

Are Modern Preparation Methods Compromising the Efficacy of Chinese Herbal Medicines? An Investigation Using Guan Ye Lian Qiao (*Hypericum Perforatum L.*)

Abstract

Using High Performance Liquid Chromatography (HPLC) and coupled techniques a comparison was made of the compounds found in Guan Ye Lian Qiao (*Hypericum perforatum L.*) when prepared as a raw herb decoction, tincture and concentrated powder tea.

Rationale

A question frequently asked of teachers of Chinese herbal medicine (CHM) is, 'How do you prescribe your herbs?'. The answer tends to vary, from the hardline of 'Raw herbs are the only way - motivate your patients through your own conviction and they will comply', to 'I get very good results with low-dose capsules'. Of course the majority of practitioners tread a middle ground, using whichever method is most appropriate for the particular patient or condition. In our modern, fast-paced world where convenience is king, time is at a premium, meals are ready in minutes and vitamins come from a jar, the allure of herbal prescriptions that comply with such an ethos is powerful. What though if efficacy is compromised? Are our patients sacrificing therapeutic effect for convenience?

In the UK practitioners predominantly use herbal capsules, tablets and concentrated powders in addition to raw herb decoctions. The use of tinctures is much more popular in the USA (Chinese Medicine Tools, 2007), although it is beginning to become more popular in the UK. The author wished to find out if three different formats - raw herb

decoction, concentrated powder and tincture - could be considered therapeutically equivalent. From a purely biochemical perspective it seemed unlikely that these three methods of preparing a herb would result in a similar profile of compounds at the point of consumption by the patient. If different compounds are present at varying concentrations then it is likely that the different preparations will have varying therapeutic effects.

Testing

In order to investigate this question a technique known as High Performance Liquid Chromatography (HPLC) was employed. Chromatography is a method that separates out the different compounds contained within a substance. Basic paper chromatography uses a special piece of paper, on to which a few drops of a specific substance are applied, and which is subsequently placed in a beaker of a solvent. As the solvent is drawn up the paper, it 'picks up' different compounds, and because of their different properties deposits them at different positions along the paper. HPLC essentially constitutes a sophisticated version of this process.

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Keywords: Guan Ye Lian Qiao (*Hypericum perforatum L.*), raw herb decoction, tincture, concentrated powder, hypericin, hyperforin, flavonoids, HPLC-MS, HPLC-UV, fingerprints.

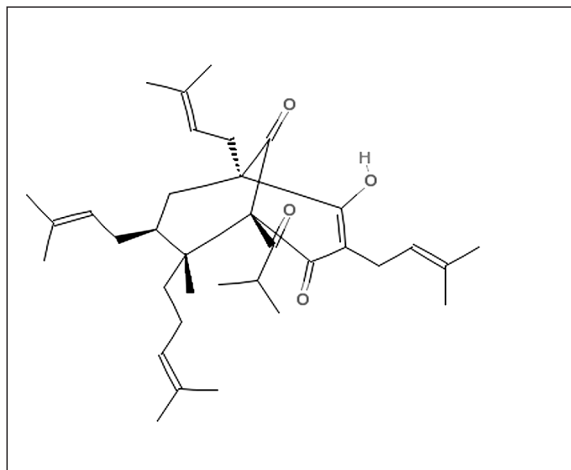


Figure 1: Hyperforin molecule

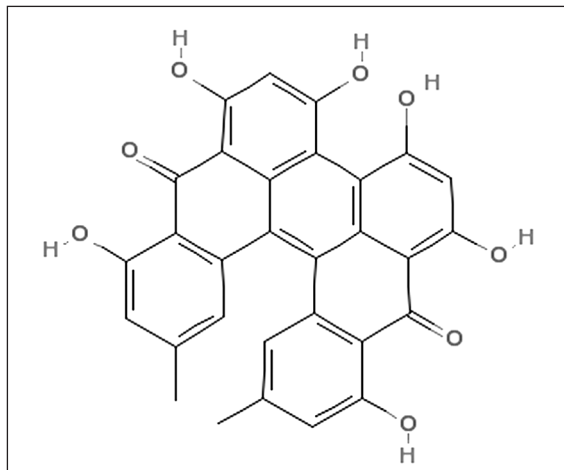


Figure 2: Protohypericin molecule

Likelihood (REML) after a Box-Cox transformation to adjust for non-normal distribution of data (NIST/SEMATECH e-Handbook of Statistical Methods, 2003). A statistically significant ($dF_6 F=11.04 p<0.05$) result was found between the levels of hyperforin found in the raw herb decoction and the concentrated powder tea. The levels of hyperforin in the powder were negligible. Given that hyperforin mediates the main therapeutic effects of Guan Ye Lian Qiao (*Hypericum perforatum*), using this herb in powder form is unlikely to produce its antidepressant or antimicrobial wound-healing actions. The difference between the relative abundance of hyperforin in tincture and raw herb form was not significant.

It can be seen that the three preparation methods produce very different fingerprints. Since it is likely that no single compound acts alone within the herb to mediate therapeutic action (Xu et al., 2008), we may infer that variability in the additional compounds could affect the herb's efficacy, although this cannot be specifically stated without further research into the nature of the interactions between the various compounds within the herb.

Limitations

This research looks at a single herb *in vitro* rather than *in vivo*, that has been removed from the complexity of a herbal formula. Research has shown that administration of a herb within a formula may affect its bioavailability (Liu et al., 2006) and pharmacokinetics (Xu et al., 2008). Further research needs to address not only the range of compounds within a herb and their effects on one another, but also the range of herbs within a formula and their interactions. This could be accomplished by looking at compounds *in vivo* using HPLC to analyse blood samples following administration of the herbal formula (Chi & Franklin, 1999).

Whilst this paper does not provide definitive answers, it is hoped that it provokes practitioners of CHM to question their chosen methods of prescribing, rather

than assuming that technological advances in methods of herbal administration are necessarily advantageous. It may be the case that, for the majority of herbs, greater patient compliance outweighs any compromise in herbal potency arising from the use of concentrated powders. It may also be the case that the most bioactive compounds are unstable: for example lingustilide in Dang Gui (*Angelica sinensis Radix*) (Lu et al., 2004), paenol in Mu Dan Pi (Moutan Cortex) (Choi et al., 1994) and ginsenosides in Ren Shen (*Ginseng Radix*) (Haijiang et al., 2003) have all been shown to be unstable. Such unstable but highly bioactive compounds may be those most likely to be denatured during the process of creating powdered herbs. In order that we provide the best for our patients and honour traditional Chinese medicine, it is the opinion of the author that we should try to answer these questions. ■

Rebecca Clarke is an acupuncturist and herbalist working in private practice in Rickmansworth and at The London Acupuncture Clinic in the UK. Her interest in herbal medicine developed after working in remote areas of Tanzania. After receiving both acupuncture and herbs whilst living in New Zealand, she decided this could be the escape from I.T. she had been seeking. Rebecca's enthusiasm for research originated during her first degree in neuroscience. She currently works as a Research Supervisor at the London College of Traditional Acupuncture.

References

- Bergonzi, M., Bilia, S., Gallori, D. & Vincieri, F. (2001). "Variability in the content of constituents of *Hypericum perforatum* L. and some commercial extracts". *Drug Development Industrial pharmacy*, 27, 491-497.
- C. De Los Reyes, G. & Koda, R. (2001). "Development of a simple, rapid, reproducible HPLC assay for the simultaneous determination of hypericins and stabilized hyperforin in commercial St. John's wort preparations". *Journal of pharmaceutical and biomedical analysis*, 26, 959-965.
- Cao, Y., Wang, L., Yu, X. & Ye, J. (2006). "Development of the chromatographic fingerprint of herbal preparations Shuang-Huang-Lian oral liquid". *Journal of pharmaceutical and biomedical analysis*, 41, 845-856.
- Chandrasekera, H., Welham, K., Ashton, D., Middleton, R. & Heinrich, M. (2005). "Quantitative analysis of the major constituents of St. John's wort with HPLC-ESI-MS". *Journal of pharmacy and pharmacology*, 57.
- Chatterjee, S., Bhattacharya, S., Wonnemann, M., Singer, A. & Muller, W. (1998). "Hyperforin as a possible

Run	Method description	Hyperforin	Protohypericin	Quercetin	Avicularin and Quercitrin	Rutin	Isoquercitrin
1	Decoction	253864	472251	1165425	171155	1279261	2141954
2	Decoction	1374341	193588	56101	344217	523344	846056
3	Decoction	137091	160726	1082212	0	1318719	1556044
1	Tincture	8064477	342095	1978543	1990601	821009	1811471
2	Tincture	7424024	348669	139932	278978	88604	198956
3	Tincture	3126093	22668	23717	753327	108146	16052
1	Powder	5337	37272	690913	1093828	855507	914230
2	Powder	0	8044	174107	206804	493433	451500
3	Powder	2831	5185	22573	148463	372621	255744

Table 1: Relative abundance of compounds in Guan Ye Lian Qiao (*Hypericum perforatum* L.)

- antidepressant component of hypericum extracts". *Life Sciences*, 63, 499-510.
- Chi, J. & Franklin, M. (1999). "Determination of hypericin in plasma by high-performance liquid chromatography". *Journal of Chromatography B*, 724, 195-198.
- Chinese Medicine Tools (2007). Retrieved on 25th October 2007 from <http://www.chinesemedicinetools.com/poll/what-preparation-of-herbals-do-you-use-most>.
- Choi, B., Kim, S., Choi, M., Chang, K. & Park, A. (1994). Retrieved on 1st June 2008 from <http://kmbase.medic.or.kr/Main.aspx?d=KMBASE&m=VIEW&i=0667719940310020592>.
- Conforti, F., Statti, G. & Tundis, R. (2005). "Comparative chemical composition and variability of biological activity of methanolic extracts from *Hypericum perforatum* L.". *Natural Products research*, 3, 295-303.
- Couceiro, M., Afreen, F., Zobayed, S. & Kozai, T. (2006). "Variation in concentrations of major bioactive compounds of *St. John's wort*: Effects of harvesting time, temperature and germplasm". *Plant science*, 170, 128-134.
- Gibbons, S., Ohlendorf, B. & Johnsen, I. (2002). "The genus *Hypericum* - a valuable resource of anti-Staphylococcal leads". *Fitoterapia*, 73, 300-304.
- Gurevich, A., Dobrynin, V., Kolosov, M., Popravko, S. & Riabova, I. (1971). "Antibiotic hyperforin from *Hypericum perforatum* L.". *Antibiotika*, 16, 510-513.
- Haijiang, Z., Yongjiang, W. & Yiyu, C. (2003). "Analysis of SHENMAI injection by HPLC/MS/MS". *Journal of pharmaceutical and biomedical analysis*, 31, 175-183.
- Harris, D. (2007). *Quantitative chemical analysis*. USA: Freeman.
- Liu, Y., Yang, J. & Cai, Z. (2006). "Chemical investigation on *Si Jun Zi* and its two major herbs *Panax ginseng* and *Glycyrrhiza uralensis* by LC/MS/MS". *Journal of pharmaceutical and biomedical analysis*, 41, 1642-1647.
- Lu, G., Chan, K., Chan, C., Leung, K., Jiang, Z. & Zhao, Z. (2004). "Quantification of ligustilides in the roots of *Angelica sinensis* and related umbelliferous medicinal plants by high-performance liquid chromatography and liquid chromatography-mass spectrometry". *Journal of chromatography A*, 1046, 101-107.
- NIST/SEMATECH (2003). *e-Handbook of Statistical Methods*. Retrieved on 3rd June 2007 from <http://www.itl.nist.gov/div898/handbook>.
- Radulovic, N., Stankov-Jovanovic, V., Stojanovic, G., Smelcerovic, A., Spitteller, M. & Asakawa, Y. (2007). "Screening of in vitro antimicrobial and antioxidant activity of nine *Hypericum* species from the Balkans". *Food Chemistry*, 103, 15-21.
- Schwob, I., Bessiere, J., Masotti, V. & Viano, J. (2004). "Changes in essential oil composition in *Saint John's Wort* (*Hypericum perforatum* L.) aerial parts during its phenological cycle". *Biochemical systematics and ecology*, 32, 735-745.
- Tatsis, E., Boeren, S., Exarchou, V., Troganis, A., Vervoort, J. & Gerothanassis, I. (2007). "Identification of the major constituents of *Hypericum perforatum* by LC/SPE/NMR and/or LC/MS". *Phytochemistry*, 68, 383-393.
- Xie, P., Chen, S., Liang, Y., Wang, X., Tian, R. & Upton, R. (2006). "Chromatographic fingerprint analysis - a rational approach for quality assessment of traditional Chinese herbal medicine". *Journal of chromatography A*, 1112, 171-180.
- Xu, M., Wang, G., Xie, H., Huang, Q., Wang, W. & Jia, Y. (2008). "Pharmacokinetic comparisons of schizandrin after oral administration of schizandrin monomer, *Fructus Schisandrae* aqueous extract and *Sheng-Mai-San* to rats". *Journal of Ethnopharmacology*, 115, 483-488.