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REVIEW (PART I)

Synergy research: Approaching a new generation of phytopharmaceuticals

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Abstract

The longstanding, successful use of herbal drug combinations in traditional medicine makes it necessary to find a rationale for the pharmacological and therapeutic superiority of many of them in comparison to isolated single constituents. This review describes many examples of how modern molecular–biological methods (including new genomic technologies) can enable us to understand the various synergistic mechanisms underlying these effects. Synergistic effects can be produced if the constituents of an extract affect different targets or interact with one another in order to improve the solubility and thereby enhance the bioavailability of one or several substances of an extract. A special synergy effect can occur when antibiotics are combined with an agent that antagonizes bacterial resistance mechanisms. The verification of real synergy effects can be achieved through detailed pharmacological investigations and by means of controlled clinical studies performed in comparison with synthetic reference drugs. All the new ongoing projects aim at the development of a new generation of phytopharmaceuticals which can be used alone or in combination with synthetic drugs or antibiotics. This new generation of phytopharmaceuticals could lend phytotherapy a new legitimacy and enable their use to treat diseases which have hitherto been treated using synthetic drugs alone.

Keywords: Phytomedicine; Synergy effects; Omic technology; Multi-target therapy; New perspectives

Introduction

Synergy research in Phytomedicine has established itself as a new key activity in recent years. It is one main aim of this research to find a scientific rational for the therapeutic superiority of many herbal drug extracts derived from traditional medicine as compared with single constituents thereof. The efficacy of these plant extracts used for centuries was verified in many cases by clinical studies. Synergy effects of the mixture of bioactive constituents and their byproducts contained in plant extracts are claimed to be responsible for the improved effectiveness of many extracts. For a long time, the mechanisms underlying these synergy effects remained unexplained. Only with exact knowledge of these mechanisms, it will be possible to develop a new generation of standardized, effect-optimized mono- and multi-extract preparations, which not only fulfill today's standards for quality, safety and efficacy of medicinal drugs but can ideally also be used for the treatment of diseases that have been treated previously only with chemosynthetics or antibiotics. The first impetus for this synergy research came from pharmaceutical legislation, which demands the verification that every component of a combined pharmaceutical preparation contributes to the claimed complete efficacy.

In the 1970s and the 1980s, it was difficult if not impossible to meet this requirement because of the lack

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of analytical high-tech- and molecular biological methods and the immense effort that would have been necessary in their absence. Many drug preparations at that time were not yet appropriate for controlled clinical studies; that is, they had not been sufficiently investigated analytically or toxicologically.

In addition, smaller pharmaceutical companies lacked adequate financial means to accomplish all necessary multiple comparative studies with analogue standard preparations. Then, as now, the major pharmaceutical companies were not interested in efficacy studies of complex herbal drug mixtures.

Two events initiated synergy research in phytomedicine: first, the new methods of analytical chemistry and molecular biology that have become available during the past decade, and second, an unexpected change of paradigm in chemotherapy that appeared without great attention.

This change of paradigm in chemotherapy involved the gradual transition away from the mono-substance therapy that had long been advocated with great vehemence toward a multi-drug therapy. Multi-drug therapy is already being practiced worldwide in the treatment of AIDS and other infectious diseases, hypertension, numerous types of cancer and rheumatic diseases.

This multi-drug concept in current cancer therapy was recently designated as biomodulatory – metronomic chemotherapy. The idea is to fight the tumor via a kind of concerted action not through direct destruction of the tumor but rather by suppression or activation of different processes which are essential for the tumor's survival (e.g. by angiogenesis and oncogene inhibition, induction of apoptosis, activation of the immune system or combating inflammatory processes). This concept complies with the multi-target therapy which will be described later in this review.

Such a change of paradigm was not necessary in phytotherapy, because therapy with drugs and their respective extract combinations had been favored from the very beginning. These practices remain the actual basis of therapy in Traditional Chinese and Ayurvedic medicine. No question that also mono-extract preparations, which in most cases contain a majority of several bioactive constituents, can also exhibit synergistic effects.

Why this preference for pharmaceutical combinations? The multi-drug strategy is based on a long awareness that many diseases have a multi-causal etiology and a complex pathophysiology. As we know from clinical studies carried out in both therapeutic disciplines, diseases can obviously be treated more effectively with well-chosen pharmaceutical combinations than with a single drug. The demonstration of improved effectiveness of drug combinations in chemotherapy is relatively simple, because they use mixtures of singular pure substances, whose pharmacology is in most instances known. Moreover, several examples of synergy effects in classical pharmacology are already known although their exact mechanisms have still not been exactly clarified.

Proving these synergy effects in phytotherapy is more difficult, because the plant extracts consist of complex mixtures of major compounds, minor concomitant agents and fibres, which can all be involved in the synergy effects.

Therefore, the research strategy in phytomedicine to prove synergy effects must be different from that of classical medicine.

Pharmacological approach

Definition and proof of synergy effects

As many publications, which are partially more theoretically than experimentally based, indicate, it is rather difficult to give an unequivocal universal definition for the term synergy effect (Loewe 1953, 1957; Greco et al. 1995; Kodell and Pounds 1985; Hewlett and Plackert 1979; Gessner 1988; Rentz 1932; Greco et al. 1995; Barrera et al. 2005; Berenbaum 1977, 1981, 1989). The ''isobole method'' of Berenbaum (1989) seems to be one of the most practicable experimentally and also the most demonstrative method among all those so far proposed for the proof of synergy effects.

Pharmacological *in vitro* or animal models can be used for the demonstration of the isoboles of a mixture of two substances. This method supplies a graphic demonstration with linearly arranged x and y axes reflecting the dose rates of the single individual components (Fig. 1). The dose combinations are represented by geometric points with coordinates matching the dose rate of the separate components in the combination. An isobole is understood to be a line

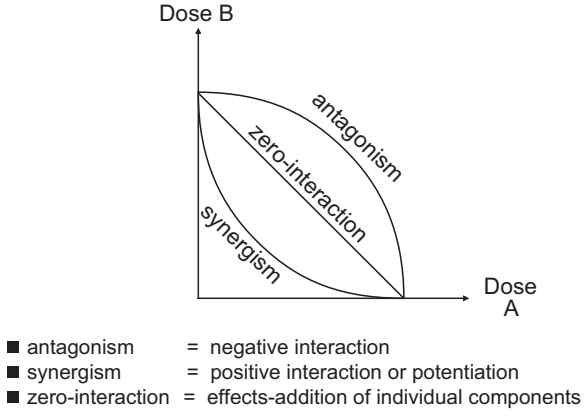


Fig. 1. Isoboles for zero-interaction, synergism and antagonism.

or curve between points of the same effect. The construction of isoboles requires the knowledge of the amounts of the individual components in the combination for one dose combination per effect level, at least, if the mechanism of interaction can be seen as independent of the amount of the single components. Data of several dose combinations are necessary for the exact drawing of the course of an isobole. In this manner, the concentrations of the substances a and b that are most responsible for the synergy effect can be inferred simultaneously from this chart. Strictly speaking, data on different effect levels are necessary for the identification of the interaction, because quality and quantity of the interaction can depend on the effect grade:

- According to Berenbaum, the zero (0) – or additive interaction means that the effect of two substances a and b is a pure summation effect (Eq. (1)). This might to be the case, for example, in heart glycosides, which all target the Na^+-, K^+-dependent ATP-ase, even though the concentrations differ.
- Correspondingly, the overall effect with antagonistic interaction is less than expected from the summation of the separate effects (Eq. (2)). A convex curve will be obtained.
- With the existence of a real synergism with potentiated or over-additive effect, the overall effect of two drugs a and b that are applied together as a mixture must be larger than it would be expected by the summation of the separate effects. The result is then a concave curve (Eq. (3)).

$$E(d_a, d_b) = E(d_a) + E(d_b) \quad (1)$$

$$E(d_a, d_b) < E(d_a) + E(d_b) \quad (2)$$

$$E(d_a, d_b) > E(d_a) + E(d_b) \quad (3)$$

E is the observed effect, d_a and d_b are the doses of agents a and b.

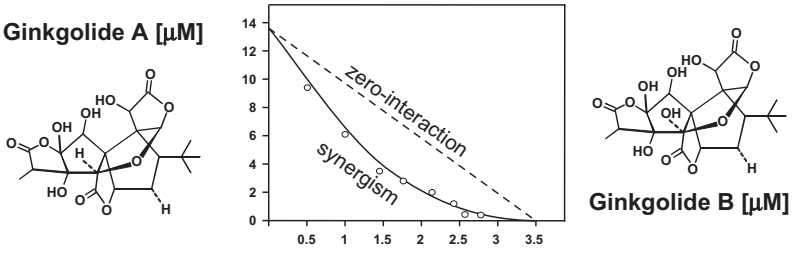
Therefore, lower amounts of agents a and b (doses $= d$) are necessary to achieve the synergy effect in the case of a present synergism. The achieved synergy effect can amount to doubling or even greater multiplication of the expected effect. Connected with this option of dose reduction, one can expect that at correctly chosen combination of a natural product a with a strongly effective synthetic product b, the potential of side effects of agent b can be reduced simultaneously.

The graphic of Fig. 2 shows a practical example for the verification of a synergy effect due to the combination of the two known natural products, ginkgolides A and B of *Ginkgo biloba*. Various ginkgolides A and B mixtures were used for this experiment and the IC_{50}-values were determined in the PAF-induced *in vitro* thrombocyte-aggregation inhibition test according to Born (1962). The IC_{50}-values are given in µg/ml and the according concentrations of the ginkgolides A and B concentrations in µg/ml and µM, respectively.

The combination effect, as shown in Fig. 2, can be also described by the means of the isobol Eq. (4):

$$I = \Sigma i(x_i/X_i) = \begin{cases} <1 \text{ synergism} \\ 1 \text{ 0-interaction} \\ >1 \text{ antagonism} \end{cases}$$

I = interaction index
x_i = dose of the individual component in the combination
X_i = dose of the individual component which generates the same effect as the combination
i = the i^{th}individual component in the combination (4)



GA : GB	IC50 [µg/ml]	Ginkgolide A [µM]	Ginkgolide B [µM]
3 : 1	2.40	4.41	1.42
2 : 1	2.20	3.60	1.72
1 : 1	1.80	2.21	2.12
1 : 2	1.55	1.27	2.43
1 : 3	1.40	0.88	2.57
1 : 10	1.30	0.29	2.79

Fig. 2. Isobol curve for 50% inhibition of a Ginkgolide AB-combination; IC_{50}-values (µg/ml) for various dose combinations of PAF-induced *in vitro* thrombocyte aggregation (Wagner and Steinke, 2005).

As shown in the chart, the interaction index is <1 and, therefore, corresponds to the isobole that is concavely curved towards the zero point. In this way, it clearly signalizes a synergistic effect. Strictly speaking, this interactive manner is valid for the analyzed combinations of ginkgolides A and B on the effect level of an inhibiting effect of 50%, only.

Of course, it cannot be stated with this experiment only, whether this proven synergy effect has any impact on the therapeutic use of Ginkgo extracts in general, because the extracts contain further ginkgolides as well as bilobalide and flavonol glycosides which could participate in further interactions. In addition, Ginkgo extracts have not only a thrombocyte-aggregation inhibiting effect, but also possess antioxidant, anti-inflammatory, neuro-protective and anti-tumoral effects which are not necessarily involved into the described synergy effect.

The synergy example of the ginkgolides A + B-mixture, therefore, is only used as an example how the existence of synergy effects can be detected for a mixture of natural products.

Mechanisms of synergy effects

Based on results of the latest investigations in classic pharmacological, molecular–biological and clinical works, the following four mechanisms can be discussed:

(1) Synergistic multi-target effects.
(2) Pharmacokinetic or physicochemical effects based on improved solubility, resorption rate and enhanced bioavailability.
(3) Interactions of agents with resistance mechanisms of bacteria.
(4) The respective elimination or neutralization of adverse effects by agents contained in the extract, added to it, or achieved by heating, so that altogether a better effectiveness than without these additions or manipulations can be achieved.

Synergistic multi-target effects

"Synergistic multi-target effects" means that the single constituents of a mono-extract or a multi-extract combination affect not only one single target, but several targets, and therefore cooperate in an agonistic, synergistic way. Imming et al. (2006) have listed possible important drug targets based on approved drug substances such as enzymes, substrates, metabolites and proteins, receptors, ion channels, transport proteins, DNA/RNA, ribosomes, monoclonal antibodies and physicochemical mechanisms. In addition also signal cascades can be targets.

The multi-target principle will be especially effective, if negative concomitant, auxiliary symptoms or "lateral damages", which have developed during a disease, can be comedicated therapeutically this way. In this context, the polyvalence effect of numerous secondary constituents, such as polyphenols and terpenoids, must be noted. The first possess a strong binding ability to different molecular structures like proteins or glycoproteins. Because of their large lipophilicity the terpenoids have great affinities for cell membranes and, therefore a high potential to permeate through cell walls of the body or bacteria. Since many plant extracts are rich in these two groups of constituents, these compounds can strongly enhance overall efficacy, if they possess a sufficiently high bioavailability.

As shown in Fig. 3, only an additive effect can be expected if a mixture of compounds in mono-extracts with binding ability to one target is present. If, on the other hand, the single constituents bind to several targets, over-additive or potentiated, synergistic effects can be obtained this way. These can amount to a multiple value of the additive effects.

Williamson (2001) was one of the first who has addressed this theme in a review article and described some interactions of natural products and extracts as "synergistic effects".

Example 1: For a long time it has been known, that the well-known cannabis and tetrahydrocannabinol (THC = Δ^9-THC) possess antispastic effects in addition to their hallucinogenic, antiemetic, anxiolytic appetite-stimulating, anti-inflammatory and analgesic effects. This has been proven in an immunogenic animal model of multiple sclerosis (MS) (Baker et al. 2000) (Fig. 4). Because there were some indications for a stronger muscle-antispastic effect of the extract than of pure THC, which today is available as Dronabinol® in Germany, Marinol in the USA and Cesamet in England, a comparative i.v. test of 1 mg/THC and 5 mg/kg *Cannabis* extract, the latter standardized on a concentration of 20% of THC, was carried out. As the graphic

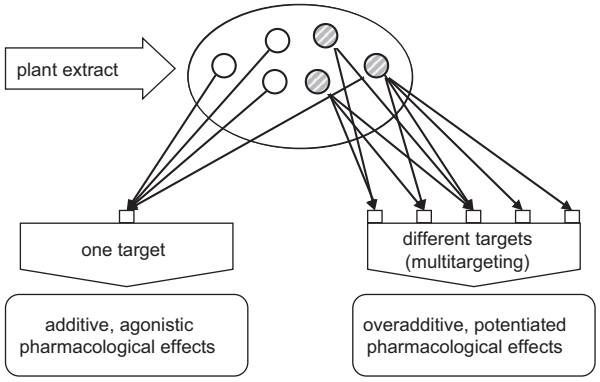


Fig. 3. A simplified and idealized graphic description of mono- and multi-target effects generated by a mono-extract containing various constituents directed to one target or different targets of a cell.

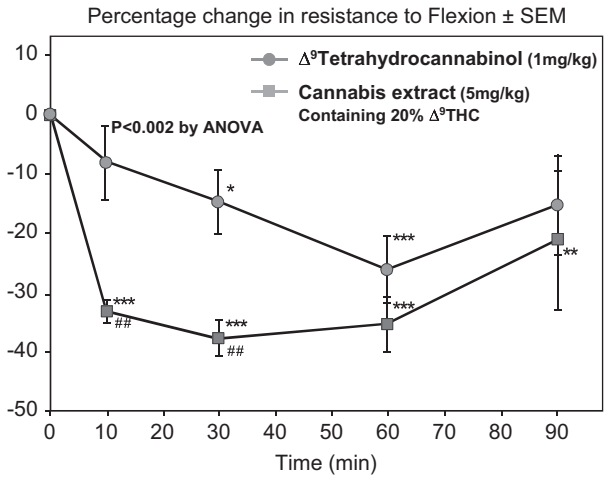


Fig. 4. Cannabis extract is a better antispastic agent than tetrahydrocannabinol at an equivalent dose (Baker et al. 2000; Williamson, 2001 with permission).

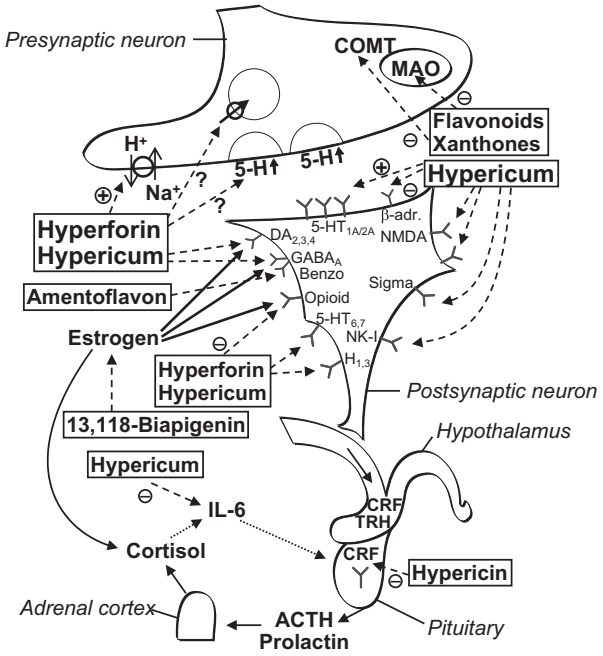


Fig. 5. Schematic scheme of proposed biological targets of *Hypericum perforatum* according to *in vitro* studies (Simmen et al. 2001) with permission of the G. Thieme Verlag Stuttgart).

shows, the cannabis extract with equimolar THC content was considerably more effective antispastically than THC alone. Since a THC-free extract in a preliminary investigation did not show strong antispastic effect, concomitant constituents of the *Cannabis* extract, probably cannabidiol, may be responsible for the synergy effects enhanced (Zuardi et al. 1982; Williamson and Evans 2000; Wilkinson et al. 2003). Cannabidiol promotes an increase in the transport of anandamide through the brain membrane not evident with THC. This could explain the stronger antispastic effect of the Cannabis extract.

Example 2: There are more than 40 placebo-controlled clinical studies of standardized *Hypericum* (St. John's Wort) extracts for the indications of mild, moderate and even moderately severe depression, among them several in comparison with synthetic psychopharmacological drugs (e.g. imipramin, flumazenil, fluoxetin or amitriptylin (Woelk 2000; Schulz 2001, 2003).

According to many of the pharmacological investigations performed to date, several constituents of *Hypericum* must be involved in its effectiveness. hyperforin, the hypercines, amentoflavon, rutin, hyperosid, xanthones and proanthocyanidines can be primarily suggested (Müller et al. 1998; Butterweck et al. 1997). This hypothesis was corroborated by neuro-chemical *in vitro* studies with different CNS receptors using radioligand-binding techniques to confirm that the beneficial antidepressant action of standardized *Hypericum* extract might be a result of the cooperation of several compounds of St. John's wort. As shown in the scheme of Fig. 5 presynaptic as well as postsynaptic neurons, the hypothalamus and the pituitary gland are involved as targets and all main compounds of Hypericum show affinities to any of the targets described (Simmen et al. 2001).

Binding inhibitions were detected for the opiod-linked G-protein as well as for serotonin (5-HT), histamin, neurokinin, corticotropin- and releasing factor (CRF) receptors, for the steroidestrogen-α and for the ligand-gated ion channel $GABA_A$-receptor. Hyperforin inhibited, through binding to opiod and serotonin (5-HT) receptors at IC_{50} values between 0.4 and 3 μM, while hypericin and pseudohypericin inhibited to a lesser extent. The mono- and biflavonoids and the xanthones also inhibited ^{3}H-estradiol binding to the estrogen-α-receptor with an IC_{50} of 1 μM.

Example 3: Another example for the multi-target principle is given by the phytopreparation Iberogast® which is composed of nine plant extracts and can be considered in Germany and Europe as a leading phytopreparation for the treatment of functional dyspepsia and motility-related intestinal disorders. Twelve clinical studies, among them two in comparison with the synthetic drugs cisapride and metoclopramide, showed a complete therapeutic equivalence of iberogast with the two synthetics, with the advantage that the phytopreparation showed fewer or no side effects in comparison to the two synthetics. Iberogast leads to a multi-target effect by balancing the disturbed gastrointestinal motility function, by alleviating gastrointestinal hypersensitivity, by inhibiting the inflammation, suppression of gastric-juice secretion and effects on gastro-intestinal autonomic afferent function. In

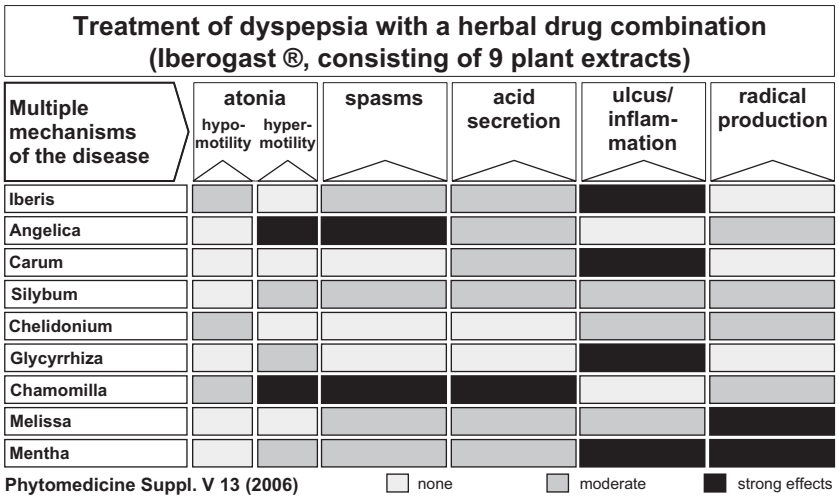


Fig. 6. Pharmacological and therapeutic approach to treat dyspepsia and motility-related disorders of the gastrointestinal tract with a herbal drug combination (Iberogast®), consisting of 9 plant extracts – none, – moderate – strong effects (Wagner, 2006).

Table 1. Selected from a table listed in the review of Williamson (2001).

Herbal drug	Investigated monoextract Mixtures and single constituents	References
Ginkgo biloba	Ginkgolide mixtures, Ginkgo extract	Chung et al. (1987)
Piper methysticum	Kava lactones/mixtures of Kava lactones and extract fractions	Singh and Blumenthal (1997)
Glycyrrhiza glabra	Licorice extract potentiates other substances and acts as detoxifier	Cantelli-Forti et al. (1994), Kimura et al. (1992), Miaorong and Jing (1996)
Cannabis sativa	Cannabis extract/THC	Zuardi et al. (1982), Baker et al. (2000)
Valeriana officinalis	Valeriana extract, individual constituents	Hölzl (1997)
Zingiber officinalis	Zingiber extract/mixture of volatile terpenoids and mixtures	Beckstrom-Sternberg and Duke (1994)

contrast to this multiphytopreparation, the synthetic monodrugs cisaprid and metoclopramid as classical proton pump inhibitors target only one symptom of functional dyspepsia. Each plant extract was examined in all relevant pharmacological *in vitro* and *in vivo* models with the result that all extracts, some of them multifunctionally or synergistically, are involved in the overall pharmacological effect (Fig. 6) (Wagner and Allescher 2006).

In Table 1, further examples of mono-extracts are given which, according to the definition of Berenbaum (1989), exhibit synergistic effects. This postulation is based on detailed pharmacological and molecularbiological investigations of sub-fractions and isolated compounds of the single extracts. It cannot be ruled out that several mechanisms described above are involved in these effects.

"Pharmacokinetic" effects based on improved solubility, resorption rate and enhanced bioavailability

The possibility is well-known in phyto-pharmacology that particular concomitant compounds in an extract, e.g. polyphenols or saponins that often do not possess specific pharmacological effects themselves may increase the solubility and/or the resorption rate of major constituents in the extract and thereby enhance its bioavailability virtually in a kind of pharmacokinetic effect, and simultaneously result in a higher effectiveness of the extract than an isolated constituent thereof. For example, the leaf extract of *Atropa belladonna* with its main agent l-hyoscyamin develops a stronger effectiveness because of presence of the concomitant flavonol-triglycosides in the extract which act as resorption catalyzer (List et al. 1969). The extract of *Ammi visnaga* gives us another example. Its main agent, Khellin, is completely bioavailable already after 10 min, in favourable comparison to pure equimolar Khellin, which is not fully resorbed until 60 min (Eder and Mehnert 2000).

A similar enhancement of the bioavailability of an agent because of polyphenolic concomitant agents of an extract was recently detected by Butterweck et al. (1997). Hypericin of *Hypericum perforatum*, which for a long time had been considered the main antidepressant agent

of this drug, possesses only a weak antidepressant (MAO-inhibiting) effect alone, because its bioavailability is extremely low. If, however, hypericin is combined with the polyphenols epicatechin, procyanidin, hyperosid or rutin, which are normally present in the extract, the plasma level of hypericin is clearly enhanced and a strong antidepressant effect is obtained, as evidenced in the *Porsolt* swimming test in mice (Butterweck et al. 2003) (Fig. 7).

This systematic investigation shows that an additional "pharmacokinetic synergy effect" may participate in the overall effect of *Hypericum* extracts. In this case, the multi-target synergism of *Hypericum* could be described as an agonistic combination of two independent synergistic mechanisms of action (see Example 2).

Interactions of agents with resistance mechanisms of bacteria

A third possibility of synergy effects has been known for many years, which occurs when antibiotics are combined with such agents that are able to partly or completely suppress bacterial resistance mechanisms. The best-known example of such a combination is the comedication of the β-lactam antibiotic (BLA) penicillin

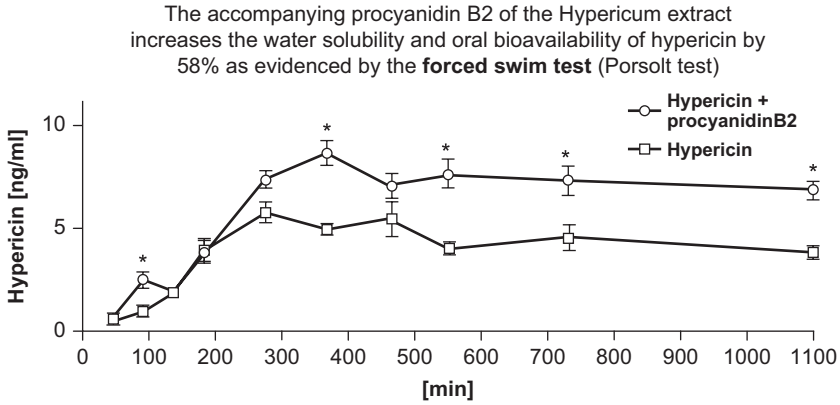


Fig. 7. Arguments for existing synergy effects of *Hypericum perforatum* extracts. The accompanying procyanidin B2 of the Hypericum extract increases the water solubility and oral bioavailability of hypericin by 58% as evidenced by the forced swim test (Porsolt test). Plasma levels of hypericin in the presence (○) and absence □ of procyanidin B 2 (Butterweck et al. 2003 with permission).

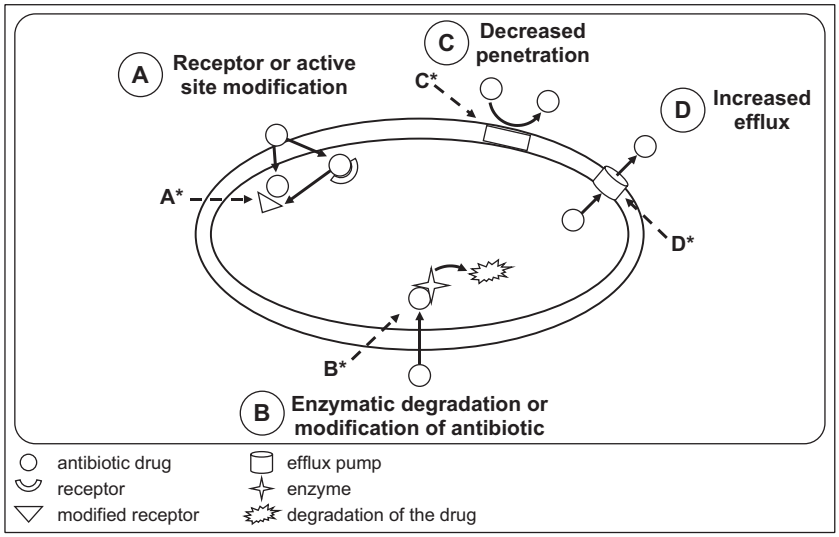


Fig. 8. Strategies of bacteria to antagonize the effect of antibiotics and natural products which can overcome resistance problems: (A*) Corilagin, tellimagrandin I, diterpene 416 and compound P inhibit PBP 2a, a modified receptor; (B*) EGCg inhibits the β-lactamase; (C*) thymol, carvacrol, gallic acid increase the outer membrane permeability and (D*) EGCg, 5′-methoxyhydnocarpin, reserpine, carnosic acid and isopirmarane derivatives inhibit the efflux pumps (see the text and Table 2) (Hemaiswarya et al. 2008 with permission).

with clavulinic acid (sulbactam or tazobactam) which successfully antagonizes the penicillinase resistance (Lee et al. 2003).

Bacteria gain antibiotic resistance due to three reasons: *(i) modification of active site of the target resulting in a reduction in the efficiency of binding of the drug, (ii) direct destruction or modification of the antibiotic by enzymes produced by the micro-organism or (iii) efflux of antibiotics from the cell (Sheldon 2005) by hindrance of the antibiotic to penetrate into the bacteria cell or after the penetration to extrude the accumulated drug out of the bacteria cell* (Hemaiswarya et al. 2008) (see Fig. 8).

(i) One target for intervention at the active site is, for example, the so-called penicillin-binding proteins (PBPs). As the literature research has revealed, a lot of natural products do exist which are specialized to overcome resistant micro-organisms (Table 2), e.g. epigallocatechin gallate (EGCg) acts together with the β-lactam antibiotic by a direct or indirect attack on the peptidoglycan part of the bacterial cell wall (Yam et al. 1998; Zhao et al. 2001). Some others act as inhibitors of the topoisomerase IV or the RNA synthesis (see details in the review of Hemaiswarya et al. 2008).

(ii) A second mechanism exists in the inhibition of lactam- or ester-cleaving enzymes that are generated for the deactivation of antibiotics by bacteria. Here, the EGCg also seems to be an adequate natural product in order to maintain the activity of penicillin versus *Staphylococcus aureus*, for instance (Zhao et al. 2002).

(iii) A third option could be the blocking of a pumping system developed by several bacteria in order to inhibit agents from penetrating into the bacteria or to extrude the antibiotics out of the bacteria cell that have already penetrated into the cell. Normally, the inhibiting agents themselves are no effective antimicrobial agents.

Example 1: Reserpin (Schmitz et al. 1998; Gibbons and Udo 2000; Stermitz et al. 2000) or carnosic acid of *Rosmarinus officinalis* (Oluwatuyi et al. 2004), for example, belong to the plant agents that have developed different mechanisms to inhibit the efflux pump of bacteria or to reduce its effectiveness.

The alkaloid Berberin is found in the plant *Hydnocarpus wightiana* together with the flavonolignan 5′-methoxy-hydnocarpin (MHC). This phenolic compound, which does not act antimicrobially itself, magnifies the effect of berberin, which acts only weakly antibiotic, by completely inhibiting the efflux of berberin from *Staphylococcus aureus* and eliminates the multidrug resistance of the bacterium. As 5′-MHC does not act as a cationic substrate, as other numerous known MDR inhibitors do, the mechanism of inhibition seems to be different. For this reason, extracts of *B. aquifolia* and *B. repens* have a better antimicrobial effect as compared with pure Berberin alone (Stermitz et al. 2000).

Example 2: Thymol and carvacrol, two main compounds of the essential oil of *Thymus vulgaris*, act as so-called "membrane permeabilizers" and that way facilitate the penetration of antibiotics into Gram-negative bacteria (Helander et al. 1998).

The leaves of the same plant further contain another compound, the 5,6,7-trihydroxyflavon baicalein present in *Scutellaria* species. This flavone exhibits two remarkable synergy effects with tetracycline and β-lactam antibiotics against methicillin-resistant *Staphylococcus aureus* (MRSA) (Fujita et al. 2005). Baicalein inhibits the outwards transport of tetracyclin of bacteria due to intervention with the responsible flavonoid-borne gene TetK. The minimum inhibitory concentration (MIC) of tetracycline against MRSA of 4 μg/ml is reduced. Since Baicalein also has a synergy effect with β-lactam antibiotics against MRSA strains that do not carry TetK-genes, another mechanism of intervention seems to be the cause of this effect. Perhaps baicalein inhibits the PB proteins 2a or affects the peptidoglycan structure of the bacteria membrane.

Example 3: A positive synergy effect was also found between the anti-bacterial constituents of hop, xanthohumol and lupulon of *Humulus lupulus* and some antibiotics (e.g. polymyxin, tobramycin or ciprofloxacin) against Gram-positive and, to a lesser extent, against Gram-negative bacteria (Natarajan et al. 2008).

Example 4: The increased survival rates are remarkable, when treating *Candida albicans*-infected mice with amphotericin B together with a grape seed extract (GSE) of *Vitis vinifera* , as compared with a control group that had received only amphotericin B (Han 2007) (Fig. 9).

Table 2. Selected from a table listed in the review of Hemaiswarya et al. (2008).

Compound	Plant source	Reference
EGCg (epigallocatechin gallate)	*Camellia sinensis*	Suresh et al. (1997)
Catechin	*Camellia sinensis*	Takahashi et al. (1955)
Tellimagrandin I, Rugosin B	*Rosa canina*	Shiota et al. (2004)
Corilagin	*Arctostaphylos uva-ursi*	Shimazu et al. (2001)
Baicalin	*Scutellaria amoena*	Liu et al. (2000)

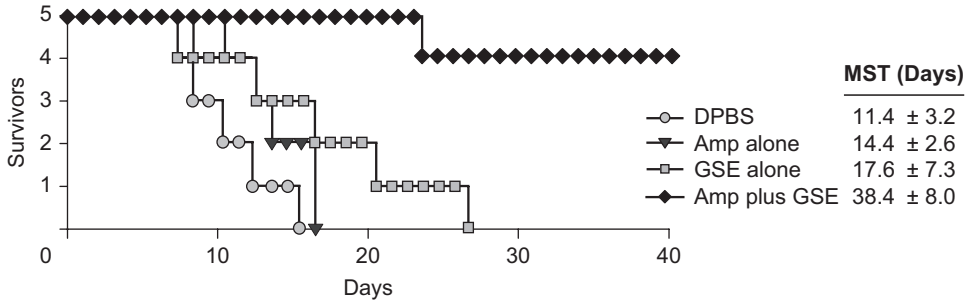


Fig. 9. Synergy effect of GSE with AmpB to the disseminated candidiasis of mice. DPBS (negative control), AmpB, GSE or AmpB plus GSE resulted in a mean survival time (MST) of 11.4 (±3.2), 14.4 (±2.6), 17,6 (±7.3) and 38.4 (±8.0) days, respectively. The doses were 0.5 mg/kg of body wt for AmpB, 2 mg/kg for GSE plus 0.5 mg/kg AmpB before the i.v. administration of the yeast cells. Mice group given AmpB or GSE at such doses had similar survival days as the control mice. The mice treated with the combination survived ~27 days longer than the DPBS-received mice during the period of 40-day observation (Han 2007) with permission.

While the negative control (DPBS), amphotericin B, GSE or AmpB resulted in mean survival times (MST) of 11.4, 14.4 and 17.6 days, the infected animals survived after the treatment with GSE + Amp an average of 38.4 days, which therefore was 27 days more during the 40-day observation time than the control group of mice, and 24 days more than with amphotericin B alone. In the combination experiment, the concentrations of amphotericin B amounted to 0.5 mg/kg body wt and the one of GSE to 2 mg/kg body wt. Mice that received a double dose of AmpB (1 mg/kg body wt without GSE survived longer, but the survival time of these mice were still less than the survival times of the combination therapy treated mice. After Koga et al. (1999) could detect polyphenols of the type of procyanidines in GSE, a direct anticandidial effectiveness is likely (Maeta et al. 2007; Okubo et al. 1991), or also an immunogenic effect, since polyphenols as e.g. EGCg or procyanidines have the ability to a Th-1 induced release of γ-interferon (Marodi et al. 1994; Domini et al. 2007).

Example 5: Interestingly, the berberin mentioned above also possesses synergy effects against *Candidiasis* of mice (Han and Lee 2005). Because a large number of essential oils with antimicrobial and antifungal effects have been and still are used internally for the supportive treatment of infections of the respiratory tracts as well as topically for the therapy of skin infections, essential oils in several recent investigations were combined with antibiotics with the aim of improving the antimicrobial effect and at the same time reducing the concentration of antibiotics. In the first *in vitro* experiments with essential oils of *Origanum vulgare*, *Pelargonium graveolens* and *Melaleuca alternifolia* in combination with norfloxacin and amphotericin B, distinct synergy effects against *Bacillus cereus*, *B. subtilis*, *Escherichia coli*, *Staphylococcus aureus* and several *Candida* strains could be detected at a simultaneous reduction of antibiotic concentrations (Rosato et al. 2007, 2008). The measured FIC[1] and FICI[2] values, according to the Isobologram criteria established by Berenbaum (1989), showed that in all cases real synergy effects could be measured.

Due to the increasing multi-drug resistance of TB strains, tuberculosis, after malaria, has become one of the most severe threats for humankind. Two million die yearly. According to statistics of the WHO (2004), the mortality rate averages 50–80% within a period of 4–16 weeks from diagnosis to death.

A concomitant impediment in combating tuberculosis is the ability of TB to persist in macrophages for a long time, i.e. that an antituberculosis static drug must be able to kill also these "dormant bacteria" in macrophages. In spite of the decades – long search for effective natural products, there has not yet been success, despite the use of high-throughput screening methods in finding combinations that are superior in effectiveness to the synthetics. Therefore, greater reliance also exists in tuberculosis therapy on the use of drug combinations to utilize synergy effects.

First experiments to combine natural products with known synthetics such as isoniozid (INH), (RMP), ethambutol (EMB), streptomycin (SM) or pyrazinamide (PZA) have been begun, and first results are available. Naphthochinon 7-methyljuglon (7-MJ), isolated from *Euclea natalensis*, which grows widely in South Africa, was combined with rifampicin and with isoniozid and the MIC and the FIC were determined for *M. tuberculosis*. The MIC for the first combination amounts to 1.25/1.025 μg/ml. The FIC was found to be at 0.5 and 0.24 μg/ml. That clearly shows, according to the Isobol method of Berenbaum, that the combination acts synergistically. Compared with streptomycin, 7-MJ is superior in its extra- and intra-cellular activity against

[1] $FIC = MIC_a$ of the combination/MIC_a alone + MIC_b of the combination/MIC_b alone.

[2] FICI = synergistic effect when $\leqslant 0.5$.

M-t. The MICs of both combinations reduce eight-fold due to the combination of 7-MJ with IN (Bapela et al. 2006). The exact synergy mechanism is not yet known. Since however, plumbagin, a 2-methyljuglon, also acts synergistically together with INH, and since it is known that plumbagin increases the superoxide concentration intracellularly and so transforms IN into its active form, the action with 7-MJ could be analogous (Bulatovic et al. 2002; Mo et al. 2004).

Furthermore, it is known, that numerous chinones possess direct antimicrobial and cytotoxic effects themselves. Another example for a synergy effect between a plant extract and rifampicin is the combination with the water extract of the seeds of *Cuminum cyminum*. The 3′, 5 dihydroxyflavone 7-O-β-D-galacturonide 4′O-β-D-glucopyranoside is held responsible for the 35% increase of rifampicin plasma level, and it seems that this glycoside increases the resorption and thereby the bioavailability of the antibiotic (Sachin et al. 2007). Conspicuous among the natural products able to antagonize bacterial resistance to antibiotics are many polyphenolics and essential oils (see Table 2 and the comprehensive list in the review article of Hemaiswarya et al. 2008).

The respective elimination or neutralization of adverse effects by agents contained in the extract, added to it, or other manipulations

This fourth effect that is associated with the field of synergy research is no real synergy effect. It can be reached when a constituent contained in a plant extract or an agent artificially added to an extract "neutralizes" or destroys a toxically acting constituent and, therefore, generates a better effectiveness as compared with the original raw drug. While this first option cannot be ascertained directly, and requires extraction and application, the addition of an auxiliary product (antidote) is based on the long-time practical implementation of a drug. Thus, we find in traditional Chinese medicine (TCM) the terms "Pretreated Drugs", which simply means that the drug undergoes a pretreatment of heating, addition of alcohol, alum or other substances (Chinese Pharmacopoeia 2005). For Radix Aconiti alone, there exist at least four methods to reduce the percentage of toxic aconitin to ~0.2%, which makes this drug therapeutically useful for treatment.

Therapeutic approach

In the preceding part of this review, pharmacological *in vitro* and *in vivo* investigations were described, through which synergy effects of herbal drug combinations can be detected and determined. The results, however, do not represent 100% evidence for the therapeutic superiority and counterparts of these drug combinations as used in humans. Therefore, these findings must also be verified in controlled clinical trials. Possible side effects of herbal drug extracts when combined with any synthetic drug or antibiotic for comedication must also be taken into consideration. Some adverse effects in combined use with synthetic drugs have been reported (Bailey et al. 1998; Ernst 2000; Hall et al. 2003; Strandell 2004). As a result of such interactions with the cytochrome 450-isoenzyme CYP 3 Ay in the intestinal wall, which are responsible for the oxidative metabolism of a drug during the resorption and the intestinal and presystemic first-pass effect, such drug mixtures can result in negative reactions.

Therefore, it is also imperative that mixtures of substances or plant extracts be subjected to the same safety studies and phase-I to phase-III studies as the chemosynthetics before they can be submitted as conventional drugs for registration. In any case, the therapeutic superiority of a drug combination must be assessed by a placebo-controlled, randomized, double-blind study, the greatest hurdle a drug has to overcome.

In this respect, the best evidence for a synergy effect are studies which are aimed at a chosen indication in comparison with one or several standard drugs, if no ethical reasons exclude it. The main criterion is a significant therapeutic equivalence and side effects equal or lesser to those of the reference drug.

Among the around 200 placebo-controlled, randomized clinical studies carried out with standardized plant extracts in the last 10 years, at least 50% have been performed in comparison with several synthetic standard substances. The results have shown, surprisingly, that most of them showed a significant therapeutic equivalence. Some of them showed also a significant superiority over the standard preparation in terms of side effects and tolerability. For example, standardized Hypericum extract possesses, in comparison to a synthetic psychopharmacological drug used in the treatment of "mild" and moderate depression (according to the Commission E), a very low rate of 1–3% side effects as compared with 30–60% with the synthetic tricyclic antidepressant psychopharmacological drugs and 15–30% with serotonin reuptake inhibitors (SSRI). Hypericum extract showed no influence on REM sleep and generated no rebound effect. In Table 4 are listed some of the most important standardized plant extract preparations that have shown complete therapeutic equivalence and fewer or no side effects in comparison

Table 3. Clinical evidences for synergy effects of combinations of two extracts (Williamson 2001).

Valeriana off. + *Humulus lupulus* (Hindmarch, 1975)
Valeriana off. + *Kava-kava* (Wheatley, 2001)
Urtica dioica + *Pygeum africanum* (Hartmann et al., 1996)
Ginseng + Ginkgo (Scholey and Kennedy, 2000)

Table 4. Therapeutic equivalence of standard plant extracts with synthetic drugs at a given indication, evidenced by comparative placebo controlled clinical studies.

Herbal extract	Chem. synth. drug	Indication	References
Crataegus flos + folium	Captopril	Working tolerance, heart insufficience grade II	Tauchert et al. (1994)
Boswellia (Incense)	Sulfasalazine	Morbus Crohn	Gerhardt et al. (2001)
Hypericum perfor. (St. John's Wort)	Imipramine, Amitripyline, Citalopram, Sertalin	Mild, moderate and moderately severe depression	Schulz (2001)
Hedera helix	Ambroxol®	Chronic bronchitis	Meyer-Wegener et al. (1993)
Iberogast® (9 extracts containing Phytopharmaceutical)	Metoclopramide Cisapride	Functional dyspepsia, irritable bowel disease	Rösch et al. (2002)
Sabal (Saw palmetto)	Proscar® (Finasteride)	Benign prostate hyperplasia I + II	Carraro et al. (1996)
Salix spez.	Aspirin	Osteoarthritis	Schmid et al. (2001)
Sinupret® (5 extracts containing Phytopharmaceutical)	Ambroxol®	Sinusitis	Richstein and Mann (1980)

with chemosynthetic compounds used for treatment of the same indications.

In Table 3, clinical evidences of synergy effects for a combination of two plant extracts are reported.

With the exception of a few plant preparations, however, it was not possible to assign the therapeutic synergy effects to defined combinations of bioactive compounds and to determine the molecular–biological mechanisms underlying the therapeutic equivalence. Nevertheless, it can be suggested that this conspicuous therapeutic equivalence must be due to synergy effects, as evidenced by extended pharmacological investigations and demonstrated in this review.

In this context, it must be noted that not only plant mono-extracts or extract combinations are able to exhibit synergy effects, but also single natural products or extracts in combinations with chemosynthetics or antibiotics. For example, the following combinations are used successfully in Thailand for the treatment of uncomplicated and severe *Falciparum* malaria: artemisinine derivatives (artesunate, artemether, arteether and dihydroartemisinine) combined with mefloquine, lumefantrine, doxycycline or tetracycline (Wilairatana et al. 2002). These comedications could become of beneficial interest also in other areas of a future therapy. The rationale for herbal drug combinations in Traditional Chinese Medicine is also gaining increasing acceptance, as evidenced by a successful clinical trial of such a multi-herbal drug combination in 37 young patients suffering from eczema (Sheehan and Atherton 1992). This trial was followed by an extensive program of pharmacological tests showing that only the complete herbal mixture produced the optimal effect (Phillipson 1994).

In part II of this review (issue 4, 2009), further methods, especially the omic technology, are described through which the synergy effects of drug combinations may be rationalized and assessed with the aim of constructing new drug combinations with optimized efficiency and which may represent a "new generation of phytopharmaceuticals".

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