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 naphtodianthrone, 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.

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

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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 *Hypericum 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 herborarios. 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

**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).
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 dicyclohexyl 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 H₃PO₄ (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 proto-pseudohypericin 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 NaH₂PO₄ x 2 H₂O; pH 2.1 with phosphoric acid), methanol and

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**Table 1**

<table>
<thead>
<tr>
<th>Reference Trade name</th>
<th>Batches</th>
<th>Packing</th>
<th>Formulation</th>
<th>Extract Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1 *</td>
<td>3</td>
<td>Aluminum-plastic blister (individually packaged)</td>
<td>Film tablets</td>
<td>250 mg Remotiv</td>
</tr>
<tr>
<td>S2 *</td>
<td>3</td>
<td>Aluminum-plastic blister (individually packaged)</td>
<td>Film tablets</td>
<td>300 mg Hiperikan</td>
</tr>
<tr>
<td>S3 *</td>
<td>2</td>
<td>Aluminum-plastic blister (individually packaged)</td>
<td>Capsules</td>
<td>300 mg Vivicaps</td>
</tr>
<tr>
<td>S4 **</td>
<td>1</td>
<td>Plastic bottle</td>
<td>Uncoated tablets</td>
<td>400 mg Hierba de San Juan Ultra soya</td>
</tr>
<tr>
<td>S5 **</td>
<td>3</td>
<td>Plastic bottle</td>
<td>Uncoated tablets</td>
<td>333 mg Deskan</td>
</tr>
<tr>
<td>S6 **</td>
<td>1</td>
<td>Plastic bottle</td>
<td>Uncoated tablets</td>
<td>100 µg Hiperfort 300</td>
</tr>
<tr>
<td>S7 **</td>
<td>3</td>
<td>Glass bottle</td>
<td>Uncoated Tablets</td>
<td>300 mg St. John’s Wort GNC</td>
</tr>
</tbody>
</table>

* Phytopharmaceutical  ** Dietary supplement
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.

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

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

*Results are average and s.d. related percent from 2 batches
**Results are average and s.d. related percent from 3 batches

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

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

*Results are average and s.d. related percent from 2 batches
**Results are average and s.d. related percent from 3 batches

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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.

References

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