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Synergy and polyvalence: paradigms to explain the activity of herbal products

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Introduction

The terms *synergy* and *polyvalence* are often used to explain the effects of the many constituents found in herbal medicinal products and their extracts, particularly where it is difficult to distinguish the 'active ingredient'.

The concept of 'active ingredient' has been useful in elucidating the chemical basis for the biological effect of a large number of medicinal and poisonous plants. Many naturally occurring compounds such as atropine, morphine, digoxin, quinine and menthol, originally taken as part of the complex mixture occurring in a solvent extract or distilled oil, now have extensive clinical use as single compounds since they give a pharmacological effect similar to that given by an extract from the plant in which they occur. It would be true to say that, to a large extent, over the last 100 years 'orthodox' 'Western' medicine has valued plants only as a source of such potent compounds, whose activity could be clearly demonstrated at low doses. Any drug discovery from natural sources carried out by the pharmaceutical industry is still very much based on this paradigm.

However, there were many medicinal plant species from which active ingredients could not be isolated and/or gave no immediately obvious effects when their extracts were tested pharmacologically. These extracts consisted of complex mixtures of many different compounds and were either dismissed as inactive, with anecdotal reports of activity being explained by the placebo effect, or they declined in use because of difficulties in analysis, standardisation or making 'user-friendly' dosage forms. It is only in the past three decades that this group of plants has re-emerged in the developed world, arousing interest from the general public as alternatives to orthodox medicines or as complementary products used to maintain health or treat aspects of diseases in which orthodox medication has had only limited success.

As well as the mainly European and North American herbal substances that have enjoyed this renaissance, there is now a vast array of medicinal plants from other parts of the world, many of which

have become globalised and become significant items of commerce. This increase in commerce and public use has stimulated much scientific and clinical interest and one aspect of research that has been generated is to explain the effects seen in terms of ingredients detected which, on their own as single compounds, have relatively weak activity. Such explanations are often couched in terms of synergy and polyvalence, but the meaning of these terms is often not fully understood, with consequent confusion in their use.

For example, synergy is often stated to occur when an extract of a plant gives a greater (or safer) response than an equivalent dose of the compound considered to be the 'active' one. An example of this is the much improved antispastic effect shown by cannabis extract compared with an equivalent dose of tetrahydrocannabinol (Baker *et al.*, 2000; Williamson, 2001). However, in such an instance, it might well be that other active compounds are present, some as yet undiscovered. In whole organism or tissue studies, other compounds present may be active against a range of targets, all contributing to the observed effect. In this instance it is polyvalence, rather than synergy, which is occurring, and this topic is discussed below.

Another situation sometimes explained by synergy is the loss of activity seen when an active crude extract is fractionated and the resulting fractions, when tested for the same activity, do not show an enhanced effect, e.g. the loss of cytotoxic activity seen when extracts of *Kigelia pinnata* were fractionated (Houghton, 2000). The explanation given in such circumstances is that activity is reduced because two compounds, which act together synergistically, have been separated into two discrete fractions. However, it should be always borne in mind that the loss may be due to the 'actives' having decomposed during the fractionation process or that they have become bound to one of the materials used, e.g. the stationary phase in chromatography.

This chapter aims to define synergy and polyvalence, discuss ways in which they can be assessed and quantified and give some examples of herbal medicinal products where these processes have been shown to occur.

Synergy

Definition

The literal meaning of synergy is 'working together' but a useful definition is 'an effect seen by a combination of substances that is greater than would have been expected from a consideration of individual contributions' (Heinrich *et al.*, 2004). More precise definitions, incorporating mathematical considerations, have been discussed by Berenbaum (1989), who favoured the use of isoboles (see below) in determining synergy, since these are concerned with the effect and not with the mechanisms involved. Synergy as applied to herbal products has been discussed well in seminal papers by Williamson (2001; 2002), who also included the attenuation of toxicity or adverse effects seen with a mixture compared with one of its constituents. Synergy in this present Chapter is used in a 'positive' sense, i.e. an increase in effect greater than that predicted, but it should not be forgotten that an unexpected decrease in activity, sometimes called 'negative synergy' or 'antagonism' may also occur, particularly in some interactions between orthodox medication and some herbal products (Barnes *et al.*, 2002).

In the case of the pharmacological or clinical effects of herbal material, two types of synergy are observed. In one case the activity of an active compound, or extract, is increased in the presence of another compound or extract which, on its own, has no effect in the system under test at the concentrations used. This has been demonstrated for the flavonoids present in *Artemisia annua*, source of the antimalarial drug artemisinin, which at 5 μmol/L, well below their IC50 value of about 25 μmol/L, gave rise to a three to fivefold reduction in the IC_{50} value of artemisinin when they were added to the in-vitro antiplasmodial test used (Chiung-Sheue Chen Liu *et al.*, 1992).

The more common situation is when all compounds concerned have activity but, in combination, this is much greater than expected. This is exemplified by the reduction in platelet aggregation observed for the total mixture of ginkgolides from *Ginkgo biloba* L., as opposed to that given by indi-

vidual compounds, because of their activity as platelet-activating factor antagonists (Williamson, 2002).

In some traditional medicine systems, mixtures of plants are used rather than one species and so the situation is even more complex, although the same concepts of synergy apply, i.e. the mixture of the two (or more) species gives a better activity than either species on its own. This has been demonstrated for a mixture of extracts of *Salvia chamelaegnea* Berg. and *Leonotis leonorus* (L.) R.Br. against Gram-positive bacteria (Kamatou *et al.*, 2006). This mixture was used traditionally in South Africa against infections.

Measurement of synergy: use of isoboles and isobolograms

Various mathematical models have been used to describe and determine synergism (Berenbaum, 1989) but the use of isobolograms is the method that is reckoned to be the best and has been used in recent publications. Although many researchers use the method without considering the mathematics involved, proof of the validity of the method was given by Berenbaum (1989) and this has provided a sound basis for its use.

The *isobole* method is applicable under most conditions and, since it is the ultimate effect which is the factor used in the measurement, it is independent of the mechanisms of action involved. The isobole is a curve, constructed by plotting coordinates consisting of values representing the *fractional effect* for each of two components. The fractional effect is the ratio of the 'effect' caused by the two compounds in combination to that of one of the compounds alone. The effect is a measure of activity, e.g. minimum inhibitory concentration (MIC) for antibacterial and antifungal compounds, concentration giving 50% inhibition (IC50) for enzyme studies or studies on cytotoxicity, 50% binding coefficient for receptor-binding studies.

The fractional effect (FE) of two compounds (or extracts) X and Y for antibacterial studies, in terms of their MIC for bacterial growth, can be expressed as follows (Schelz *et al.*, 2006):

$$FE^X = \frac{MIC_{(X \text{ with } Y)}}{MIC_{(X \text{ alone})}}$$

$$FE^Y = \frac{MIC_{(Y \text{ with } X)}}{MIC_{(Y \text{ alone})}}.$$

The sum of the two fractional effects is known as the FE index and is the correlation between the two test substances. Different authors interpret the FE index in different ways. According to Berenbaum (1989) synergism is said to occur if the FE index is ≤ 1.0, if 1.0, an additive effect is reckoned to occur, and antagonism if the FE is greater than 1.0. Schelz (2006), however, stated that if the FE index is ≤ 0.5, the effect is synergistic; if >0.5 to 1.0, the effect is additive, if >1.0 to 4.0 it is indifferent and if ≥ 4.0 the effect of the two substances is antagonistic.

If FE^X and FE^Y are calculated for a series of the two compounds in different ratios, then an isobologram (Figure 6.1) can be constructed using the two values as coordinates to express the FE index for each combination of the two compounds. If no synergism exists, the coordinate points form a straight line but, if synergism exists, the points occur between the straight line and the origin in a concave shape, whereas if they are 'outside' the straight line, and give a convex curve, antagonism exists. Note that for a set of different mixtures of the same compounds, not all the combinations may give the same effect, i.e. some may show synergy while others may not.

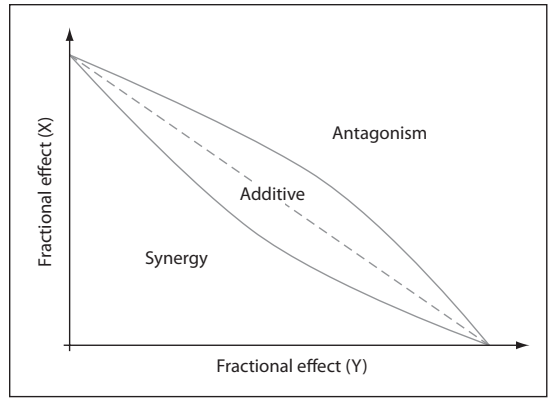

Figure 6.1 Isobologram to show areas of synergy and antagonism.

This is exemplified in Table 6.1 in data for the antibacterial activity (expressed as MIC) of volatile (X) and non-volatile (Y) fractions from the South African herb *Tarchonanthus camphoratus* L. (Asteraceae) (van Vuuren, 2007). The data are plotted as an isobologram on Figure 6.2. The mixtures consisting of concentration ratios 12.8 : 3.2, 11.2 : 4.8, 9.6 : 6.4 and 8.0 : 8.0 all show synergism as shown by their being in a concave distribution in Figure 6.2. The 14.4 : 1.6 mixture shows an additive effect while the 6.4 : 9.6, 4.8 : 11.2, 3.2 : 12.8, 1.6 : 14.4 mixtures all show antagonism since they are 'outside' the straight line, representing a mere additive response. These set of results demonstrate the point noted above; that some concentration ratios may display synergy while others do not. Such a situation demonstrates how complicated it is to try to predict the activity of a herbal extract where relative concentrations of compounds may vary widely between batches or chemical races of the plant.

Polyvalence

Polyvalence can be defined as the range of biological activities that an extract may exhibit which contribute to the overall effect observed clinically or *in vivo*. It is often confused with synergism but the distinction lies in the fact that synergism is strictly concerned with only one pharmacological function, rather than a range of activities resulting in an overall effect. As mentioned above for synergy, Williamson (2001) discussed the role played by polyvalence in the scientific evidence for the claims often made for herbal traditional medicines, that the overall effect is greater, and sometimes different, than might be predicted from the activities of the individual components.

It is unusual for a disease to be due to, or to be corrected by, a single factor, so it is unlikely that one compound alone would successfully treat the disease in the patient, even when the disease and its cause are unequivocally certain. This 'silver bullet' concept, which has driven the drug discovery process for the past 100 years, is now increasingly viewed as inadequate in many clinical situations (Walker, 2007). As a result, in orthodox Western medicine, a cocktail of drugs is now commonly employed against diseases such as HIV infection and cancer. A similar situation arises in the treatment of a disease such as hypertension, where symptoms may be reduced by using a diuretic or beta-blocker, but other drugs have to be employed to compensate for their side-effects, e.g. potassium supplements are given to overcome the hypokalaemia induced by diuretics. Similarly antiemetics are commonly given as part of the regime in cancer chemotherapy.

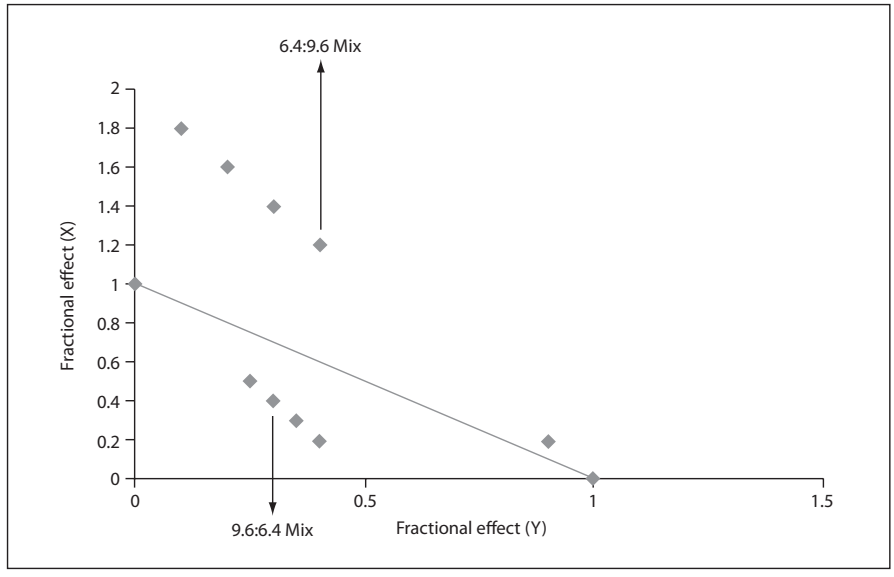

Figure 6.2 Isobologram to demonstrate synergy, additive and antagonistic antibacterial effects with different ratios of volatile and non-volatile fractions of the South African herb *Tarchonanthus camphoratus* L.

Table 6.1 Data for antibacterial activity (minimum inhibitory concentration, MIC) against *Escherichia coli* for different ratios of volatile (X) and non-volatile (Y) fractions from the South African herb *Tarchonanthus camphoratus* L.

Concentration (mg/mL)		MIC (mg/mL)		Fractional effect*		FE index
X	Y	X	Y	FEX	FEY	
16	0	8.0	–	1	–	
14.4	1.6	7.2	0.8	0.90	0.20	1.1
12.8	3.2	3.2	0.8	0.40	0.20	0.6
11.2	4.8	2.8	1.2	0.35	0.30	0.65
9.6	6.4	2.4	1.6	0.30	0.40	0.7
8.0	8.0	2.0	2.0	0.25	0.50	0.75
6.4	9.6	3.2	4.8	0.40	1.20	1.6
4.8	11.2	2.4	5.6	0.30	1.40	1.7
3.2	12.8	1.6	6.4	0.20	1.60	1.8
1.6	14.4	0.8	7.2	0.10	1.80	1.9
0	16	–	4	–	1	

*In this instance the FE is calculated as follows:

$$FE^X = \frac{\text{MIC(X with Y)}}{\text{MIC(X alone)}}$$

$$FE^Y = \frac{\text{MIC(Y with X)}}{\text{MIC(Y alone).}}$$

Worked example for 9.6 : 6.4 ratio mixture:

$$FE^X = \frac{2.4}{8} = 0.3$$

$$FE^X = \frac{1.6}{4} = 0.4$$

A similar situation can be seen in many different traditional systems where herbs are used. In some systems, e.g. TCM and Ayurvedic medicine, such adjuvant herbs are clearly recognised and classified. Thus, in a Chinese prescription of, say, ten different herbs, one is recognised as providing the major activity, i.e. 'the monarch', while two or three others have a similar activity, i.e. 'the ministers', one or more others are included to aid absorption and delivery to the site of action, i.e. 'the guides' and the fourth group are chiefly utilised because they reduce unwanted side-effects caused by the major active herbs, i.e. 'the assistants' (Xue *et al.*, 2003).

In many traditions however, such differentiation has not been codified, and knowledge is pragmatic or empirical. The need for a herbal plant to be collected at a specific time of year, or processed in a particular way, may be associated with a high level of the actives, but may also coincide with the presence (or absence) of other compounds that affect the activity. Even where such matters are of little concern, pharmacological and biochemical research over the past 30 years has increasingly shown that most herbs have several types of constituents and a corresponding range of biological effects. It is too simplistic to talk about a herb having one 'active ingredient' and one 'activity'. The spectrum of activities, i.e. the polyvalence and the variety of compounds responsible for the effects seen are discussed in the following paragraphs.

Polyvalence can be due to the following:

- A variety of types of chemical compound is present, each type having a different biological effect.
- Compounds of one particular chemical type are present which have more than one biological effect relevant to treating the disease and/or improving the health of the patient.
- Compounds are present, which do not affect the cause or symptoms of the disease itself, but which modify the side-effects, absorption, distribution, metabolism and excretion of active constituents.

Each of these three points is considered in more detail below.

A variety of types of chemical compound being present

The leaves and flowers of many *Buddleja* species throughout the world are used to treat wounds and inflamed tissues (Houghton, 1984). In a study on some Chinese species of *Buddleja*, compounds extracted from the leaves of *B. asiatica* and flowers of *B. officinalis* were tested for their inhibitory effects on eicosanoid synthesis, a key part of the inflammatory process (Liao *et al.*, 1999). Four major types of compounds triterpenes, phenylethanoids, flavonoids and carotenoid glycosides were found to be present in the extracts. The triterpene δ-amyrone, the flavonoid aglycone acacetin and the carotenoid glycoside crocetin displayed inhibition of cycloxygenase, while acacetin also inhibited lipoxygenase. These results suggested that an aqueous extract or poultice of the plant material, which is likely to contain these compounds, and is the form used traditionally, might well include anti-inflammatory effects as part of its portfolio of properties that would help wounds to heal.

Another example of polyvalence is the widely used anxiolytic and sleep-promoting herb valerian (*Valeriana officinalis*). This contains sesquiterpenes such as valerenic acid, which appear to inhibit γ-aminobutyric acid (GABA) breakdown, thus causing a net increase of this CNS-depressant neurotransmitter *(Riedel et al., 1982)*; the monoterpenes known as valepotriates which relax smooth muscle (Wagner and Jurcic, 1979); large amounts of free GABA, which might bind to the GABA receptors (Santos *et al.*, 1994); lignans, e.g. 1-hydroxypinoresinol, which inhibit binding of serotonin to receptors (Bodesheim and Hölzl, 1997); and flavonoids, e.g. 6-methylapigenin, which have been shown to bind to benzodiazepine receptors (Wasowski *et al.*, 2002). All of these effects contribute to depression of CNS activity and to overall relaxation, thus aiding reduction in anxiety and promoting the onset of sleep (Houghton, 1989).

Ginkgo (the leaves of *Ginkgo biloba* L.) is an ancient Chinese medicinal plant which is now widely sold throughout the world to preserve good memory and cognitive function (Barnes *et al.*, 2007). Clinical studies have given a considerable evidence base to its efficacy, which is generally ascribed to an improved blood flow in the microcirculation of the CNS and a concomitant reduction in damage caused by reactive oxygen species associated with ischaemia. Ginkgo contains flavonoids such as ginkgetin which are neuroprotective because of their antioxidant and anti-inflammatory activities (Kang *et al.*, 2005), the diterpenoid ginkgolides, which are potent anticoagulants because of their ability to inhibit platelet aggregation, thereby increasing blood flow (Braquet *et al.*, 1991) and the sesquiterpene biolobalide which has antioxidant properties (Joyeux *et al.*, 1995). In addition, the ginkgolides are antioxidant as well as anticoagulant (Joyeux *et al.*, 1995). All of these properties would increase blood flow and protect against oxidative damage.

St John's Wort (*Hypericum perforatum L.*) contains a variety of compounds and work still needs to be done to fully ascertain the roles played by each. Its major use in modern phytotherapeutics is for treating mild-to-moderate depression. Extensive research into the compounds responsible for this has revealed that this herb is a good example of polyvalent complexity. It contains the naphthodianthrone hypericin, prenylated phloroglucinols (e.g. hyperforin) and flavonoids. Hyperforin has been shown to inhibit serotonin re-uptake, an activity common to synthetic antidepressants such as fluoxetine (Prozac) (Chatterjee *et al.*, 1998), while there is evidence that hypericin inhibits binding to some subtypes of dopamine receptors (Butterveck *et al.*, 2002).

Compounds of one particular type with more than one biological effect

An interesting example of polyvalency being due to the same group of compounds having different effects, all of which might contribute to the treatment of a disease, is afforded by the tanshinone diterpenes present in *Salvia miltiorrhiza*. This herb is used extensively in TCM and has been investigated as potentially useful in Alzheimer's disease. The tanshinones were shown to have acetylcholinesterase inhibitory activity (Ren *et al.*, 2004), but are also potent cyclo-oxygenase inhibitors and therefore likely to have anti-inflammatory properties, thought to associated with a reduction in incidence of Alzheimer's disease (Paulus and Bauer, 1999). It should be noted that the tanshinones have some antioxidant effects, as have the group of compounds known as salvianolic acids, e.g. salvianolic acid B 16, which are also present in *S. miltiorrhiza* (Du *et al.*, 2000), and such antioxidants are thought to play a part in reducing the incidence of Alzheimer's disease (Maxwell, 1995).

European *Salvia* species have been investigated for possible use in Alzheimer's disease, based on traditional usage for poor memory in old age. The oil of *S. lavandulaefolia* has acetylcholinesterase-inhibitory effects because of the monoterpenes (Perry *et al.*, 2001), but also some anti-inflammatory and antioxidant action (Perry *et al.*, 2002; Houghton *et al.*, 2007a).

The phenylpropanoid compounds from south-eastern Asian species of *Alpinia* also display at least two activities that support their traditional uses for prevention or treatment of cancer. Some of these compounds, especially 1′-acetoxychavicol, display direct cytotoxic activity against cancer cell lines (Lee and Houghton, 2005). However, they also up-regulate the cell's self-defence systems, the increased production of the antioxidant glutathione (GSH) and the up-regulation of glutathione-*S*-transferase (GST), an enzyme involved in phase 2 metabolism and removal of reactive oxygen species and other harmful compounds. Using in-vitro methods for detecting these activities, it has been shown that five species from Malaysia showed activity in at least one of the bioassays, the two most active being *Alpinia officinarum* and *A. galanga,* with 1′-acetoxychavicol being found to be the most active compound (Houghton *et al.*, 2007b).

Compounds present that modify the pharmacokinetics of active constituents

Pharmacokinetics is the term used to describe the absorption, metabolism and excretion of chemical substances in the body. These aspects are well-researched for single chemical entity pharmaceuticals since the data has to be presented for registration of the drug. Deviation from the norm can be due to genetic factors in the patient, e.g. 'fast' and 'slow' metabolisers, dietary factors and interactions with other medication. The same factors can apply with herbal products, but an extra dimension can occur because the plant material or extracts used can, in themselves, contain substances that modify the pharmacokinetics of the 'actives' present.

If the herbal material is taken orally, which is the most common situation, absorption occurs across the wall of the gastrointestinal (GI) tract. This can be slowed down if the plant contains a large amount of polysaccharide polymers, a factor exploited in the use of guar gum in diabetes to reduce the absorption rate of glucose. The presence of high levels of tannins can also reduce the rate of absorption. A more rapid effect is noted with coffee than tea, even though a cup of each contains about the same amount of caffeine, because tea contains high levels of tannins which reduce absorption and provide a slower increase in caffeine levels in the blood by complexing with the caffeine (Heinrich *et al.*, 2004).

It has been shown that some hot spices, e.g. black pepper *Piper nigrum* and ginger *Zingiber officinale*, increase the transfer of many chemical substances across the gastrointestinal wall and it is of interest that black pepper is not only used as a drug in Ayurvedic medicine in its own right, but is also often added to mixtures containing other drugs (Atal *et al.*, 1981). Piperine 18, the major constituent of black pepper, has been shown to affect the uptake of several conventional drugs in humans (Bano *et al.*, 1991).

Once absorbed, the plasma levels of any drug are affected by its metabolism and excretion. Metabolism chiefly takes place in the liver, especially by the group of enzymes known as cytochrome P450

(CYP), of which many sub-types exist. If these are up-regulated, plasma levels of active substances decrease more rapidly than might be expected, whereas the reverse may be seen if the enzymes are inhibited or the liver cells damaged in some way. Both effects have been noted with plant constituents, e.g. St John's wort (*Hypericum perforatum*) is known to up-regulate CYP4A, leading to loss in efficacy of a wide range of drugs such as the immunosuppressant cyclosporin, the anti-HIV drugs and the contraceptive steroids (Mannel, 2004), while the furanocoumarins present in grapefruit juice are thought to be responsible for the high levels of the cardiotoxic antihistamine terfenadine which have had serious effects in some patients (Barnes *et al.*, 2007). Most of the concern which has arisen over these effects has come from herb–drug interaction studies, but it should not be forgotten that the types of compounds affecting metabolism may coexist in a herb with the actives, or be constituents of a herb added to the 'active' according to a herbal prescription.

In addition, the constituents of a herb may not be very active *per se*, but may need to be metabolised to more active forms by gut or liver enzymes. This is true for many of the laxative herbs containing anthracene-derivative glycosides and it has been demonstrated that the activity of senna (*Cassia acutifolia, Cassia angustifolia*) is due to rheins formed by the activity of intestinal bacteria (Kobashi *et al.*, 1980). Many flavonoids are probably also metabolised by gut bacteria but it should also be remembered that the CYP enzymes in the liver may also act as conversion agents from the 'prodrug' form found naturally to more active metabolites. Other compounds present in a particular plant, or another plant when the herbal substance is given in a mixture, may affect the enzyme activity of gut bacteria or liver enzymes, and consequently also the conversion process.

The duration of activity of a particular herb is also affected by the excretion which occurs in the urine through the kidneys, or by sweating. Reduced absorption might also take place if gastric transit time is decreased due to any laxative effect. If the glomerular filtration through the kidneys is increased due to a diuretic effect, then levels will drop rapidly and the same result will be obtained if sweating increases due to changes in temperature regulation or increased blood flow to the skin. It should be noted that, in European herbal treatment, increased diuresis and/or sweating is a major approach in eliminating 'toxins' from the body to restore health, so it would not be surprising to find herbs used which exert these effects, but which would also decrease the time over which the herb constituents reach effective levels in the blood. A recent review (Wright *et al.*, 2007) has highlighted the large number of diuretic herbs in use, which must be seen as potential modifiers of pharmacological activity.

Polyvalency and testing of herbal extracts for activity

If polyvalency occurs, it should be noted that the use of only one bioassay for in-vitro testing for clinically relevant activity should be treated with great caution, since this approach is usually much too reductionist (Houghton *et al.*, 2007a). In cases of polyvalence, a battery of bioassays covering different aspects of the condition should be used, to elucidate a greater range of possible activities that could explain traditional usage. This must certainly be the case when a specific disease is diagnosed because of its presenting symptoms, but where these may be caused by a variety of factors. It would not be unusual to find that two different herbs, used for the same condition, have differing portfolios of activities.

Conclusions

Plant material or herbal extracts display much chemical complexity and it is only recently that advances in analytical procedures, coupled with sensitive bioassays, have allowed some teasing out of the factors that contribute to any overall effect. The interaction with such a complex mixture with the complicated array of biochemical pathways and other physiological processes, that exist in a delicate balance to maintain a healthy state in the human body, is a challenge for systems biology. The use of its approaches and techniques in seeking to understand what happens when a herb is taken is in its infancy but it appears likely that a greater understanding and demonstration of synergy and polyvalence in herbal extracts will be a feature of research in the next few years.

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