Indian Journal of Experimental Biology
Vol. 38, November 2000, pp. 1077-1085

Review Article

Hypericum perforatum: Nature's mood stabilizer

Vikas Kumar[1, 2], P N Singh[1], A V Muruganandam[2] & S K Bhattacharya[1*]

[1]Pharmacology Laboratory, Department of Pharmaceutics, Institute of Technology,

[2]Neuropharmacology Laboratory, Department of Pharmacology, Institute of Medical Sciences,

Banaras Hindu University, Varanasi 221 005, India

Hypericum perforatum (HP), better known as St. John's Wort, has been used clinically for centuries. Modern usage is still quite diverse and includes kidney and lung ailments, insomnia and depression. Standardised extracts of HP are widely used in the treatment of psychovegetative disorders and especially for mild forms of depression. Several bioactive constituents of this plant may play important role in its well-known antidepressant activity, which are discussed in the present article. Furthermore, emphasis is also given on its botany, chemistry, pharmacology and clinical efficacy.

The name *Hypericum* is a derivation of two Greek words, *hyper* and *eikon* which translate "over" and "icon" as in "over an apparition," alluding to its use in ancient times for protecting against demonic possession and its reported ability to protect one from "evil spirits." The species name *perforatum* is based on the perforated appearance of the leaves due to their translucent leaf glands which can be observed when held up to light. *Hypericum perforatum* (HP) is also known as St. John's Wort because its flowers bloom around St. John's Day (June 24) and red pigments which are exuded when the buds and flowers are squeezed were associated with the blood of St. John the Baptist[1].

HP is a perennial plant belonging to the Guttiferae family. Some taxonomists classify the genus *Hypericum* in a separate family, the Hypericaceae. The genus *Hypericum* encompasses approximately 400 species, of which ten morphologically and chemically distinct species grow in central Europe[2]. HP is distributed in Europe, Asia, North Africa and North America. Indian HP (IHp) is a rhizomatus perennial herb growing up to a height of 3 feet distributed in the western Himalayas at altitudes of 3000-10,500 feet.

Hypericum species were already known to ancient communities as useful medicinal plants. HP has been known since Greek and Roman times. Closer to our times, Mattioli also wrote of *Hypericum in Discorsi*[3]. The use of HP in particular, as a remedy was described and recommended throughout the Middle Ages. The primary ancient medical herbalists, including Hippocrates, Pliny, Dioscorides, Theophrastus and Galen wrote about the medicinal properties of St. John's wort, noting its use as a vulnerary (wound healing) and for treatment of neuralgic conditions such as sciatica and hip pain. Mattioli wrote of its use as an emmenagogue, diuretic and antimalarial[4]. The most common use of *Hypericum* has been for the treatment of depression and various psychological and neuralgic disorders, as an anthelmintic for worms, a vulnerary for minor hemorrhages, for bedwetting in children, and as a diuretic[5-8]. Besides it has been also used as balm for wounds, burns, ulcers and bites[9].

Botanical description

HP is glabrous throughout, green or sometimes glaucescent; the stems are erect, branched at top and 30 to 100 cm long; the leaves are oval or elliptic, sucordate, or rather narrow, oblong-linear, subobtuse, revolute-marginated with numerous pellucid black glandular dots. The flowers are numerous, forming a broadly paniculate, almost corymbose inflorescence, 7-11cm long and 5-11 cm broad. The bracts are lanceolate, 0.5 cm long and acute. The calyx is deeply parted, 5 mm long and about two to three times shorter than corolla; sepals are lanceolate 4-5 mm long, 1 mm broad, as long as ovary, acute or acuminate, with black glandular mostly oval dots. The petals are oblong to oblong elliptic, in equilateral, 1.2 to 1.5 cm long, 0.5 to 0.6 cm broad, with numerous black glandular dots and lines on margin of upper part, surface with numerous yellow glandular dots. The stamens are numerous, in 3

*Correspondent author
E-mail: salil@banaras.ernet.in

bundles; the ovaries ovoid, 3-5 mm long; there are three styles. The seed is 1 mm long, cylindric, brown, minutely pitted longitudinally.

Chemistry

Hypericum contains numerous compounds with documented biological activity. Most researchers consider its effects to be due to a variety of constituents rather than any single component. Constituents that have stimulated the most interest include the napthodianthrones hypericin and pseudohypericin, a broad range of flavonoids, including quercetin, quercitrin, amentoflavone and hyperin, the phloroglucinols hyperforin and adhyperforin, essential oils and xanthones. Recently hyperforin has been considered as an important antidepressive constituent of HP[10,11]. The following major groups of bioactive constituents will summarise the constituents of HP:

Napthodianthrones—The napthodianthrones present in HP include hypericin (1.8%), pseudohypericin (relative abundance 3.9%), isohypericin and emodin-anthrone (precursor of hypericins). Protohypericin and protopseudohypericin were also detected in fresh material, which on exposure to light yields hypericin and pseudohypericin, respectively. An oxidation product of pseudohypericin, cyclopseudohypericin was also cited[12,13].

Flavonoids—Flavonoids present in the HP include, flavonol glycosides, viz., rutin (0.3%), quercitrin (0.3-0.524%), isoquercitrin (0.3%)[14,15], hyperin/hyperoside (0.7-1.1%)[16], and aglycones, viz., kaempferol, luteolin, myricetin and quercetin (2%).

Biflavones—The following biflavones: 3,8″-biapigenin (0.1-0.5%)[16,17], amentoflavone/ 3',8″-biapigenin (0.01-0.05% in flowers), were reported from the HP.

Proanthocyanidins—The proanthocyanidins, consisting of dimers, trimers, tetramers, and high polymers of catechin and epicatechin, represent approximately 12% of the dried weight of the aerial portion of the plant[3,18], the highest values were observed during the flowering stage.

Phenyl propanes—HP contain phenyl propanes in the form of esters of cinnamic acids, such as *p*-coumaric and caffeic acids (0.1%). Chlorogenic acids have been detected and found to less than 1%. Ferulic, isoferulic and gentisic acids alongwith leucocyanidin have also been described[19-21].

Phloroglucinols—Two closely related phloroglucinol derivatives; hyperforin and adhyperforin, were reported from the HP[22]. The amount of hyperforin was found be 2% in flowers and 4.5% in the unripe fruits while the amount of adhyperforin was found to be 0.2% (flowers) and 1.6% (unripe fruits)[23,24]. These hyperforins were lipophilic, unstable toward heat and light either on storage or in solution. One of the products of degradation was 2-methyl-3-buten-2-ol, probably generated by oxidative cleavage of isoprenyl side chains of hyperforin and adhyperforin. Recently, an unusual compound with combined structure of cadinan sesquiterpene and hyperforin, hydroperoxy-cadiforin (0.0006%)[25], and furohyperforin (ca. 5% of hyperforin concentration)[26] were isolated and characterized from HP.

Xanthones—A xanthanolignoid, kielkorin (0.01% in roots)[27], and 1,3,6,7-tetrahydroxy xanthone (in trace amounts in leaves and stems)[28] were reported in HP.

Essential oil—The essential oil consists mostly of monoterpenes (pinenes) and sesquiterpines[19]. Of these, the primary compounds include the saturated hydrocarbons, 2-methyl-octane (16.4% and a-pinene (10.6%). Also present were traces of 2-methyl-decane, 2-methyl-butenol and undecane, b-pinene, a-tocopherol, geraniol, myricine, linolene, caryophillene, humulene, C16 and C24 *n*-alkanes, and C24, C26 and C28 n-alkanols[29-31].

Additional compounds include choline, carotenoids (lutein, luteoxanthin, violaxanthin, cis-throllixanthin, throllichromone), b-sitosterol, pectin, phlobaphene and rhodan; isovalerianic, lauric, myristic, nicotinic (0.12% in leaves, palmitic and stearic acids, amino acids; including cysteine, GABA (0.7 mg/g), glutamine, leucine, lysine, ornithine, proline, threonine, scopoletin, umbelliferone, and vitamin C[16,19,31-34].

The pharmacological activity of various constituents are summarised in Table 1.

Biological properties

Hypericum perforatum has been widely researched for its antidepressant effects. Recent interest has focused on its potential as an antiviral agent. There is some data confirming its traditional wound-healing effects. *Hypericum* contains several compounds of biological interest, including the napthodianthrones, hypericin and pseudohypericin, and a broad spectrum

Table 1—Pharmacological activity of constituents of *Hypericum*

Constituent	*Activity*
Amentoflavone (13', 118-biapigenin)	Anti-inflammatory, anti-ulcerogenic[35]
GABA	Sedative[31]
Hyperforin	Antibacterial against gram-positive bacteria, wound healing[22,23,36]; neurotransmitter inhibitor[31], potential anticarcinogenic[37]
Hypericin	Antiviral[38-40]
13, 118-biapigenin	Probably sedative[35]
2-methyl-butenol	Sedative[31]
Proanthocyanidins	Antioxidant, antimicrobial, antiviral, vasorelaxant[31]
Pseudohypericin	Antiviral[39]
Quercitrin	*In vitro* MAO inhibiting activity[28]
Xanthones	Antidepressant, antimicrobial, antiviral, diuretic, cardiotonic, MAO_A inhibitor[28,31,41,42]

of flavonoids. Although investigations continue, these are considered to be primarily responsible for HP's activities.

Antidepressant activity

A commercial standardized extract of HP (Psychotonin®) was tested in several animal models predictive of psychotropic activity[43]. These activities included two, used for antidepressants, increased activity in water wheel test in mice and reduced aggressiveness in isolated male mice. The pharmacological models most commonly used to demonstrate a potential antidepressive activity of *Hypericum* extracts are the examination of the behaviour of mice in an unknown environment[43], forced swim test[44], and the measurement of biogenic amines metabolites in urine[45]. A correlation can be established between the excretion in urine of 3-methoxy-4-hydroxy phenyl glycol (MHPG), the main metabolite of noradrenaline, and the initiation of the therapeutic activity of HP as an antidepressant agent.

Butterweck *et al.*[44,46] compared the *Hypericum* extract, LI 60, with bupropion, a synthetic antidepressant. Results indicated similar effects of both the drugs in the tail suspension test (mice) and the forced swim test (rats). Since *Hypericum* treatment was antagonized by drugs known to reduce dopamine functional activity (haloperidol, sulpiride, a-methyltyrosine and g-butyrolactone) the authors concluded that *Hypericum* exerted its activity via dopaminergic activation. *Hypericum* extract, LI 160, subchronic treatment (250 mg/kg for two weeks) resulted in a 15% down regulation of β-adrenergic receptors in the rat frontal cortex. In the same study, a 25% down regulation was observed after the imipramine treatment[47].

Many questions exist about the composition, pharmacology and mechanisms of action of HP. In fact the active constituent is still unclear. While previous studies report that hypericin inhibits MAO at concentrations of 50 μg/mL[48], others have failed to confirm this effect[49-51]. Some of the research work on the identification of the active antidepressant constituent has been performed using computer modelling. Out of the flavonoids, xanthones and hypericins compared best. Overlap was obtained with flavonoid derivatives and suggest flavonoids as the most likely MAO inhibitor fraction, due to structural similarity to toloxone and brofaromine, two known inhibitors of MAO-A[52]. Bladt and Wagner[49] reported that the *Hypericum* fractions with the greatest MAO inhibition contains the highest concentration of flavonoids. In another study, the xanthone fraction was particularly strong inhibitor of MAO-A *in vitro*[28]. However, the MAO inhibition shown by *Hypericum* may not be pharmacologically relevant since it has not been confirmed *in vivo*. Bladt and Wagner[49] reported that no MAO inhibition was seen *in vivo* after administration of 300 mg/kg *Hypericum* extracts to rats.

Other proposed mechanisms involve effects on serotonin. Muller and Rossol[53] reported that *Hypericum* extract inhibits serotonin receptor expression at 50 μM (~ 25 μg/mL) and Perovic and Muller[54] reported inhibition of serotonin uptake (IC_{50} = 6.2 μg/ml). The concentration required for the former effect could never be achieved in the whole animal and even the latter concentrations seem unlikely. As a reference comparison, Muller *et al.*[47] reported an IC_{50} for the synthetic antidepressant, clomipramine, of 0.9 nM (~0.3 ng/ml) for serotonin uptake inhibition. In addition, an inhibition of both synaptosomal GABA uptake (IC_{50} = 1 μg/ml, LI 160) and $GABA_A$-receptor binding (IC_{50} = 3 μg/mL) was noted.

Through an National Institute of Mental Health (NIMH, USA) screening contract (Novascreen, Baltimore, Maryland) a commercially available crude extract from the fresh flowers and buds of HP was subjected to *in vitro* assays in a battery of 39 receptor types and two enzyme systems. The receptor assays showing 50% displacement of radioligand (or 50% inhibition of MAO) were considered "hits". Concentration-response curves (IC_{50}) were then performed for the hits. The crude extract of HP had significant receptor affinity for adenosine, $GABA_A$, $GABA_B$, serotonin, benzodiazepine, inositol triphosphate (IP_3), and $MAO_{A,B}$[55]. The inhibition of MAO by crude *Hypericum* extracts is consistent with previous reports[48,49,51]. Unlike the crude extract, synthetic hypericin (95%) lacked significant MAO_A or MAO_B inhibition at concentrations up to 10 μM. Hypericin had affinity only for NMDA receptors (Ki~1 μM) and this may play a role in its reported antiviral activity since NMDA antagonists prevent gp 120-induced neurotoxicity[56]. These data are consistent with the recent pharmacological evidence suggesting that other constituents of this plant may be more important for the reported psychotherapeutic activity.

More recently, hyperforin, a prenylated phloroglucinol present in this plant, has been focused as primarily responsible for the antidepressant activity of the HP[10,11,57-60]. Many of the experimental and clinical studies[60] have confirmed the antidepressant activity of hyperforin. Hyperforin was shown to inhibit uptake of serotonin (5-HT), dopamine (DA), noradrenaline (NA), GABA and L-glutamate with IC_{50} values of about 0.05 - 0.10 μg/ml (5-HT, NA, DA, GABA) and about 0.5 μg/ml (L-glutamate) in synaptosomal preparation[11]. Recently, Indian *Hypericum perforatum* (IHp) extract standardised for hyperforin lacked MAO A and B inhibitory activity[61]. Additionally, IHp showed antidepressant[62], anxiolytic[63] and nootropic[64,65] activities.

Another novel proposal is that *Hypericum* extract reduces cytokine expression (interleukin-6)[66]. The hypothesis is that interleukins can induce depression in susceptible individuals[67]. The link between depression and the immune system is well established[67]. In addition to the antidepressant effects, *Hypericum* has historically been used for a wide variety of neurological conditions, including anxiety, insomnia due to restlessness, irritability, neuralgia, trigeminal neuralgia, neuroses, migraine headaches, fibrosis, dyspepsia (oil), and sciatica[69-71,74].

Wound healing properties

Hypericum has historically been one of the most relied upon botanicals for the treatment of wounds. Part of this activity is due to *Hypericum*'s antimicrobial activity, which is attributed to the essential oil, phloroglucinols and flavonoids. The essential oil and the water-soluble fraction of an alcoholic extract exhibit minor antifungal and significant antibacterial activity. A resin fraction of the alcoholic extract has also been shown to be effective against gram-positive organisms[72]. The tannins and flavonoids were reported to inactivate *Escherichia coli* at dilution of 1:400 or 1:200[73,74]. Hyperforin and adhyperforin have been reported to possess an chemotherapeutic effect greater than that of sulfanilamide[75].

An ointment prepared by extracting the fresh flowers (5 g) with olive oil (100 g) (for 10 days at 20.C) was used in the treatment of 1st, 2nd, and 3rd degree burns. First degree burns healed in 48 hours. Second and third degree burns healed atleast three times faster than burns treated with conventional methods and keloid formation was inhibited[76]. A commercial preparation containing 0.412% quercitrin (Novoimamin) was found to be effective against *Staphylococcus aureus* infection[75] and its effects have been reported to be greater than conventional treatment with sulfanilamide[77]. A tincture (1:10) of *Hypericum* was studied for its wound healing properties and compared with calendula, another widely used wound healing herb. The effect of orally administered tincture of *Hypericum* was more pronounced than topical application of Calendula tincture in the healing of incision, excision and dead space wounds, as evidenced by an increase in epithelisation and wound-breaking strength[7].

Antiviral activity

Hypericin is currently in early clinical trial in the USA as an antiviral agent[4,39]. In an open pilot study, 18 patients with acquired immune deficiency syndrome (AIDS classifications; 3 with CDC II, 8 with CDC IVB and 3 with CDC IVCI) were treated with an i.v. *Hypericum perforatum* preparation (Hyperforat; 2×2ml weekly) plus additional *Hypericum* tablets of undefined dosage. Sixteen out of 18 patients with good study compliance showed

increasing counts of absolute CD4 values over a 40-month period. Also observed were improvements in CD4/CD8 ratios in the majority of patients. In addition only 2 of the 16 patients experienced an opportunistic infection during the 40-month observation period. The other 14 of the 16 patients remained clinically stable[78].

Studies have shown that two of HP's primary components, hypericin and pseudohypericin, inhibit a variety of encapsulated viruses, including herpes simplex types 1 and 2[40,79] and the human immunodeficiency virus type 1 (HIV-1) virus associated with AIDS[39,80,81]. While the later researchers have concluded that hypericin and pseudohypericin display a unique and effective antiviral activity, Weber *et al.*[40] suggest that it may be due to non-specific association with cellular and viral membranes. *In vitro* antiviral activity has also been reported against murine cytomegalovirus, para-influenza 3 virus, Sindbis virus[81], vesicular stomatitis virus[79], and equine infectious anemia virus[82].

The antiviral activity appears to involve a photoactivation process[81-85] which forms singlet oxygen and inactivates viral fusion and syncytia formation[86-88]. While hypericin does show antiviral activity *in vivo* (mice), these photodynamic properties may limit its potential usefulness as an antiretroviral agent[89]. However, besides singlet oxygen production, hypericin can photoreduce oxygen to superoxide radicals and can form semiquinone radicals in the absence of light[38]. These authors speculate that this ability to form semiquinones might account for the antiviral activity.

Protein kinase C inhibition

Hypericin has been reported inhibit the growth of glioma cell lines *in vitro* and to be a potent inducer of glioma cell death due to inhibition of protein kinase C (PKC) as measured by [^{3}H] thymidine uptake[90]. The anti-PKC activity doses are reported to be below those associated with clinical hypericism[90].

Other researchers report a PKC-inhibiting activity with both hypericin and pseudohypericin (IC_{50} of 1.7 µg/ml and 15 µg/ml, respectively)[91]. Receptor tyrosine kinase activity of epidermal growth factor has also been reported to be inhibited hypericin[92]. These effects have been linked to both the antiviral and antineoplastic activities[38,93]. In addition, the PKC inhibition may also contribute to the anti-inflammatory effects historically associated with *Hypericum* as hypericin has been found to inhibit the release of arachidonic acid and leukotriene B_4[93].

Other effects

Hypericum has been reported to have number of additional effects. In one study the procyanidin fraction of *Hypericum* was tested in an isolated guinea pig heart preparation[94] and found to enhance coronary flow in the same way as the procyanidin from Crataegus (Hawthorn). The procyanidins fractions also antagonized histamine or prostaglandin $F_{2\alpha}$-induced atrial contractions in porcine isolated coronary arteries[95].

In another study, a significant increase in the production of nocturnal melatonin was observed after administration of 90 drops of the commercial preparation, Hyperforat. These effects were observed after a three week period[96]. Other researchers report that *Hypericum* may be useful in the treatment of chronic tension headaches[97], while a hepatoprotective activity of a water/alcohol extract has also been reported in animals[98]. Pain and inflammation of nerve origin may also respond to *Hypericum* which can be administered both topically and orally[99-101]. Recent study with IHp in our lab confirms its anti-inflammatory and analgesic activity[102].

Clinical studies

Hypericum has become increasingly popular in Germany, where physicians routinely prescribe herbal medicines. In 1994, 66 million daily doses of HP were prescribed there for use in the treatment of depression[103]. This phytomedicine has been tested in more than 3,000 patients against placebo and various active medications[104-109]. German researchers recently published a meta-analysis of 23 randomized trials of *Hypericum* with a total of 1,757 outpatients with mild to moderately severe depressive disorders. They concluded that the herb was significantly superior to placebo and appeared comparably effective to standard antidepressants (maprotiline, imipramine and amitriptyline) while producing fewer side effects[6].

Pharmacokinetic studies

Detailed pharmacokinetic studies have been performed with the standardized *Hypericum* extract, LI 160 (Jarson® 300), one of several officially recognized formulations (containing 300 mg of the dried extract of HP, yielding 0.24-0.32% total

hypericin) in Germany for the treatment of mild to moderate depression. Oral administration of this preparation was given to twelve healthy male subjects[110]. Hypericin and pseudohypericin were determined in plasma using an HPLC technique (limit of detection 0.2 ng/ml) after oral administration of single doses of 300, 900 and 1800 mg total extract. Blood levels were measured for up to three days after a single dose. Peak plasma concentrations were seen with hypericin after 2.0 to 6.0 hours and with pseudohypericin between 0.4 to 0.6 hours. Peak concentrations of hypericin were 1.5, 7.5 and 14.2 ng/ml, respectively, for the three doses of the extract. Pseudohypericin attained concentrations of 2.7, 11.7 and 30.6 ng/ml, respectively. The elimination half-life of hypericin was between 24.8 and 26.5 hours. The elimination half-life of pseudohypericin ranged from 16.3 to 22.8 hours.

Repeated doses over 14 days (3×300 mg/day) resulted in steady state (level at which the constituents accumulate to reach a steady concentration) after four days. The peak concentration of hypericin was 8.5 ng/ml and 5.8 ng/ml for pseudohypericin. Trough concentrations (14 hours after the last dose) were between 5.3 and 3.7 ng/ml for both compounds[110].

Earlier pharmacokinetic data on mice report that maximum plasma concentrations of hypericin and pseudohypericin were reached at 6.0 hours and maintained for atleast 8.0 hours. The aqueous-ethanolic extract used contained 1.0 mg of hypericin. The same researchers also tested the concentration of hypericin in one human test subject after a single dose of the same extract. The concentrations found in human serum were comparable to those found in the mice[111].

Pharmacokinetic studies of *Hypericum* extracts containing hyperforin revealed the half-life and clearance values as 6 hour and 70 ml/min/kg respectively. After oral administration of 300 mg/kg *Hypericum* extract (WS 5572, containing 5% hyperforin) to rats maximum plasma levels of approximately 370 ng/ml (approx. 690 nM) were reached after 3 h, as quantified by HPLC and UV detection method.

Conclusion

Hypericum is a clearly one of the leading psychotherapeutic phytomedicines for the treatment of mild to moderate depression. The shortcomings of the current controlled clinical trials of *Hypericum* have been pointed out in the review by Linde *et al.*[6] and by Ernst[112]. They include lack of well-characterised severely depressed patients populations, heterogeneity of diagnoses, lack of intent-to-treat analyses, lack of long term studies, lack of control over compliance, and low dosages of comparison medications.

Despite the extensive use of this plant extract as an antidepressant , the chemical nature of its bioactive principles still remains unclear. Earlier, *in vitro* studies with hypericin and pseudohypericin as MAO inhibitory agents suggested that hypericin may be responsible for the antidepressant activity of this plant. Consequently, the pharmaceutical preparations were standardized on the basis of their hypericin contents. However, in later studies, these earlier results could not be confirmed. Flavonoids present in the HP were also shown to inhibit MAO-A, but a therapeutic efficacy of this class of compounds appeared doubtful due to their low plasma levels after oral administration[113]. Recently, hyperforin, a prenylated phloroglucinol present in this plant, has been focused as the major component responsible for the antidepressant activity of HP. The lack of viable pharmacological mechanism or the assurance of which components within the plant are critical for therapeutic effect (necessary in order to standardise formulations) will continue to create skepticism among psychopharmacologists and regulatory authorities. However, continued research is needed to identify the constituents most responsible for *Hypericum's* activity so that preparations can be optimally standardised. While determining the pharmacological profile of all major components of *Hypericum* will be a difficult task, it will surely add to the body of knowledge regarding the biochemistry of depression. The presence of significant anxiolytic activity of HP extract[63], suggest that co-existence of anxiolytic-antidepressant activity may help in its clinical profile since anxiety and depression often co-exist.

References

1 Upton R, St. John's wort monograph, American Herbal Pharmacopoeia, Santa Cruz, CA, USA, 1997, 4.
2 Hoelzl J, Inhaltsstoffe und Weitschrift des johanniskratutes, Zeitschrift fur Phytotherapie, 14 (1993) 255.
3 Mattioli I, *Discorsi-Venezia*, (1557) 447.
4 Bombardelli E & Morazzoni P, *Hypericum perforatum*. *Fitoterapia*, 66 (1995) 43.

5 Fernie WT, *Herbal Simples*, John Wiely & Co, Bristol, 1897.

6 Linde K, Ramirez G, Mulrow CD, Pauls A, Weidenhammer W, Melchart, D : St. John's wort for depression - an overview and meta-analysis of randomized clinical trials, *British Medical Journal*, 313 (1996) 253.

7 Rao SG, Laxminarayan AU, Saraswathi LU, Padma GM, Ganesh R & Kukarni DR, Calendula and *Hypericum*: two homeopathic drugs promoting wound healing in rats, *Fitoterapia*, 6 (1991) 508.

8 Vickery AR, Traditional uses and folklore Hypericum in British Isles, *Economic Botany*, (1981) 289.

9 Gerard J, *The Herbal*, Revised and enlarged T Johnson, Reprinted by Dover Publications Inc, New York, 1975, 1633.

10 Muller WE, Singer A, Wonnemann M, Hafner U, Rolli M & Schafer C, Hyperforin represents the neurotransmitter reuptake inhibiting constituent of hypericum extract, *Pharmacopsychiatry*, 31 (1998) 16.

11 Chatterjee SS, Bhattacharya SK, Wonnemann M, Singer A & Muller WE, Hyperforin as a possible antidepressant component of *Hypericum* extracts, *Life Sciences*, 63 (1998) 499.

12 DAC, *Deutscher Arzneimittel - Codex*, 3rd Supplement 91 ed, 1986.

13 ESCOP, *Monograph St. John's wort*, European Scientic Cooperative for phytomedicines, The Netherlands, Meppel, 1996.

14 Dorosseiv I, Determination of flavonoids in *Hypericum perforatum*, *Pharmazie*, (1985) 585.

15 Koget TA, Determination of amount of quercitrin in *Hypericum perforatum*, *Khimiya Prirodnykh Soedinenii*, 2 (1987) 185.

16 Hansel R, Keller K, Rimpler H & Schneider G, *Hagers Handbuch der Pharmazeutischen Praxis*, vol 5, (Springer-Verlag, Berlin), 1993.

17 Berghofer R & Holzl J, Biflavonoids in *Hypericum perforatum*, Part I. Isolation of I3, II8 - biapigenin. *Planta Medica*, (1987) 216.

18 Akhtardzhiev K & Kitanov G, Catechin composition of *Hypericum perforatum*, *Farmatsiya (Sophia)*, 24 (1974) 17.

19 Benigni R, Capra C & Cattorini PE, *Hypericum*, *Piante Medicinali: Chimica, Farmacologia e Terapia*, Milano, Inverni and Della Beffa, 1971.

20 Seabra RM, Vasconcelos MH, Costa MA & Alves AC, Phenolic compounds from *Hypericum perforatum* L and H undulatum, *Fitoterapia*, 63 (1992) 473.

21 Stoyanova A, Popova M & Georgiev E, Thin-layer chromatography of extracts of *Hypericum perforatum*, *Farmatsiya (Sofia)*, 1(1987) 8.

22 Bystrov NS, Chernov BK, Dobrynin VN & Kolosov MN, The structure of hyperforin, *Tetrahedran Lett*, 32 (1975) 2791.

23 Maisenbacher P & Kovar AK, Adhyperforin: a homologue of hyperforin for *Hypericum perforatum*, *Planta Med*, (1992) 291.

24 Maisenbacher P & Kovar AK, Analysis and stability of Hyperici oleum. *Planta Med*, (1992) 351.

25 Ruecker G, Manns D, Hartmann R & Bonsels U, A C-50-hydroper-oxide from *Hypericum perforatum*, *Arch Pharm (Weinheim)*, 328 (1995) 725.

26 Verotta L, Appendino G, Bellora E, Jakupovic J & Bombardelli E, Furohyperforin, A prenylated phloroglucinol from St. John's Wort (*Hypericum perforatum*), *J Nat Prod*, 62 (1999) 770.

27 Nielsen H & Arends P, Structure of the xanthone lignoid kielcorin, *Phytochemistry*, 17 (1978) 2040.

28 Sparenberg B, Demisch L & Holzl J, Investigations of the antidepressive effects of St. John's wort, *Pharm Ztg Wiss*, 6 (1993) 50.

29 Brondz I & Greibrokk T, *n*-1-Alkanols of *Hypericum perforatum*, *J Nat Prod*, 46 (1983) 940.

30 Mathis C & Ourisson G, Etude chimio-taxonomique du genre *Hypericum* IV; Repartition des sesquiterpenes des alcools monoterpineques et des aldehydes satures danes les huiles essentielles D *Hypericum*, *Phytochemistry*, 3 (1964) 37.

31 Nahrstedt A & Butterweck V, Bilogically active and other chemical constituents of herb of *Hypericum perforatum* L. *Pharmacopsychiatry*, 30 (Suppl 2) (1997) 129.

32 Bennett GJ & Lee HH, Xanthones from Guttiferae, *Phytochemistry*, 28 (1989) 967.

33 Costes C & Thomas C, Carotenoid p[igments of petals of the inflorescence of St. John's wort (*Hypericum perforatum*), *Ann Physiol Veg*, 9 (1967) 157.

34 Karryev MO & Komissarenko NF, Phytochemical study of *Hypericum* L plants of Turkmenian flora, *Izv Akad Nauk Turkm SSR*, (1980) 52.

35 Berghofer R & Holzl J, Isolation of I3', II8 - biapigenin (amentoflavone) from *Hypericum perforatum*. *Planta Medica*, (1989) 91.

36 Gurevich AI, Dobrynin VN, Kolosov MN *et al.*, Hyperforin, an antibiotic from Hypericum perforatum, *Chem Abstracts*, 75 (1971) 95625t, *Antibiotiki* (Moscow) 971 (16) 510.

37 Oittmann J, Herrmann HD & Palleske H, Normalizing glucose metabolism in brain tumore slices by hyperoside, *Arzneim-Forsch*, 21 (1971) 1999.

38 Lavie G, Mazur Y, Lavie D & Meruelo D, The chemical and biological properties of hypericin- a compound with a broad spectrum of biological activities, *Medicinal Research Reviews*, 15 (1995) 111.

39 Meruelo D, Lavie G & Lavie D, Therapeutic agents with dramatic antiretroviral activity and little toxicity at effective doses: aromatic polycyclic diones hypericin and pseudohypericin, *Proc Natl Acad Sci*, USA, 85 (1988) 5230.

40 Weber ND, Murray BK, North AJ & Wood SG, The antiviral agent hypericin has *in vitro* activity against HSV-1 through nonspecific association with viral and cellular membranes, *Antiviral chemistry & Chemotheraphy*, 5 (1994) 83.

41 Holzl J, demisch L & Gollnik B, Investigations about antidepressive and mood changing effects of *Hypericum perforatum*, *Planta Medica*, 55 (1989) 643.

42 Kitanov GM & Blinova KF, Modern state of the chemical study of species of the genus *Hypericum* species, *Khimiya Prirodnykh Soedinenii*, 2 (1987) 185.

43 Okpanyi SN & Weischer ML, Animal experiments on the psychotropic action of *Hypericum* extract. *Arzneim Forsch*, 37 (1987) 10.

44 Butterweck V, Winterhoff H & Nahrstedt A, An extract of *Hypericum perforatum* L decreases immobility time in rats in the forced swimming test, *2nd International Congress on Phytomedicine*, Munich, 1996.

45 Muldner H & Zoller M, Antidepressive Wirkung eines auf den Wirkstoffkomplex Hypericin standardisierten Hypericum-Extraktes, *Arznemittel Forchung*, 34 (1984) 918.

46 Butterweck V, Wall A, Lieflandes-Wolf U, Winterhoff H & Nahrstedt A, Effects of total extract and fractions of *Hypericum perforatum* in animal assays for antidepressant activity, *Pharmacosychiatry*, 30 (Suppl 2) (1997) 117.

47 Muller WE, Schafer C, Rolli M & Wonnemann R, Effects of *Hypericum* extract LI 160 on neurotransmitter uptake systems and adrenergic receptor density, *2nd International Congress on Phytomedicine*, Munich, 1996.

48 Suzuki O, Katsumata Y, Oya M, Bladt S & Wagner H, Inhibition of monoamine oxidase by hypericin, *Planta Med*, 50 (1984) 272.

49 Bladt S & Wagner H, Inhibition of MAO by fractions and constituents of *Hypericum* extract, *J Geriatric Psychiatry Neurology*, 7 (1994) S57.

50 Demisch L, Holzl J, Golnik B & Kacynarczyk, Identification of MAO-type-A inhibitors in *Hypericum perforatum* L (hyperforat), *Pharmacopsychiat*, 22 (1989) 194.

51 Thiede HM & Walper A, Inhibition of Mao and COMT by *Hypericum* extracts and hypericin, *J Geriatric Psychiatry Neurology*, 7 (1994) S54.

52 Holtje J & Walper A, Molecular modelling of the antidepressive mechanism of *Hypericum* ingredients, *Nervenheilkunde*, 12 (1993) 339.

53 Muller WE & Rossol R, Effects of *Hypericum* extracts on the suppression of serotonin receptors, *J Geratric Psychiatry Neorol*, 7 (1994) S63.

54 Perovic S & Muller WEG, Pharmacological profile of *Hypericum* extract: effect on serotonin uptake by post synaptic receptors, *Arzneim-Forsch*, 45 (1995) 1145.

55 Cott J & Misra R, in Medicinal Plants : a potential source for new psychotherapeutic drugs, *New Drug Development from Herbal Medicines in Neuropsychopharmacology*, edited by Kanba S & Richelson E, (Brunner/Mazel, Inc, New York) 1997.

56 Diop AG, Lesort M, Escaire F, Sindou P, Couratier P & Hugon J, Tetrodotoxin blocks HIV coat protein (gp120) toxicity in primary neuronal cultures, *Neuroscience Letters*, 165 (1994) 187.

57 Bhattacharya SK, Chakrabarti A & Chatterjee SS, Activity profiles of two hyperforin-containing hypericum extracts in behavioural models, *Pharmacosychiatry*, 31 (1998) 22.

58 Chatterjee SS, Nolder M, Koch E & Erdelmeier C, Antidepressant activity of *Hypericum perforatum* and hyperforin: the neglected possibility, *Pharmacopsychiatry*, 31 (1998) 7.

59 Biber A, Fischer H, Romer A & Chatterjee SS, Oral bioavilability of hyperforin from hypericum extracts in rats and human volunteers, *Pharmacopsychiatry*, 31 (1998) 36.

60 Laakmann G, Schule C, Baghai T & Kieser M, St. John's wort in mild to moderate depression : the relevance of hyperforin for the clinical efficacy, *Pharmacopsychiatry*, 31 (1998) 54.

61 Kumar V, Singh PN & Bhattacharya SK, Neurochemical studies on Indian *Hypericum perforatum* Linn, *17th Annual Conference of Indian Academy of Neurosciences & Annual Meeting of Neuroscience Society of India "Neuroscience 2000 and Beyond"*, Gwalior, (1999).

62 Kumar V, Singh PN, Jaiswal AK & Bhattacharya SK, Antidepressant activity of Indian *Hypericum perforatum* Linn in rodents, *Indian J Exp Biol*, 37 (1999) 1171.

63 Kumar V, Jaiswal AK, Singh PN & Bhattacharya SK, Anxiolytic activity of Indian *Hypericum perforatum* Linn : an experimental study, *Indian J Exp Biol*, 38 (2000) 36.

64 Kumar V, Singh PN, Jaiswal AK & Bhattacharya SK, Effect of Indian *Hypericum perforatum* Linn on animal models of cognitive dysfunctions, 32nd annual conference of Indian Pharmacological Society *"International Congress on Frontiers in Pharmacology and Therapeutics in 21st Century"*, New Delhi, (1999).

65 Kumar V, Khanna V, Seth PK, Singh PN & Bhattacharya SK, Brain neurotransmitter receptor binding and nootropic studies on Indian *Hypericum perforatum* Linn, *Phytotherapy Research*, (2000) In Press.

66 Thiele B, Brink I & Ploch M, Modulation of cytokine expression by *Hypericum* extract, *J Geriatric Psychiatry Neurology*, 7 (1994) S60.

67 Smith RS, The macrophage theory of depression, *Med Hypotheses*, 35 (1991) 298.

68 Kook AI, Mizruchin A, Odnopozov N, Gershon H & Segev Y, Depression and immunity: the biochemical interrelationship between the central nervous system and the immune system, *Biol Psychiatry*, 37 (1995) 817.

69 Bluementhal M, Grunwald J, Hall T, Riggins CW & Rister RS (eds); Klein S & Rister RS(Trans), *German commission E Monographs: Therapeutic Monographs of Medicinal plants for Human Use*, American Botanical Council, TX, 1997, 214.

70 Bradley P, *British Herbal Pharmacopoeia*, Bournemouth, Dorset, British Herbal Medicine Association, 1983.

71 Hahn G, *Hypericum perforatum* (St John's wort- a medicinal herb used in antiquity and still of interest today), *J Naturopathic Med*, 3 (1992) 94.

72 Gaind KN & Ganjoo TN, Antibacterial principle of *Hypericum perforatum* Linn, *Indian J Pharm*, 21 (1959) 172.

73 Khosa RL & Bhatia N, Antifungal effect of *Hypericum perforatum* Linn, *J Sci Res Plants Med*, 3 (1982) 49.

74 Shakirova KK, Garagulya AD & Khazanovich RL, Antimicrobiol properties of some species of St. John's wort cultivated in Uzbekistan, *Mikrobiol Zh*, 32 (1970) 494.

75 Negarsh AK & Pochnok PY, Comparative study of chemotherapeutic and pharmacological properties of antimicrobiol preparations from common St. John's wort, *Fitontsidy, Mater Seveshch*, 1972, 6th meeting, 198.

76 Saljic J, Ointment for the treatment of burns, *Ger Offen*, 2 (1975) 406.

77 Aizenman BE, Antibiotic preparations from *Hypericum perforatum*, *Microbiol Zh*, 31 (1969) 128.

78 Steinbeck KA & Wernet P, Successful long-term treatment over 40 months of HIV-patients with intravenous hypericin, *International Conference on AIDS*, Germany, 1993, Abstract # Po-B26-2012.

79 Wood S, Huffman J, Weber N, Anderson D, North AJ, Murray BK, Sidwell R & Hughes B, Antiviral activity of naturally occurring anthraquinones and anthraquinone derivatives, *Planta Med*, 56 (1990) 651.

80 Lavie G, Valentine F & Levin B, Studies of the mechanisms of antiretroviral agents hypericin and pseudohypericin, *Proc Natl Acad Sci*, 86 (1989) 5963.

81 Lopez-Bazzocchi I, Hudson JB & Towers GH, Antiviral activity of the photoactive plant pigment hypericin, *Photochem Photobiol*, 54 (1991) 95.

82 Carpenter S & Krays GA, Photosensitization is required for inactivation of equine infectious anemiavirus by hypericin, *Photochem Photobiol*, 53 (1991) 169.

83 Degar S, Lavie G & Meruelo D, Photodynamic inactivation of radiation leukemia virus produced from hypericin-treated cells, *Virology*, 197 (1993) 796.

84 Hudson JB, Lopez-Bazzocchi I & Towers GH, Antiviral activities of hypericin, *Antiviral Res*, 15 (1991) 101.

85 Kraus G, Pratt D, Tossberg J & Carpenter S, Antiretroviral activity of hypericin and related analogs, *Biophys Comm Res*, 172 (1990) 149.

86 Lenard J, Rabson A & Vanderoef R, Photodyanamic inactivation of infectivity of human immunodeficiency virus and other envoloped viruses using hypericin and rose bengal: inhibition of fusion and syncytia formation, *Proc Natl Acad Sci*, 90 (1993)158.

87 Thomas C, MacGill Miller GC & Pardini RS, Photoactivation of hypericin generates singlet oxygen in mitochondria and inhibits succinoxidase, *Photochem Photobiol*, 55 (1992) 47.

88 Yip L, Hudson JB, Kowalik EG, Zalkow LH & Towers GHN, Antiviral activity of derivative of photosensitive compound hypericin, *Phytomedicine*, 3 (1996) 85.

89 Stevenson NR & Lenard J, Antiretroviral activities of hypericin and rose bengal: photodynamic effects on Friend leukemia virus infection of mice, *Antiviral Res*, 21 (1993) 119.

90 Couldwell WT, Gopalakirshna R & Hinton DR, Hypericin: a potential antiglioma therapy, *Neurosurgery*, 35 (1994) 705.

91 Takahnishi I, Nakanishi S, Kobyashi E, Nakano H, Suzuki K & Tamaoki T, Hypericin and pseudohypericin specifically inhibit protein kinase C: possible relation to their antiretroviral activity, *Biochem Biophys Res Comm*, 165 (1989) 1207.

92 De Witte P, Agostinis P, Van Lint J, Merlevede W & Vandenheede JR, Inhibition of epidermal growth factor receptor tyrosine kinase activity by hypericin, *Biochem Pharmacol*, 46 (1993) 1929.

93 Panossian AG, Gabrielian E, Manvelian V, Jurcic K & Hagner H, Immunosuppressive effects of hypericinon stimulated human leucocytes: inhibition of arachidonic acid release, leukotriene B4 and interleukin -1 production and activation of nitric oxide formation, *Phytomedicine*, 3 (1996) 19.

94 Melzer R, Fricke U, Holzl J, Podehl R & Zylka J, Procyanidinidins from *Hypericum perforatum*: effects on isolated guines pig hearts, *Planta Medica*, 55 (1989) 655.

95 Melzer R, Fricke U & Holzl J, Vasoactive properties of procyanidinidins from *Hypericum perforatum* L. in isolated porcine coronary arteries, *Arzneim-Forsch*, 41 (1991) 481.

96 Demisch L, Nispel J, Sielaff T, Gebhart P, Kohler C & Pflug B, Influence of subchronic Hyperforat administration on melatonin production, *Pharmacopsychiatry*, 24 (1991).

97 Heinze A & Gobel H, *Hypericum* perforans in the treatment of chronic tension-type headache, *2nd International Congress on Phytomedicine*, Munich, (1996).

98 Ozturk Y, Aydin S, Baser KHC, Kirimer N & Kurtar-Ozturk N, Hepatoprotective activity of *Hypericum perforatum* L. alcoholic extract in rodents, *Phytother Res* (UK), 6 (1992) 44.

99 Boericke W, *Pocket Manual of Homeopathic Materia Medica*, Boericke and Tafel, Philladelphia, 1927.

100 Felter HW, *Eclectic Materia Medica, Pharmacology and Therapeutics*. John K Scudder, Cincinnati, 1922.

101 Priest AW & Priest LR, *Herbal Medication*, LN Fowler & Co. Ltd., London, 1982.

102 Kumar V, Singh PN & Bhattacharya SK, Anti-inflammatory and analgesic activity of Indian *Hypericum perforatum* L., *Phytomedicine*, (2000) Communicated.

103 De-Smet PA & Nolen WA, St. John's wort as an antidepressant, *British Med J*, 313 (1996) 241.

104 Hansgen KD, Vesper J & Ploch M, Multicenter double-blind study examining the antidepressant effectiveness of the *Hypericum* extract LI 160, *Nervenheilkunde*, 12 (1994) 285.

105 Harrer G, Hubner WD & Padzuweit H, Effectiveness and tolerance of the *Hypericum* extract LI 160 in comparison with imipramine: randomised double-blind study with 135 outpatients, *J Geri Psychiat Neurol*, 7 (1994) S 24.

106 Hubner WD, Lande S & Podzuweit H, *Hypericum* treatment of mild depression with somatic symptoms, *J Geri Psychiat Neurol*, 7 (1994) S 12.

107 Sommer H & Harrer G, Placebo-controlled double-blind study examining the effectiveness of a *Hypericum* preparation in 105 mildly depressed patients, *J Geri Psychiat Neurol*, 7 (1994) S 9.

108 Vorbach EU, Hubner WD & Arnoldt KH, Effectiveness and tolerance of the *Hypericum* extract LI 160 in comparison with imipramine: randomised double-blind study with 135 outpatients, *J Geri Psychiat Neurol*, 7 (1994) S 19.

109 Wolk H, Burkard G & Grunwald J, Benefits and risks of the *Hypericum* extract LI 160: drug monitoring study with 3250 patients, *J Geri Psychiat Neurol*, 7 (1994) S 34.

110 Staffeldt B, Kerb R, Brockmoller J, Ploch M & Roots I, Pharmacokinetics of hypericin and pseudohypericin after oral intake of the *Hypericum perforatum* extract LI 160 in healthy volunteers, *J Geriatric Psychiatry Neurol*, 7 (1994) S47.

111 Stock S & Holzl J, Pharmacokinetic tests of [^{14}C]-labelled hypericin and pseudohypericin from *Hypericum perforatum* and serum kinetics of hypericin in man, *Planta Medica*, 57 (Suppl 2) (1991) A61.

112 Ernst E, St. John's wort, an anti-depressant? A systematic criteria-based review, *Phytomedicine*, 2 (1995), 47.

113 Konig C & Meier B, Johanniskrant – ein pflanzlicher Stimmungsant heller, *Phytotherapie*, (1992) 1691.