pharmacological, toxicological and technological aspects. In this review article, it was noted that for many years the phytochemistry studies on the species concentrated their efforts around essential oils, highlighting the monoterpene thujone, which is attributed to the pharmacological and toxicological activities. Recently, preclinical studies have identified the presence of polysaccharides, flavonoids, tannins and proteins in the ethanol fraction, which showed antioxidant, hepatoprotective, antitumor, hypolipidemic, antidiabetic and antiulcerative activity. The clinical studies proving its immunostimulant and action against warts, however no need to isolate the constituents responsible for the therapeutic action and to establish of quality parameters for plant drug and its derivatives, capable of ensuring the effectiveness, safety and quality of products from *T. occidentalis*.

INTRODUCTION: The genus Thuja of exotic origin belongs to the Cupressaceae family and covers five species [1], including *Thuja occidentalis* L., commonly known as the tree of life. It is endemic in the eastern part of North America and is grown in northern Europe and Brazil as an ornamental shrub [2], being especially appreciated by landscape designers [1].

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The mother tincture (MT) diluted or hydro-alcoholic have been widely used in homeopathy and human and veterinary phytotherapy, one of the main uses being the treatment of acute and chronic infections of the upper respiratory tract, and as an adjuvant to antibiotics for severe bacterial infections, such as bronchitis, angina, pharyngitis, otitis media and sinusitis. Its effects are attributed primarily to its immunostimulant and antiviral properties, which increase the proliferation of T lymphocytes and the production of interleukin-2. There are also reports of its association with other plants that modify the immune system, such as *Echinacea purpurea*, *Echinaceae pallida* and *Baptisia tinctoria* [3].

In the form of a tincture, it is used in the lower genital tract for the treatment of warts, papillomas, condylomas, and various types of excrescence, especially those related to *Human papillomavirus* (HPV) [4]. There are reports that the cure rate for warts is 84.2% [5]. Adverse reactions reported in clinical trials are generally mild or moderate and often associated with constituent of essential oil thujone [3]. However, there were seen no sign of mutagenesis or synthesis of β-galactosidase in tinctures analyzed [6].

The most important of the metabolites present in this medicinal plant are the flavonóides [3,7], lignans [2,8], polysaccharides [3] and constituents of the essential oils, characteristic of the Thuja genus [9], including diterpenes, monoterpenes (α-thujone, β-thujone, fenchone), and a sequiterpene [2]. The polysaccharides as well as flavonoids (kercetin, campherol), tannins and proteins were identified by phytochemical investigations in ethanolic fraction of aerial part of *T. occidentalis* used in several pharmacological studies conducted by Dubey e Batra for hepatoprotective, antidiabetic, anti-ulcerative, antioxidant and hypolipidemic activities [10-14]; addition of flavonoids also are involved with the anti-tumor activity [15,16].

According to Naser *et al* [3], there are various reviews and monographs that describe the botanical aspects, the metabolites, and some pharmacological properties of this plant. However most of these reports are old, written in German and often not available in the data bases. In view of this and given the therapeutic importance of the species under study, it was necessary to update the data with recently published studies and those not included in previous reviews, including the description of the general, botanical and phytochemical aspects of *T. occidentalis*, beyond its pharmacological properties and data on safety and efficacy, based on clinical and pre-clinical studies, along with the technological aspects involved.

General description, botany and cultivation: Thuja belongs to a small genus of the Cupressaceae family and covers five species, including *T. occidentalis* [17]. The tree has been given various names, including arbor vitae, white cedar of the east, white cedar of the north, the tree of life, white cedar, swamp cedar or yellow cedar. The medicinal parts of this plant are composed of oils extracted from the leaves and extremities of the branches, which must be young, dry and fresh[18]. The four main varieties of *T. occidentalis* cited by Naser et al. [3] are *T. occidentalis* cv. Aureospica, *T.occidentalis* cv. Lutea, *T.occidentalis* cv. Vervaeneana and *T. occidentalis* cv. Wareana.

This plant is originally from the eastern part of North America, and is found in Europe mainly as an ornamental shrub. The trees are characteristically coniferous and monoecious, measuring 12-21 m in height [3,18], have a pyramid-shaped summit with monopodial branchings to the trunk, which is erect with a reddish-brown cortex and relatively highly ramifying branches [19]. The branches are flattened, short, horizontals, with an ascending extremity and covered with small rigid leaves, which overlap one another. The leaves are oval-shaped, persistent, greens, with scales crossing from opposite sides and the extremities tapering towards a convex dorsal surface [18, 19].

On the upper branches, the leaves present an angular oval glandular structure containing oil resin with a characteristic intense odor, a sharp taste, which is balsamic and camphoric. On the extremities of the branches there are also small oval cones (6-8 mm in length), which are microsporophyll, greenish yellow in color and covered in yellow leathery foliage [18, 19]. These cones are made up of 8 or more scales, with 1-3 ovules each [1]. The flowers appear in separate (monoecious) cones, composed of male flowers (dark-brown) and female flowers (greenish-yellow), which are almost perfectly star-shaped. The seeds are yellowish-brown, 3-5 mm in length and around 1 mm in width [18]. The fruit is an oblong sub-conic chestnut-green megasporophyll [19]. It is recommended that young branches be used for production, with harvest in the spring in the Northern hemisphere, when the content of the active agents is ideal. After drying, the plants should be handled with care [18].

This species shows great tolerance to the availability of water and can be found in various habitats, from dry to swampy. There is evidence that *T. occidentalis* adjusts osmotically in response to water shortage. This osmotic adjustment response is common when faced with the lack of

water in some herbaceous species and species of woody plants [20].

In relation to its production, based on technical visits to nurseries and small rural producers in four municipalities of Rio Grande do Sul (Brazil), it was found that there was a preference for the multiplication of *T. occidentalis* by planting them out, with healthy trees chosen to furnish the branches and cultivated in the field, where they remain for two to four years to be subsequently transplanted into pots and sold, compared to the system of cultivation in pots, which requires greater experience. The total average cost of a sapling is R$ 2.79 [1].

As for factors that affect the regeneration of *T. occidentalis* after partial cutting of the trees, Larouche [21] notes that upward growth of the trees is influenced by the region and the availability of light, while diameter growth is related to partial cutting, preferably of low intensity or natural regeneration. Subsequently, the study outlined by Larouche et al. [22] confirms previous results, also showing that the initial growth of planted saplings and the production of biomass were proportional to the availability of light.

Phytochemistry: Studies that cite the general phytochemical composition of *T. occidentalis* provide evidence of the existence of a series of compounds, including saponins, phenols, tannins, amines, mucilages, bitter principles, lactonic compounds, carotenes, essential oils, triterpenes, steroids [7], reducing sugars, cumarines (p-cumarice and umbelliferone acid), tannic acid, polysaccharides, proteins and minerals [3].

The flavonoids [3, 7] and lignans [2,8] are representative groups of the species, and of the flavonoid group, the following have been descried (+/-)-catechin, (-)-gallocatechin, mearusitrin, myricetin, procyanidin B-3, prodelphinidin, quercetina, quercitrin, campherol and campherol-3-O-α-rhamnoside [3]; in addition to mearusitrin and, of the bioflavonoids, bilobetin and amentoflavone, among others [18].

The amentoflavone notable for being present in high amounts in specie [23], exhibiting potent antifungal activity against several pathogenic fungal strains [24] and antiviral activity against respiratory syncytial virus (RSV) [25].

In terms of therapeutic use, stands out the polysaccharides [3] since they have been shown as the active constituents responsible for the antiviral and immunostimulant activities in several studies [3, 26, 27]. Furthermore, these compounds as well as flavonoids, tannic acid and proteins were identified by phytochemical investigations in ethanolic fraction of aerial part of *T. occidentalis* (EFTO) in several pharmacological studies conducted by Dubey e Batra [10-14].

Although there are no reports in the literature of polysaccharides in the composition leaves the species, Willför et al. [29] analysed the content and composition of carbohydrates comprising polysaccharides in sapwood and heartwood of 12 species, including *T. occidentalis,* content industrially pulpwood important. The content of cellulose (predominant polysaccharide type) in *T. occidentalis* was 442 and 440 mg/g in sapwood and heartwood, respectively.

The carbohydrate composition suggested that the main non-cellulose polysaccharides were xylans and mannans whose content was the same range in heartwood and sapwood. As cited by Willför et al. [29], Hans [30] found mannose to be the main carbohydrate unit in *T. occidentalis* stemwood and in works of Timell [31, 32] also were isolated and characterised mannans in the specie. Additionally the amount and composition of water-soluble carbohydrates from ground wood samples were also analysed. *T. occidentalis* released mainly arabinogalactans and pectins.

Most of the plant lignans are related to antioxidant, antifungal, antibacterial, insecticide, nematicide, allelopathic activities (inhibit growth of other plant species different), among others outras [33]. The lignans isolated of *T. occidentalis* are represented by the following constituents: (-)-matairesinol, (-)-thujaplicatin methyl ether, (-)-wikstromol, 8-hidroxi-thujaplicatin methyl ether, (-)-4-O-demethyliateina, epi-pinoresinol, pinoresinol and secoisolariciresinol, all isolated from the xylem of the branches. The neolignan, dihydrodehydrodiconiryl alcohol, was also isolated from the hydrolized extracts of the leaves [8].

In study of Willfor *et al* [28], thujalignanas (several unidentified lignans, probably related to thujaplicatin and 4-Odemethylyatein and small

amounts of matairesinol and lignan and oligomers were identified as major groups in hydrophilic extracts and fractions of extracts of specie. In later work, thujalignanas and lignans were the compounds most representative hydrophilic extract of *T. occidentalis*, which stood out for its antioxidant activity. This extract also showed significant amounts of sugars [34].

Despite the broad phytochemical profile, the constituents of the essential oil are the characteristic metabolic group of the Thuja genus [9], being found in quite significant quantities in the species. Chang et al. [2] cite various phytochemical studies that result in the isolation of various compounds, including diterpenes (dehydroabietane, neothujic acids III e IV), lignans [(-)-matairesinol, (-)-thujaplicatin methyl ether, (-)-wikstromol, epi-pinoresinol], monoterpenes (α-thujone, β-thujone, fenchone), and a sequiterpene alcohol [(+)-occidentalol]. Furthermore, in this same study, the authors isolated six substances, two of them brand new [(+)-7-oxo-13-epi-pimara-14,15-dien-18-oic acid (diterpene acid) and (+)-isopicrodeoxypodophilotoxina (lignan)] and four that had already been previously elucidated [(+)-7-oxo-13-epi-pimara-8,15-dien-18-oic acid, (+)-isopimaric, acid (-)-deoxypodophilotoxin and (-)-deoxypodorizone].

Also noted was the presence of others constituents of essential oils present in the species, such as borneol, camphene, limonene, miricene, α-terpine, terpinolene, thujyl alcohol, carvotanacetone, origanol, origanes, mircene and camphene (Naser et al. 2005a), beyond the sesquiterpene occidenol [35].

For this reason, various researchers have evaluated the chemical composition of the essential oils present in *T. occidentalis* in a more specific manner, principally for analysis of distinct varieties and ecotypes. Svajdlenka et al. [9], for example, conducted a comparative evaluation of the presence of 33 essential oils in two forms (cultivars) of *T. occidentalis*, namely: *T. occidentalis 'malonyana'* and *T. occidentalis 'malonyana'* (selecton Misak). The analyses were carried out using Gas Chromatography (GC) with samples collected during distinct periods (May and October) and showed a similar profile for both the cultivars for all collection periods.

Of the components investigated, the most striking were α-thujone, β-thujone and fenchone, as these represented more than 50% of the composition of the oils.

Tsiri *et al* [17], in turn, investigated the chemical composition of the essential oils of four varieties of Thuja species grown in Poland, namely: *T. occidentalis 'globosa'*, *T. occidentalis 'aurea'*, *T. plicata* and T. plicata 'gracialis'. The authors identified 31 compounds in *T. occidentalis 'globosa'* and 37 in *T. occidentalis 'aurea'*, both as principal constituents of α-thujone (50.14 and 51.60%, respectively), beierene (8.54% and 11.28%, respectively), sabinene (4.55% and 3.43% respectively) and camphor (4.47 and 3.09% respectively). On the other hand, a characteristic difference was the high levels of β-thujone and fenchone in *T. occidentalis 'globosa'*, and high levels of rimuene diterpene in *T. occidentalis 'aurea'*.

Sulea and Leca [36] examined the volatile components of the tincture of *T. occidentalis* var. columnaris using GC and obtained 39 peaks, of which 36 were identified. The terpenes and terpenoids accounted for 88.4% of the volatile compounds in the tincture, with 47.4% α-thujone, 8.3% β-thujone and 10.3% fenchone. The remaining 11.6% consisted of pimaric acid (5.6%), phytol (4.4%) and fatty acid esters (oleic, palmitic and linolenic) (2.6%), as well as small quantities of vitamin A (1%) and E (0.6%) acetates, a hormone of the gibereline A1 plant (0.4%) and cyclohexanone (1.5%). In study of Yatagai et al. [37], the main components of the leaf oil of *T. occidentalis* were cis- and trans-thujone and fenchone.

Studies have also identified the presence of monoterpenes and diterpenes in cell cultures of *T. occidentalis* [38, 39], relating even the thujone-rich fraction with an anti-cancer activity potential on A375 cells [40].

Some differences observed in the composition of the essential oils may be related to a series of factors, such as origin of the plant, harvest time, drying method, intensity and duration of lighting, temperature, altitude, season, soil, nutrition, conservation of vegetable material and the extraction process [36,41].

Pozetti *et al* [41], for example, analyzing the phytochemical profile of the MT of *T. occidentalis* obtained from different laboratories in Brazil and in European countries confirmed the presence of qualitatively and quantitatively distinct phytochemical compositions, including thujone that showed higher levels in samples from France and Germany.

Khomasurya [42], in study using samples collected in eastern Ontario (Canadian), detected the lowest yield of essential oils of *T. occicdentalis* in the month of July/1998 (0,56%) and the highest yield was in December/1998; so the summer was about 30% less profitable compared to winter months. In this study, the main components of the oil were thujone and fenchone. Recently, samples of *T. occidentalis* grown in Iran showed a higher content of volatile oils at 7 am [43].

Despite these differences, the content of the essential oils, especially thujone, is a critical factor for the use of *T. occidentalis* as a medicinal plant, as this compound, which occurs in nature as a mixture of α- and β- isomers (85% α-thujone and 15% β-thujone) has been described as a toxic agent for many plants that form part of the human diet, such as *Salvia folium* and *Artemisia pontica*.

However, its content in finished products containing *T. occidentalis* depends on the processing of the extract. Extracts obtained by distillation result in higher thujone content than in extracts obtained by percolation. Moreover, it has been reported that percolation with 30% ethanol (v/v) may significantly reduce the thujone content, when compared with percolation with 90% ethanol (v/v) and purified water [3].

These essential oils of *T. occidentalis* have also been reported as a potential insecticide. In relation to this application, by way of confirmation of the presence of α-thujone (49.64%), fenchone (14.06%) and β-thujone (8.98%), the oils were used against adult insects of *Callosobruchus maculatus* (bean weevil), which commonly infest seeds in the field. This assay was used to obtain satisfactory results for lethal doses (DL_{50}) with 1.1; 0.7; 0.5 and 0.2 mL/insect after 3, 6, 9 and 12 h, respectively, with a significant difference in relation to the control group treated only with an odorizer [44].

Pharmacological properties:

Preclinical *in vitro* and *in vivo* studies:

Antiviral and immunostimulant activities: Previous studies have shown the antiviral and immunostimulant properties of the *in vitro* polysaccharides of *T. occidentalis* regarding inhibition of HIV and influenza Type A [3]. Action on HIV cells has been previously reported by Gohla *et al* [26], who pointed out the ability of the high molecular weight polysaccharide fraction isolated from *T. occidentalis* (TPS) to inhibit HIV-1 at a concentration of 625 µg/mL without showing any toxicity towards MT-4 cells not infected by HIV-1. Furthermore, the expression of the specific HIV-1 antigen in recently infected MT-2 cells was inhibited at a concentration of 6.25 µg/mL, along with the reverse transcriptase (RT) of HIV-1. TPS is related to proliferation of $CD4^+$ T lymphocytes (LT $CD4^+$), interleukin-1, -2, -3, -6, granulocyte colony-stimulating factor (G-CSF) and interferon-gamma. These cytokines have been shown to enhance hematopoietic reconstitution after high-dose chemotherapy [45].

In relation to influenza A, the effects of oral administration of hydro-alcholic extract with a 30% hydro-alcoholic solution (w/w) obtained by percolation from *T. occidentalis, Baptisiae tinctoria, Echinaceae purpureae* and *Echinaceae pallidae* was tested in mice, with this extract being administered for 14 days, beginning six days before intranasal infection with the influenza virus. The extract was capable of bringing about a statistically significant increase in survival rate, prolonged the mean survival time and reduced consolidation and virus titers. The plant, in this case, exercises an immune-modulator effect six days before exposure and is a potent inhibitor of the influenza virus [46].

Generally speaking, fractions of polysaccharides were shown to be highly mitogenic in relation to peripheral leukocytes in the blood, with the induction of T-cells, especially LT $CD4^+$ [3, 26].

Homeopathic medicine with *T. occidentalis* at various centesimal dilutions (CH) was tested *in vitro* with macrophages obtained from mice, showing stimulation of the cytokines through Th1/Th2 and NO.

The relation between active macrophages and residents was statistically significant at a potency of CH30 and CH200; while there was little significance in the CH6 group, which also showed certain toxicity. At a potency of CH12 there was no morphological alteration in the macrophages. The author thus concludes that the CH6 and CH30 dilutions were shown to have triggered the release of IFN-γ cytokine, which, in turn may trigger the immune cell response or Th1 and, in general, the CH30 dilution presented results on morphological activation and production of cytokines simultaneously. Later these activities were supported by other additional ones related to homeopathic *T. occidentalis*, namely: an increase in the release of nitric oxide (NO) *in vitro*, a reduction in the superoxide anion (O_2^-) *in vitro* by macrophages and an increase in bone marrow cells, such as CD3, CD45R and CD11 [48]

The immunomodulating potential of the association between seven medicinal plants that make up the "Mais Vida" compound [Babaçu (*Orbignia martiana* Rodr.), Ipêroxo (*Tabebuia avellanedae* L.G.), Bardana (*Arctium lappa* L.), Rose (*Rosa centifolia* L.), Espinheira Santa (*Maytenus ilicifolia* Mart.), Boldo baiano (*Vernonia condensata* Baker) and *T. occidentalis*], were tested, showing that, in 25 samples, the mean production of O_2^- by mononuclear and polymorphonuclear cells increased significantly, indicating its dose-dependent immunostimulant effect with, in this case, less adverse effects, when compared to conventional treatment with chemotherapy and radiotherapy [49].

Likewise, *T. occidentalis* has been cited as one of the alternative treatments used against the poxvirus, which specifically affects sheep, causing cutaneous lesions (of the mouth and upper respiratory tract). In this case, *T. occidentalis* is generally used for the treatment of domestically raised chickens and ornamental birds. In a study conducted by Chaves et al. [50], which aimed to evaluate the activity of a dilute solution of *T. occidentalis* in water (5 mL/L), obtained from a commercial aqueous solution of 18% of *T. occidentalis* alcohol extract, orally administered for ten days in poultry (chicken) after infection, no detectable differences were found in the evolution and severity of the lesions in the test and control groups. However, under the conditions used for the test, there was no regression of the lesions, requiring additional studies to evaluate the interference of the means of administration, the immunological status and the environmental conditions of the poultry rose in different settings.

Hepatoprotective, antiulcerative and antidiabetic properties: In an analysis of hepatoprotective properties, the EFTO, with confirmed presence of flavonoides, tannins and polysaccharides, in a dose of 400 mg/kg, provided significant protection against acute and chronic liver damage in models involving rats with acute and chronic liver damage induced by CCl_4. The effects of this dose were also pronounced with the antagonism of an increase in the aspartate transaminase and alanine transaminase enzymes. The histological examination carried out after treatment confirmed the good hepatoprotective properties, which impede degenerative alterations [10].

Subsequently, to correlate the protective mechanisms of the phenolic compounds in this EFTO, studies by Dubey and Batra [12] revealed in a pre-medication test in rats that this compound provides a significant protective effect against gastric lesions brought about by aspirin, stress, alcohol, and HCl; protection against ulcers induced by ethanol in rats with a pillor ligature; antidiabetic activity at a dose of 200mg/kg and a significant increase in levels of glutathione in blood owing to the high quantity of phenol compounds (17.12 mg/g in the 90% ethanol extract and 25.20 mg/g in EFTO).

The antidiabetic properties were demonstrated by testing the EFTO for evaluation of glycemia on fasting, levels of glutathione in blood and biochemical analyses of the serum of albino Wistar rats with diabetes induced by aloxane, producing significant anti-hyperglycemic activity in the 200mg/kg dose, with a significant increase in glutathione, which has an antioxidant effect. Improvements were also observed in the body weight and lipid profiles, which also contributed to protecting against oxidative cell damage. Phytochemical investigations carried out on this fraction demonstrated the presence of flavonoids (kercetin, campherol), tannic acid, polysaccharides and proteins.

Efforts are currently being taken to isolate the active components of this fraction and confirm the mechanism involved [11].

Antioxidant and hypolipidemic activities: As for antioxidant activity, that of the EFTO was increased in a dose-dependent manner in the presence of various systems that point to the use of EFTO as a potential source of natural anti-oxidant. Using 100, 150, 200, 250 and 300 mg of EFTO, inhibited lipid peroxidation induced by $FeSO_4$ revealing an inhibitory concentration (IC_{50}) of 195.60 µg/mL. In DPPH (1, 1-diphenyl-2-picryl-hydrazyl), the elimination of radicals showed the value of IC_{50} for the extract of 202.45µg/mL. EFTO also inhibited the hydroxyl radical generated by the Fenton reaction with an IC_{50} of 158.59 µg/mL, and of O_2^- generated by the PMS/NADH-NBT system presenting an IC_{50} of 124.11µg/mL [13].

In another analysis, the antioxidant and hypolipidemic potential of EFTO in rats fed with cholesterol indicated the inhibition of lipid peroxidation induced by $FeSO_4$ in a dose-dependent manner (100, 150, 200, 250 and 300 mg/kg) demonstrating an IC_{50} of around 195.60 µg/mL. The hypolipidemic activity in the 200mg/kg and 400mg/Kg doses significantly reduced the cholesterol level of the serum (77% and 92%), the LDL (53 and 84%) and triglycerides (27% and 46%), with an increase in HDL in relation to total cholesterol and a reduction in the atherogenic index in the groups treated. This provides strong evidence of the antiatherosclerotic properties of *T. occidentalis*. The results obtained thus show that EFTO is a potential source of natural antioxidant and activities related to this [14]. Phytochemical investigations in these studies also showed the presence of flavonoids (quercetin, kaempherol), tannic acids, polysaccharides and proteins.

The study by McCune and Johns [51] also points out that the antioxidant activity of phenolic compounds of *T. occidentalis* is crucial to its use as a plant traditionally used by certain indigenous peoples to treat the symptoms of diabetes and its complications.

Treatment of warts: Taking advantage of the use of this medicinal plant for the treatment of condylomas in animals, preparations of *T. occidentalis* were tested in 40 cows, dairy crossbreed of dutch, created semi-intensively, with varying degrees of cutaneous papillomas. In this case, no partial and complete regression in the groups that received *T. occidentalis* MT at 30% and *T. occidentalis* MT at 30% with propolis. However, with the use of 10 mL of *T. occidentallis* CH6 daily for 63 days, there was a decrease of warts in 20% of animals and partial regression in 80% in the other, with gross changes color and size of the warts confirmed by histopathology. This finding contributes to the indication *T. occcidentalis* CH6 for bovine cutaneous papillomatosis, highlighting the need for studies for periods exceeding 63 days. The serum revealed that the plant did not produce liver and kidney during this period [52].

Antifungal and antimicrobial activities: It's *in vitro* antifungal potential in homeopathic dilutions shows activity against *Aspergillus flavous*, which causes cutaneous aspergilosis and *Aspergillus niger*, which causes otomycosis in humans, as evaluated by the inhibition of sporulation and exudation [53].

The methanolic extract of *T. occidentalis* was tested *in vitro* for activity against Gram-positive and Gram-negative bacteria (*Escherichia coli*, *Citrobacter*, *Shigella flexenari*, *Yersinia aldovae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*) and fungi (*Saccharomyces cereviciae*, *Aspergillus parasiticus*, *Trichophyton rubrum*, *Yersinia aldovae* and *Candida albicans*), suggesting strong activity against all micro-organisms, including resistant ones. These benefits point to the potential of medicinal plants and their products in emerging countries that enjoy a high degree of biodiversity and serve as the basis for future studies explorig its still unknown potential [54].

In another study, after confirmation of the presence of oils of sabinyl acetate (16.55%), fenchone (12.87%), sabinene (12.14%), β-thujone (9.48%), α-pinene (3.33%) and terpinen-4-ol (3.32%), the results for antimicrobial activity showed *T. occidentalis* to be active against *S. aureus*, *E. coli* and *E. faecalis*; and α-thujone and β-thujone very active against the Gram(-) bacteria *P. aeruginosa* and *K. pneumoniae*, and moderately active against *S. aureus*, *E. coli* and *C. albicans*.

Other studies, however, suggest that not only one or two of these compounds alone are responsible for the antimicrobial activity, the whole composition, with the alternance of synergies and antagonistic effects of each volatile oil [55].

In a study carried out by Castellón et al. [7] with tinctures of *T. occidentalis* in a concentration of 65% ethanol, it was observed that these were only active against strains of *S. aureus* and *Bacillus subtilis*.

A study conducted by Tsiri et al.[17], tested the essential oils of four varieties of *Thuja*, including *T. occidentalis 'globosa' and T. occidentalis 'aurea'*, against six Gram-positive and Gram-negative bacteria (*Staphylococcus aureus, S. epidermidis, Pseudomonas aeruginosa, Enterobacter cloacae, Klebsiella pneumoniae, Escherichia coli*) and three pathogenic fungi (*Candida albicans, C. tropicali*s and *C. glabrata*). They showed significant antimicrobial activity, with this being especially powerful, in the isolates of the mixture of α and β-thujone and beierene. The main constituents of these oils have already been described.

Antimetastatic and neuropharmacological activities: An evaluation was carried out of the potential anticancer activity of homeopathic MT of *T. occidentalis* and its thujone-rich fraction (TRF), which pointed to maximum cytotoxicity over malignant A375 melanoma cells. Furthermore, exposure of TRF to A375 cells *in vitro* showed greater cytotoxic, antiproliferative and apoptotic activity, in comparison with MT, but minimal growth in the inhibitory response when exposed to normal cells (mononuclear cells of peripheral blood). Both, however, caused intimate effects related to the generation of apoptosis in A375 cells. Thus, TRF and its correspondents showed all the responses to cancer of MT, and may therefore be the principal bioactive fraction. The use of MT in traditional anti-tumor medicines thus has a scientific basis [44].

In vivo experiments were conducted to test the preventive role of the leaves of *T. occidentalis* against breast cancer induced by DMBA [dimethylbenz(a)anthracene]. In this case, the extract of this vegetable produce with ethyl acetate (10mg/Kg) was tested against DMBA in rats with a mammary carcinoma, taking as a point of reference doxorubicin, which is the standard drug used in animals. This achieved a reduction in weight of 39% and in volume of 50% in the tumors, a reduction of 83% in glutathione (GSH) (83%) and malignant cancerous cells compared to the control group. This extract has been the subject of detailed chromatographic separation to reveal the most potent fraction of the *flavonoid* units, correlating this with activity against DMBA-induced mammary carcinoma [15].

In the case of metastatic cancer, in a preliminary study, the effects of *T. occidentalis* extract on cells with melanoma induced by B16F-10 in C57BL/6 mice were examined. The extract was administered simultaneously, prophylactically and after development of the tumor, achieving 74.4%, 71.5% and 60.2% reductions respectively in tumor nodules. The control of metastases was significantly reduced in treated animals and the time of life increased with treatment [56]. These results were then extended to evaluation of the extract of *T. occidentalis* and its polysaccharides, which cause a prior increase in metastatic cells of activity NK cells, of antibody-dependent cytotoxicity and complement-dependent cytotoxicity in relation to the control, as well as reducing levels of pro-inflammatory cytokins (IL-1β, IL-6, GM-CSF and TNF-α) in the serum of animals with tumors and an increase in the levels of anti-tumor factors IL-2 and TIMP [27].

Likewise, the potential for antimetastatic activity against cells with melanoma was proved to be related to the monoterpene thujone which, when administered at a dose of 1mg/kg of body weight, inhibited the proliferation and growth of tumor nodules in the lungs (59.45% and 57.54%, respectively) and led to an increase in the survival rate (33.67% and 32.16%, respectively) in animals with metastatic tumors. These results, correlated with biochemical and enzymatic parameters proves the antimetasthetic effect of the substance [57].

In a study carried out by MacLaughlin *et al* [58], the antiproliferative effect of prepared homeopathics of *Sabal serrulata, T. occidentalis* and *Conium maculatum* in mice with prostate cancer. The mother tincture was prepared with 67% alcohol, and these homeopathically diluted solutions were used to obtain a potential of greater than 200 CH.

In this study, only treatment with *Sabal serrulata* produced a positive result and no effect was observed with *T. occidentalis* and *Conium maculatum.*

Madhuri and Pandey 16 also highlighted the anticancer activity of T. occidentalis, among other medicinal plants, citing as the major active constituents flavonoid, tannin, volatile oil and mucilage.

During evaluation of neuropharmacological activity the aqueous extract obtained by decoction of the aerial parts of *T. occidentalis* at concentrations of 100, 200 and 400 mg/Kg, after administration in rats and mice, improved memory in animal models with amnesia induced by scopolamine and significantly repressed SNC, and acted as an anticonvulsant and muscular relaxant. The study also led to the recommendation that the drug not be administered during the day or before going to sleep, owing to the way it acts on SNC. In this case, additional studies are needed to isolate the constituents responsible for this activity [59].

Clinical Efficacy Studies: The MT of *T. occidentalis* is usually prepared by crushing its aromatic leaves and branches [60]. In popular medicine, this plant has been used to treat bronchial catarrh, cystitis, psoriasis, carcinoma of the uterus, amenorrhea and rheumatism [2], and it has been proven to be an effective treatment for acute respiratory infections, the common cold and to help with the treatment of bacterial infections [3, 61]. However, other properties have not been confirmed by clinical trials.

Immunostimulant: *T. occidentalis* in combination with other species as a component of homeopathic medicine composed of *Aconitum napellus*, *Arsenicum album*, *Asa foetida*, *Bryonia alba*, *Calcarea carbnica*, *Lachesis muta*, *Pulsatilla nigricans*, *Ricinus communis*, in various dilutions and formulations (including injectable, inhalable, vaginal creams and gels), provide immunomodulation of macrophages, lymphocytes and cytokines in cases of immune disorders, such as tumors, inflammation and infectious diseases, as described in the patent. This medicine was shown to be absorbed by the mucous membrane and, as there is no lethal dose of the compound, has been found to be safe for intravenous use.

In cancer patients, it provides a reduction in painful symptoms and an increase in quality of life, reduces the size of the humor and halts its development. The homeopathic compound obtained is not toxic and no interactions with other medicines have been reported and there is no interference in patients with heart conditions, diabetes, convulsions and mental disorders [62].

The immunostimulant role played by cytokines and leukocytes, however, was not found in a study using the Echinacea complex, comprising *Echinacea angustifolia*, *Eupafofiurn perfoliaturn* and *T. occidentalis*. Oral administration of the extract for four weeks, in patients undergoing curative surgery for a solid localized malignant tumor, did not lead to significant variations in the production of cytokines and populations of leukocytes in relation to the control group [63].

Treatment of condilomas: According to a previous in vivo study carried out by the European Academy of Dermatology, the efficacy of treatment of warts caused by HPV with full healing over three months was 90%. Since then, 30 patients aged between 7-40 years, with a mean age of 16 years, were randomized for a placebo-controlled study. The proportion of men to women in this study was 18 to 12 and the duration of the lesions was 17 months. With weekly application of an extract for three weeks, the results proved to be significant with 80% of active cases of warts cleared up and 33% of the control cases up to the first evaluation, with no evidence of recurrence during a six-month follow-up period and no signs of recidivism in more than one year. This follow-up study showed the benefits for treatment of warts caused by HPV, and efficacy against various types of HPV superior to the placebo with spontaneous resolution [64]. The extraction procedure was not described.

Furthermore, Leal et al. [65] submitted 18 outpatients (aged between 17 and 39 years, one of whom was male), with various degrees of condyloma and an evolution time of 15 days and six months, for localized treatment with *T. occidentalis* (balms and baths in MTs diluted in water) and by way of oral administration (CH 1, 10 drops twice a day) for a variable period, according to the regression of the lesions. The preparation procedure for MTs was again not described. Evaluation of efficacy was based on the disappearance of the lesions.

The results were excellent in all cases, with total regression in ten cases in up to 30 days, in four cases in up to 60 days and in the others in up to 106 days. The trials provided no evidence of side-effects. The cautious use of homeopathic treatments is therefore recommended as an alternative in cases of condylomas, which are frequently the cause of clinical dermatological and tocogynecological disorders.

However, according to a bibliographical survey carried out by Simonart at al.[66], homeopathic treatments for skin diseases, including preparations of *T. occidentalis* at dilutions of CH30 and CH6, did not achieve sufficiently conclusive results to justify the use of homeopathic substances for any skin conditions. In this case, it was seen that the concentrations tested were not adequate for the desired efficacy.

In contrast to this result, Bergo et al. [60] found benefits in the homeopathic use of *T. occidentalis*. Since 1993 they had begun to make the first clinical observations of the action of this treatment on HPV lesions, which were carried out at the Public Homeopathy Outpatients Clinic, in partnership with the Cervical Pathology Outpatients Clinic (both linked to the Municipal Health Secretary of the city of Campinas, in the Brazilian State of São Paulo). The initial results were presented at the 54th PanAmerican Homeopathy Congress, in Ribeirão Preto, in the State of São Paulo. Given the impact of this work, the authors presented it in various seminars, lectures and reviews, as well as in two important publications in 1997 and 2000. Over the years, authors have come to recognize the need for systematization of the use of this preparation, as a way of meeting growing demand from patients. A treatment protocol has thus been drawn up for use at various outpatient clinics, for the treatment of genital lesions caused by HPV.

The treatment protocol suggests concomitant oral and topical use. For oral use, one MT was prepared for crushing at a proportion of 1:10, at different dilutions (CH12, CH30, CH200), according to the German pharmacopeia. For local use, it is recommended the vaginal ovules delivered in cocoa butter once a day for 15days and the application of cotton wool steeped in MT twice a day in area vulvar /penile for fifteen days.

If this stings, it is suggested that the MT be diluted in water to a final concentration of 50%. The MT for topical use was obtained with 20% from the dry plant in ethanol 90% [60].

According to a previous study by the authors, the idea of treating HPV patients with homeopathic medicine arose from the need to find treatments that are less aggressive than current allopathic medicines. This study involved for a period of 12 months, 198 female patients carrying the virus. The women returned for evaluation after 30, 60, and 90 days, according to availability and the seriousness of cases. A colposcopy diagnosis was carried out and all patients underwent a cytological examination. Treatment was initiated only in those whose cytology tested positive for suspected HPV lesions. Once a definitive diagnosis had been reached all received *T. occidentalis*, treated according to homeopathic techniques [67]. After 90 days of patient follow-up the following results were obtained: 84.2% receive tested negative on oncotic cytology and colposcopy, while 15.5% tested positive for viral condyloma. This study concluded that *T.occidentalis* treatment was more effective than other allopathic medicines (Metacresol sulfonic acid – 37.5%; Podophiline 4% Gel – 64.7%; Podophiline 2% Gel – 44.4%; Fluoruracyl 5% - 60.2%; Interferon Gel – 64%), in addition to the fact that Thuja has virtually no side effects.

Treatment of respiratory infections: The commercial preparation Esberitox®N (Aspen Australia), containing *Echinacea purpurea*, *Echinaceae pallida*, *Baptisia tinctoria* and *T. occidentalis*, presents in several clinical studies proven action for acute and chronic respiratory infection (viral or bacterial), including for children [68, 69, 70] and as adjuvant antibiotics, in case of a serious bacterial infection [61, 71].

Results of a prospective, randomized, placebo-controlled clinical study confirms the efficacy and safety of this drug for treatment of the common cold, indicating that the time to symptom improvement was 1.1 days, 0.76 days and 0.52 days in compared with placebo, the groups of low dose and high dose respectively, ie the effectiveness varies in a dose-dependent and furthermore, no evidence of adverse effects, even at higher dosages [70].

Treatment of urethral caruncle: The assessment for this activity was described in prospective, longitudinal descriptive analytic, with group of 46 women diagnosed with urethral caruncle, which were followed from 2000 to 2005 using the homeopathic remedy *T. occidentalis* 12CH. As a result, the treatment increased the result of disappearance of the lesion before three weeks of treatment in 92.8% of patients. In 85.7% of these results exceeded one year of evolution, without the appearance of recurrence and associated symptoms. Thus, this therapy is efficient, non-invasive, reliable and low cost, eliminating the usual surgical procedures and long periods of treatments with estrogen [72].

Toxicity: The symptoms of being poisoned by the fresh plant include vomiting, stomach ache, diarrhea and gastroenteritis, followed by absorption disorders, headaches, nervous agitation chronic convulsions and liver and kidney poisoning, leading to atrophy, arrhythmia and myocardial bleeding. In cases of overdose and abuse, oral ingestion of extracts of *T. occidentalis* may bring on severe metabolic disorders, irritating the gastrointestinal tract, the liver, the uterus and the kidneys. In cases of babies who have ingested the leaves and branches of the fresh plant mild gastrointestinal disorders and vomiting are found [3, 73].

Despite all the studies suggesting the effectiveness of *T. occidentalis*, which is much used in herbal medicine and homeopathy, it is not used that much in medicine in general because of the toxic properties of the constituent of essential thujone oil. The following information has been reported on the acute toxicity of thujone (DL_{50} in mg/kg): orally in rats, 500; IV in rabbits, 0.031; subcutaneously in rabbits, 5000. A lethal dose was reached at 0.2 mL/Kg body weight. Up to a single daily dose of 75 mg has been reported to be safe in human beings [3].

In general, according to a survey carried out by Naser *et al* [3] regarding adverse effects observed in clinical trials, adverse reactions were generally found to be slight or moderate, and the preparations obtained from this plant considered safe for respiratory infections. Furthermore, there is no direct or indirect abortive effect, although, given the absence of conclusive results, the general recommendation is that, during pregnancy and lactation, preparations based on this medicinal plant not be administered without the consent of a physician. The toxicity of *T. occidentalis* was also tested by Valsa and Felzenszwalb [6] using samples of three tinctures to evaluate *in vitro* genotoxicity by way of two quick tests (the Ames test - Mutatest and the SOS-chromotest - β-galactosidase induction in *Escherichia coli*). This study led to the conclusion that none of the extracts positively induce mutagenesis or synthesis of β-galactosidase (a general and early sign of DNA damage), even with metabolization.

This result has recently been confirmed by Nikolić *et al* [74], who investigated the potential antigenotoxic properties of *T. occidentalis* monoterpenes, camphor, eucalyptol and thujone, on procariot and eucariot cells, demonstrating its effect in terms of DNA repair. The results indicate that small quantities of camphor, eucalyptol and thujone may stimulate DNA repair and act as bioantimutagenics. However, larger quantities of monoterpenes may rupture DNA filaments.

Technological Aspects: In a study evaluating the quality control of nine batches of commercial homeopathic MTs in Cuba, using the method described in the French pharmacopeia, analysis was carried out of: organoleptic characteristics, the refraction index (1.3), relative density (0.89), pH (5,4), ethanol (around 68%) and dry residue (over 1.3%), apart from identification reactions and the phytochemical profile of the tinctures. These results showed that, in terms of the organoleptic characteristics, the tinctures were translucent, with no brownish-green coloration or an aroma characteristic of vegetal drugs.

All the batches responded to reactions to identify secondary metabolites and the phytochemical profile identified the presence of lactanic compounds, carotenes, essential oils, triterpenes and steroids, reducing sugars, saponins, phenols, tannins, amines, flavonoids, mucilages, and bitter principles. In terms of the other physico-chemical controls cited, the results obtained were capable of reproduction.

The preliminary stability study also showed that the tinctures have suitable characteristics for conservation in 30 mL colored glass ambar flasks at ambient temperature for a year [7].

Pozetti *et al* [41] carried out thin-layer chromatography for six samples of *T. occidentalis* MT from European countries (two tinctures from foreign laboratories and one from Portugal, prepared in Brazil) and from Brazil (three national tinctures of the plant grown in Brazil). It was concluded that there is a significant difference in the qualitative and quantitative composition of samples, with those of foreign origin having more constituents. These differences may be explained by the different origin, the collection time, the drying method, the material used for conservation, the parts used (young or old branches), the extraction process and so forth. According to this author, the efficacy of these preparations in clinical practice may change, thereby compromising the expected results.

Phytochemical and physico-chemical investigation of leaves of *T. occidentalis* of Indian origin, carried out by Meenu et al. [75] included evaluation of total ash (6.14%, p/p); water soluble ash (4.5%, p/p); the determination of constituents obtained by hot extraction (83.1 mg/g), cold extraction (39.67 mg/g), and with ether (4.7 mg/g); determination of volatile compounds and water (150 mg); spume index (18.18), according to the Dutch method.

The metabolites common to the methanol and hydro-alcohol extracts obtained by microwave extraction included carbohydrates, saponins, phenolic and tannic compounds (with a 5% solution of iron chloride), non-reducing polysaccharides and flavonoids. Thin-layer chromatography was also carried out for two extracts with different mobile phase systems, obtaining maximum separation of constituents and Rf values clearly defined in the toluene system: ethyl acetate: formic acid: water (100: 36: 36: 10).

SUMMARY: About the specie mentioned above, there are very ancient scientific records reflecting its traditional use by populations for the treatment of various symptoms, particularly respiratory infections of the upper tract and the common cold, to help with the treatment of bacterial infections, treatment of warts, Attributed primarily to its immunostimulant and antiviral properties. Thus, to elucidate the chemical composition of this species and its metabolites associated with these activities, many studies over time have given prominence to the constituents of the essential oil, which, in fact, have contributed to the pharmacological properties presented by *T. occidentalis*. However, a measure that certain majority monoterpenes were being disclosed as responsible also for the toxicity of the plant, preclinical studies latest revealed the presence of other metabolites of relevance, such as flavonoids, tannins and polysaccharides associated with various pharmacological activities, apart from those previously reported (anti-cancer, hypoglycemic, hepatoprotective, anti-ulcer, anti-diabetic and antioxidant).

Over the years, clinical trials, particularly for the treatment of condyloma, confirm the efficacy of *T. occidentalis* for the treatment of lesions caused by HPV, but it is still necessary to establish more detailed methods for performing a more accurate assessment of efficacy and safety.

Another important point to be explored concerns the need to isolate the components responsible for the therapeutic activity of *T. occidentalis*, especially the constituents of EFTO whose preclinical studies indicate results of relevant pharmacological activities. Thus, the discovery of these markers can corroborate for standardization of herbal drugs, which allows initially set quality parameters for tinctures, extracts and other products obtained from *T. occidentalis*, some of which are already available on the market, such as Esberitox®N as well as their subsequent efficacy and safety.

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