

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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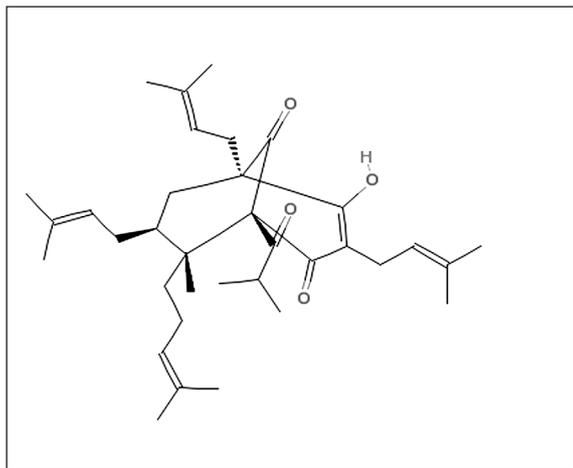


Figure 1: Hyperforin molecule

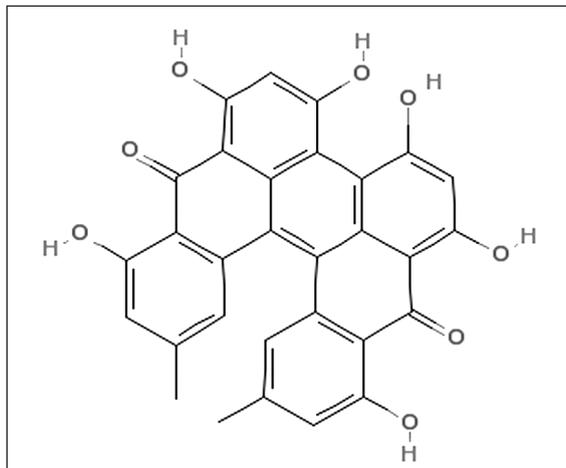


Figure 2: Protohypericin molecule

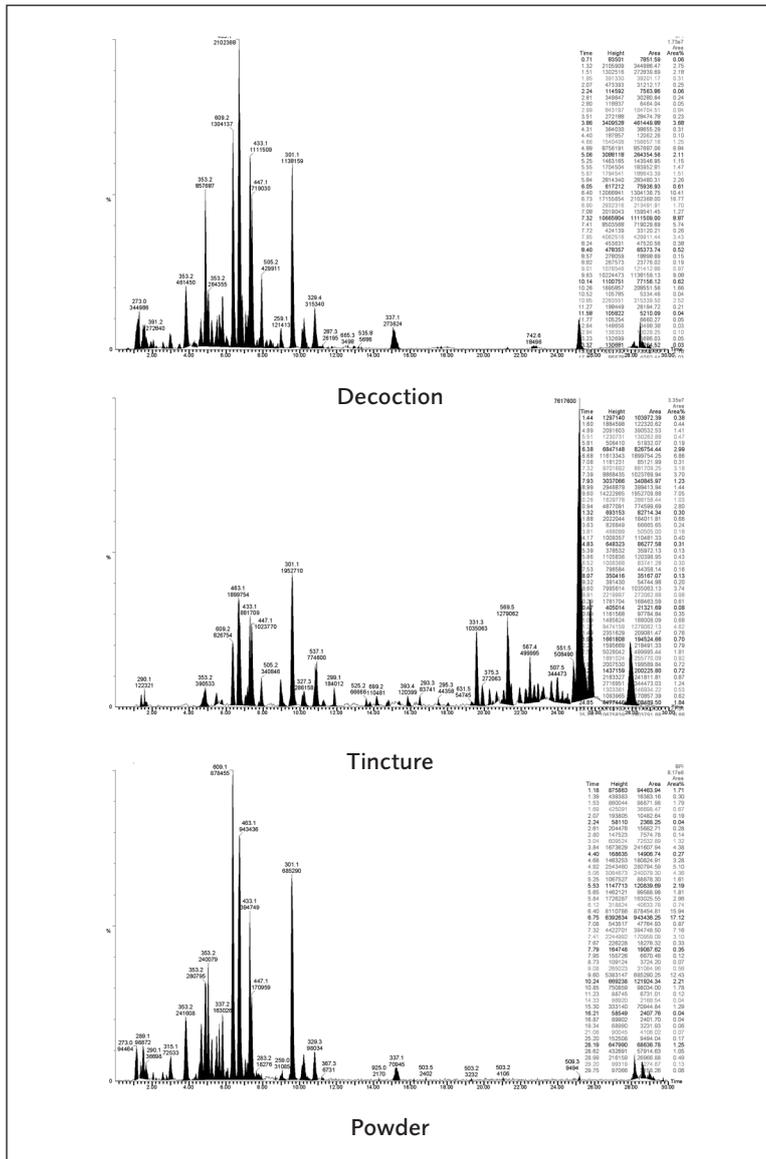


Figure 3: Chromatographic fingerprints of three methods of preparation of Guan Ye Lian Qiao (*Hypericum perforatum*)

After the compounds have been separated it becomes possible to use coupled techniques to find out more about the individual compounds (Cao et al., 2006). The coupled techniques used here were HPLC-Ultra Violet (UV) and HPLC-Mass Spectrometry (MS). HPLC-UV was used to identify compounds through their UV refraction properties, and HPLC-MS was used to determine their relative abundance (Xie et al., 2006; Harris, 2007). Relative abundance is a measure that is used for comparing the numbers of molecules within a sample; it is not an absolute measure, but is similar to quantity in that it allows a comparison of the amounts of different compounds.

Guan Ye Lian Qiao (*Hypericum perforatum*) was chosen as a suitable herb to test because of the large body of extant research into its active compounds. It has long been used both in European and Chinese

herbal medicine for the treatment of infection (Couceiro et al., 2006) and recent research focuses on its use as an antidepressant. The main compound – hyperforin - that exerts its antidepressant action (Chatterjee et al., 1998) is also that which appears to mediate much of its antimicrobial actions (Gurevich et al., 1971; Gibbons et al., 2002). It is not the only compound in Guan Ye Lian Qiao with antimicrobial properties; there are also flavonoids which can have this effect (Radulovic et al., 2007), although these tend to be found in much larger amounts in commonly eaten foods.

There is typically variation in the profile of compounds detected within a plant, dependant on where and when it was grown (Bergonzi et al., 2001; Schwob et al., 2004; Conforti et al., 2005). To exclude these effects, the raw sample of Guan Ye Lian Qiao (*Hypericum perforatum*) used here was obtained from the same batch of herbs from which the concentrated powder was subsequently produced.

The aim in creating the samples was to replicate the preparation methods used by practitioners and patients. For the raw herb, five milligrams of the herb was added to five millilitres of tap water, which was then decocted over a Bunsen burner. For the powder, five millilitres of boiling tap water were added to five milligrams of powdered herb. This was then diluted 1:15 with distilled water (because the powder was a 15:1 concentration). For the tincture, five millilitres of ethanol was added to five milligrams of the raw herb. The samples were centrifuged to remove any plant materials and then placed in vials for testing in the HPLC-UV and HPLC-MS machines. This process was repeated three times, creating the sample fluids from scratch and retesting to obtain the relative abundance of the identified compounds.

Many studies are available detailing HPLC analysis of Guan Ye Lian Qiao (*Hypericum perforatum*) (C. de los Reyes & Koda, 2001; Chandrasekera et al., 2005; Tatsis et al., 2007). From these analyses a list was constructed of which compounds to identify in the samples, which were: hyperforin (see Figure 1), which mediates the herb's main antidepressant and antimicrobial actions, protohypericin (see Figure 2), which is a precursor form of hypericin, and five flavonoids. Table 1 shows the relative abundance of the compounds in each sample following each of the three runs. In addition to these metrics, a chromatographic fingerprint was obtained (see Figure 3). This is a signature unique to each sample that shows the relative abundance of all compounds, including those that were detected but not identified (Xie et al., 2006).

Conclusions

The differences between relative abundance of the compounds were tested using Restricted Maximum

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 ($dF6 F=11.04 p<0.05$) 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.

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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.)

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