

DETERMINATION OF HYPERICIN AND HYPERFORIN CONTENT IN DIFFERENT PHYTOPHARMACEUTICALS AND DIETARY SUPPLEMENTS CONTAINING *HYPERICUM PERFORATUM* EXTRACT

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SUMMARY

Background: Phytopharmaceuticals have long been used as official medicine in different countries around the world. In Mexico, they were recently introduced. One of the most prescribed phytopharmaceuticals is derived from the *Hypericum perforatum* species. This plant, commonly known as St. John's Wort, is widely used in traditional herbal medicine for wound healing and as an antidepressant. Several clinical and pharmacological works have demonstrated the efficacy and safety of extracts prepared from the aerial parts of this plant in the treatment of mild to moderate depression. This activity has been attributed to hyperforin, hypericin and flavonic compounds. For many years, the naphthodianthrone, hypericin, was considered as the main active constituent. Nevertheless, it has been demonstrated that the acylphloroglucinol, hyperforin together with hypericin and some flavonic compounds are the active antidepressant compounds. Since depressive disorders are very common in clinical practice, with approximately 11.3% of all adults being afflicted by these disorders during any one year, different preparations of St. John's Wort are available as phytopharmaceuticals and dietary supplements in different countries, including Mexico.

Methods: In order to compare different phytopharmaceuticals and dietary supplements containing *H. perforatum* extract available in Mexico, the total content of hypericin and hyperforin was determined. Three products were purchased in pharmacies as phytopharmaceuticals (S1, S2, S3), while four products were available as dietary supplements (S4, S5, S6, S7).

Results: Phytopharmaceuticals were found as coated tablets or gelatin capsules with individual packing in aluminum blisters, while dietary supplements were all found as uncoated tablets in glass or plastic bottles. With the exception of one product (S4), in all samples detectable amounts of hypericin were found. Products S2, S3, and S7 had a higher hypericin content (between 0.2% and 0.26%). Most of the samples analyzed had no detectable amounts of hyperforin. The products S1 and S7 contained only trace quantities of hyperforin, while products S2 and S3 had the highest amount with more than 4.0% and 2.5%, respectively.

Conclusions: Although all of the products studied contained

the extract of *H. perforatum* as a main constituent, there were marked differences between them. It was observed that the formulation (coated tablets) packing (individual packing in aluminum blisters) and active constituents content (hypericin and hyperforin) of the phytopharmaceuticals differ significantly from that of the dietary supplements.

Key Words: *Hypericum perforatum*, hyperforin, hypericin, phytopharmaceutical, dietary supplement, complementary medicine, antidepressant.

RESUMEN

Antecedentes: Durante muchos años, los fitofármacos han formado parte de la medicina oficial en diferentes países alrededor del mundo, mientras que en México estos productos obtuvieron reconocimiento oficial hasta hace poco. Uno de los fitofármacos más prescritos en el mundo se prepara a partir de la especie vegetal *Hypericum perforatum*. Esta planta, conocida popularmente como "hierba de San Juan", se ha utilizado ampliamente en las medicinas tradicionales de diferentes países para curar heridas y como antidepresivo. Numerosos trabajos clínicos y farmacológicos han demostrado la eficacia y la seguridad de los extractos preparados a partir de las partes aéreas de esta planta en el tratamiento de la depresión leve a moderada. Esta actividad ha sido atribuida a diferentes constituyentes como son la hiperforina, la hipericina y los compuestos flavonoides. Por muchos años, se consideró que la naftodiantrona, hipericina, era el principal constituyente activo. Sin embargo, se ha demostrado que el floroglucinol denominado hiperforina, junto con la hipericina y algunos compuestos flavonoides, son en realidad el conjunto de compuestos con actividad antidepresiva. En apoyo a lo anterior, se demostró que la hiperforina es capaz de antagonizar las contracciones inducidas con serotonina en el ileon aislado del cobayo, además de inhibir la recaptura de este neurotransmisor en las terminales nerviosas. Este compuesto ha demostrado efectos antidepresivos en el modelo *in vivo* de desesperanza aprendida en ratas, mientras que en un estudio clínico doble ciego se comparó el efecto producido

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por un extracto de *Hypericum perforatum* que contenía hiperforina contra otro extracto similar que carecía de ella. Con lo anterior se demostró que el efecto terapéutico de este extracto sobre la depresión depende de su contenido de hiperforina. Los trastornos depresivos son muy comunes en la práctica clínica y afectan a cerca de 11.3% de los adultos durante un año. En el mercado existen ahora diferentes preparaciones de *Hypericum perforatum* que se pueden usar para tratarlos. Estas se encuentran registradas como suplementos alimenticios o como fitofármacos en diferentes países, incluido ahora también, México.

Método: Con el fin de comparar diferentes fitofármacos y suplementos alimenticios preparados con el extracto de *H. perforatum* disponibles en el mercado en México, se determinó, por procedimientos analíticos basados en la cromatografía de líquidos de alta resolución, el contenido de hipericina e hiperforina. Tres productos fueron adquiridos en farmacias como fitofármacos (S1, S2, S3), mientras que otros cuatro fueron hallados como suplementos alimenticios (S4, S5, S6, S7).

Resultados: Los fitofármacos fueron encontrados como tabletas laqueadas o cápsulas de gelatina, empaçadas en forma individual en *blister* de celopolial, mientras que los suplementos alimenticios fueron hallados como tabletas no cubiertas, introducidas en frascos o botes de plástico. Con excepción de uno de los productos (S4), en todas las muestras estudiadas se encontraron cantidades detectables de hipericina. Los productos S2, S3 y S7 tuvieron el mayor contenido de hipericina (entre 0.2 y 0.26%). La mayoría de las muestras analizadas no presentó cantidades detectables de hiperforina. Los productos S1 y S7 presentaron sólo trazas de este compuesto, mientras que los productos S2 y S3 tuvieron la cantidad más alta, con más de 4.0% y 2.5% respectivamente.

Conclusiones: A pesar de que todos los productos incluidos en este estudio contenían como principal constituyente el extracto de *H. perforatum*, se encontraron marcadas diferencias entre ellos. Se observó que la formulación (tabletas laqueadas), el empaque (individual en *blister* de celopolial) y el contenido de compuestos activos (hipericina e hiperforina) de los fitofármacos difiere ostensiblemente de los suplementos alimenticios.

Palabras clave: *Hypericum perforatum*, hiperforina, hipericina, fitofármaco, suplemento alimenticio, medicina alternativa o complementaria, antidepresivo.

INTRODUCTION

Phytopharmaceuticals have long been used as official medicine in different countries around the world. In Mexico, however, they were only recently introduced as medication based on the regulations related to drugs in "The General Law of Health" (18).

One of the most prescribed phytopharmaceuticals in the world is derived from the *Hypericum perforatum* (*Clusiaceae*) species. This plant is widely known as St. John's Wort, and has a long historical use in traditional herbal medicine for wound healing and as an antidepressant (10). Depressive disorders are more common in clinical practice, with approximately 11.3% of all adults afflicted during any one year (13).

Several research works related to this species can be found in specialized scientific magazines. From 1979 to 1997, 31 clinical studies involving a total of 2,254 patients were reported. In one year (1993), nine clinical projects for the evaluation of standardized extracts of *H. perforatum* were developed, in which antidepressant efficacy of St. John's Wort extract in mild to moderate depression was fully demonstrated (23). This antidepressant activity was evaluated against a placebo or other well known antidepressants such as maprotiline (11), imipramine (15, 24), and amitriptyline (25), as well as a selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine (15). These and other recent studies have revealed that St. John's Wort extracts have better safety and tolerability profiles than synthetic antidepressants (14). Based upon a multi-centre clinical study applied on children under 12 years, *Hypericum* was found to be a potentially safe and effective treatment for children with depression symptoms (12).

Most of the products of St. John's Wort which are commercially available contain hydroalcoholic dry extracts, prepared from the upper aerial parts of the plant with either 60% (W/W) ethanol or 80 vol.% methanol. Such extracts contain a spectrum of six major natural product groups: naphthodianthrones, acylphloroglucinols and flavonol glycosides, biflavones, proanthocyanidins and phenylpropanes (7).

For many years, the naphthodianthrone hypericin was considered as the main active constituent. Today, however, some studies explain the antidepressant activity of St. John's Wort solely upon hypericin (19), which is the basis for standardization of commercially available hypericum preparations.

It has been demonstrated that the acylphloroglucinol hyperforin together with hypericin and some as yet undetermined flavonic components of the extracts could be the active antidepressant compounds. Different experiments clearly demonstrate that the antidepressant efficacy of hypericum extracts depends on the presence of all three of these different classes of active constituents (4, 5).

Finally, extracts of St. John's wort have proven to be a useful additional tool in the treatment of mild to moderate depression, avoiding the well-known side effects of standard antidepressant agents like tricyclics or SSRIs (8). Its different products are available as phytopharmaceuticals and dietary supplements in different countries, including Mexico. Phytopharmaceuticals are known in Mexico as *fitofármacos* or *medicamentos herbolarios*. These are drugs in which the active principle is derived exclusively from plants, parts of plants or preparations obtained from them, in a specific galenic form. The efficacy and safety of modern phytopharmaceuticals must be proved by clinical

TABLE 1
Description of different samples of *Hypericum perforatum* extracts obtained from products available in the Mexican market

Reference Trade name	Batches Tested	Packing	Formulation	Extract Amount	
S1*	3	Aluminum-plastic blister (individually packaged)	Film tablets	250 mg	Remotiv
S2*	3	Aluminum-plastic blister (individually packaged)	Film tablets	300 mg	Hiperikan
S3*	2	Aluminum-plastic blister (individually packaged)	Capsules	300 mg	Vivicaps
S4**	1	Plastic bottle	Uncoated tablets	400 mg	Hierba de San Juan Ultrasoya
S5**	3	Plastic bottle	Uncoated tablets	333 mg	Deskan
S6**	1	Plastic bottle	Uncoated tablets	100 µg	Hiperfort 300
S7**	3	Glass bottle	Uncoated Tablets	300 mg	St. John's Wort GNC

* Phytopharmaceutical ** Dietary supplement

research and the active compounds must be standardized.

In order to compare different dietary supplements and phytopharmaceuticals available in the Mexican market containing St. John's wort extracts, we used a validated methodology to determine the total hypericin and hyperforin content. When possible, different batches were obtained to evaluate the reproducibility.

MATERIALS AND METHODS

Samples tested

All products studied here were purchased in the Mexican market, in or near Mexico City. The main constituent reported in these products was the standardized extract of *Hypericum perforatum*. As shown in Table 1, only three products were available in pharmacies as phytopharmaceuticals (S1, S2, S3). S1 and S2 were formulated as tablets coated in sugar, while S3 was found as capsules, all packed in plastic blisters. S1 had a label strength of 250 mg of St. John's wort (measured as dry extract), while S2 and S3 had 300 mg. The rest of the products (S4–S7) were purchased in different natural products stores as dietary supplements, and were all found in glass or plastic bottles prepared as uncoated tablets.

Reference substances

The reference standards were hypericin and dicyclohexil ammonium salt of hyperforin. Both were obtained from Dr. Willmar Schwabe Arzneimittel Co., and stored in dry conditions at -20°C until preparation.

Samples preparation and analysis

All the products under analysis were submitted

simultaneously to the following process in order to determine the total amount of hypericin and hyperforin contained in every formulation.

Ten dosage forms from every product and batch were used. All of them were first individually weighted and then milled as a pooled sample. With basis on the total amount of *H. perforatum* extract contained in every formulation, an amount corresponding to 75 mg of the extract was transferred to analytical glasses and diluted in 25 ml of methanol. All samples were maintained 20 minutes in an ultrasonic bath at room temperature in order to improve the hypericin and hyperforin extraction. The resulting products were centrifuged 10 min at 4000 rpm and the clear supernatant was then filtered and transferred into brown HPLC vials.

The analysis was made with Merck-Hitachi HPLC equipment with a L6200 pump and a diode-array detector. In both cases, hyperforin and hypericin determination standard curves were built.

For total hyperforin determination, a Merck LiChrospher 100 RP-18, 5µ (125 x 4 mm) column and LiChroCart 4 x 4 precolumn were used. The mobile phase was acetonitrile, water and phosphoric acid H_3PO_4 (85%), with an isocratic system (86 : 14 : 0.3) and flow of 1.2 ml/min. The volume injection was 20 µl and detection at 273 nm.

For total hypericin determination, the method described by Gaedcke F. (9) was followed. The samples were exposed to light for 10 min in the SUN test apparatus to convert protohypericin and protopseudohypericin to the corresponding hypericin. In this case, for HPLC analysis a Kromasil 100-RP18, 5µ (125 x 5) column (Macherey Nagel) was used. The mobile phase was phosphatbuffer (15.6 g NaH_2PO_4 x 2 H_2O ; pH 2.1 with phosphoric acid), methanol and

ethylacetate (410 : 1600 : 390), while the flow was 1.0 ml/min. The volume injection was 20 µl and detection at 590 nm.

RESULTS

The main component of the products studied had an extract of *Hypericum perforatum*. With the exception of the product S4, in all samples detectable amounts of hypericin were found. Products S5 and S6 had less than 0.1%, the lowest concentration of hypericin. The product S1 had a total hypericin content of 0.16%, with a good reproducibility between different batches, while products S2, S3 and S7 had higher hypericin content, between 0.2 and 0.26%, showing uniformity between samples.

Most of the products studied had no detectable amounts of total hyperforin (0.01 mg minimum detectable). The products S1 and S7 contained only trace quantities of hyperforin (<0.3% of extract or <0.1 mg/unit), while product S2 and S3 had the highest amount with more than 4.0% and 2.5% in the extract, respectively.

All products available in different batches (S1, S2, S3, S5, S7) showed excellent batch-to-batch reproducibility for hypericin and hyperforin content.

DISCUSSION

In general, the results show that although all products studied have as a main constituent the extract from *Hypericum perforatum*, there are in relation with the content of active principles notorious differences among them. Since it cannot be expected that the concentration of active compounds in different extracts will be the same, it is highly recommended to have as a minimum the adequate standardization of active compounds in order to produce a more homogeneous product that can be prescribed in a sufficient dosage.

TABLE 2
Total hypericin content in phytopharmaceuticals and dietary supplements containing *Hypericum perforatum* extracts obtained in the Mexican market

Product	Extract (mg/unit)	Total Hypericin (mg/unit)	s.d. rel. (%)	% in extract
S1**	250	0.41	1.5	0.16
S2**	300	0.64	6.5	0.20
S3*	300	0.77	1.4	0.25
S4	400	0.00	0.0	0.00
S5**	333	0.29	4.3	0.08
S6	33	0.005	0.0	0.01
S7**	300	0.69	2.3	0.23

*Results are average and s.d. related percent from 2 batches

** Results are average and s.d. related percent from 3 batches

In the proposal for USP monograph, with relation to the powdered St. John's wort extract, a minimum total hypericin content of 0.2% is required, while the minimum content of hyperforin is 3% (22). With basis on the results obtained, only one of the products studied complies with these requirements. At the same time, it was observed that the galenic formulation (coated tablets), packing (individual packing in aluminum blisters) and active constituents content (hypericin and hyperforin) of the phytopharmaceuticals differ ostensibly from that of the dietary supplements. These details could be very important, especially when some of the active compounds, as is the case of hyperforin, are unstable and decompose their chemical structure under light and air (3).

Hyperforin is the principal component of the acylphoroglucinol-type compounds, that is, the largest group of compounds in the fresh species of *H. perforatum* (7). It has been repeatedly speculated that this compound, being a labile substance, cannot be expected to be present in therapeutically used hypericum extracts in sufficiently high concentrations and therefore cannot be their active antidepressive ingredient (6). Nevertheless, degradation and transformation can occur to hypericin, hyperforin and many other active compounds, in which their presence and stability depend on different factors that must be controlled. In this case, these factors could be primarily: appropriate extraction, dried, pharmaceutical preparation, and packing.

With regard to the antidepressant effects of St. John's wort, hyperforin, rather than hypericin as was originally thought, has emerged as one of the major constituents responsible for antidepressant activity (1). In support of hyperforin as one of the active constituents of *H. perforatum* extracts, it was recently reported that this compound is able to antagonize serotonin-induced contractions on the Guinea pig ileum and can inhibit the uptake of this neurotransmitter. At the same time, this compound has shown antidepressant activity in the *in vivo* behavioral despair test in rats (6, 20, 21).

TABLE 3
Total hyperforin content in phytopharmaceuticals and dietary supplements containing *Hypericum perforatum* extracts obtained in the Mexican market

Product	Extract (mg/unit)	Total Hyperforin (mg/unit)	s.d. rel. (%)	% in extract
S1 **	250	<0.20	0.0	<0.10
S2 **	300	13.52	4.8	4.40
S3 *	300	7.66	4.3	2.50
S4	400	0.00	0.0	0.00
S5 **	333	0.00	0.0	0.00
S6	33	0.00	0.0	0.00
S7 **	300	0.80	6.6	0.30

*Results are average and s.d. related percent from 2 batches

** Results are average and s.d. related percent from 3 batches

Moreover, in a double-blind clinical trial, the effect produced by hypericum extract with hyperforin versus an hypericum extract without hyperforin was compared on patients suffering depression. The results showed that the therapeutic effect of St. John's wort in mild to moderate depression depends on its hyperforin content (17). Independently of that, the clearer advantage of *H. perforatum* extracts over synthetic antidepressants is the lower frequency of adverse drug reactions (8, 14).

Finally, in addition to efficacy, safety, tolerance and toxicology, pharmacokinetics can be applied to phytopharmaceuticals. By using validated analytical methods, plasma concentrations have been recorded for hyperforin and hypericine (2, 16). In conclusion, this work shows that although all products studied, phytopharmaceuticals or dietary supplements, contained as a main constituent the extract of *H. perforatum*, even though the standardized contents of the products were markedly different.

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