Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
https://www.phytojournal.com
JPP 2023; 12(6): 48-58
Received: 08-10-2023
Accepted: 16-11-2023

Rui Aguiar
1. Escola de Medicina Tradicional Chinesa, Lisboa, Portugal
2. IPLuso-Instituto Politécnico da Lusofonia, NICiTeS - Núcleo de Investigação em Ciênciase Tecnologias da Saúde, Lisboa, Portugal

Maria do Céu Costa
1. IPLuso-Instituto Politécnico da Lusofonia, NICiTeS - Núcleo de Investigação em Ciênciase Tecnologias da Saúde, Lisboa, Portugal
2. Universidade Lusófona, CBIOS, Research Center for Biosciences & Health Technologies, Portugal

Corresponding Author:
Rui Aguiar
1. Escola de Medicina Tradicional Chinesa, Lisboa, Portugal
2. IPLuso-Instituto Politécnico da Lusofonia, NICiTeS - Núcleo de Investigação em Ciênciase Tecnologias da Saúde, Lisboa, Portugal

Active compounds and biological activities of *Hypericum androsaemum* L.: A review

Rui Aguiar and Maria do Céu Costa

DOI: https://doi.org/10.22271/phyto.2023.v12.i6a.14770

Abstract
Hypericum androsaemum L. is a plant renowned in the Mediterranean basin for diuretic, hepatoprotective, anti-hypertensive, anti-hemorrhoidal, anti-depressant and skin-protective effects. This review makes a critical summary of published pharmacognostical data. *H. androasemum* contains significant amounts of phenolic compounds, namely chlorogenic acids, flavonoids, triterpenoids, polycyclic polyprenylated acylphloroglucinols (PPAPs) and xanthones. Extracts of the plant have shown radical scavenging activity. The fruits' extracts have shown cytotoxic activity against some cancer cell lines. The plant has some immunomodulatory, DNA-protective, anti-glycation, antidepressant and anti-inflammatory effects. Given its effect on fibroblasts proliferation and migration, collagenase and tyrosinase inhibitory action, immunomodulatory effect, UV-protective and lack of phototoxicity, it has potential in skin formulas. It has shown biocidal activity against some bacteria, fungi, nematodes, mollusks and insects. At low concentrations, the extracts were nontoxic on normal cells. However, safety issues may arise with oral administration of high hyperforin *H. androasemum* preparations.

Keywords: *Hypericum androsaemum*, phytoactive compounds, biological activities, hyperforin

1. Introduction
Hypericum androsaemum L. is a small shrub common in the north of Portugal that displays yellow flowers in the summer, followed by berry-like red fruits that mature to black [1]. It is popularly known as tutsan, sweet amber, and hipericão-do-Gerês (Gerês being a mountain region in Portugal), endemic to Western and Southern Europe, and the Near East beyond the Mediterranean Region [2].
In contrast to *H. perforatum* and most of the *Hypericum* species, that have dry capsules as fruits, *H. androsaemum* produces fleshy berry-like capsules [3] (Figure 1). These capsules undergo a color transformation from red to black as they reach maturity [4]. Additionally, *H. androsaemum* does not have the dark leaf nodules that synthesize naphtodianthrones like hypericin and its derivatives, very abundant in *H. perforatum* [5].

Fig 1: Image of *Hypericum androsaemum* by Coli, Jardim Botânico UTAD, Flora Digital de Portugal

Due to its upright growth form, leaf variegation, flashy flowers and attractive fruits, *H. androsaemum* is cultivated extensively as an ornamental plant [5-6]. It has also been used in several regions for its medicinal properties. The popular name "tutsan" derives from "toutsaine", meaning 'all-heal'. It was one the four *Hypericum* species recommended by Dioscorides, who prescribed it for sciatica and burns [2].In Portuguese folk medicine, it is known for its diuretic effect, that granted it the name 'erva-mijadeira' ('diuretic herb'). Infusions are used for kidney and bladder ailments. It has also been used for its hepatoprotective and antihypertensive effects [1, 3, 7, 8].

Although this species does not contain hypericin, the compound to which the anti-depressant effect of *H. perforatum* is mainly credited, *H. androsaemum* has been used as an antidepressant and anxiolytic in Portuguese and Spanish folk medicine [5, 9, 10]. That may be supported by the neuroprotective and antidepressant activities of the phenolics and other compounds [5, 9, 10].

It has also been traditionally used as anti-haemorrhoidal, and to prepare ointments to treat cuts and wounds in England and in Iran [4, 11, 12].

2. Materials and Methods

A PubMed search was performed in August 2023 looking for the term "*Hypericum androsaemum*" in the full text, without any date restriction. 37 papers were found. This search was complemented by a similar one on Google Scholar, which returned an unbound number of results. The first 100 results were scanned and, after the exclusion of duplicates from Pubmed and of articles not related with pharmacological activities, 8 were added to the library. The data presented in this work summarizes the main reports on its phytochemical compounds, ethnomedicinal uses and pharmacological activities. During the writing phase, 10 articles about pharmacological properties of individual chemical compounds were included.

3. Results and Discussion

3.1 Phytochemical compounds: *H. androsaemum* contains several types of compounds, like organic acids, phenolic compounds, flavonoids, triterpenoids, vitamins, minerals, and fatty acids. Its chemical composition has been studied by many authors through the chromatographic analysis of several types of extracts of different parts of the plant.

In a recent study, the most abundant secondary metabolites found in the leaves were caffeic acid (3313±80 µg/g) in the leaves water extract, and (-)-epicatechin (6538±235 µg/g), (-)-epigallocatechin gallate (3035 ± 82 µg/g), myricitrin (3657 ± 113 µg/g) and hyperoside (3713 ± 111 µg/g) in the leaves methanol extract [11]. In the aerial parts, quinic or caffeoylquinic acid, quercetin derivatives, catechin and kaempferol are the major compounds, according to several studies [13, 16].

The red fruits contain a high amount of shikimic acid, as high as 12.799 mg/g DW (Dry Weight). Still, chlorogenic acid and neochlorogenic acid are the most abundant phenolic acids in the fruits, reaching as much as 15 g/g DW and 6.6 g/g DW respectively in the red fruits, being much smaller or even undetected in some samples of the black fruits [1]. In a study focusing only on the red berries, the most abundant compounds found both in the water and methanol extracts were shikimic acid (115 901±8 284 mg/kg), chlorogenic acid (57 002±94), catechin (5 770±27), hyperoside (2 831±136), epicatechin (1 887±185), rutin (1411±207) and neochlorogenic acid (944±s30). Similar results were obtained in other studies [4, 9].

The differences between the compositions of red berries, black berries and aerial parts and leaves are explained by the transformations that occur during the phenological transitions. For instance, shikimic acid is the precursor of aromatic amino acids and phenolic compounds, being more abundant in the early stages. The main sites of chlorogenic acid accumulation were found to be the leaves, and the amount was found to decrease linearly with the progression of phenological stages, with the top level being attained at the vegetative stage [17, 18].

H. androsaemum is devoid of the black nodules that are the typical secretory structures where naphtodiantrones such as hypericin and pseudohypericin are produced in other *Hypericum* species [1, 19]. The fact that *H. androsaemum* is a hypericin-free plant, can be considered as an advantage in some cases, especially skin-related activities, since hypericin can cause phototoxicity, and can also induce activation of hepatic enzymes [11].

H. androsaemum contains several types of phenolic compounds, namely phenolic acids, lignans, quinones, xanthones and polycyclic polyprenylated acylphloroglucinols (PPAPs) [1, 14, 17, 20, 21].

Compared with *H. perforatum*, *H. androsaemum* showed a higher total phenolic contents in almost all the samples analyzed in a study [22]. Among 30 *Hypericum* species screened in another study, *H. androsaemum* had one of the highest contents in phenolic acids, but the lowest flavonoid amount [4, 22, 23].

3.1.1 Phenolic acids

Chlorogenic acids are the most abundant phenolic acids in this species. They include several esters of caffeic acid and quinic acid, like 5-O-caffeoylquinic acid (chlorogenic acid), 3-O-caffeoylquinic acid (neochlorogenic acid) and 3,5-dicaffeoylquinic acid [1, 5, 9, 17, 24]. These compounds have been reported to exhibit antidepressant, antioxidant and anti-inflammatory effects and to have high potential in the treatment of depression, and improvement of learning and memory [17, 25].

H. androsaemum also contains ferulic, fumaric, gallic and rosmarinic acids [5, 9, 11].

3.1.2 Flavonoids

Several types of flavonoids have been found in *H. androsaemum*: flavanols (catechin) and their derivatives, flavonols (quercetin, kaempferol) and their derivatives, flavones (7-O-glucosyl luteolin, biapigenin and aumentoflavone), flavanones (eriodictyol-O-glucoside and taxifolin) and several proanthocyanidins [1, 5, 9, 14, 15, 17, 20, 24, 26].

The total flavonoid contents in dichloromethane extracts of the plant has been measured recently as 86.47 µg/g in the leaves leaves, and 9.24 µg/g in the fruits [11].

Another study found the flavonoids to be better extracted with water infusions than with methanol, reaching a total of 2463 mg rutin Eq/Kg in red fruits. In black fruits the highest content was 1140 mg rutin Eq/Kg [1].

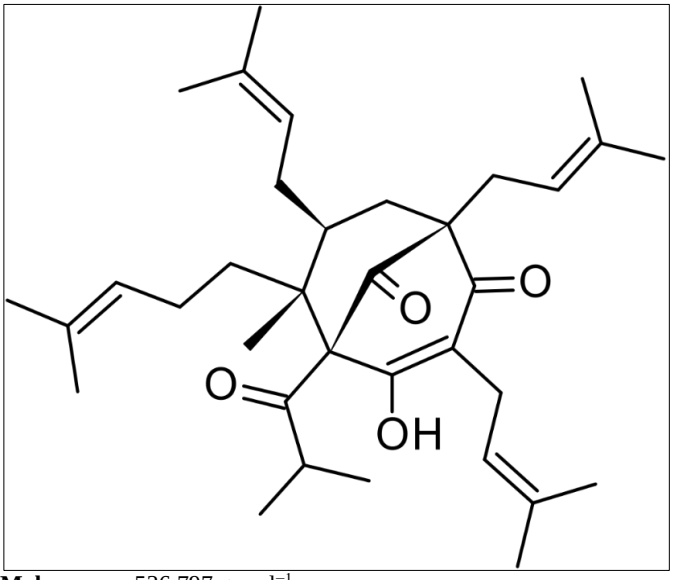
The most abundant flavonoid aglycones in methanol extracts were found to be apigenin, quercetin and rhametin [27].

Quercetin has various biological properties, including antioxidant, anti-inflammatory, antibacterial, antiviral, radical-scavenging, gastroprotective, and immune-modulatory activities [28]. Catechin has been studied for its anti-cancer potential, showing anti-oxidative, pro-oxidative, and anti-inflammatory activities, among others [29].

3.1.3 Polycyclic Polyprenylated Acylphloroglucinols (PPAPs): PPAPs contain an acylphloroglucinol core, which consists of a phloroglucinol moiety (a benzene ring with three hydroxyl groups) bearing one or more acyl groups, along with multiple polycyclic and polyprenylated structures.

The total acylphloroglucinols content in the ethanol extract of *H. androsaemum* flowering tops was measured as 10.69 mg/g DW, around four times less than that of *H. perforatum* or *H. perfoliatum*. In these extracts, hyperforin and adyperforin were measured at 9.34 and 1.35 mg/g DW, respectively [14]. In the methanol extract of aerial parts, the hyperforin content varied with the phenological stage of the plant, ranging between 0.39 and 2.37 mg/g DW [17]. Androforin A and hyperandrone A were other PPAPs detected in *H. androsaemum* [20].

PPAPs have well-reported biological activities, such as anti-HIV, antidepressant, antibacterial, antimalarial, antioxidant, anti-neurodegenerative, antiulcer, wound healing, and anti-inflammatory. Hyperforin (Figure 2) is one of the main characteristic bioactive compounds in the *Hypericum* genus [30].



Molar mass: 536.797 g·mol^{-1}

Fig 2: Molecular structure of Hyperforin, $C_{35}H_{52}O_4$

3.1.4 Xanthones

Xanthones are a class of compounds that tend to be less abundant in nature than other phenolic compounds. The pigment giving the red color to the unripe capsules of *H. androsaemum* has been identified as 1, 2, 3, 5-tetrahydroxyxanthone [1]. 1, 3, 6, 7-tetrahydroxy-8-prenylxanthone, 1, 3, 5, 6-tetrahydroxy-2-prenylxanthone, y-mangostin, 1, 7-dihydroxyxanthone and cudraxanthone K have also been detected [21]. Previously, hydroxy and methoxy substituted xanthones had been isolated from roots, and oxygenated xanthones and prenylated xanthone aglycones and their glucosides were also found in the plant [9].

In cultures of cells of the plant, xanthone accumulation was found to parallel cell growth and to be repressed by light and strongly influenced by the culture medium [21].

These heterocyclic compounds containing oxygen have many interesting pharmacological and biological properties, such as monoamine oxidase inhibition and antioxidant, antifungal, cytotoxic, and hepatoprotective activities [31].

3.1.5 Triterpenoids

The presence of acetyloleanolic acid, β-Amyrin, putranjivic acid and friedelin has been detected in the methanol extract of the aerial parts. Some of these compounds have exhibited cytotoxicity against several human cancer cell lines [20]. Hederagenin and quillaic acid were also detected [11]. Red fruits have higher content of essential oil and monoterpenes hydrocarbons [32].

3.1.6 Other secondary metabolites

The lignan 1, 4-O-diferuloylsecoisolariciresinol was identified in the aerial parts of the plant. The strong cytotoxic activity of this compound against several cancer cell lines had been reported, and was found in a recent study to be comparable to that of cisplatin [20].

Hernianin, or 7-methoxycoumarin, was found in substantial amounts in the fruits [11]. It has been found to have cytotoxic and anti-inflammatory activities [33, 34].

3.1.7 Vitamins and minerals

The unripe fruits are a good source of ascorbic acid, reaching 136 mg/100g DW. This is only 3 times lower than the fruits of *Rosa canina* L. and about 2 times higher than orange. The fresh fruits also contain 1.2% of vitamin K, and less than 1% of Ca, Si and Cu [35].

3.1.8 Fatty acids

The fruits contain large amounts of fatty acids, namely linoleic acid (reaching 46.9% of DW), linolenic acid (reaching 23.03%), oleic acid (14.96%), stearic acid (11.8%) and palmitic acid (10%). The ratio of PUFA n6/n3 is 2.2 in red fruits and 1.8 in black fruits [35].

3.1.9 Essential oil composition

More than 100 components have been identified in the essential oil of *H. androsaemum* in different studies. These studies found sesquiterpenoids to be the most representative group in the leaves and flowers, with estimates of between 43% and 98.5% of the oil composition. [12, 36, 37]. In the dried leaves from Iran the most representative molecules were found to be caryophyllene oxide (35.8%), ishwarane (30.5%) and humulene epoxide II [12]. In the flowers from Iran they were α-guaiene (40.2%) and caryophyllene oxide (28%) [12]. In plants from Turkey, germacrene B (31.50%), α-zingiberene (22.75%), α-curcumene (15.21%) β-caryophyllene (14.34%) and naphtalene (11.87%) were recorded as major volatile components depending on phenophases [17]. In plants from the North region of Portugal, the most abundant were (E)-caryophyllene, β-gurjunene and γ-Elemene [36, 37]. Sesquiterpenes are considered to be responsible for the specific essential oil olfactroscopic pattern of *H. androsaemum* [37].

In the fleshy red and black fruits from wild and cultivated plants in central Italy, the most representative group was found to be the monoterpenoids, accounting for between 78.8% and 84.0%, with limonene (42.9–50.9%), β-pinene (18.8–20.0%) and α-pinene (9.3–12.7%) being the most abundant. Oil yield by dry weight was 0.3% ± 0.03 and 0.1% ± 0.01 for red and black berries, respectively. Red fruits had a higher oil yield (0.3% vs 0.1%), higher amount of monoterpene hydrocarbons (84.0 vs 78.8%, respectively) and lower amount of sesquiterpene hydrocarbons (11.9 vs 16.7%) when compared with black fruits [35].

Many factors can account for differences in the volatile composition, including collection time, chemotypes, drying conditions, mode of distillation, geographic and climatic factors [12]. The floral budding and flowering phases were characterized by higher accumulation levels of majority of phenolic and volatile compounds in the plant [17].

3.2 Antioxidant activity

Free radicals, among them reactive oxygen species (ROS), play an important role as triggers in a wide variety of diseases, such as degenerative and metabolic disorders, and cancer [26]. Oxidative stress is widely acknowledged as a significant harmful process that occurs in various liver conditions, spanning from metabolic issues to alcoholic liver disease, chronic viral hepatitis, autoimmune liver diseases, and non-alcoholic steatohepatitis, leading to degenerative liver diseases and hepatocellular carcinoma. The search for antioxidant compounds is an important ongoing quest. Many phenolic compounds have showed important antioxidant properties.

The protective effect of *H. androsaemum* ethanol-water extract of aerial parts against several oxygen and nitrogen reactive species was found to be comparable or better than that of compounds like ascorbic acid, mannitol, rutin, cysteine and Trolox [8].

In another study, the ethanol-water extract showed lipid peroxidation inhibitory activity, DPPH scavenging and reducing power comparable to that of Trolox. The β-carotene bleaching inhibitory activity was found to be more than one order of magnitude inferior to that of Trolox [26].

The methanol extract of aerial parts has exhibited a radical scavenging capability just 3x lower than that of propyl gallate in the anion and DPPH tests [38].

A study with the water extracts of leaves against the superoxide radical, found a much higher scavenging activity on an enzymatic radical generation system (2.9 µg/mL) than on a non-enzimatic generation system (25.6 µg/mL). A part of the radical reduction effect observed may thus have been due to the observed moderate noncompetitive inhibitory effect on the enzyme generating the radical, and not solely to scavenging activity [39].

In the CUPRAC assay, the methanol extract of leaves has showed better results than the control substance alpha-TOC [11].

In a study of the ethanol extracts of 6 Hypericum species (*H. acutum*, *H. androsaemum*, *H. barbatum*, *H. hirsutum*, *H. maculatum*, and *H. richeri*), all of them exhibited higher DPPH scavenging activity than the lyophilized water extract of green tea, although a weaker anti-lipid peroxidization activity. Compared with the other species, *H. androsaemum* had the highest neutralization action upon superoxide anion radical (EC_{50} = 125 µg/mL), in the same level as the lyophilized water extract of green tea, and approximately just the double of Trolox's. In contrast to the DPPH• and ABTS•+, superoxide anion radical is a reactive oxygen species that can be found in biological systems [24].

The observed radical scavenging activity is attributed to the phenolic compounds of the plant, the most abundant being chlorogenic acids, catechin and its derivatives and quercetin and its derivatives. It should be noted, however, that different factors can influence the mechanisms involved in the individual and collective action of the compounds, including the concentrations used, the synergies among components and the environmental conditions.

3.3 Biological activities *in vitro* and *in vivo*

3.3.1 Cytotoxic and pro-apoptotic activity

The highest cytotoxic activity of the plant, with IC_{50} values below 20 µg/mL, has been obtained with methanol extracts of black fruits against cultures of A375 (malignant melanoma), MDA-MB 231 (breast adenocarcinoma) and CT116 (colon carcinoma), with the latest being the most relevant (IC_{50} = 8.4 µg/mL). The red fruits have shown similar activity, especially against CT116 (IC_{50} = 19.4 µg/mL) [1].

Against colorectal cancer cells, a mild 40% apoptosis in CO115 cells has been obtained with the total methanol extract at 40 µg/mL, with a significant reduction in the expression of BRaf, pErk and pAKT. The results with individual fractions did not explain the levels of apoptotic activity reached with the total extract, suggesting that synergistic effects among several constituents may be at play [40].

The water extract of aerial parts inhibited cell proliferation and induced apoptosis through several mechanisms in 2 different colon cancer cell lines, HCT15 (KRAS mutation) and CO115 (BRAF mutation). It decreased BRAF and phospho-ERK expressions in CO115, but not in HCT15. It decreased Akt phosphorylation in CO115 and induced p38 and JNK in both cell lines. It induced cell cycle arrest at S and G2/M phases as well as caspase-dependent apoptosis in both cell line [41].

The cytotoxic effect of the ethanol extract against A1235 (glioblastoma) cells, reaching 43.9% cell death after 72 hours, was one of the highest among 16 *Hypericum* species tested in a study. Given that microscopic examination of the cells showed apoptosis as the dominant type of cell death, and the long incubation times or doses required, the authors imputed the cytotoxic effects to alternations or interruptions in the cell cycle. The higher activity of *H. androsaemum* was due to the higher amount of phenolic acids and lower amount of flavonoids. Hypericin is known to have an important cytotoxic effect, but it is absent from this species. However, hyperforin, which is present in substantial amounts, has been reported to have pro-apoptotic and tumor inhibitory activity. None of the species tested had cytotoxic effect on MDA MB-231(breast cancer cells) [42].

A weak induction of apoptosis by the methanol extract of ripe fruits on PC-3 (prostate adenocarcinoma cells), with 64.75% cell death at 50 µg/mL was observed in another study. The cytotoxic activity of this extract was also weak, with IC_{50} = 74 µg/mL. The result with leaves extracts were even lower, with IC_{50} > 50 µg/mL in the dichloromethane fraction of the extract and apoptosis rate of 10.54% at 200 µg/mL of the total methanol extract [11]. Weak effects were observed against HepG2 (hepatocellular carcinoma) cells, with the best cytotoxic activity observed in the methanol extract of ripe fruits (IC_{50} = 32 µg/mL) and the best pro-apoptotic activity observed in the methanol extract of leaves (28% cell death at 25 µg/mL) [11].

The results with the ethanol-water extracts of aerial parts against this cell line as well as against MCF-7 (breast adenocarcinoma), HeLa (cervical carcinoma) and NCI-H460 (non-small cell lung cancer) were very weak, all with GI_{50} > 100 µg/mL, which was around 1/100 of that of ellipticine [26].

Two compounds isolated from the plant extracts were tested against five human cancer cells: HL-60, SMMC-7721, A-549, MCF-7, and SW480. 1, 4-O-diferuloylsecoisolariciresinol had high cytotoxic activity against all these cell lines, comparable to cisplatin's, especially in HL-60 and MCF-7. Acetyloleanolic acid also had IC_{50} values bellow 20 µg/mL against HL-60, SMMC-7721 and A-549 [20].

Several compounds of *H. androsaemum* have already been identified as cytotoxic against several cancer cell lines: the phenolics chlorogenic and shikimic acids, the flavonoids quercetin, catechin, epicatechin and rutin, and the xanthones. The triterpenoid quillaic acid had been shown to have apoptosis induction potential in several human cancer cells, including colon and gastric cancer [1, 41].

But the biological action of plants' extracts cannot be reduced to the sum of isolated actions of its top compounds. Synergistic or counteracting effects will occur, and they can involve compounds found in smaller quantities. For instance, their antioxidant activity may undermine the desired cytotoxic effect, since the oxidative damage induced by the cytotoxicity could partially be antagonized through different mechanisms, including scavenging ROS or competing for the light energy. The actual action will depend on the characteristics of the cell, the environmental conditions and other factors. For instance, flavonoids cannot be considered only as antioxidants, since under certain reaction conditions they can also display prooxidant activity, resulting on cytotoxicity [14, 43]. The amount of substance used can also change the effect drastically, the most obvious being that at very high concentrations they will typically be toxic [1].

Even the tests with isolated compounds do not always yield the same conclusion. For instance, chlorogenic acid alone didn't have a significant cytotoxic effect against colon cancer cells in one study [41], but in a different one it strongly inhibited cell viability, through ROS production, S-phase arrest and extracellular signal-related kinase inactivation [44].

The factors influencing these outcomes require more detailed investigation, to assess the potential of *H. androsaemum* or its active compounds in cancer.

3.3.2 Immunomodulatory

The effect of red berries methanol extract on the proliferation response of peripheral blood mononuclear cell (PBMC) of pigs activated by phytohemagglutinin (PHA, a polyclonal activator of T cells) and pokeweed mitogen (PWM) was studied. In PWM activated cells, the extract enhanced the proliferation response, increasing with concentrations from 0.4 to 6 µg/mL. In PHA activated cells, the proliferation increased at lower concentrations in the same range. This suggests that red berries of *H. androsaemum* may modulate the immune system, although the mechanisms of action remain to be clarified. The extract also elicits an inhibitory/cytotoxic reaction that, at high doses (10 µg/mL), resulted in the death of all cells [1]. A modulatory effect on the cytokine production by PBMCs has also been observed [4].

3.3.3 DNA protective

H2O2 is one of the main reactive oxygen species (ROS) that cause DNA damage in cells mainly due to its conversion into hydroxyl radicals through the Fenton reaction. Hydroxyl radicals are highly reactive with biomolecules such as DNA inducing DNA strand breaks. The protective effect on DNA against damage induced by H2O2 was tested for *H. androsaemum* water extract, along with the extracts of *H. perforatum* and *H. undulatum*, quercetin and rutin, in colon cells (HT29, a line of human colorectal adenocarcinoma cells with epithelial morphology). At low concentration (1 µg /ml), *H. androsaemum* achieved 40% protection, a value similar to those of *H. undulatum*, quercetin and rutin, and higher than *H. perforatum*. The antioxidant activity of the extracts may be attributed to the phenolic constituents like quercetin and rutin.

In this study, chlorogenic acid by itself did not show significant protective effect. Against alkylating damage to DNA, the effect of *H. androsaemum* was not significant [45].

In this study, *H. androsaemum* and the substances also increased the repair activity of cell extracts of alkylation damage previously inflicted to DNA molecules. Only base excision repair (BER) activity induction was observed, with no significant effect on nucleotide excision repair (NER) by any of the substances. Previous studies had found that polyphenols like luteolin, luteolin-7-glucoside, quercetin, rosmarinic acid and other phytochemicals were inducers of DNA repair activity. The observed effects should not be exclusively explained based on the effects of the major compound because it may also include the response to other bioactive compounds present in smaller concentrations, along with synergistic effects and the effects of metabolites of the initial compounds [45].

Diet may induce colon carcinogenesis through oxidative or alkylating DNA damage. However, diet may also contain anticarcinogenic compounds that contribute to cancer prevention. DNA damage prevention and/or induction of repair are two important mechanisms involved in cancer chemoprevention by dietary compounds, so the consumption of *H. androsaemum* infusions can contribute to a healthy regime [45].

3.3.4 Antiglycation

The effect of the methanol extract of aerial parts and its fractions on the glycation of bovine serum albumin (BSA) was studied. A high inhibitory effect of the process was obtained with the ethyl acetate fraction (62.8% inhibition). The n-butanol fraction and the total methanol extract also had an anti-glycation activity above 50%. The phenolic and flavonoid contents of the extracts was significantly correlated with their antioxidant and antiglycation activity [38].

Many complications of diabetes, such as retinopathy, nephropathy, neuropathy, and atherosclerosis, result from glycation processes and its end products. The metabolic abnormalities of diabetes cause mitochondrial superoxide overproduction in endothelial cells of blood vessels and in the myocardium that lead to microvascular and cardiovascular complications [46]. Crude plant extracts and plant derived compounds possessing both antiglycation and antioxidant activities have a high therapeutic potential for treating these complications [38].

3.3.5 Skin protective

An elaborate study on the potential skin-related activities of *H. androsaemum* was conducted, focusing on its effect on fibroblasts proliferation and migration, collagenase inhibitory action, immunomodulatory effect on PBMCs, protective action against UV and against APPH-induced hemolysis [4].

Fibroblasts are the key cells responsible for initiating angiogenesis, epithelization, and collagen formation during the wound healing process. Both aqueous and methanol extracts increased fibroblasts proliferation more than 190% after 6 hours of incubation at 10 µg/mL. High doses (up to 200 µg/mL) resulted in cell damage and long incubation times (up to 42h) resulted in reduced or negative effect, implying a dose-dependent toxicity [4].

Both aqueous and methanol extracts significantly increased fibroblasts migration to the site of an artificial scratch made on a fibroblast's colony. Chlorogenic acid was known to have wound healing properties but, in this study, shikimic acid also

exhibited this activity. The acceleration of wound gap reduction was greater with the aqueous extract than with any of the isolated compounds, suggesting a synergistic effect [4].

Degradation of major components of the extracellular matrix like collagen by enzymes like collagenase accelerates skin aging. The methanol and water extracts of red fruits evidenced inhibitory action on the collagenase activity (IC_{50} = 105.9 µg/mL and 88.2 µg/mL). The effect of the water extracts was close to that of chlorogenic acid. Catechin and epigallocatechin gallate are other components known for their collagenase inhibition effect. The analysis of the reaction indicated that the inhibitory activity happened through a non-competitive mechanism [4].

IL-6 has an important role in wound healing, by stimulating acute phase responses, osteoclast differentiation, B-cell proliferation, and T-cell differentiation. It is also an important pro-inflammatory cytokine, important during the inflammatory phase of wound healing. In this study, treatment with *H. androsaemum* increased IL-6 production when cells were not activated, but reduced its production in face of mitogens (80.5% (PHA) and 50.5% (PWM)), suggesting an immunomodulatory activity [4].

The first factors causing human skin aging are UV irradiation and oxidative stress, both of which affect the integrity and function of skin lipids, proteins and DNA. Dermis erythrocytes are hemolyzed by UVB photons and easily damaged by radical oxygen species (ROS).The methanol and water extracts of the red berries proved to be photostable in the UV range 280-380nm. Given their maximum absorbance wavelength, the compounds most likely responsible for this absorbance are chlorogenic and neochlorogenic acids, with a minor contribution by catechin and epicatechin. For sunscreen use, they would qualify as broad spectrum UV filters [4].

The extracts of red berries had protective effect on red blood cells (RBC) from hemolysis caused by 2, 2′-azobis (2-methylpropionamidine) dihydrochloride (AAPH), a peroxyl radical initiator. The highest level of membrane stabilizing activity was exhibited by the aqueous extract, which inhibited RBC hemolysis to an extent of 57% at a concentration of 35 µg/mL. The methanol extract exhibited a lower activity, 25% at a concentration of 15 µg/mL. However, on increasing the concentration of the methanol extract above 15 µg/mL, hemolysis occurred. Components present in higher amount in the methanol extract (e.g. xanthones) must have a pro-oxidant effect responsible for this result [4].

Given all these properties, *H. androsaemum* red berries extracts have a high potential as cosmeceutical skin-protective agents. The tyrosinase inhibitory activity adds to this potential, since this enzyme catalyzes several reactions that convert melanin into brown and red pigments [9]. The anti-inflammatory activity and cytotoxicity against melanoma add to the clinical potential for skin problems [26, 30]. The prevention of skin infections caused by bacteria like *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*, along with the control of oxidative damage caused by the infection and the leucocytes activity also comes as very useful in this domain [27]. Given that *H. androsaemum* is one of the few *Hypericum* species that does not contain hypericin, which is a potent photosensitizing agent, it is one of the most suitable for skin use.

3.3.6 Hepatoprotective

The metabolization of t-BHP in the hepatocytes follows 2 pathways, one involving cytochrome P450 that leads to toxic radicals that can damage the liver through lipid peroxidation, and a detoxification reaction that converts free gluthathione (GSH) to oxidized gluthathione (GSSG), leading to the formation of reactive oxygen species (ROS) [2,16].

In an *in vitro* study with rats' hepatocytes, pre-incubation of the isolated cells with *H. androsaemum* infusion resulted in a concentration-dependent effective inhibition of the t-BHP-induced lipid peroxidation. However, the infusion induced alterations on glutathione homeostasis, as an increase in glutathione oxidized form (GSSG) and depletion in total glutathione levels were observed. The increased depletion of GSH may be explained by the inductive action on cytochrome P450, as the data did not point to an inhibitory action on the GSH producing enzymes. Given these effects, the plant's infusions should not be considered a generalized way of treating pro-oxidant-related diseases [2].

In the *in vivo* study with rats, the consumption of a dietary infusion instead of having a liver protective effect from a t-BHP injection, potentiated the hepatotoxicity. It increased the depletion of total glutathione and GSH, and the liver damage, as assessed by the number of vacuoles in the hepatocytes. The sole administration of the infusion resulted in some liver damage, as assessed by the number of vacuoles in the hepatocytes, which leads the authors to question the traditional use as beneficial to the liver [16].

3.3.7 Anti-depressant

The *in vitro* antidepressant-like effects of red berries were studied by measuring the inhibitory activity of the methanol extract in 3 nervous system enzymes, MAO-A, tyrosinase (TYR), and acetylcholinesterase (AChE). MAO-A is known to inactivate serotonin and catecholamines, TYR interferes with levodopa increasing dopamine neurotoxicity and AChE promotes acetylcholine break down [9].

The extract showed no relevant effect on AChE but inhibited MAO-A and TYR in a clear dose dependent manner (IC_{50} = 9.3 µg/mL and 229.1 µg/mL). The high amounts of chlorogenic acid, together with catechin and epicatechin might explain the inhibitory activity on MAO-A. Caffeic acid, a metabolite of chlorogenic acid, is known to be a modulator of α1-adrenergic receptors. Rutin and hyperoside have been reported to be involved in antidepressant effects. The fruit pigment, 1, 2, 3, 5-tetrahydroxyxanthone, may also be taken into consideration for this effect. Although hypericin and hyperforin have traditionally been considered responsible for the antidepressant effect of *H perforatum*, the activity of *H. androsaemum* implies that other compounds are also involved [9].

In a previous study, the water extract of the plant was found to have high AChE inhibitory activity (IC_{50} = 0.62 mg DE/mL), stronger than *H. undulatum* and *H. perforatum*. The different plant part used, the different extract type or its concentration may explain the difference in results. The effects were attributed to the flavonoids rutin, isoquercitrin, hyperoside and quercitrin, but also to chlorogenic acid which has a less strong activity but exists in much higher quantity in this species [22].

A study was conducted on the anti-depressant activity of the methanol extracts of aerial parts of *H. androsaemum*, *H. foliosum* and *H. perforatum*. No effect was found for any of the species according to the forced swimming test (FST). In the tail suspension test (TST), only *H. foliosum* provoked a significant improvement, although *H. androsaemum* also had some action. *H. foliosum* is a species also without hypericin, and this action is attributable to the phenolic acids, hyperforin and flavonoids it contains [13]. Indeed, recent results from

preclinical trials indicate the potential of phenolic acids to reduce depressive-like behavior by regulating factors associated with oxidative stress, neuroinflammation, autophagy, and deregulation of the hypothalamic–pituitary–adrenal axis, stimulating monoaminergic neurotransmission and neurogenesis, and modulating intestinal microbiota [25].

Many pharmacological studies have been conducted with extracts and isolated constituents of *Hypericum* spp *in vivo* and *in vitro*. The mechanisms of action as well as the responsible compounds of *Hypericum* extracts are still under discussion. Some of the actions reportedly contributing to their clinical efficacy include blockade of the reuptake of serotonin (5-HT), noradrenaline and dopamine; upregulation of postsynaptic 5-HT1 and 5-HT2 receptors and of dopaminergic receptors; and increased affinity for GABAergic receptors. Some constituents that may contribute to the activity are hypericin, pseudohypericin, flavonoids, and oligomeric procyanidins. The relevance of hyperforin is discussed controversially. As a consequence the entire extract has to be considered as the active substance [47].

The antidepressant effect of the red fruits on mice subjected to bilateral common carotid artery occlusion (as models of post-stroke depression) was studied by observing improvements in behavior. All the measured parameters improved: water consumption was reduced and sucrose consumption increased towards normal levels. Immobility decreased and climbing and swimming increased even above normal levels, as well as the activity in the tail suspension test (TST) and despair swimming test (DST). This study also focused on the oxidative stress and antioxidant activity in the brain tissue of the mice after sacrifice. The levels of TBARS had significantly decreased, the levels of glutathione (GSH) had increased and the activity of superoxide dismutase (SOD, and catalase had also increased. A significant correlation between behavior improvement and antioxidant activity was found, leading to the conclusion that the anti-depressive effects found was probably due to the antioxidant activity of the plant. The anti-depressant action was attributed to chlorogenic acid, rutin, hyperoside, shikimic acid, gallic acid and the xanthones, which had been reported to have relevant effects in this situation, like antioxidant, immunomodulatory, neuroprotective and antidepressant [5].

3.3.8 Anti-inflammatory

Nitric oxide is an important marker of inflammation and immune responses. The methanol extract of the aerial parts of *H. androsaemum* was effective in inhibiting nitric oxide production in a macrophage-like cell line from rats (RAW264.7) activated by lipopolysaccharide (LP) (EC_{50} = 179 µg/mL). It has been reported that substances with significant antioxidant properties, also exert anti-inflammatory effects, and that seems to be the case with chlorogenic acid [26].

The anti-inflammatory activity of *H. androsaemum* and 4 other species (*H. barbatum*, *H. richeri*, *H. hirsutum* and *H. perforatum*) was tested *in vivo* by observing the reduction in extent of edemas induced by carrageenan in the paw of rats. With a mean effective anti-inflammatory dose (ED_{50}) of 148 mg/kg PO, *H. androsaemum* had the lowest activity among the species tested, but effective in a dose-dependant manner. The anti-inflammatory effect of *H. perforatum* was attributed, at least in part, to the content in hypericin and pseudohypericin, and the effect of *H. androsaemum* imply that other compounds in these species also have anti-inflammatory action [19, 48].

3.3.9 Anti-bacterial

In a recent study comparing the anti-bacterial activities of 4 Hypericum species (*H. androsaemum*, *H. ericoides*, *H. x moserianum* and *H. olympicum*), *H. androsaemum* had a more modest activity than the others. The total antibacterial activity (TAA, quantity extracted from 1 g of plant material divided by the MIC value) on *H. androsaemum* against all the strains tested (*S. aureus*, *B. subtilis*, *E. aerogenes*, *E. coli* and *P. aeruginosa*) was 840.33 mL/g, and the MIC was 0.1 mg/mL, with no difference between Gram-positive and Gram-negative species [27].

A previous study had found high antibacterial activity (higher than gentamicin) in the ethanol extracts of flowering aerial parts of the plant against *S. aureus* and *E. faecalis*. The antibacterial activity, measured by the diameter of the inhibition zone was lower than gentamicin against *Escherichia coli*, *Salmonella typhi*, *Shigella dysenteriaee* and *Yersinia enterocolitica*, and no effect was detected against *Pseudomonas aeruginosa*. The water and methanol extracts had a weak effect against any of the species [49].

Another study, however, found no activity against *E. coli* and *S. aureus*. This study tested 15 *Hypericum* species against drug-resistant strains, drug-resistant clinical isolates, mycobacterium species and bacterial species and found *H. androsaemum* active only against *S. faecium* (MIC = 50 µg/mL) [50].

3.3.10 Anti-fungal

Biofilm-associated *Candida* cells are resistant to a wide spectrum of available antifungal drugs. In a study of the anti-fungal activity of the plant against *Candida albicans*, *C. glabrata*, *C. parapsilosis* and *C. tropicalis*, the MIC values varied between 1.56 mg/mL (for *C. glabrata*) and 12.5 mg/mL (for *C. albicans*), more than 10 times higher than fluconazole. At high doses (> 12.5 mg/mL), the extract only significantly reduced the fungal population in *C. tropicalis* and *C. glabrata*. This weak activity should not discourage the investigation of the relevant anti-*Candida* compounds in the plant, since plant extracts are not pure substances and thus their activity is more diluted, complex and synergistic [26].

Analysis of the time-kill curves showed an inhibitory effect against *C. tropicalis*, *C. parapsilosis* and especially *C. glabrata*, in a time-dependent manner. By observing the cell internalization of a fluorescent probe, the damage inflicted on the fungal cells membranes was found to be significant, after incubation periods of 4 h with 25 mg/mL, against *C. tropicalis* and *C. glabrata*. The extract, at concentrations of 12.5 and 25 mg/mL, also achieved a reduction > 90% on the biofilms of these two species. The anti-fungal activity should be attributed to the major phenolic constituents, namely 3-O-caffeoylquinic acid and 5-O-caffeoylquinic acid [26].

3.3.11 Nematoxic

The infusion of *H. androsaemum* showed 2 types of activity against the plant nematode *Meloidogyne javanica*. Egg eclosion inhibition was higher for 10 mg/ml than for 4 mg/ml, although it apparently prevented any eclosion in the first 24 h. The mortality directly dependent on the concentration of the extract. Being observed in the first 24 hours for 6, 8 and 10 mg/ml but only after 72 h with 4 mg/ml. The plant has some potential for biocontrol of this pest [51].

3.3.12 Molluscicide

The extracts of *H. androsaemum* proved to be toxic against the air-breathing freshwater snail *Radix peregra*, which is

responsible for transmitting fasciolosis. The highest activities were recorded with the hexane extract, with a median lethal concentration (LC_{50}) of 30.47 ppm against the adult population and 73.25 in the juvenile. The water extract had the highest anti-ovicidal activity, attaining 1.85% of hatching at 500 ppm [52].

3.3.13 Insecticidal

Ethanol extracts proved to be able to control the larvae of the *Thaumetopoea solitaria* moth. A high deterrence from feeding was observed, with 18.3% decrease in body weight, as well as high toxicity, with 7 dead specimens out of 10 [53].

Today, the environmental safety of an insecticide is considered of paramount importance, and extracts from plants like *H. androsaemum* have a high potential in this field.

3.4 Toxicological data

Negative results on cytotoxicity upon normal cells also mean safeness for use with other purposes.

Extracts of aerial parts proved to have low toxicity (IC_{50}> 30 µg/mL) upon NIH3T3 mouse fibroblast cells [27]. In another study, the toxicity against non-tumor porcine liver cells (GI_{50} > 400 µg/mL) was much lower than against the cancer cell lines tested (GI_{50} between 100 and 215 µg/mL) [26]. However, high doses of extracts have a significant cytotoxic effect, as observed with concentrations above 10 µg/mL of methanol extracts of red berries which, after 5 days of culture, destroyed all the cells in a culture of pig's PBMC [1].

The naphtodiantrones hypericin and pseudohypericin are known to be highly phototoxic. Although *H. androsaemum* does not contain these compounds, its potential phototoxic effect, along with other *Hypericum* species, was studied. The cytotoxic effect was very similar in the dark and under light (LC_{50}= 148 ± 37 µg/mL and 142 ± 47 µg/mL, respectively), yielding that this species is not phototoxic [14].

In the study previously mentioned about the DNA protective effects, the water extract did not induce strand breaks in the DNA of HT29 cells [45].

Possible risks with the oral administration of preparations of *Hypericum* spp related with pharmacokinetic interactions may occur caused by hyperforin induction of CYP enzymes and P-glycoprotein, via activation of the pregnane X receptor [54, 58]. The extent of the induction of the metabolic enzymes is dose-dependent and time-dependent. The induction of the mentioned enzymes is reversible within approximately 1 week after stopping ingestion of the *Hypericum* preparations. Thus, the oral use for traditional preparations should be limited with 2 weeks. According to the EMA recommendation for the *Hypericum perforatum* L., herba traditional used medicines, this duration of use may be considered sufficient for the induction of the activity of the CYP-enzymes in the case of high-hyperforin preparations. In cases where the daily intake of hyperforin is higher than 1 mg the full information regarding enzyme induction, contraindications and warnings related to interactions should be included in the product information (EMA Assessment report on *Hypericum perforatum* L., herba, EMA/HMPC/244315/2016 Committee on Herbal Medicinal Products (HMPC) 23 November 2022, In: https://www.ema.europa.eu/en/documents/herbal-report/final-assessment-report-hypericum-*perforatum*-l-herba-revision-1_en.pdf)

The content of hyperforin of the herbal preparation should then be specified in any product available to the consumer.

3.5 Commercial products in Portugal containing *H. androsaemum*

In Portugal, the herbal tea of *H. androsaemum* is sold for general liver, urinay, nervous, digestive and skin conditions. It is frequently announced as an antidepressant and sedative, perhaps mistakenly associating it with *H. perforatum*, which activity in that area is well known. The possible neuroactive effects of *H. androsaemum* are of different nature since it does not contain hypericin. The high polyphenolic, PPAP, flavonoid in synergy with other compounds may give it some activity in that field, as shown in some of the reported studies.

Table 1: Some of the herbal teas and Food Suplements based on HA in the Portuguese market

Name and presentation	Ingredients	Proposed indications
Antiga Ervanaria's Hipericão-do-Gerês Herbal tea	*Hypericum androsaemum* dried aerial parts	For liver disease and kidney colic. Externally for burns and bruises.
MC's Hipericão Androsemo Herbal tea	*Hypericum androsaemum* dried aerial parts and fruits	For nervous disturbances and insomnia, Improves digestive system. Regulates urinary system, Fights inflammation in the trachea, Antidepressant properties
Chás do Mundo's Hipericão do Gerês	*Hypericum androsaemum* dried aerial parts	For depression, anxiety and insomnia, Digestive, diuretic, sedative, wound-healing, hepatoprotective and antiseptic
Dietmed 's Cynasine: Tablets, oral solution and drops	*Agrimonia eupatoria:* 4% *Cynara scolymus:* 3% *Peumus boldus*: 2% *Cochlospermum angolense*: 2% *Silybum marianum*: 2% *Chelidonium majus*: 2% *Ginkgo Biloba*: 2% *Hamamelis virginiana*:2% *Hypericum androsaemum:* 2% *Parietaria officinalis*: 2% *Salvia officinalis*: 2%	Hepatic protective and regenerating action, Detoxifying, Benefits digestion, Normal gastric function, Promotes the elimination of gases
Quality of Life Labs' MemoStress Ampoules	*Cynara scolymus*: 225 mg *Rosmarinus officinalis*: 225 *Taraxacum officinale* (root): 150 mg *Sylibum marianum*: 150 mg *Passiflora incarnata*: 150 mg *Turnera difusa*:150 mg	Fights physical and emotional fatigue, Fights stress with mental blockage, Improves the ability to concentrate and intellectual function, Invigorate and fortify the body, * Additional claims are presented for the actions of most of the plants individually, but not for H. androsaemum

	Lepidium meyenii:150 mg *Hypericum androsaemum*: 150 mg *Ribes nigrum* (fruit): 150 mg *Crataegus oxyacantha* (fruit): 150 mg *Ginkgo biloba:* 150 mg L-glutamic acid: 57 mg Vitamins B and E, Mg, Cu	
Naturodiet's Hepatisan Tablets	*Cochlospermum angolense*: 300mg *Peumus boldus*: 60mg *Cynara scolymus*: 60mg *Taraxacum officinale*: 60mg *Fumaria officinalis*: 60mg *Buxus sempervirens*: 30mg *Vitis vinífera*: 30mg *Hypericum androsaemum*: 15mg	Hepato-protective, Bile secretion stimulating, For chronic liver diseases and biliary problems
Eparmine	*Peumus boldus* *Cynara scolymus* *Hypericum androsaemum*	Claims made specifically for HA: Widely used in the treatment of body diseases, Effective in relieving colic and cystitis (bladder inflammation), Notable diuretic, Digestive action, very useful in digestive problem, Reduces the acidity of the gastric fluid and increases the secretion of bile ducts. Slightly sedative because it exerts a mild toning effect on the nervous system.

In hepato-gastric formulas, *H. androsaemum* role tends to be presented as a coadjutant of the more well-known plants. When specifically mentioned, its activities are told in a vague way. For example, in 'Effective in relieving colic and cystitis; notable diuretic' the anti-cystitis role is not clear, suggesting it could be just as diuretic.

Some claims seem to be out of both scientific evidence and traditional use ('reduces the acidity of the gastric fluid') while some others seem a bit contradictory ('slightly sedative because it exerts a mild toning effect on the nervous system').

4. Conclusions

In this review, we reported the ethnobotanical uses, phytochemical composition and biological activity investigations of *H. androsaemum*.

This plant used is used in the Mediterranean basin for its diuretic, hepatoprotective, anti-hypertensive, anti-hemorrhoidal, antidepressant and skin-protective actions.

Although it does not contain the naphthodianthrone compounds to which is attributed the anti-depressant activity of the most popular plant of its genus, *H. perforatum*, this species proved to have some activity in that area, suggesting that other compounds may also play an important role.

It contains several sub-classes of compounds including lignans, quinones, xanthones and polycyclic polyprenylated acylphloroglucinols (PPAPs), flavonoids and triterpenoids.

Extracts of the plant have shown important radical scavenging activity, but the hepatoprotective activity was not confirmed.

The fruits have shown some cytotoxic activity against colon carcinoma, breast adenocarcinoma and malignant melanoma cell lines. Other cells lines were also tested, with poor results.

The plant has shown biocidal activity against some bacteria, fungi, nematodes, mollusks and insects. The number of studies, however, is low and some results contradictory.

A very comprehensive case has been made in one study for its use in skin conditions, given its effect on fibroblasts proliferation and migration, collagenase and tyrosinase inhibitory action, immunomodulatory effect, protective action against UV and against APPH-induced hemolysis, to which the lack of the phototoxic hypericin adds up as an advantage.

Different extracts of the plant have exhibited immunomodulatory, DNA-protective, anti-glycation, antidepressant and anti-inflammatory properties. The number of studies, however, is still low and further tests are required to confirm these effects and to understand their mechanisms.

Although there is not any pharmacopeial monograph, herbal preparations based on HA should be quantified regarding to hyperforin, since possible risks with the oral administration of preparations of *Hypericum* spp. are related with pharmacokinetic interactions which are caused by the constituent hyperforin. Because the extent of the induction of the metabolic enzymes is dose-dependent and time-dependent, the oral use for the traditional *Hypericum* preparations should be limited to 2 weeks.

5. References

1. Caprioli G. Polar Constituents and Biological Activity of the Berry-Like Fruits from *Hypericum androsaemum* L. Front Plant Sci. 2016;7:232.
2. Valentão P. Protective activity of *Hypericum androsaemum* infusion against tert-butyl hydro peroxide-induced oxidative damage in isolated rat hepatocytes. J Ethnopharmacol. 2004;92:79-84.
3. Guedes AP, Franklin G, Fernandes-Ferreira M. Hypericum sp.: essential oil composition and biological activities. Phytochem Rev. 2012;11:127-152.
4. Antognoni F. Polar extracts from the berry-like fruits of *Hypericum androsaemum* L. as a promising ingredient in skin care formulations. J Ethnopharmacol. 2017;195:255-265.
5. Nabavi SM. The water extract of tutsan (*Hypericum androsaemum* L.) red berries exerts antidepressive-like effects and *in vivo* antioxidant activity in a mouse model of post-stroke depression. Biomed Pharmacotherapy. 2018;99:290-298.
6. Aziz N, Sauve RJ, Long D, Cherry M. Genetic and Phytochemical Diversity Assessment Among Eleven *Hypericum* Accessions via AFLP and HPLC Analyses. Journal of Herbs, Spices & Medicinal Plants. 2007;12:97-105.
7. Gião MS, Pereira CI, Pintado ME, Malcata FX. Effect of technological processing upon the antioxidant capacity of aromatic and medicinal plant infusions: From harvest to packaging. LWT - Food Science and Technology. 2013;50:320-325.
8. Almeida IF, Fernandes E, Lima JLFC, Costa PC, Bahia MF. *In vitro* protective effect of *Hypericum androsaemum* extract against oxygen and nitrogen reactive species. Basic Clin Pharmacol Toxicol. 2009;105:222-227.

9. López V, Les F, Iannarelli R, Caprioli G, Maggi F. Methanolic extract from red berry-like fruits of *Hypericum androsaemum*: Chemical characterization and inhibitory potential of central nervous system enzymes. Industrial Crops and Products. 2016;94:363-367.
10. Calvo MI, Cavero RY. Medicinal plants used for neurological and mental disorders in Navarra and their validation from official sources. Journal of Ethno pharmacology. 2015;169:263-268.
11. Yazici Bektaş N. Cytotoxic and apoptotic effects of *Hypericum androsaemum* on prostate adenocarcinoma (PC-3) and hepatocellular carcinoma (Hep G2) cell lines with identification of secondary metabolites by LC-HRMS. Turk J Chem. 2021;45:1621-1638.
12. Morteza-Semnani K, Saeedi M. The essential oil composition of *Hypericum androsaemum* L. leaves and flowers from Iran. Flavour and fragrance journal. 2005;20:332-334.
13. Ramalhete N. Comparative study on the *in vivo* antidepressant activities of the Portuguese Hypericum foliosum, *Hypericum androsaemum* and *Hypericum perforatum* medicinal plants. Industrial Crops and Products. 2016;82:29-36.
14. Napoli E. Phytochemical profiles, phototoxic and antioxidant properties of eleven Hypericum species - A comparative study. Phytochemistry. 2018;152:162-173.
15. Valentão P. Variability in phenolic composition of *Hypericum androsaemum*. Nat Prod Res. 2003;17:135-140.
16. Valentão P. *Hypericum androsaemum* infusion increases tert-butyl hydro peroxide-induced mice hepatotoxicity *in vivo*. J Ethnopharmacol. 2004;94:345-351.
17. Cirak C, Seyis F, Özcan A, Yurteri E. Ontogenetic changes in phenolic contents and volatile composition of *Hypericum androsaemum* and *Hypericum xylosteifolium*. Biochemical Systematics and Ecology. 2022;102:104429.
18. Bruňáková K. Phytochemical profiling of several Hypericum species identified using genetic markers. Phytochemistry. 2021;187:112742.
19. Savikin K, Dobrić S, Tadić V, Zdunić G. Anti-inflammatory activity of ethanol extracts of *Hypericum perforatum* L., *H. barbatum* Jacq., *H. hirsutum* L., *H. richeri* Vill. and H. *androsaemum* L. in rats. Phytother Res. 2007;21:176-180.
20. Wang K. Polycyclic Polyprenylated Acylphloroglucinols and Cytotoxic Constituents of *Hypericum androsaemum.* Chemistry & Biodiversity. 2012;9:1213-1220.
21. Schmidt W, Abd el-Mawla AM, Wolfender JL, Hostettmann K, Beerhues L. Xanthones in cell cultures of *Hypericum androsaemum*. Planta Med. 2000;66:380-381.
22. Hernandez MF, Falé PLV, Araújo MEM, Serralheiro MLM. Acetylcholinesterase inhibition and antioxidant activity of the water extracts of several Hypericum species. Food Chemistry. 2010;120:1076-1082.
23. Pilepić KH, Males Z, Crkvencić M. Quantitative analysis of total flavonoids and total phenolic acids in thirty Hypericum taxa. Nat Prod Commun. 2013;8:347-349.
24. Zdunic G, Godjevac D, Savikin K, Petrovic S. Comparative Analysis of Phenolic Compounds in Seven *Hypericum* Species and Their Antioxidant Properties. Natural Product Communications. 2017;12:1934578X1701201.
25. Cordeiro MLDS. Phenolic Acids as Antidepressant Agents. Nutrients. 2022;14:4309.
26. Jabeur I. Bioactive properties and functional constituents of *Hypericum androsaemum* L.: A focus on the phenolic profile. Food Res Int. 2016;89:422-431.
27. Saddiqe Z, Naeem I, Hellio C, Patel AV, Abbas G. Phytochemical profile, antioxidant and antibacterial activity of four Hypericum species from the UK. South African Journal of Botany. 2020;133:45-53.
28. Kim JK, Park SU. Quercetin and its role in biological functions: an updated review. EXCLI Journal; 17:Doc856; ISSN; c2018. p. 1611-2156. DOI:10.17179/EXCLI2018-1538.
29. Isemura M. Catechin in Human Health and Disease. Molecules. 2019;24:528.
30. Silva AR, Taofiq O, Ferreira ICFR, Barros L. Hypericum genus cosmeceutical application – A decade comprehensive review on its multifunctional biological properties. Industrial Crops and Products. 2021;159:113053.
31. Demirkiran O. Xanthones in Hypericum: Synthesis and Biological Activities. In Bioactive Heterocycles III (ed. Khan, M. T. H.). (Springer Berlin Heidelberg). 2007;9:139-178.
32. Iannarelli R. Phytochemical Characterization of the Berry Like Fruits of Tutsan (*Hypericum androsaemum* L.).
33. Delkhah AMD, Karimi E, Farivar S. Herniarin-loaded solid lipid nanoparticles: promising molecular mechanism and therapeutic potential against pancreatic cancer line. Mol Biol Rep. 2023;50:6469-6479.
34. Liliana Porras-Dávila S. Anti-Inflammatory and Neuroprotective Effects of Standardized Fractions in Herniarin and Daphnoretin from *Distictis buccinatoria*. Chemistry & Biodiversity 20, e202200969; c2023.
35. Caprioli G. Volatile profile, nutritional value and secretory structures of the berry-like fruits of *Hypericum androsaemum* L. Food Research International. 2016;79:1-10.
36. Guedes AP, Amorim LR, Vicente A, Fernandes-Ferreira M. Variation of the essential oil content and composition in leaves from cultivated plants of *Hypericum androsaemum* L. Phytochem Anal. 2004;15:146-151.
37. Guedes AP, Amorim LR, Vicente AMS, Ramos G, Fernandes-Ferreira M. Essential oils from plants and *in vitro* shoots of *Hypericum androsaemum* L. J Agric Food Chem. 2003;51:1399-1404.
38. Saddiqe Z, Maimoona A, Abbas G, Naeem I, Shahzad M. Pharmacological screening of *Hypericum androsaemum* extracts for antioxidant, anti-lipid peroxidation, antiglycation and cytotoxicity activity. Pak J Pharm Sci. 2016;29:397-405.
39. Valentão P. Antioxidant activity of *Hypericum androsaemum* infusion: scavenging activity against superoxide radical, hydroxyl radical and hypochlorous acid. Biol Pharm Bull. 2002;25:1320-1323.
40. Sousa D, Xavier C, Lima C, Pereira-Wilson C, Fernandes-Ferreira M. Cytotoxic activity of water extract fractions of *Hypericum androsaemum* L. on colorectal cancer cells. Planta Medica. 2015;81:78.
41. Xavier CPR, Lima CF, Fernandes-Ferreira M, Pereira-Wilson C. *Hypericum androsaemum* water extract inhibits proliferation in human colorectal cancer cells through effects on MAP kinases and PI3K/Akt pathway. Food Funct. 2012;3:844-852.
42. Madunić J, Matulić M, Friščić M, Pilepić KH. Evaluation of the cytotoxic activity of Hypericum spp. on human glioblastoma A1235 and breast cancer MDA MB-231

cells. J Environ Sci Health A Tox Hazard Subst. Environ Eng. 2016;51:1157-1163.
43. Procházková D, Boušová I, Wilhelmová N. Antioxidant and prooxidant properties of flavonoids. Fitoterapia. 2011;82:513-523.
44. Hou N, Liu N, Han J, Yan Y, Li J. Chlorogenic acid induces reactive oxygen species generation and inhibits the viability of human colon cancer cells. Anti-Cancer Drugs. 2017;28:59-65.
45. Ramos AA, Marques F, Fernandes-Ferreira M, Pereira-Wilson C. Water extracts of tree Hypericum sps. protect DNA from oxidative and alkylating damage and enhance DNA repair in colon cells. Food Chem Toxicol. 2013;51:80-86.
46. Giacco F, Brownlee M. Oxidative Stress and Diabetic Complications. Circ Res. 2010;107:1058-1070.
47. Leuner K. Hyperforin modulates dendritic spine morphology in hippocampal pyramidal neurons by activating Ca^{2+} -permeable TRPC6 channels. Hippocampus. 2013;23:40-52.
48. Ku S.-K, Kwak S, Kwon O.-J, Bae J.-S. Hyperoside Inhibits High-Glucose-Induced Vascular Inflammation *In vitro* and *In vivo*. Inflammation. 2014;37:1389-1400.
49. Mazandaran M, Yassaghi S, Rezaei MB, Mansourian AR, Ghaemi EO. Ethnobotany and Antibacterial Activities of Two Endemic Species of Hypericum in North-East of Iran. Asian J of Plant Sciences. 2007;6:354-358.
50. Nogueira T. Profile of antimicrobial potential of fifteen Hypericum species from Portugal. Industrial Crops and Products. 2013;47:126-131.
51. Guedes AP, Luques RP, Ferreira P, Almeida M, Ferreira MF. Essential oil components of *Hypericum androsaemum* infusions and their nematotoxic effects against *Meloidogyne javanica* (Treub) Chitwood; c2009.
52. Teixeira T, Rainha N, Rosa JS, Lima E, Baptista J. Molluscicidal activity of crude water and hexane extracts of Hypericum species to snails (*Radix peregra*). Environmental Toxicology and Chemistry. 2012;31:748-753.
53. Ertürk Ö. Antifeedant and Toxicity Effects of Some Plant Extracts on *Thaumetopoea solitaria* Frey. (Lep.: Thaumetopoeidae).
54. Mueller SC. The extent of induction of CYP3A by St. John's wort varies among products and is linked to hyperforin dose. Eur. J Clin Pharmacol. 2006;62:29-36.
55. Mueller SC. No clinically relevant CYP3A induction after St. John's wort with low hyperforin content in healthy volunteers. Eur. J Clin Pharmacol. 2009;65:81-87.
56. Pal D, Mitra AK. MDR- and CYP3A4-mediated drug-herbal interactions. Life Sciences. 2006;78:2131-2145.
57. Izzo AA. Interactions between Herbs and Conventional Drugs: Overview of the Clinical Data. Med Princ. Pract. 2012;21:404-428.
58. Izzo AA, Ernst E. Interactions Between Herbal Medicines and Prescribed Drugs: An Updated Systematic Review. Drugs. 2009;69:1777-1798.