Synergy in Herbal Medicines: Part 1

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ABSTRACT

In the following paper, we will review the available literature on synergy and additive effects involving medicinal herbs and herbal extracts. Several types of synergistic interactions are discussed, including apparently inactive constituents enhancing the effects of apparently active constituents within and between herbal medicines, various herbal compounds altering the absorption of others, reduction in toxicity of some herbal constituents by others, and direct synergistic therapeutic effects when active constituents are combined within and between many medicinal herbs. Species discussed include *Artemisia annua* (sweet Annie, *qīng hāo*), *Ammi visnaga* (khella), *Glycyrrhiza glabra* (licorice), *G. uralensis* (Chinese licorice, *gān cǎo*), *Panax ginseng* (Asian ginseng, *rén shēn*), *Mahonia aquifolium* (Oregon grape), *Berberis aetnensis* (Mt. Etna barberry), *B. trifoliolata* (algerita), *B. fendleri* (Colorado barberry), and *Coptis chinensis* (goldthread, *huáng lián*). Part 2 of this article will continue this review on other medicinal herb species.

Keywords: Synergy; Medicinal plants; *Artemisia annua; Glycyrrhiza; Coptis*

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INTRODUCTION

Medicinal plants contains hundreds if not thousands of unique compounds. Models based on single molecular entities do not accurately describe or capture the complexity of interactions among the constituents in medicine plants and multiconstituent extracts made from them.

In the mainstream pharmaceutical view, there is a single active constituent in a plant that explains its activity and which can be isolated and used as a conventional drug. This idea is reinforced by the fact that many widely-used drugs are either single molecules isolated from plants or semisynthetic variants of natural molecules.[1, 2] However, this still does not prove that the single compounds are superior to complex mixtures, particularly when possible beneficial effects of various compounds that indirectly support the main desired action have not been assessed.

In many studies, an assay (such as receptor activation or inhibition, or killing of a microbe or cancer cell *in vitro*) can be used to identify a single molecule or several molecules that appear to provide most or all the effect of a crude plant extract for that assay. For example, one fractionation assay ultimately determined that berberine was the single most active antimalarial constituent found in 14 different Vietnamese medicinal plants.[3] This would seem on the face of it to support the conventional pharmacological model for medicinal plants. However, these assays do not evaluate the complex activities of other compounds in the plant that could directly affect the outcome being assessed. This can lead to severe oversights and a failure to recognize "inactive" constituents as providing important functions. As cited below in depth, flavonoids found in the same plant as berberine have been shown to inhibit resistance to berberine in microbes. Thus, while berberine may be the most important antimicrobial, removing the flavonoids could result in microbial resistance developing toward berberine and its failure as an antimicrobial. Activity-guided modeling misses the benefit of other plant constituents on the overall outcome, as well as other outcomes not being assessed.

This article will review the evidence on synergy of multiple compounds in complex herbal extracts, including those involving multiple herbs. Many instances are reviewed where various types of synergy have been studied with both supportive and some negative results. The term synergy will be used loosely in this review to include effects greater than the sum of the parts (true synergy) and simple additive effects. Overall, however, the current research on many important medicinal herbs underappreciates the value of complex mixtures compared with single isolated constituents.[4]

ARTEMISIA ANNUA (SWEET ANNIE)

The prolific and widespread weed *Artemisia annua* (sweet Annie, *qīng hāo*) illustrates the problem of activity-guided research overlooking useful effects beyond the "active" constituents. The sesquiterpene lactone artemisinin (Figure 1) from *A. annua* is a potent antimalarial schizonticide.[5] Artemisinin (qinghaosu in Chinese) and various semisynthetic derivatives of it are among the most widely-used antiplasmodial drugs in the world. It seems to be largely assumed in mainstream medicine that this is the beginning and ending of the sweet Annie story: artemisinin is the silver bullet, and there is nothing else worth investigating further in the herb. However, a clinical trial showing a crude decoction of *A. annua* that effectively eliminated symptoms and dramatically lowered parasite burden in adults in the Democratic Republic of the Congo with chronic malaria, with cure rates on average of 74%, despite providing far lower levels of artemisinin than are used as an isolated drugs, suggesting that there is more to *A. annua* than just artemisinin.[6]

Polymethoxylated flavonoids in *A. annua* also have physiological activity (Table 1), though they are supposedly not directly antiplasmodial (note that at least one *in vitro* study did find artemetin directly antiplasmodial).[24] These flavonoids have been shown to decrease resistance of *Plasmodium* spp. to artemisinin by inhibiting efflux pumps in the parasite.[25] Chrysosplenol D (see Figure 1), chrysosplenitin, circilineol, casticin, and artemetin have all been shown to significantly lower (by 20%–50%

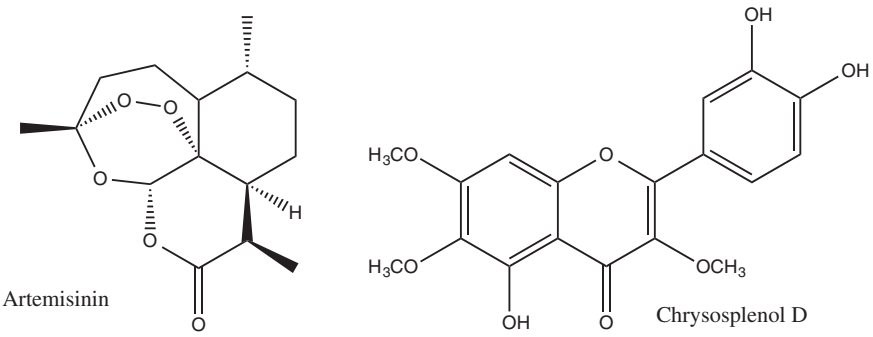


Figure 1: Artemisinin and chrysosplenol D.

depending on the compound) the half minimal inhibitory concentration (IC_{50}) of artemisinin against *Plasmodium* spp. *in vitro*.[26, 27] These flavonoids did not reduce *Plasmodium* resistance to chloroquine.[26, 27]

While artemisinin is readily and stably extracted by aqueous decoction, flavonoids are best extracted by alcohol.[28] This is interesting in light of *in vitro* evidence that only ethanolic and not aqueous crude extracts of *A. annua* are antiplasmodial, and an absence of evidence of synergy in *A. annua* aqueous extracts *in vitro*.[29, 30] It is also confusing given the human research on aqueous infusions of *A. annua* which shows they are effective.[6] It should be noted that traditional methods of preparation of *A. annua* in Chinese medicine by soaking combined with wringing or pounding followed by juicing lead to extracts with 20 times

Table 1: Select other actions reported for polymethoxylated flavonoids found in *Artemisia annua.*

Flavonoid	Action	Model	References
Chrysosplenol D	Antioxidant	*In vitro*	Xie *et al*., 2014[7]
	α-Glucosidase inhibitor	*In vitro*	Ezzat and Salama, 2014[8]
	Antiangiogenic	*In vitro*	Zhu *et al*., 2013[9]
Casticin	Antiangiogenic	*In vitro*	Zhu *et al*., 2013[9]
	Growth inhibition by mitotic spindle disruption	Cervical cancer cells *in vitro*	Kobayakawa *et al*., 2004[10]
	Antineoplastic and pro-apoptotic by many mechanisms	Breast, ovarian, lung, colon, liver cancer cells *in vitro*	Liu *et al*., 2014[11]; Jiang *et al*., 2013[12]; Ono *et al*., 2002[13]; Yang *et al*., 2011[14]
	Inhibit prolactin secretion by inhibiting ERα and stimulating ERβ	Metoclopramide-treated rats	Ye *et al*., 2010[15]
	Co-stimulates IL-1β secretion	*In vitro* Toll-like receptor 2-activated cells	Lim *et al*., 2013[16]
	Cyclooxygenase-2 inhibition, iNOS inhibition, NF-κB inhibition	*In vitro*	Liou *et al*., 2014[17]
	Lipoxygenase inhibition	*In vitro*	Choudhary *et al*., 2009[18]
Artemetin	Lipoxygenase inhibition	*In vitro*	Choudhary *et al*., 2009[18]
	Anti-inflammatory	Carageenan-induced edema in rat paws	Sertié *et al*., 1990[19]
	Antibacterial against Gram positive bacteria	*In vitro*	Michielin *et al*., 2009[20]
	Angiotensin converting enzyme inhibition	*In vitro*; normotensive rats	de Souza *et al*., 2011[21]
	Hepatoprotective	Carbon tetrachloride-treated rats	Sridevi *et al*., 2012[22]
	Antiperoxidative	*In vitro*	Dugas *et al*., 2000[23]

ER, estrogen receptor; IL, interleukin; iNOS, inducible nitric oxide synthase; NF, nuclear factor.

higher levels of artemisinin than just infusion.[31] Artemisinin content alone did not explain the antimalarial activity of these traditional juiced extracts *in vitro*; the IC_{50} concentrations were up to 18 times lower for the crude juices than pure artemisinin. One mouse study found that a crude ethanol extract of *A. annua* had a median effective dose (ED_{50}) of 35 mg/kg vs. 122 mg/kg for pure artemisinin.[32] Short-term (3 day) use of this product in an open human trial was as effective, if not more so, than chloroquine, though only 217 mg of artemisinin were delivered over the total treatment period, but had a high recrudescence rate (recurrent disease due to incomplete parasite killing). Recrudescence was lowered by extending duration of treatment or combining the *A. annua* capsule with primaquine.[32]

An *in vitro* analysis found that *A. annua* infusion with very low artemisinin concentrations (0.18% dry weight) was equally active against chloroquine-resistant and -sensitive strains of *P. falciparum* as pure artemisinin.[33] The authors speculated these results are due either to the presence of other antiplasmodial compounds or other synergistic effects.[33] Presumably these are compounds other than the polymethoxylated flavonoids given the low solubility of such flavonoids in water. Ongoing work is needed to identify all the synergistic compounds in *A. annua*.

Other compounds in *A. annua* have activities that may enhance its clinical utility for people with malaria and other conditions beyond direct antimalarial activity. For instance, a mixture of many sesquiterpene lactones from *A. annua* besides artemisinin showed promising analgesic activity in rodents.[34] Qinghao acid, a sesquiterpene precursor to artemisinin in *A. annua*, and the coumarin scopoletin were shown to contribute to the bacteriostatic and inflammation-modulating effects of *A. annua* in another report.[35] Cinnamic acid derivatives (rosmarinic and chlorogenic acids in particular) from *A. annua* inhibited the pro-inflammatory cytokines IL-6 and -8 *in vitro*.[36]

Polymethoxylated flavonoids from *A. annua* reduce resistance to other natural antimicrobials. Combining chrysosplenol D or chrysosplenetin from *A. annua* with subinhibitory concentrations of the isoquinoline alkaloid berberine was effective in killing *Staphylococcus aureus in vitro*.[37] Both flavonoids by themselves had minimal direct antibacterial activity. This suggests another kind of synergy: that which occurs when multiple herbs are mixed together. Such formulation is a common practice in most systems of herbal medicine around the world and it has been suggested that various other natural antimalarials should be combined and tested in humans.[38] Other herbal mixtures have been shown to have synergy against malaria *in vitro*.[39] Other examples of formulation synergy are presented elsewhere in this paper.

AMMI VISNAGA (KHELLA)

There is evidence of two types of synergy in the native Mediterranean medicinal plant *Ammi visnaga* (khella). First, there is evidence of improved absorption of furanocoumarins from crude extracts than when they are given in isolation. Second, there is evidence of activity from other constituents in the plant besides furanocoumarins, and these other compounds enhance the activity of furanocoumarins.

The most well-known spasmolytic furanocoumarin of *Ammi visnaga* is called khellin (Figure 2).

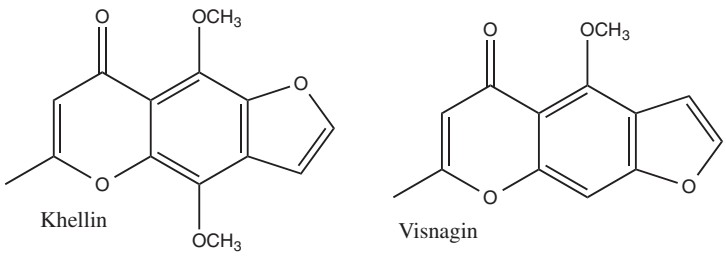


Figure 2: Khellin and visnagin.

Khellin is absorbed more rapidly and completely when administered as part of a whole *Ammi visnaga* extract than when given in isolation.[40] Another important furanocoumarin in *Ammi visnaga*, visnagin (see Figure 2), has also been shown to have its absorption significantly enhanced when administered to 12 male Sprague-Dawley rats as a whole plant extract compared with a pure compound, with a ten-fold increase in area under the curve in serum visnagin levels at the lowest dose tested (containing 0.625 mg visnagin/ml extract) of the extract compared to pure visnagin.[41] This difference was highly statistically significant ($P=0.005$). Equal concentrations of visnagin were administered in both forms.

Whole extracts of *Ammi visnaga* were more effective at inhibiting the mutagenicity of 2-aminoanthracene, 1-nitropyrene, and daunomycin than isolated khellin *in vitro*.[42] The identity of other constituents that were synergistic in this model were not identified. In male Sprague-Dawley rats ($n=8$ per group) with hyperoxaluria induced by injection of ethylene glycol and ammonium chloride, a crude aqueous extract of *Ammi visnaga* fruits, but not isolated khellin or visnagin, reduced urine oxalate levels compared with untreated controls ($P<0.01$).[43] The crude extract and isolated khellin and visnagin all reduced calcium oxalate crystal deposition compared with untreated controls ($P<0.05$). However, the crude extract also increased urinary citrate levels while decreasing urine oxalate excretion, but the isolated constituents did not have these effects. *In vitro*, an aqueous *Ammi visnaga* extract was superior to khellin or visnagin alone at protecting renal epithelial cells from lysis due to calcium oxalate.[44] These results mostly suggest other compounds in *Ammi visnaga* are active with different and beneficial additive or synergistic effects besides khellin or visnagin.

GLYCYRRHIZA GLABRA (LICORICE) AND GLYCYRRHIZA URALENSIS (GĀN CǍO)

The European/Central Asian *Glycyrrhiza glabra* (licorice) and its close cousin from China, *G. uralensis* (*gān cǎo*, Chinese licorice), are two of the most important and widely used herbs in the world. One of their major uses, particularly in Chinese medicine, is to act as a corrective assistant, meaning to decrease the toxicity of other herbs with which they are combined. This is an intriguing type of formulation synergy that deserves further research, though several examples can be cited involving *G. uralensis*.

A classic example of this corrective assistant effect is pairing *G. uralensis* with *Aconitum* spp. (aconite, *fù zǐ*) prepared lateral roots to prevent cardiotoxicity. One *in vitro* study using rat cardiomyocytes found that *G. uralensis* prevented the tendency of *Aconitum* to increase pulse frequency of the cells, and also inhibited the increase in lactate dehydrogenase induced by *Aconitum*.[45] Adding *Zingiber officinale* (ginger) rhizome extract to these two herbs further enhances the protective effect of *G. uralensis*. This creates a formula known in traditional Chinese medicine as *sì nì tāng* or "frigid extremities decoction," first described in the *Shāng Hán Lùn* (*Discussion of Cold Damage*) from circa 220 CE that is actually cardioprotective.[46–48] Another observation is that *Aconitum* by itself induced cytochrome 3A4 in Sprague-Dawley rats ($n=3$), resulting in a significantly decreased absorption of buspirone, with the area under the curve of the drug reduced by 52.8% ($P=0.02$).[49] This effect was eliminated when *G. uralensis* was added to *Aconitum*. One possible mechanism for protection against the toxicity of *Aconitum* is by increasing the rate of metabolism of the potentially toxic alkaloids by *G. uralensis*.[50]

There are clear chemical differences between isolated extracts of *Aconitum* and a combination of *G. uralensis* and *Aconitum*, suggesting that the protective effect of *G. uralensis* begins during preparation and is not only due to pharmacologic effects.[51, 52] There is also evidence of pharmacokinetic changes in *Aconitum* alkaloids such as hypoaconitine when taken in combination with *G. uralensis* and *Zingiber* that could explain some of the protective effects of this formulation.[53]

Another example of the corrective assistant effect of *G. uralensis* is that it decreases hepatonephrotoxicity of *Dioscorea bulbifera* (*huáng yào zǐ*, air potato) tuber. When the two are boiled together, the resulting mixture has lower levels of toxic

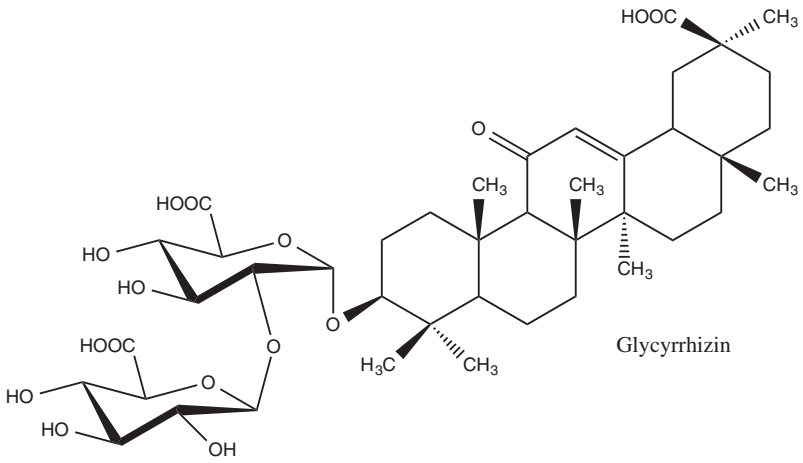


Figure 3: Glycyrrhizin.

compounds.[54] Even better documented is both the reduction of toxicity of the highly toxic immunosuppressive herb *Tripterygium wilfordii* (thunder duke vine, *léi gōng téng*) decorticated root and potentiation of its antiarthritic effects in a randomized clinical trial by the addition of *G. uralensis*, compared with *T. wilfordii* alone.[55] Although this trial was not blinded, it still provides supporting evidence for of this type of formulation synergy.

G. glabra and *G. uralensis* contain triterpenoid saponins, notably glycyrrhizin (Figure 3). It is believed that these constituents are the chemical basis for the historical use of these herbs as solubilizing agents in herbal formulas to increase absorption of other constituents, an important and distinctive synergistic feature of herbal medicine. This property is referred to as a guiding action in traditional Chinese medicine, and *G. uralensis* is the king of guide herbs. It appears in 50% of the 283 formulas in one of the most important materia medica of Chinese medicine, the *Shén Nóng Běn Cǎo Jīng* (*Divine Husbandman's Classic of the Materia Medica*), compiled between 300 BCE and 200 CE.[56]

In one *in vitro* study, adding glycyrrhizin to an aqueous solution increased the solubility of saikosaponin A from *Bupleurum falcatum* (thorowax, *chái hú*) from 0.1 mg/mL to 5 mg/mL at standard temperature and pressure.[57] Saponins from other plants have this same solubilizing effect. In the same study, ginsenoside Ro from *Panax ginseng* (Asian ginseng, *rén shēn*) increased the solubility of saikosaponin A in water from 0.1 mg/mL to 3.4 mg/mL. A combination of ginsenoside Ro and the dammarane saponins of 20(*S*)-protopanaxadiol from *P. ginseng* were more effective than ginsenoside Ro by itself at solubilizing multiple saikosaponins in another *in vitro* study.[58] Many other studies confirm the solubilizing properties of ginsenoside Ro, which may in part explain its extremely widespread incorporation into traditional Chinese herbal formulas; a similar explanation for the widespread incorporation of *G. uralensis* in such formulas is also likely.[59–61]

Another mechanism whereby *G. uralensis* (and likely other saponin-rich herbs) can increase absorption of various other herbal compounds is by inhibition of P-glycoprotein (Pgp, also known as multidrug resistance protein 1, or MDR1) in the intestines. Pgp is a common efflux pump responsible for removal of a number of drugs and herbal compounds from intestinal epithelial cells, thus preventing their absorption. Glycyrrhizin significantly enhanced oral absorption of aconitine (AUC of aconitine increased 61% with glycyrrhizin, $P<0.01$ compared with aconitine alone) from *Aconitum* through inhibition of Pgp in male Sprague-Dawley rats ($n=5$).[62] Other licorice compounds such as licochalcone A (Figure 4) also inhibit Pgp.[63] This is a clinical concern given the potential toxicity of aconitine, but may be reconciled given all the other information reviewed above about how *G. uralensis* offsets the toxicity of *Aconitum*. Note that this property of *G. uralensis* has been leveraged to reduce drug resistance and amplify the utility of various antimicrobial drugs.[64] The licorice flavonoid glabridin (see Figure 4) is itself a Pgp substrate

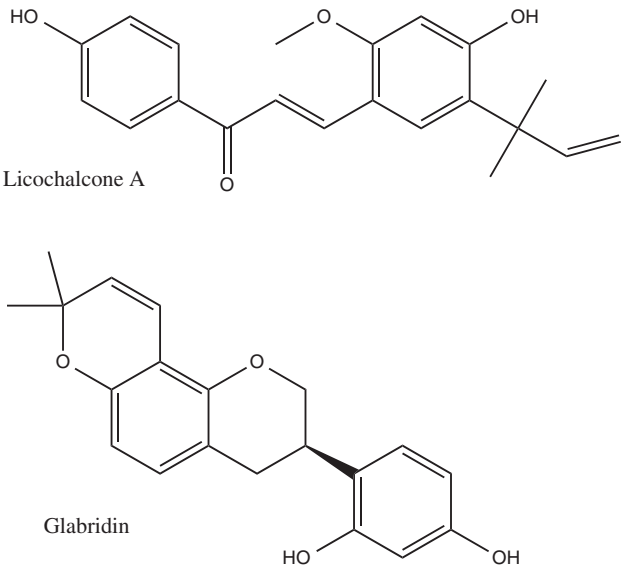


Figure 4: Licochalcone A and glabridin.

and thus a competitive inhibitor of Pgp, based on a study looking at absorption of digoxin, another Pgp substrate.[65] Another study found that *G. uralensis* decoction *in vitro* had no effect on Pgp, but after oral ingestion by Wistar rats (n=3) it mildly increased absorption of rhodamine 123 (P<0.05 compared with controls) and thus confirming a mild Pgp inhibitory effect.[66] This study suggests some process occurred during digestion that activated compounds in the decoction.[66] Other saponin-rich herbs such as *P. ginseng* have also been shown to be Pgp inhibitors.[67]

In combination with at least two herbs, crude Chinese licorice (using the alternative species *Glycyrrhiza inflata*) extracts have been shown to synergistically inhibit Pgp *in vitro* in the case of *Daphne genkwa* (genkwa, *yuán huā*) and in Wistar rats in the case of the endangered species *Euphorbia kansui* (kan-sui, *gān suì*).[68, 69] Both herbs are powerful cathartic laxatives and the authors of these studies commented that the synergistic inhibition of Pgp by these herbs could actually increase the toxicity of these herbs by keeping the laxative compounds in the gut longer allowing them to act more potently. Both herbs are among the very few that are traditionally regarded as becoming more toxic in combination with licorice, a type of toxic synergy. There is other evidence that *G. uralensis* enhances the extraction of potentially toxic diterpenoids and other terpenoids from *E. kansui* and this might mediate the enhancement of its toxicity.[70] Whatever the mechanism, these herbs should not be combined.

Whole root aqueous extracts of *G. glabra* have been shown to result in lower and more delayed absorption of glycyrrhizin (described below) and its aglycone glycyrrhetinic acid, and thus to lower toxicity of the crude extract compared with glycyrrhizin given in isolation.[71, 72] Unidentified lipophilic constituents appear to be responsible for the effect of the whole root extract on glycyrrhizin pharmacokinetics.

There are many other aspects of synergy in licorice and *G. uralensis*. Table 2 lists a selection of many other intriguing reports in this vein, including at least one negative report.

MAHONIA AQUIFOLIUM (OREGON GRAPE) AND BERBERIS SPP. (BARBERRY)

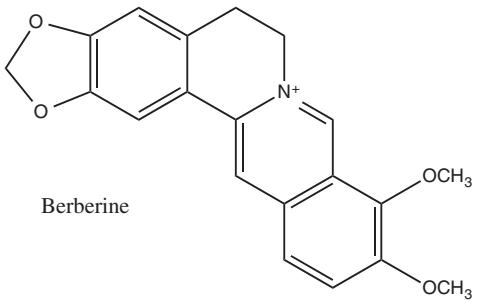
Berberine (Figure 5), an isoquinoline alkaloid, is traditionally regarded as the active constituent from the roots of *Mahonia aquifolium* (Oregon grape) and the many species in the genus *Berberis*. However, multiple alkaloids are found in these species and multiple studies have found crude extracts

Table 2: Other reports of synergy in *Glycyrrhiza* spp.

Comparators	Model	Result	References
Liquiritin apioside, liquiritin, liquiritigenin, or all three combined	Capsaicin-induced cough in guinea pigs	Combination inhibited cough significantly more than any compound in isolation	Kamei *et al*., 2005[73]
Glycyrrhizin, glycyrrhetinic acids, licorice extract	Multiple *in vitro* mutagenicity assays	All were antimutagenic but licorice extract had broadest effects	Zani *et al*., 1993[74]
Various flavonoids and chalcones, *G. uralensis* extract	Granulomatous inflammation in rodents	Isoliquiritin many times more potent inhibitor than whole root extract	Kobayashi *et al*., 1995[75]
Glycyrrhizin, 18β-glycyrrhetinic acid (18BGA), aqueous licorice extract	Respiratory syncytial virus inhibition *in vitro*	Aqueous extract and 18BGA very active while isolated glycyrrhizin inactive	Feng Yeh *et al*., 2013[76]

of the roots from various species more effective than isolated alkaloids. Furthermore, non-alkaloid compounds, including those found in the leaves, have been shown to reduce bacterial resistance to the alkaloids, suggesting a potential type of synergy between different parts of the same medicinal plant.

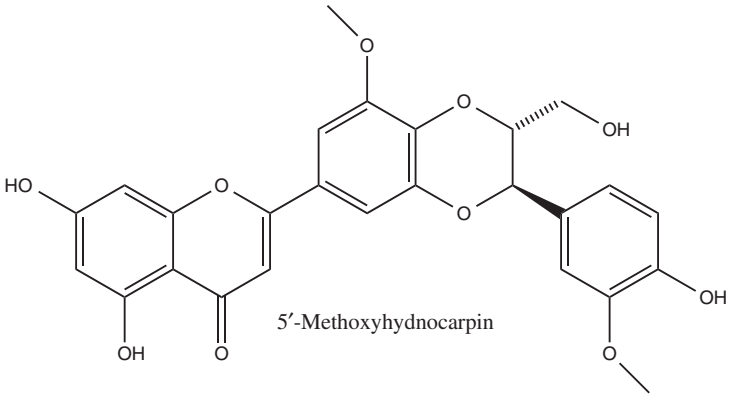
Methyoxylated flavonolignans such as 5′-methoxyhydnocarpin (Figure 6) primarily found in the leaves of *M. aquifolium* (Figure 7) with no intrinsic antistaphylococcal activity have been shown to enhance this activity in berberine from roots of the plant through inhibition of the NorA efflux pump in *Staphylococcus aureus*.[77] Another compound found in *M. aquifolium* leaves, pheophorbide A (Figure 8), a natural degradation product of chlorophyll, was also a strong resistance pump inhibitor in *S. aureus*, greatly magnifying the antibacterial activity of berberine *in vitro*.[78] The flavonolignan silymarin from *Silybum marianum* (milk thistle) seed was also very active as a resistance pump inhibitor enhancing the efficacy of berberine in this study.



Berberine

Figure 5: Berberine.

B. trifoliolata (algerita) leaf also contains 5′-methoxyhydnocarpin and *B. fendleri* (Colorado barberry) leaf also contains pheophorbide A with drug efflux pump-inhibitor effects in *S. aureus*.[79] *B. aetnensis* (Mt. Etna barberry) has also been shown to contain similar pump inhibitors in its



5′-Methoxyhydnocarpin

Figure 6: 5′-Methoxyhydnocarpin.

Figure 7: *Mahonia aquifolium* leaves.

leaves as that of *M. aquifolium* and to also inhibit the efflux of ciprofloxacin from drug-resistant *S. aureus*, thereby enhancing the efficacy of the drug.[80] This research suggests that combining some fruit or leaf of *M. aquifolium* with the root would optimize efficacy, at least when treating patients with staphylococcal infections.

A crude ethanol extract of *B. vulgaris* (barberry) was more effective than isolated alkaloids or alkaloid fractions in rodent models of acute and chronic inflammation.[81] A crude methanol extract of the root of *B. aetnensis* was more active against *Candida albicans* and other pathogenic species of *Candida in vitro* than an alkaloid fraction or pure berberine.[82] These findings suggest a need for human research on the synergistic effects of not only whole root extracts, but also root/leaf extracts of *Berberis* and *Mahonia* spp.

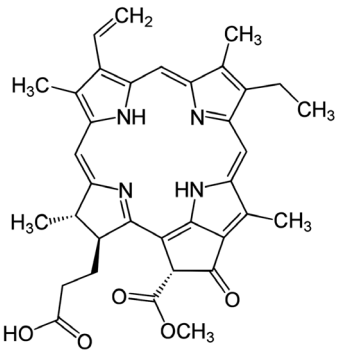


Figure 8: Pheophorbide A.

COPTIS CHINENSIS (GOLDTHREAD)

Another berberine-containing medicinal species studied for synergistic properties that is frequently used in Chinese medicine, is *Coptis chinensis* (goldthread, *huáng lián*) rhizome. Total extracts of the rhizome are more active hypoglycemics than an alkaloid-only fraction of *C. chinensis*.[83] A combination of four *C. chinensis* alkaloids (berberine, coptisine, palmatine, and epiberberine) were more hypoglycemic and less toxic to liver cells than berberine by itself.[84, 85] An alkaloid-enriched extract of *C. chinensis* killed 100% of male and female Kunming mice (n=20) at a dose of 0.28 g/kg; no mice were killed by a total ethanol extract of *C. chinensis* at this dose.[86] The LD_{50} of the total extract was 2950 mg/kg in rats, but it was 160–210 mg/kg for the alkaloid-enriched extract used in this study. Statistical significance was not reported. While berberine and total aqueous extract of *C. chinensis* rhizome both limited hepatic damage from carbon tetrachloride in male Sprague-Dawley rats, the total extract was more effective (statistical significance not stated).[87] In a similar study, the aqueous rhizome extract and berberine had similar efficacy at preventing carbon tetrachloride liver damage in rats.[88] The reason for the differences between these two studies is unknown, as the extracts and experimental designs were very similar.

The mixture of six parts *C. chinensis* and one part *Tetradium rutaecarpa* (evodia, *wú zhū yú*) fruit is known as *Zuǒ Jīn Wán*, "Left Metal Pill," in Chinese medicine. It originated in 1481 CE in *Dān Xī Xīn Fǎ* (*Essential Teachings of Dan-Xi*) and is used to treat a wide range of digestive problems ranging from ulcers and esophageal reflux to nausea and vomiting. Administration of this formula to rats resulted in a complex pharmacokinetic interaction, notably an increase in absorption of major important compounds from *T. rutaecarpa* and a decrease in bioavailability of alkaloids from *C. chinensis*, notably coptisine and berberine.[89, 90] Pretreatment of rats with *T. rutaecarpa* fruit aqueous extract for 2 weeks also resulted in significant reduction in berberine bioavailability from *C. chinensis*.[91] The mechanism appeared to be through the constituents of *T. rutaecarpa* inducing hepatic UGT1A1, increasing first pass metabolism of berberine. *C. chinensis* is commonly combined with *T. rutaecarpa* as a

corrective assistant because *C. chinensis* is "cold" while *T. rutaecarpa* is "hot."[92] This pharmacokinetic outcome could be the desired result of the combination though it is difficult to be certain at this time.

One other line of evidence suggesting a balancing effect of these two herbs in combination comes from an *in vitro* study looking at the effects of *C. chinensis* and *T. rutaecarpa* separately and together on bovine adrenal medullary cells.[93] The two herbs had diametrically opposed effects, with the alkaloids of *C. chinensis* inhibiting secretion from the cells and the alkaloids of *T. rutaecarpa* stimulating it. While this might suggest they simply cancel each other out and it would make little sense to combine them, it is more likely given the endurance of this herb pair in Chinese herbal medicine that the effect is balancing and beneficial in some way.

There are other hints in the literature that combining these two herbs as *Zuǒ Jīn Wán* may be superior to either alone. Evodiamine is an alkaloid found in *T. rutaecarpa* with multiple antineoplastic actions against gastric cancer cells *in vitro*, but it also increases IL-8 secretion by the cells, which is associated with increased risk of metastasis. Berberine blocked this effect.[94] Berberine and evodiamine in combination were significantly better at inducing apoptosis in human hepatocellular carcinoma cells *in vitro* than either compound alone.[95] All these results suggest a possible benefit of combining these two herbs in cancer patients.

CONCLUSION

Numerous lines of inquiry demonstrate synergistic and additive effects among and between medicinal herbs. Some of these are straightforward, and with multiple compounds with similar actions showing greater efficacy together than any one of them in isolation, such as the hypoglycemic activities of alkaloids in *C. chinensis*.[16, 17] Others are more subtle and complex; for instance, showing that compounds can indirectly enhance the efficacy of other compounds with direct effects, such as the methyloxylated flavonolignans in *M. aquifolium* leaf reducing resistance in *Staphylococcus aureus* to berberine in *M. aquifolium* root.[9] Effects on pharmacokinetics between herbal compounds can be beneficially inhibitory (as in the case of the interaction between *T. rutaecarpa* and *C. chinensis*)[21–23] or enhancing (as is the case with *G. uralensis* and many compounds).[78] Another type of synergy is reduction of toxicity of one herb by another, as demonstrated with *G. uralensis* and several very different types of herbs.[62, 71, 72]

Further research is warranted to determine the extent and importance of these effects, particularly in humans, as much of the existing research is preclinical. Currently herbal medicine is still practiced by many practitioners based on the idea that these synergistic effects are relevant, as herbal formulas are still in widespread use. Further verifying that this approach is clinically useful and determining optimal formulations would be of great benefit to many patients around the globe.

DISCLOSURE OF INTEREST

Dr. Yarnell reports a financial interest in Heron Botanicals, outside the submitted work.

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