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Antinociceptive Properties of St. John's Wort (*Hypericum perforatum*) and Other *Hypericum* Species

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Dedicated to Prof. Dr. Wilhelm Fleischhacker on account of his 85th Birthday.

This review aims at a coherent summary of the results obtained from various studies that concern analgesic-like activity of the extracts of *H. preforatum* L. and thirteen other *Hypericum* L. species (Hypericaceae). Botanical origin, plant organs and extraction modes of the plant material, experimental models, routes of administration and doses used for animal treatment are summarized. Mechanisms of action and substances (and even the synergy thereof) proposed so far to be responsible for the observed activity have also been discussed. Even though St. John's wort (*H. perforatum*) is the most renowned plant species of this genus, it is neither the only nor the most potent one in inducing pain relief.

Keywords: Analgesic-like activity, *Hypericum*, *H. caprifoliatum*, *H. grandifolium*, Opioid receptors.

Over the past few decades, phytomedicine has gained global importance, having an impact on both world health and international trade. A great majority of the indigenous populations in developing countries depend on ethnopharmacology and medicinal plants as their primary source of healthcare. *Hypericum* species have been used as medicinal plants for centuries. St. John's wort (*Hypericum perforatum* L., SJW) has recently gained popularity as an alternative treatment for mild to moderate depression [1a]. Given the current widespread use of this herbal remedy, it is important to have a firm overview of all other less known pharmacological and toxicological aspects of this species, as well as of other taxa belonging to this genus.

Hypericum L. is a genus of about 400 species of flowering plants in the family Hypericaceae (Clusiaceae, Guttiferae). Plants from this genus are annual or perennial shrubs or small trees that possess pale to dark yellow flowers. The secondary metabolites of the taxa belonging to this genus have been extensively studied and this subject was reviewed on several occasions [1a-1c]. There are even reports of more than 100 successfully identified (volatile) constituents of a single *Hypericum* species in only one study [2a-2c]. St. John's wort is the well-known and preferred in medicine representative of this genus, and it is supposed to possess sedative and astringent properties. The up to date known secondary metabolites of this taxon are naphthodianthrones, flavonoids, xanthones, tannins, essential oil constituents and phloroglucinols [3a-3f]. Commercial herbal products containing any species of this genus, most often comprised of SJW alone, are formulated from quantified extracts which contain a standardized amount of active compounds including a broad range of flavonols (based on quercetin aglycone), naphthodianthrones (hypericin and pseudohypericin) and phloroglucinol derivatives such as hyperforin, adhyperforin and others. This herb is traditionally being used for the treatment of liver diseases, irritable bowel, stomach ulcers, chronic bladder inflammation, menopausal neurosis, menstrual pain, sciatica, fibrositis, neuralgia, anxiety, excitability, depression, treatment of wounds (promotes healing), and inflammation [1b,4]. Today, SJW is best known for its use in the treatment of mild-to-moderate severe depressive disorders. The only known side-effects of the herb are that it makes the skin more sensitive to sunlight (phototoxicity in animals) [5]. Owing to such popularity, convincing evidence has been built up providing justification for the numerous uses of this and other *Hypericum* taxa in ethnomedicine (antidepressant [6], hypnotic, anxiolytic [7], antimicrobial, antioxidant [3a,8], antiviral, anti-inflammatory [9], antispasmodic, bronchodilatory, cardiovascular-modulatory activity [10], enzyme-inhibitory activity [11a,11b]).

Analgesia or alleviation of pain, which is achieved through interference of a wide range of neurotransmitter systems, can be in a form of either neuropathic (lesions of the central and peripheral nervous system), inflammatory (a consequence of tissue damage and inflammation) or nociceptive pain (an activation of the peripheral terminals of nociceptors) [12]. The central control of pain is subject to descending modulation by brainstem cell groups such as locus coeruleus/subcoeruleus and raphe complex [13]. There are two pathways in the central nervous system (CNS) that are responsible for antinociception. The first stretches from locus coeruleus, whereas the second one from midbrain and both ends in the superficial dorsal horns (spinal cord). The endings of the neuron pathways can release noradrenaline (*locus coeruleus*) and serotonin (raphe complex) which inhibit the release of substance P. The second pathway, which involves midbrain and medulla, is rich in opioid peptides and opioid receptors, and the serotonin discharge can induce the release of opioid peptides (enkephalin, endorphins or dynorphin in the human body) [14a-14c]. On the other hand much less is known about the modulation of peripheral pain, though recently, a number of promising targets for peripheral pain control have been put forward and these include the transient receptor potential family of neuronal ion channels, the family of proteinase-

activated receptors, GABA receptors, glycinergic transmission, cannabinoids and opioids [15].

Several methods have been applied for the assessment of antinociceptive properties of various naturally occurring or synthetic compounds. These experimental procedures investigate both peripheral (e.g. formalin test, writhing test) and central analgesia (e.g. tail flick, hot plate). The formalin test is a model which involves two phases of nociception; the first phase corresponds to acute neurogenic pain probably induced by direct chemical stimulation of nociceptors and C fibers, whereas the second phase is related to inflammatory pain responses [16a-16c]. Although "the writhing test" (abdominal writhing induced by acetic acid) is not a specific model for neurogenic pain, it is the most repeatedly used test to detect general (either neurogenic or inflammatory) antinociceptive properties of compounds. Quite a lot of drugs, such as opioid analgesics, adrenergic blockers, antihistamines, and muscle relaxants, can inhibit acetic acid-induced writhing (or of any other kind of chemical that can induce abdominal contortions, such as acetylcholine and *p*-benzoquinone) and this makes this test inappropriate for the determination of the mechanism of action of the tested substances. Only specific assays that utilize selective antagonists can shed light onto the underlying mechanism(s). The tail flick response is predominantly a spinal reflex, and is considered to be selective for centrally acting analgesic compounds [17]. The modulating properties of potential analgesic compounds, acting on the spinal cord level, may be expressed by the inhibition of, for example, a specific nitric oxide synthetase (NOS) or the action on opioid class receptors [12]. The hot plate test is considered to be a selective test for drugs that affect supraspinally integrated responses, such as the opioid-derived analgesics, and represents a specific model of central analgesia [18]. The aim of this review was to systematize and possibly correlate all of the up to date published studies on the antinociceptive activity of *Hypericum* spp. extracts and of the pure compounds isolated therefrom in different animal models. The probable mechanisms of pain relief, by which the extracts of these species, purified fractions and single compounds work, are also discussed.

Utilized methodologies: This paragraph is an overview of the materials and methods utilized in the scientific articles concerning the evaluation of antinociceptive properties of extracts of SJW [19a-19l] and eleven other different *Hypericum* species [20a-n] (*H. brasiliense* Choisy [20a,b], *H. calycinum* L. [20c], *H. canariense* L. [20d], *H. caprifoliatum* Cham. & Schltdl. [20e-20g], *H. cordatum* (Vell. Conc.) N. Robson. [20b], *H. empetrifolium* Willd. [20h], *H. glandulosum* Ait. [20d], *H. grandifolium* Choisy [20i], *H. myriathum* Cham. & Schltdl. [20j], *H. organifolium* Wild. [20k], *H. polyanthemum* Klotzsch ex Reichardt [20e, l], *H. reflexum* L. fil. [20m], and *H. triquetrifolium* Turra. [20n]). Tables 1 and 2 summarize the data on the botanical origin (specific species of the genus), plant organs and solvents used for extraction (water, methanol, ethanol, *n*-butanol, dichloromethane, diethyl ether, chloroform, cyclohexane, light petroleum, given in an approximate decreasing order of polarity), types of extraction (maceration, Soxhlet apparatus, ultra-turrax apparatus), animals tested (mice, rats), experimental models, doses (from 10 to 1000 mg/kg) and administration routes (*p.o.*, *i.p.*, *i.t.*, *i.c.v.*) employed [19-20]. Commercial samples (Table 1) used in the cited studies were obtained from: Indena Research Laboratories (Settala, Milan, Italy) with purity 96.25% [19a, g, l], Safamood, ATOS Pharma, ARE [19d], and Medics Laboratories, Karachi, Pakistan (Deprisin) [19f].

Observed activity of* Hypericum *taxa extracts: Extractable matter from *H. perforatum* was shown to possess analgesic-like activity in six different models of nociception in animals, in a dose dependent manner in the range from 10 to 250 mg/kg [19a-19l]. For example, an aqueous-ethanol extract of SJW inhibited the number of writhings at 10 and 20 mg/kg. This effect was not reversed by naloxone [19g].

Also, an extract of SJW produced a long-lasting increase in the thermal-pain threshold, which appeared in the interval of 30-60 min, peaked at 90 and slowly diminished at 180 minutes after the administration of the extract [19a]. The same group of authors showed that the SJW antinociception can be reversed by naloxone and by the administration of the protein kinase C activator 12-*O*-tetradecanoylphorbol-13-acetate; on the other hand, atropine and yohimbine had no effect on the antinociceptive effect of SJW [19a].

One of the frequent chronic complications that occurs in diabetes mellitus is the so-called diabetic neuropathy that is the cause of diabetic pain [21]. Current treatment of this disorder is comprised of tricyclic antidepressants, calcium channel ligands (gabapentin and pregabalin), topical lidocaine, opioid analgesics and tramadol [21]. In addition to the mentioned antinociceptive activities of SJW extracts, the antihyperalgesic efficacy of SJW extracts was demonstrated to be comparable with that of the clinically used drugs (carbamazepine, lamotrigine, L-acetyl-levocarnitine) for the treatment of diabetic neuropathy [19k].

Table 1: Plant organs, types of extraction, solvents used for extraction, animals tested, experimental models, doses given, and administration routes for *H. perforatum*.

Plant organ	Type of extraction, solvents	Experimental animal, models (dose(s) (mg/kg) and administration route)	Ref.
commercial sample	maceration for 3 h with chloroform, methanol	mice, hot plate test (10–60 *p.o.*[a]) mice, writhing test (10–30 *p.o.*)	[19a]
aerial parts, dried and powdered	Soxhlet apparatus extraction for 16 h with methanol	/[b], formalin test (25–250 *i.p.*[c]) /, hot plate test (25–250 *i.p.*) /, writhing test (25–150 *i.p.*)	[19b]
/	/, 50% aqueous-ethanol	rat, tail flick test (100, 200 for 3 days *p.o.*) rat, hot plate test (100, 200 for 3 days *p.o.*) rat, writhing test (100, 200 for 3 days *p.o.*)	[19c]
commercial sample	/, /	rat, hot plate test (25, 50,100 *p.o.*) rat, tail electric stimulation test (50, 150, 300 *p.o.*) rat, capsaicin-induced hind paw licking (25, 50 *p.o.*) rat, writhing test (25, 50 *p.o.*)	[19d]
aerial parts, dried and powdered	maceration for 3 days with 70% aqueous-ethanol	mice, writhing test (10, 20 *i.p.*)	[19e]
commercial sample	/, /	mice, writhing test (30–100 *i.p.*) mice, formalin test (30–100 *i.p.*)	[19f]
commercial sample	/, /	mice, formalin test (100–1000 *p.o.*) mice, tail flick test (1000 *p.o.*)	[19g]
aerial parts, dried and semi-crushed	maceration for 24 h at 37°C with 50% aqueous-ethanol	mice, tail flick test (250 *i.p.*)	[19h]
/	/, water	/, formalin test (/, *i.p.*, *i.t.*[d], *i.c.v.*[e]) /, tail flick test (/, *i.p.*, *i.t.*, *i.c.v.*)	[19i]
aerial parts, dried and powdered	maceration with 50% aqueous-ethanol (1:10) for one night, water extraction (40°C) for 8 h	rat, tail-pinch test (125, 250 *i.p.*) rat, tail flick test (125, 250 *i.p.*)	[19j]
flowers, leaves and seeds	maceration with 70 and 50% aqueous-ethanol for 6-8 h (50°C), filtered, evaporated and freeze-dried	rat, diabetic neuropathy-paw-pressure test (20–1000 *p.o.*)	[19k]
commercial sample	/, /	mice, meningeal nociception – hot and cold plate (5 *p.o.*)	[19l]

[a]) *p.o.* – substances given *per os* (orally given). [b]) / – the data are missing in the cited work. [c]) *i.p.* – substances given intraperitoneally. [d]) *i.t.* – substances given intrathecally [e]) *i.c.v.* – substances given intracerebroventricularly

Table 2: Plant species, plant organs, types of extraction, solvents used for extraction, animals tested, experimental models, doses given and administration routes for thirteen different *Hypericum* species

Species	Plant organ	Type of extraction, solvents	Experimental animal, models (dose(s) (mg/kg) and administration route)	Ref.
H. brasiliense Choisy	leaves and flowers, air-dried	maceration at room temperature, /[a]	rat, writhing test (50, 250 *p.o.*[b]) rat, hot plate test (50, 250 *p.o.*)	[20a,b]
		turbolysis technique (hydroalcoholic extracts 50%)	mice and rats, hot plate test (10, 50 *i.p.*[c], 100, 500 *p.o.*) mice and rats, tail-flick test (10, 50 *i.p.*, 100, 500 *p.o.*)	
H. calycinum L.	aerial parts, dried and semi-crushed	maceration for 24 h at 37°C with 50% aqueous-ethanol	mice, tail flick test (250 *i.p.*)	[20c]
H. canariense L.	aerial parts, dried in an oven at 40°C and powdered.	maceration for 3 days at room temperature with methanol, partitioned with different solvent mixtures of chloroform/ water and *n*-butanol/water	mice, writhing test (500 *p.o.*) mice, tail flick test (500 *p.o.*)	[20d]
H. caprifoliatum Cham. & Schltdl.	aerial parts, air-dried, powdered	Ultra-turrax apparatus with cyclohexane, Soxhlet apparatus with light petroleum, chloroform, methanol,	mice, hot plate test (90, 180 *p.o.* and *i.p.*) mice, writhing test (90, 180 *p.o.*)	[20e] [20f]
		maceration for 24 h with dichloromethane followed by methanol	mice, hot plate test (10, 20 *i.p.*) mice, writhing test (20, 40 *p.o.*)	[20g]
H. cordatum (Vell. Conc.) N. Robson.	aerial parts	turbolysis technique (hydroalcoholic extracts 50%)	mice and rats, hot plate test (10, 50 *i.p.*, 100, 500 *p.o.*) mice and rats, tail-flick test (10, 50 *i.p.*, 100, 500 *p.o.*)	[20b]
H. empetrifolium Willd.	aerial parts, crushed	maceration for 4 days with methanol	rat, hot plate test (50 ,100 *i.p.*) rat, writhing test (100 *i.p.*)	[20h]
H. glandulosum Ait.	aerial parts, dried in an oven at 40°C and powdered	maceration for 3 days at room temperature with methanol, partitioned with different solvent mixtures of chloroform/ water and *n*-butanol/water	mice, writhing test (500 *p.o.*) mice, tail flick test (500 *p.o.*)	[20d]
H. grandifolium Choisy	aerial parts, dried in an oven at 40°C and powdered	maceration for 3 days at room temperature with methanol, partitioned with chloroform/ water, and *n*-butanol/water	mice, writhing test (500 *p.o.*) mice, formalin test (500 *p.o.*) mice, tail flick test (500 *p.o.*)	[20i]
H. myriathum Cham. & Schltdl.	aerial parts, dried and powdered	maceration for 3 days at 20°C with n-hexane	mice, writhing test (5-90 *p.o.* and *i.p.*) mice, hot plate test (5-90 *p.o.* and *i.p.*)	[20j]
H. organifolium Wild.	aerial parts, dried	maceration for one night with 50% ethanol and extraction for 8 h at 46°C, three times repeated	mice, hot plate test (50, 100, 250 *p.o.*) mice, tail-clip test (50, 100, 250 *p.o.*) mice, tail flick test (50, 100, 250 *p.o.*)	[20k]
H. polyanthemum Klotzsch ex Reichardt	aerial parts, air-dried, powdered	Ultra-turrax apparatus with cyclohexane	mice, hot plate test (90, 180 *p.o.* and *i.p.*) mice, writhing test (90, 180 *p.o.*)	[20e]
		maceration for 24 h at 20°C with cyclohexane	mice, hot plate test (45, 90, 180 *p.o.*) mice, writhing test (60 *p.o.*)	[20l]
H. reflexum L. fil.	aerial parts, dried in an oven at 40°C and powdered	maceration for 3 days at room temperature with methanol	mice, writhing test (500, 1000 *p.o.*) mice, formalin test (500, 1000 *p.o.*) mice, tail flick test (500, 1000 *p.o.*)	[20m]
H. triquetrifolium Turra.	aerial parts, dried and powdered	Soxhlet apparatus at 80°C with methanol	mice, formalin test (10, 25, 50, 60 *i.p.*) mice, tail flick test (10, 25, 50, 60 *i.p.*)	[20n]

[a]) / – the data is missing in the cited work. [b]) *p.o.* – substances given *per os* (orally given). [c]) *i.p.* – substances given intraperitoneally

Can and coworkers [19j] investigated for the first time the effect of SJW ethanolic extract on nociceptive perception of streptozotocin-diabetic animals. Their results pointed out that the extract can produce a dose dependent prolongation of response latencies in both antinociceptive tests (tail-pinch and tail flick tests) [19j]. The administered extracts of SJW did not show any behavioral side-effects or signs of altered locomotor activity [19l]. This confirmed the rationale behind the ethnomedicinal usage of this plant for the treatment of diabetes [19j].

The capability of SJW and hypericin to relieve meningeal nociception in an animal model induced by administration of the nitric oxide (NO) donors was demonstrated in a recent publication [19k]. A single oral dose of SJW dried extract (5 mg/kg *p.o.*) counteracted the nociceptive behavior and the overexpression of IL-1β and iNOS. A number of the cellular pathways were found to be involved: the expression of protein kinase C (PKC) and downstream effectors was detected; NO donors increased expression and phosphorylation of PKCγ, PKCε and transcription factors, such as nuclear factor (NF)-κB, cyclic AMP response element binding protein (CREB), and Signal Transducer and Activator of Transcription (STAT)-1. These results might suggest SJW as an innovative and safe perspective for migraine pain [19k].

Extracts from other *Hypericum* taxa gave comparable results in related antinociception assays. *H. brasiliense* standardized extract showed both peripheral and central analgesic activity in a dose-dependent manner, starting from 50 mg/kg as the lowest tested dose [20a]. An effect of *H. canariense* and *H. glandulosum* infusions, organic solvent extracts and extract fractions on nociception in mice was demonstrated in two models (acetic acid-induced writhings and tail flick test). Although both plant extracts turned out to have the same most effective dose (500 mg/kg), only the methanol extract of *H. glandulosum*, as well as its *n*-butanol fraction significantly prolonged the reaction time of mice 1 h after the treatment in a tail flick assay [20d]. The differing polarity of solvents used to extract *H. caprifoliatum* and *H. polyanthemum* had little effect in altering the analgesic-like activity in the applied doses of 90 and 180 mg/kg, respectively. In the hot plate test, these plant extracts showed analgesic activity in the doses of 90 and 180 mg/kg, with the same doses of the plant extracts of both plant taxa inhibiting equally the number of abdominal writhings [20e]. The hot plate test revealed no

analgesic activity of the methanolic extract of *H. empetrifolium* administered *i.p*. (50 and 100 mg/kg), whereas during the writhing test, at a dose of 100 mg/kg, the extract produced a significant inhibition of the contractions induced by acetic acid [20h]. The chloroform fraction of an extract of *H. grandifolium*, at a dose of 500 mg/kg, provoked a maximal decrease in the number of writhings induced by chemical noxious stimuli (acetic acid and formalin) and modified tail flick reactions of rats [20i]. Low doses (ranging from 10 to 60 mg/kg) of an extract of the aerial parts of *H. triquetrifolium* had an effect throughout the biphasic pain response in the formalin test and increased the tail flick latencies [20n].

Synergism is very common between substances in plant extracts [22] and *Hypericum* taxa extracts are no exception. Alone the polar and non-polar (cyclohexane) fractions of a methanolic extract of *H. caprifoliatum* had no influence on nociception. On the other hand, mixtures of the same fractions showed a notable effect indicating a synergistic effect of substances rather than the effect of one single compound [20f]. Another obvious example of such synergism is given by the chloroform fraction of the extract of *H. grandifolium,* containing benzophenone derivatives as major components. Being the most effective one in an antinociceptive test, this fraction was further separated into three sub-fractions (F1, F2, F3) that were evaluated again for antinociceptive activity. The results showed that separately only two of them (F2, F3) had only a mild activity in comparison with the original chloroform fraction, i.e. they indicated a synergism between compounds in the abovementioned fraction [20i].

Hypericum *secondary metabolites associated with antinociceptive activity:* In an attempt to explain the observed antinociceptive activity of the various extracts of *Hypericum* taxa a number of researchers have investigated the analgesic effect of pure major and/or minor constituents isolated during their phytochemical studies. These further pharmacological evaluation studies revealed that the naphthodianthrone hypericin and the acylphloroglucinol hyperforin (Figure 1), the most abundant compounds of SJW methanolic extract, possess certain antinociceptive properties, whereas the flavonoids hyperoside and quercetin (Figure 1) showed no antinociceptive properties, but potentiated the hypericin-induced antinociception [18,19a].

Extracts and the sub-fraction of SJW containing the two active plant metabolites produced a bell-shaped dose response curve for both the abdominal-constriction test and the hot plate test [19a] and these follow the same bell-trend of the well-known antidepressant activity of SJW extracts [23]. Based on these findings, it appears that the hypericin (and possibly pseudohypericin) content in the plant material could be correlated with the pain-relieving effect that different *Hypericum* taxa produce. A previous study [24], dealing with the content of the two naphthodianthrones in different taxa of this genus, could possibly provide us with helpful data in the estimation of which taxa (their extracts to be precise) should be expected to have a greater antinociceptive effect when compared with SJW. Hence, if we should judge the antinociceptive potential of a *Hypericum* taxon only according to the relative content of (pseudo)hypericin, we could anticipate that species of the section *Drosocarpium* should show several times greater effect in alleviating pain than SJW. For example, *H. boissieri* with 0.51% of the naphthodianthrones in the dry herb (cf. with the 0.125% content of the same compounds in *H. perforatum*) should be a good candidate for future studies in this field. Only one additional plant species (*H. empetrifolium*), besides *H. perforatum*, screened for the content of hypericin in the work of Kitanov [24], has been tested for antinociceptive activity. The extract of this species showed in an antinociceptive evaluation, as expected from the low abundance of hypericin (0.009% of the weight of dry plant material), and no pseudohypericin, no effect on centrally modulated nociception. However, it did show a significant inhibition of contractions induced by acetic acid and an impact on inflammatory processes (carrageenan-induced paw edema) [20h]. From these facts one can see that not only hypericin is to be considered responsible for the overall antinociceptive effect of the plant extracts and that (pseudo)hypericin is most possibly responsible for central analgesic activity.

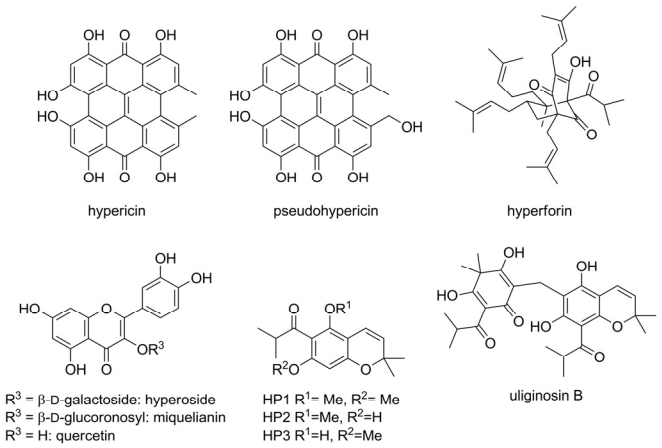

Figure 1: Secondary metabolites of *Hypericum* taxa from the extracts showing antinociceptive activity

Different extracts of different *Hypericum* species had an analgesic effect both in rats and mice, but none exceeded the activity of *H. polyanthemum* secondary metabolites. Benzopyrane HP1 (Figure 1), isolated from the cyclohexane extract of this plant species, was assessed for possible antinociceptive activity in two models, hot plate and writhing test. It possessed a dose-dependent antinociceptive effect (30-60 mg/kg), but it did not influence mice performance on a rota-rod apparatus [20l]. The same group of authors [20g] also investigated the antinociceptive activity and influence on CNS of hyperoside, one of the most abundant components of the methanol extract of *H. perforatum* and other South Brazilian *Hypericum* species. Hyperoside was shown to have pain-relieving properties (10-40 mg/kg), using the hot plate and writhing tests, whereas its influence on CNS was demonstrated through several models (gross behavior, open field test, pentobarbital-induced sleeping-time, and forced swimming test) in various doses (1.8-40 mg/kg) indicating an antidepressant activity of this compound. Alongside the mentioned compounds, a newly isolated compound from *H. myriathum*, uliginosin B (Figure 1), was proven to possess a dose-depended antinociceptive activity in the hot plate and abdominal writhing tests [20j]. However the application of higher doses of this compound produced an ataxic effect observed during a rota-rod test. Also, the interaction of uliginosin B with drugs used for treating pain in clinical practice, morphine, amitriptyline and clonidine, in an array of analgesic assays was studied [25]. The effect of the mixtures of drugs was studied using an adapted isobologram analysis at the effect level of 50% of the maximal effect observed. The analysis showed that the interactions between uliginosin B and morphine was synergistic, while the interactions between uliginosin B and amitriptyline or clonidine were additive. These findings point to uliginosin B as a potential adjuvant for pain pharmacotherapy, especially for opioid analgesia. [25].

Possible mechanisms of action: Up to date a number of mechanisms of action of the extracts and pure compounds from *Hypericum* taxa have been put forward, and these, summarized below, generally can be grouped as: an opioid-like mechanism, influence on the catecholaminergic system, neurotransmitter uptake blocking activity, ion channels modulating and enzyme-inhibiting activity.

The opioid mechanism of action of several solvent extracts originating from different *Hypericum* spp., as well as of the isolated active compounds from these extracts, was demonstrated on numerous occasions [19a,e, 20e,h,k,l]. The opioid receptor agonists and antagonists can act due to three subtypes of this receptor (μ, δ or κ) that are coupled to heterotrimer Gi/o proteins [14c]. Naloxone, the non-selective opioid antagonist, is believed to act due to all three receptor subtypes. When naxolone was administered together with the extracts of either SJW or other *Hypericum* taxa, the antinociceptive effect of these extracts was inhibited or reversed [19a,e, 20e,h,k,l]. Furthermore, the opioid antinociception of an extract of SJW and its chloroform fraction was shown to be mediated by an unselective stimulation of μ, δ and κ-opioid receptor subtypes. This was concluded from the fact that the κ-opioid antagonist nor-binaltorphimine, the μ-opioid antagonist CTOP (D-Phe-Cys-Tyr-D-Try-Orn-Thr-Pen-Thr-NH_2), the δ_1-opioid antagonist, 7-benzylidene naltrexamine, and the δ_2-opioid antagonist, naltriben, were all able to prevent the antinociception of the mentioned extract and fraction [19a].

Antinociceptive benzopyrans isolated from *H. polyanthemum*, shown in Figure 1, are also believed to have a mechanism of action through the opioid receptor system (similar to cannabinoids). More than a few substances which contain a benzopyran skeleton, either naturally occurring or synthetic, have been established to have a certain potential as pharmacologically active compounds. Thus, one can expect that not only the benzopyran-containing *Hypericum* species are to have relevant activities on the CNS, but also those taxa which contain other related compounds such as benzophenones, xanthones, and dimeric phloroglucinol derivatives that have a widespread occurrence in this genus [20l]. The phloroglucinol derivative, hyperforin, was proven to possess the opioid-like activity since it inhibited the agonist/antagonist binding to the opioid receptors [26]. However, some scientists [19g] also suggested that the extract of SJW is not acting through the opioidergic system (effect of the extract was unaffected by naloxone) because a relatively low dose of SJW extract significantly potentiated the antinociceptive effect of morphine in the second phase of the formalin test.

The extractable lipophilic matter from SJW has been shown to block noradrenaline, serotonin, dopamine, γ-aminobutyric acid and glutamate uptake, in cortical synaptosomes, by a mechanism not directly related to binding at the uptake sites [27a-c]. The extracts of SJW have also been proven to inhibit monoamine oxidase enzyme activity which is, together with the previously mentioned blocking activity, in good agreement with the well-known effect of SJW on the central nervous system and its most frequent usage for the treatment of depression [28a-c]. The anti-noradrenaline and serotonin-uptake mechanism is similar to the one of tricyclic antidepressants (TCAs), but it does not fit into the action of classic TCAs because of the other mentioned neurotransmitter uptake blocking activities [20i-20l]. The underlying mechanism of both antinociceptive and antidepressant activity of uliginosin B was evaluated in great detail [20j, 29a,b]. Its antinociceptive action was studied using various antagonistic agents and monoaminergic and glutamatergic neurotransmissions were shown to be responsible for its action [20j, 29a]. The antidepressant action of uliginosin B was demonstrated to encompass the inhibition of synaptosomal uptake of dopamine, serotonin and noradrenalin without binding to any of the monoamine transporters [29b].

Hypericum calycinum and *H. triquetrifolium*, along with SJW, were demonstrated to possess analgesic effects that can possibly be attributed to an action at the catecholaminergic system level [20c,n]. A similarity with TCAs can also be observed here, due to the potentiating action of both TCAs and *Hypericum* extracts on the effect of biogenic amines in endogenous pain-relieving systems and that the enhancement of noradrenaline and serotonin function may contribute to the noted analgesic effect. It has been reported that TCA drugs potentiate the antinociceptive effect of morphine in both animals and humans and possess clinical efficacy in the treatment of chronic pain states as adjuvant analgesics [30].

A P-type Ca^{2+} channel-blocking activity has been demonstrated for SJW extract, via the interaction with calmodulin or through calmodulin-activated pathways involving at least one second messenger [31], which might be in connection with the antinociceptive action since there are a number of calcium channel blockers that are well-known for their pain-reliving effect (reduce chest pain caused by angina pectoris) [32]. An opposite type of action was noted for hypericin, one of the major constituents of SJW and other *Hypericum* species extracts. It inhibited guanylate cyclase and, as a consequence, increased the L-type Ca^{2+} channel (CaV1) conductance leading into prolonged action potential in cardiac myocytes [33]. Additionally, the results of another study indicated that hypericin, when applied extracellularly, increases the duration of the action potential in hippocampal neurons and that this effect might be explained by its modulation of voltage-gated K^+ currents [34]. Nevertheless, hyperforin is believed also to significantly contribute to the overall activities observed for SJW with its influence on sodium conductive pathways and these affect the physicochemical properties of neuronal membranes and act on the hypothalamic-pituitary-adrenal axis [35].

Enzyme assays performed on rat brain (periaqueductal grey area, PAG) homogenates established that hypericin and pseudohypericin are potent and selective inhibitors of protein kinase C (PKC), which belongs to a family of enzymes involved in numerous important cellular signal transduction cascades, including pain modulation [36]. However, not all major constituents of the extracts of *Hypericum* taxa have such a PKC-inhibiting action [36]. A single oral administration of SJW produced a significant decrease of the PKCγ and PKCε phosphorylation in the PAG area due to the presence of hypericin (Figure 1). Furthermore, SJW showed a dual mechanism of action since hyperforin (Figure 1) antinociception involves an opioid-dependent pathway [19a, 36]. The presence of hypericin in the extracts was fundamental to induce both thermal and chemical antinociception through the inhibition of PKC activity, whereas hyperforin selectively produced a thermal opioid antinociception. Possible mechanisms that involve inhibition of prostaglandin generation, mediators of both pain and inflammatory processes, were also suggested for SJW extract [19a, 20h].

Conclusions: The up to now conducted studies have shown that all *Hypericum* species exert an antinociceptive effect at spinal and central levels (with the spinal effect more pronounced). Although *H. perforatum* represents the most renowned plant species of this genus for its medicinal usage, it is certainly not the only one that possesses antinociceptive properties. There are several proposed mechanisms explaining the pain-relieving properties of *Hypericum* plant extracts so-far put forward and a number of them seem to be operational

simultaneously. The most repeatedly demonstrated *modus operandi* involving the major constituents of these extracts (hypericin, pseudohypericin, hyperforin and others) is the one concerning the opioid receptor system, suggesting the agonist/antagonist effect of these extracts. However, pro arguments can be, likewise, found in the literature for other mechanisms of action, such as the neurotransmitter reuptake-blocking activity or ion channel-modulating activity. Having all this in mind and the work reported on the antidepressant effect of SJW on CNS, one can say that these plants rightfully earned their highly esteemed place in the ethnomedicine of many nations and deserve further study.

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