

Herbal products: active constituents, modes of action and quality control

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Abstract

An overview is given of the current position of medicinal herbs in general in relation to usage, market and production, types of pharmacological activity and how they differ from conventional drugs. The increasing importance of quality and manufactured products is also discussed. A more detailed consideration of these issues is given in relation to echinacea, valerian and St John's wort as these herbs are well studied, are market leaders and have widespread community usage.

Herbal products: Echinacea: Valerian: St John's wort

General overview

Background

Medicinal herbs were the primary health care agents over the many centuries before the advent of modern medicine. Their usage had, however, been in decline in most developed Western countries from about the beginning of the 20th century up to the 1970s. This decline in popularity coincided with industrialization and urbanization and their associated rejection of traditional values and systems. It is of interest that a similar decline in the use of medicinal herbs did not occur in the more developed Asian countries such as Japan, despite considerable industrialization. It was estimated by the World Health Organization that about 70% of the world population currently use plants for medicinal purposes, with high usage mainly in Asia, South America and Africa (Bannerman *et al.* 1983).

The medicinal herb industry in the Western world was nurtured during the mid 20th century in central Europe, particularly in Germany and France where the medical fraternity remained sympathetic to the co-use of modern and traditional therapies. North America, despite being the source of many medicinal herbs, showed little interest in medicinal herbs and there was little interaction between conventional and traditional medicine. The international resurgence of medicinal herbs in Western culture, especially in the USA, over the last 20 years coincides with the 'greening' of society, enhanced interest in natural systems and the questioning of an over-dependence on synthetic drugs to maintain health.

Abbreviation: GABA, γ -aminobutyric acid.

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Regulations governing the manufacture and sale of medicinal herbs vary between countries. In many parts of Europe and in Australia herbal products are regarded as medicines and must be manufactured under the pharmaceutical code of good manufacturing practice, whereas in the USA, food good-manufacturing-practice standards currently apply. Where herbal products are medicines, therapeutic claims are allowed, although these are often restricted to mild or self-limiting disorders for over-the-counter products. Levels of proof for these claims range from traditional use to well-conducted, controlled clinical trials. In the USA, therapeutic claims are permitted only for the few products which are registered as over-the-counter medicines; the remainder are regarded as dietary supplements and are represented by structure–function claims, e.g. antioxidants maintain cell integrity, fibre maintains bowel regularity.

The consumer is becoming increasingly well informed about the properties of herbs and the majority of over-the-counter sales are self-prescribed. However, registered health care professionals such as medical practitioners, pharmacists and naturopaths (registered in the USA and Germany) prescribe or recommend herbal treatments. In addition, non-registered practitioners including herbalists and naturopaths often prescribe herbal treatments for their patients. Depending on the legal environment, prescriptions are purchased either directly from the practitioner or from a pharmacist.

Medicinal herbs are also increasingly being sold into the nutraceutical food sector; nutraceuticals are food ingredients consumed for their health-giving properties. They are now added as healthy ingredients to conventional foods such as cereals, confectionery, snack foods and beverages and promoted as the reason to purchase the product.

Market and production data

Data on the international trade in medicinal herbs are sparse and market estimates can vary widely, making comparisons between countries difficult. In a review of the European market, Grimwald & Buttlet (1996) reported that world retail trade in medicinal herb preparations was valued in 1994 at about \$US 12 billion with the European Union accounting for about half of this total. Greatest sales were in Germany with 45 % of the European Union market and France (30 %) followed by Italy (11 %), the UK and Spain (each at 5 %). Each inhabitant of the European Union was estimated to spend about \$US 17 per annum on phytotherapeutics. Asian sales were estimated at \$US 4.4 billion with Japan contributing about half this total while North American sales were only \$US 1.5 billion. The annual growth rate of these regions was estimated to be 12–15 % in all regions except in the European Union, where it was only 5 %, but this was from a much higher baseline. The growth rate of phytotherapeutics in Europe was considered to be greater than for pharmaceuticals.

The leading medicinal herb product sold in the European Union in 1993 was manufactured from *Ginkgo biloba* with wholesale sales of \$US 200 million. The next most popular products were manufactured from ginseng (\$US 50 million), garlic (\$US 40 million), evening primrose (*Oenothera biennis*) and echinacea (each \$US 40 million). In Germany, phytotherapeutics accounted for 30 % of all non-prescription drugs, with about 55 % comprising products which can only be purchased through pharmacies and are covered by health insurance and 45 % as over-the-counter products. *Ginkgo biloba* is the most frequently prescribed herbal product (retail sales of DM 460 million), with garlic the leading non-prescription product (DM 140 million) (Grimwald & Buttlet, 1996).

Brevoort (1998) has reviewed the US market and estimated medicinal herbs to be worth about \$US 4 billion retail in 1998. This represents an annual growth of about 40 % over the 4

years from her 1994 estimate of \$US 1.6 billion. The growth has been aided by expansion of retail sources for herbal products from the traditional natural foods stores and naturopaths to be ubiquitous in the retail and mail-order food chain. The natural foods sector still retains the largest market share with sales of \$US 1.2 billion but now represents only 30 % of total sales. The rise has been aided by strong advertising campaigns made possible by the entry of multinational groups and considerable media attention. Mass-market sales, such as through supermarkets, have seen the greatest rate of increase, with sales rising from \$US 360 million in 1997 to \$US 660 million in 1998, an 80 % increase to secure 17 % of the market.

Brevoort (1998) has also summarized sales data published by a range of commercial agencies and trade magazines. The greatest-selling medicinal herb in 1997 by value was echinacea (9 % of total market, i.e. \$US 320 million) followed by ginseng (8 %), ginkgo (7 %), garlic (6 %) and St John's wort (*Hypericum perforatum*) (6 %). Sales by volume showed echinacea to be the market leader, with 10 % of total sales, followed by St John's wort (9 %), ginkgo (7 %) and garlic (7 %). The market is characterized by rapid changes in popularity, with the 'in-herb' for 1998 being St John's wort, which recorded a 2800 % increase in sales in the mass-market trade over 1997; its sales volume ranking rose from 17th to 2nd position. Surveys of consumers showed that about one-third of consumers used a medicinal herb, with garlic by far the most-used herb (20 % of users) followed by ginseng, ginkgo, echinacea and St John's wort (each at 10 % or less).

Phytochemicals in plants

Pharmacologically active constituents of plants are mostly secondary metabolites generated from the shikimate, acetate–malonate and acetate–mevalonate pathways (Kuc & Rush, 1985; Tyler *et al.* 1988). The chemically distinct, but often overlapping, classes of constituents are mainly terpenoids (such as sesquiterpenes, saponins, iridoids, carotenoids and steroids), phenolics (such as tannins, quinones, salicylates and lignins), and their glycosides (such as flavonoids, glucosinolates and cyanogens), alkaloids, polysaccharides (such as gums and mucilages) and peptides. Also of interest are essential oils and resins, which often contain several of the above constituent classes (Tyler *et al.* 1988; Pengelly, 1996).

Secondary metabolites were originally considered as peripheral to the essential metabolism of the cell and often as merely waste products of metabolism. They are now believed to fulfil many important plant functions, although the full function of most is not completely understood (Williams *et al.* 1989; Bennett & Wallsgrove, 1994). The role of secondary metabolites includes:

1. Protection against attack or the environment. Protection may be provided against predators including insects, animal and marine herbivores, micro-organisms and parasites. According to Verpoorte (1998), defence compounds can be: expressed in certain cells, for example, cyanogens, glucosinolates, sesquiterpenes and alkaloids to assist protection against insect or herbivore feeding (Bennett & Wallsgrove, 1994); expressed by biochemical action, for example, cyanogens which, under the action of glucosidases, yield hydrogen cyanide; induced by environmental, metabolic or mechanical stress, for example phytoalexins, which are synthesized after infection of the plant with pathogenic micro-organisms.
2. Protection against u.v. radiation. Excess radiation induces accumulation of compounds such as the flavonoids which absorb at specific wavelengths but do not diminish photosynthetic yield. Polyamines, waxes, diterpenoids and specific alkaloids have also been suggested to

- contribute to u.v. tolerance. The free-radical scavenging activity of flavonoids may also offer additional protection (Langenheim, 1994; Jansen *et al.* 1998).
3. Participation in allelopathy. Allelopathy is the release of a chemical by an organism into the environment that affects the growth, health, behaviour or population of another organism. Such chemicals usually act to inhibit seed germination or growth of other plants, thereby reducing competition, or inhibit growth of soil bacteria. Compounds implicated in plant allelopathy include phenolics (Inderjit, 1996), terpenoids (Langenheim, 1994), glucosinolates (Brown, 1995), coumarins (Macias, 1993), saponins (Birk & Reri, 1980) and quinones (Inoue, 1992).
 4. Metabolism. Secondary metabolites may function in overflow storage or in disposal of waste products from primary metabolism (Mitchell-Olds *et al.* 1998), or may be recycled into primary metabolism during leaf senescence (Langenheim, 1994). In addition, secondary metabolites may be involved in detoxification processes (Bussotti *et al.* 1998) and certain alkaloids participate in storage and growth regulation (Bruneton, 1995).
 5. Other plant–animal interactions. Some herbivorous insects use plant secondary metabolites to protect themselves from predators and parasitoids (Bennett & Wallsgrave, 1994). In some cases, plants may offer both food and protective chemicals to their insect and bird pollinators (Langenheim, 1994). Various insects sequester secondary metabolites which are converted to male pheromones (Nahrstedt, 1989; Harborne, 1993), while there may be pheromonal insect-induced reactions between plants (Dajoz, 1992).

While the production of secondary metabolites by plants was not designed to benefit humans, the stimulatory biochemical action of certain metabolites has effects on the human systems that generate a beneficial effect. The art of the original herbalists was in the recognition of this benefit through a trial-and-error process for specific human conditions. Despite the widespread use of medicinal herbs, much research is still required to identify the active constituents and understand their mode of action. Progress on this aspect will be explored later for specific medicinal herbs.

How do medicinal herbs differ from conventional drugs?

While many conventional drugs or their precursors are derived from plants, there is a fundamental difference between administering a pure chemical and the same chemical in a plant matrix. It is this issue of the possible advantage of chemical complexity which is often rejected as having no basis in fact and avoided by most researchers as introducing too many variables for comfortable research.

Is there any advantage in chemically complex medicines? Life is chemically complex, and science is only beginning to grasp the subtle and varied mechanisms involved in processes such as inflammation and immunity. Taking an evolutionary perspective, it could follow that the human body is better adapted to complex medicines, but hard proof of an advantage is difficult to establish.

Synergy is an important concept in medicinal plant use. In the context of chemical complexity it applies if the pharmacological action of a chemical mixture is greater than the arithmetic sum of the actions of individual components. A well-known example of synergy is exploited in the use of insecticidal pyrethrins. Piperonyl butoxide has little insecticidal activity but interferes with the insect's ability to break down the pyrethrins, thereby substantially increasing their toxicity. Hence, a particular chemical extracted from a medicinal herb might in pure form have only a fraction of the pharmacological activity that it has in the plant matrix.

The basic issues of an advantage from chemical complexity leading to enhanced solubility or bioavailability of key components were discussed by Eder & Mehnert (1998). Some advantages arising from chemical complexity are as follows: the isoflavone glycoside daidzin in a crude extract of *Pueraria lobata* achieves much greater concentrations in plasma than equivalent doses of pure daidzin (Keung *et al.* 1996); co-administration of procyanidins from St John's wort significantly increases the *in vivo* antidepressant effects of hypericin and pseudo-hypericin. The latter effect was attributed to the observed enhanced solubility of hypericin and pseudohypericin in the presence of procyanidins and indicates that pure hypericin and pseudohypericin have considerably less antidepressant activity than their equivalent amounts in St John's wort extract (Butterweck *et al.* 1998).

Synergy can also have a pharmacodynamic basis. One example is the antibacterial activity of major components of essential oils from lemon grass. While geraniol and nerol individually elicited antibacterial action, the third main component, myrcene, did not exhibit any activity. However, myrcene enhanced activities when mixed with either of the other two main components (Onawunmi *et al.* 1984). Sennoside A and sennoside C from senna have similar laxative activities in mice. However, a mixture of these compounds in the ratio 7:3 (which somewhat reflects the relative levels found in senna leaf) has almost two-fold laxative activity (Kisa *et al.* 1981).

Quality issues

The quality of medicinal herbs has traditionally been based on appearance. An important early visual quality evaluation was to ensure that the plant was of the required species. The medicinal efficacy of many herbs varies greatly between plants of different species of the same genus and can extend to different plant parts being of different medicinal value, as illustrated by skullcap where the commercial plant part of *Scutellaria baicalensis* is the root while for *Scutellaria lateriflora* it is the aerial section.

The modern trend towards value-added products, where the native plant structure has been destroyed, eliminates visual assessment for species identification. Such products can range from ground dried raw plant material to manufactured liquid or solid extracts or finished products, including formulations containing more than one herb. It then becomes impossible to detect species misnaming or fraudulent adulteration using macroscopic or organoleptic techniques. Positive species identification can be achieved with chromatography. TLC is often used as a relatively cheap but rapid species identification test but HPLC is a more powerful tool. DNA fingerprinting would seem to also offer advantages in species identification, but is currently not used to any great extent.

The ultimate quality criterion of medicinal herbs, however, is the presence of chemical constituents that confer a health benefit. For most medicinal herbs, a range of compounds have been ascribed with pharmacological activity with often unresolved synergistic effects between individual compounds. These active constituents are often complex labile compounds that traditional analytical techniques had difficulty in extracting and quantifying. It is only since the advent of HPLC in the 1970s that serious consideration could be given to setting quality standards based on levels of active constituents or marker compounds that can signify the presence of active constituents.

Progress has been slow in the setting of chemical quality standards, owing to lack of conclusive clinical evidence for the activity of specific compounds, multiple active constituents in a product and probable synergistic effects, and reluctance of some health authorities to

accord recognition to medicinal herbs as valid therapeutic agents. Where an individual compound is chosen as the marker for activity in a herb, there must be some meaningful consistent relationship between the amount present and a quantitative therapeutic benefit. The situation is changing slowly and can be expected to accelerate in the coming years, not least as a result of consumer pressure to guarantee the efficacy of retail medicinal herb products.

Manufactured products

The traditional use of medicinal herbs was for a family or village unit to harvest wild-crafted plants from the forest, typically dry the plant and store it until required for use, when a portion was transformed into the desired powder, tincture, poultice or decoction and consumed by the patient. Such practices became increasingly difficult to maintain in urbanized society, but were sustained by the emergence of the professional naturopath or herbalist, who kept a stock of dried medicinal herbs or liquid extracts and prepared mixtures for patients as required.

The emergence of the synthetic pharmaceutical industry after World War II with a multitude of products targeted at numerous ailments created the mass market for prescription and over-the-counter medicines that are sold in a stable form and in a dose-specific package from numerous retail outlets. The marked increase in consumption of medicinal herbs by communities throughout the Western world, coupled with customer product expectations of uniformity and convenience, has seen the rise of a substantial medicinal-herb manufacturing industry. This process is being accelerated by the recent entry of pharmaceutical multinationals into the industry.

The advent of a sophisticated manufacturing industry has seen the move to higher value-added products such as standardized extracts and phytomedicines. If such products are based on a small number of active constituents and involve their excessive isolation and concentration from the plant material, and they are given at high doses, then additional safety and efficacy data need to be generated. Many medicinal herbs have not been subjected to rigid clinical trials but rely on evidence from traditional usage as to efficacy and dosage safety. Ingestion of a selection of ingredients at elevated dosage may have previously unknown side effects, or the product may lose synergistic benefits from the multiple active constituents in the plant.

The following three examples of medicinal herbs have been chosen because they are good examples of the issues involved in the modern production of herbal products. These herbs are well studied, are market leaders and have widespread usage. However, many issues pertaining to active constituents, modes of action and quality control are still to be fully elaborated.

Echinacea

Echinacea is a member of the Compositae (daisy) family. It is commonly known as purple coneflower (Lust, 1974; Bauer & Wagner, 1990). Three species of echinacea are used medicinally: *Echinacea angustifolia* DC. (narrow-leafed purple coneflower), *E. purpurea* (L.) Moench. (common or broad-leafed purple coneflower), *E. pallida* (Nutt.) Nutt. (pale purple coneflower).

Echinacea purpurea has become the most cultivated and widely used of the three species, because the whole plant (root, leaf, flower, seed) can be used and also because it is more easily cultivated. The root and rhizome of *E. angustifolia* and *E. pallida* are used medicinally. In the past, *E. pallida* preparations have been incorrectly labelled as *E. angustifolia*, particularly in

Europe. *Parthenium integrifolium*, the Missouri snakeroot, is a documented adulterant of commercial echinacea.

Historical background

Echinacea is an indigenous plant of North America and was used extensively by many native American tribes against a wide range of ailments and illnesses. The use of echinacea passed to the European settlers in the 19th century and was adopted by the Eclectics, a group of medical practitioners prominent around the late 19th to early 20th centuries. Echinacea (specifically the root of *E. angustifolia*) became the most popular treatment prescribed by Eclectic physicians. Its use was based on tribal knowledge and accumulated clinical experience during usage (Felter & Lloyd, 1983; Ellingwood, 1993). With the advent of modern medicine, a range of traditional treatments came under question and, in 1909, echinacea was removed as an approved therapeutic agent by the American Medical Association. Echinacea seeds were taken to Europe and became widely cultivated, particularly in Germany and France. Scientific research into the composition and pharmacological properties of echinacea was commenced in Germany in the 1930s and studies over the next 30 years have provided a solid base which has supported the revival of echinacea throughout the western world.

Usage of echinacea

Early texts list an extensive range of conditions for which echinacea was prescribed: these included snake bite, syphilis, typhus, septic wounds, diphtheria, scarlet fever, dysentery and even cancer. The conditions treated were mainly infections and envenomations of various kinds, although they also included tuberculosis and disorders related to autoimmunity such as diabetes, exophthalmic goitre, psoriasis and renal haemorrhage.

Modern usage of echinacea is primarily as an immunostimulant acting on non-specific immunity. Hence it may modulate immune function in allergy and autoimmunity and enhance resistance to infections, particularly those of the upper respiratory tract. It is also attributed with antibacterial and antiviral activities, probably as an indirect effect of immune enhancement. It has anti-inflammatory properties, particularly by topical application. Echinacea is sometimes prescribed as adjunct therapy during cancer treatment (Grieve, 1971).

Echinacea has found application in a range of mainstream manufactured products such as lip balms and toothpaste (Leung & Foster, 1996) and skin- and hair-care products, including facial toners, creams and lotions especially for damaged skin (Smeh, 1995). It is increasingly found in food products such as breakfast cereals, confectionery and teas.

Preparations of *E. purpurea* include liquid extracts of fresh or dried whole plant or aerial parts, fresh or dried preparations of root and rhizome, stabilized juice of the flowering tops, mixtures of any of the above preparations, and tablets and capsules based on any of the above plant parts or corresponding extracts. For *E. angustifolia* and *E. pallida*, similar preparations are used, although the roots of these species are generally preferred.

Active constituents

A wide range of compounds in echinacea has been reported to possess pharmacological activity (Hobbs, 1989; Bauer & Wagner, 1991). From these identified components, the active con-

stituents considered to be of most interest can be divided into three major groups, namely the caffeic acid phenols, polysaccharides and unsaturated lipophilic components. There is, however, still debate as to the relative importance of these groups, with some having the view that substantial synergistic effects are occurring between the groups.

Caffeoyl phenols. The basic chemical structure of caffeic acid derivatives consists of one or two caffeoyl moieties with a link molecule such as tartaric acid, quinic acid or a sugar residue, for example cichoric acid in Fig. 1. The first caffeoyl phenol identified was echinacoside, which was isolated from the roots of *E. angustifolia* by Stoll *et al.* (1950) but was later also found in *E. pallida*. Cichoric acid is the predominant caffeoyl phenol in *E. purpurea* and is also found in *E. pallida*. A range of other caffeoyl phenols are found at lower levels in the three echinacea species, especially in the aerial parts (Bauer & Wagner, 1991). The differing caffeoyl phenols in the echinacea species might be expected to confer different pharmacological properties to each species.

Polysaccharides. Elucidation of the polysaccharides in echinacea with pharmacological activity is much less advanced than for other active constituents. Bauer & Wagner (1991) isolated two polysaccharides with immunostimulatory properties from the aerial parts of *E. purpurea*. Polysaccharide I was found to be a 4-*O*-methyl glucurono-arabinoxylan while polysaccharide II was an acidic arabinorhamnogalactan. A xyloglucan has also isolated from the leaves and stems of *E. purpurea*.

Most of the studies on echinacea polysaccharides have, however, been on those derived from tissue cultures of *E. purpurea*, which are fucogalactoxyloglucans and arabinogalactans. As expected, the structures of the tissue culture polysaccharides differ from those of the aerial parts of the naturally grown plant, since cells in culture possess only primary cell-wall components.

Lipophilic components. The unsaturated lipophilic components of echinacea comprise two main groups: the alkylamides and the polyacetylenes. Alkylamides are not common plant constituents and most compounds have been found to occur in two tribes of the Asteraceae. Many alkylamides (particularly isobutylamides) have been isolated from *E. angustifolia* and *E. purpurea* roots and aerial parts, but they are largely absent from *E. pallida*. The alkylamides in echinacea are composed of a highly unsaturated carboxylic acid (often with triple C–C bonds)

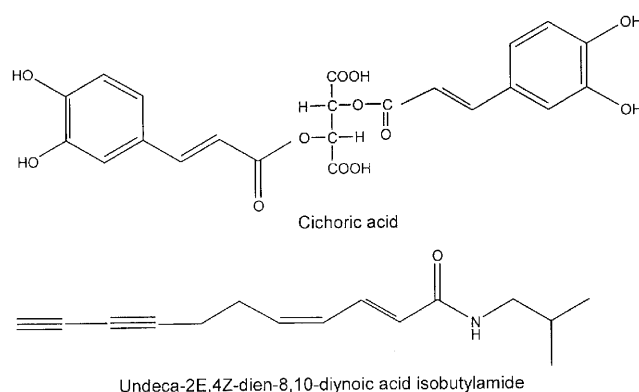


Fig. 1. Structure of cichoric acid and an alkylamide.

and an amine compound, either isobutylamine or 2-methylbutylamine (Fig. 1). It is possible that this bond between the acid and the amine will be broken during digestion, and the true active entity from these compounds is the carboxylic acid (Bauer & Wagner, 1991).

The occurrence of polyacetylenes is typical of the Asteraceae family, and *E. pallida* root contains significant levels of ketoalkynes and ketoalkenes, but they do not occur in the other echinacea species (Bauer & Wagner, 1991).

Laboratory studies

Since the three echinacea species differ in composition of active constituents, and echinacea can be administered orally in the form of tablets, liquids (mainly as ethanol–water mixtures), capsules and spray-dried powders (in tablets or capsules) or by intramuscular injection of a liquid, it would be reasonable to expect that different echinacea preparations have different pharmacological effects in the human body. Interpretation of research into the mode of action of echinacea needs to take these factors into account. Most of the research on echinacea is on stabilized juice extracts of the aerial parts of fresh *E. purpurea*, a product commonly used only in Germany, and is probably irrelevant to common usage in the English-speaking world of oral preparations of dried root material of *E. angustifolia* and/or *E. purpurea*.

Immune-modulating activity. A common property of all three echinacea species is to effect an increase in phagocytic activity. This was shown in early *in vitro* studies in Germany with echinacea juice on human cells and micro-organisms (Brandt, 1967; Krause, 1984; Coeugnet & Elek, 1987; Wildfeuer & Mayerhofer, 1994; Burger *et al.* 1997) and later with ethanolic extracts after oral administration in the C-clearance test, with *E. purpurea* the most active species (Bauer *et al.* 1988, 1989; Bauer & Wagner, 1991). Extracts of the aerial parts of the three species demonstrated lower activity than the roots (Bauer *et al.* 1989). Enhanced cellular immune function of blood cells from normal individuals and patients with depressed cellular immunity from chronic fatigue syndrome and acquired immune-deficiency syndrome has also been demonstrated (See *et al.* 1997).

Oral doses of fractions containing cichoric acid and the alkylamides from *E. angustifolia* and *E. purpurea* root have increased phagocytic activity. Stimulation of granulocyte phagocytosis was observed in healthy male subjects who received an alcoholic extract of *E. purpurea* root standardized to cichoric acid and the alkylamides. The rate of immune stimulation was much higher than that observed with administration by intramuscular injection (Jurcic *et al.* 1989). In contrast, echinacoside from *E. angustifolia* and *E. pallida* (which is often used as a quality marker for these species) has not demonstrated immune-enhancing activity (Bauer *et al.* 1988).

Immune-enhancing activity has been demonstrated for echinacea polysaccharides *in vitro* (Stimpel *et al.* 1984; Wagner *et al.* 1985; Luettig *et al.* 1989; Bauer & Wagner, 1991), but the polysaccharides are probably not present in pharmacologically significant quantities and would not be absorbed in levels sufficient to achieve the concentrations used in the *in vitro* studies. Moreover, the quantity of polysaccharides present in preparations containing about 500 g ethanol/l will be low. In addition, some of the polysaccharides used in these studies were isolated from tissue culture and differ from those found naturally in echinacea.

Antiviral activity. A range of extracts from the root and expressed juice of aerial parts from the three echinacea species have shown antiviral activity towards herpes simplex virus and influenza virus *in vitro*. An indirect antiviral effect was also observed via stimulation of α - and β -interferon production. Viral resistance did not develop in the presence of hyaluronidase,

hence the virucidal activity was indirect (Orinda *et al.* 1973; May & Willuhn, 1995; Wacker & Hilbig, 1978; Beuscher *et al.* 1995). Cichoric acid has demonstrated antiviral activity against vesicular stomatitis viruses *in vitro* (Cheminat *et al.* 1988).

Anti-inflammatory and wound healing activity. Alkylamides from echinacea have shown inhibitory activity against cyclooxygenase (EC 1.14.99.1) and 5-lipoxygenase (EC 1.13.11.12) *in vitro* with the effect varying between different alkylamides (Wagner *et al.* 1989; Wagner & Jurcic, 1991). An anti-inflammatory effect was observed after the topical application of a crude polysaccharide fraction from *E. angustifolia* roots in the croton oil mouse ear test (Tubaro *et al.* 1987). Application of patches containing expressed juice of *E. purpurea* (aerial parts) to experimental wounds reduced oedema and subcutaneous haemorrhage and the rate of necrosis of skin flaps (Meissner, 1987). An ointment made from the expressed juice of *E. purpurea* (aerial parts) significantly improved wound healing in an experimental model (Kinkel *et al.* 1984).

Antihyaluronidase activity. Early research found that expressed juice of *E. purpurea* (aerial parts) inhibited hyaluronidase *in vitro* (Büsing, 1952), and may exert an indirect anti-hyaluronidase activity. Caffeoyl phenols obtained from *E. angustifolia* root have demonstrated antihyaluronidase activity *in vitro* (Facino *et al.* 1993). The possible antihyaluronidase activity may help increase the resistance of tissue to the spread of certain infections and, in conjunction with the increased presence of fibroblasts, facilitate connective tissue regeneration. This effect would most likely be observed with topical application of echinacea preparations.

Antimicrobial activity. In early research, echinacoside demonstrated weak antimicrobial activity *in vitro* against *Staphylococcus aureus* (Stoll *et al.* 1950) while polyacetylenes from *E. angustifolia* and *E. purpurea* root showed bacteriostatic and fungistatic activity against *E. coli* and *Pseudomonas aeruginosa* (Schulte *et al.* 1967). Pure acidic arabinogalactan isolated from *E. purpurea* plant cultures caused destruction of 90 % of Leishmania parasites by intracellular lysis (Bauer & Wagner, 1991). *E. angustifolia* extract showed weak inhibitory activity against *Trichomonas vaginalis* (Samochowiec *et al.* 1979), and *E. purpurea* extract inhibited the growth of *Epidermophyton interdigitale* (Jung & Schröder, 1954).

Clinical trials

Whilst many clinical trials have been conducted, many did not have adequate experimental methodology, which meant that results were not able to be analysed or interpreted adequately. The following are examples of properly constructed trials using a randomized, double-blind placebo-controlled approach with adequate numbers of subjects. The findings are summarized in Table 1.

Upper respiratory tract infections. A number of trials have shown that patients with upper respiratory tract infections, receiving the equivalent of at least 900 mg/d *E. purpurea* or *E. pallida* root as a tincture, experienced significant relief of symptoms (Bräunig *et al.* 1992; Bräunig & Knick, 1993). Ingestion of a stabilized expressed juice of *E. purpurea* tops resulted in a 50 % more rapid recovery of patients with initial symptoms of a common cold (Hoheisel

et al. 1997). Beneficial effects have also been obtained in trials where echinacea was used in conjunction with *Eupatorium perfoliatum*, *Baptisia tinctoria*, *Arnica montana* (Dorn, 1989; Schmidt *et al.* 1990), *Thuja occidentalis* and *B. tinctoria* (Reitz, 1990).

Table 1. Summary of clinical trials with echinacea preparations

| Study design and herbal preparation | Results | Reference |
|--|---|---------------------------------|
| Four-armed, randomized, double-blind, placebo-controlled (<i>E. purpurea</i> herb, <i>E. purpurea</i> herb concentrate, <i>E. purpurea</i> root) | Two echinacea herb preparations were significantly more effective than the <i>E. purpurea</i> root preparation or placebo in reducing symptoms of common cold | Brinkeborn <i>et al.</i> (1999) |
| Randomized, double-blind parallel, placebo-controlled (<i>E. purpurea</i> tops, stabilized juice; magnesium supplement) | Decreased incidence of upper respiratory tract infections in athletes taking echinacea compared with both the supplement and placebo groups | Berg <i>et al.</i> (1998) |
| Randomized, double-blind, placebo-controlled (<i>E. purpurea</i> root) | Significant relief of symptoms of upper respiratory tract infection | Bräunig <i>et al.</i> (1992) |
| Randomized, double-blind, placebo-controlled (<i>E. pallida</i> root) | Significant improvement of symptoms of upper respiratory tract infection; significantly shortened duration of illness | Bräunig & Knick (1993) |
| Randomized, double-blind, placebo-controlled (<i>E. purpurea</i> tops, stabilized juice) | More rapid recovery from common cold for treatment group | Hoheisel <i>et al.</i> (1997) |
| Randomized, double-blind, placebo-controlled (Tinctures (1 : 10, w/v) of <i>E. angustifolia</i> , <i>Eupatorium perfoliatum</i> , <i>Baptisia tinctoria</i> and homoeopathic <i>Arnica montana</i>) | Significant improvement of symptoms of upper respiratory tract infection | Dorn (1989) |
| Randomized, double-blind, placebo-controlled (Tinctures (1 : 10, w/v) of <i>E. angustifolia</i> , <i>Eupatorium perfoliatum</i> , <i>Baptisia tinctoria</i> and homoeopathic <i>Arnica montana</i>) | Most reduction in the frequency of infections was observed in the treatment group | Schmidt <i>et al.</i> (1990) |
| Randomized, double-blind, placebo-controlled (<i>E. angustifolia</i> and <i>E. pallida</i> root, <i>Thuja occidentalis</i> and <i>Baptisia tinctoria</i>) | Majority of symptoms were significantly better in the test group | Reitz (1990) |
| Randomized controlled low dose (<i>E. angustifolia</i> and <i>E. pallida</i> root, <i>Thuja occidentalis</i> and <i>Baptisia tinctoria</i>) | No significant improvement in peripheral blood count or incidence of infections in fifty female patients undergoing irradiation following breast cancer | Bendel <i>et al.</i> (1988) |
| Randomized controlled (<i>E. angustifolia</i> and <i>E. pallida</i> root, <i>Thuja occidentalis</i> and <i>Baptisia tinctoria</i>) | Recuperation of the haematopoietic system was promoted and there was a tendency to reduced infections, but only in cases of minor damage to the bone marrow | Bendel <i>et al.</i> (1989) |
| Open comparative (<i>E. purpurea</i> tops, stabilized juice) | Reduced recurrence rate and significant normalization of cell-mediated immunity in treatment group for recurrent candidiasis, compared with those using only the antifungal cream | Coeugniet & Kühnast (1986) |
| Open comparative (<i>E. angustifolia</i> and <i>E. pallida</i> root, <i>Thuja occidentalis</i> and <i>Baptisia tinctoria</i>) | X-ray and global assessment was much better for the group receiving the herbs compared with those acute sinusitis patients receiving only doxycycline | Zimmer (1985) |

Anti-neoplastic therapy. Results from patients with breast cancer have not shown conclusive benefits from echinacea use. Patients undergoing irradiation following breast cancer receiving a preparation containing extracts of *E. angustifolia* and *E. pallida* root, *Thuja occidentalis* and *Baptisia tinctoria* showed no significant change in peripheral blood count and incidence of infections from the control group. A study with patients with advanced breast cancer who received a similar dose of a similar preparation in conjunction with chemotherapy showed a tendency for the echinacea treatment to reduce infections, but only in cases of minor damage to the bone marrow (Bendel *et al.* 1988, 1989).

Other diseases. The expressed juice of *E. purpurea* (aerial parts) was administered orally in conjunction with an antifungal cream for the treatment of recurrent candidiasis. A 17% recurrence rate was observed in the treatment group compared with 60% for those receiving only antifungal cream and a significant normalization of cell-mediated immunity was also observed (Coegniet & Kühnast, 1986).

Patients suffering from acute sinusitis receiving extracts of *E. angustifolia* and *E. pallida* root, *T. occidentalis* and tincture of *B. tinctoria* together with doxycycline (a tetracycline antibiotic), or doxycycline alone in an open comparative study showed much better X-ray and global assessment of their condition (Zimmer, 1985).

In a post-marketing surveillance study, 4500 patients were examined by 500 doctors from all over Germany investigating the effect of an ointment containing echinacea juice on a variety of skin complaints. In 86% of cases a favourable result was obtained (Corrigan, 1994).

Safety issues

Echinacea is usually prescribed for short-term usage although there is no evidence to suggest that long-term usage will have an adverse effect on immune function. There have been recent reports that echinacea can trigger a strong allergic response (Mullins, 1998). Extracts or expressed juice of the aerial parts may contain pollen proteins. Hence caution should be exercised in those with a tendency to allergic reactions, especially against Compositae (daisy). Sharp (1997) has cautioned that echinacea is a danger to asthmatics, apparently based on the concern that echinacea increases the cytokine tumour necrosis factor- α which increases the inflammatory process in asthma. Since the information for tumour necrosis factor- α comes from *in vitro* tests on echinacea juice or polysaccharides such studies are likely to have little relevance to normal oral use of echinacea. This has been recently confirmed in a clinical study which found that oral therapy with echinacea had no detectable effect on cytokine production by lymphocytes (Elsasser-Beile *et al.* 1996).

Quality considerations

Quality considerations are becoming much more important in the echinacea industry. As knowledge of the differences in composition of the different echinacea species and differences between plant parts are more closely linked to pharmacological effects, it will become more critical to ensure that the required species and plant part is obtained. This is made feasible by HPLC testing becoming more widely available as a commercial service in many countries.

With the move of medicinal herbs into the mainstream food and pharmaceutical marketing scene, consumers and regulatory authorities are becoming more concerned that the retail product contains adequate levels of active constituents to ensure consistent medical efficacy. A study in Australia found that about 20% of retail echinacea products contained near-zero levels of caffeoyl phenols and alkylamides (Wills & Stuart, 1998). Whilst most manufactured pro-

ducts list the amount of added echinacea in the product, this was not a reliable guide to the level of active constituents in the final product.

Wills & Stuart (1999a) recently reported that substantial losses of active constituents can occur at many points in the postharvest handling, drying and processing chain that converts the freshly harvested echinacea to a manufactured product. They found that the greater the total heat load imposed during drying, the greater the loss of alkylamides and cichoric acid. The efficiency of drying to preserve the active constituents was therefore: hot-air drier < heat-pump drier < vacuum drier. They even suggested ambient-air drying could be an efficient drying method under certain conditions. A national survey of sixty-two dried echinacea samples traded commercially by Australian growers found that levels of active constituents were consistent with the use of hot-air driers (Wills & Stuart, 1999b).

Wills & Stuart (1999a) also reported that processing of dried echinacea with alcoholic solutions gave highly variable extraction rates under differing processing variables, with a substantial proportion of active constituents either degraded during processing or not extracted from the raw material. Use of 600 g ethanol/l (a common industrial extractant) in the laboratory system extracted 60 % of alkylamides and 40 % of cichoric acid. Other factors found to influence processing efficiency were: increasing temperature of the extracting solvent decreased the yield of alkylamides but increased the yield of cichoric acid; increasing the solvent:substrate ratio increased the extraction of both alkylamides and cichoric acid; decreased particle size increased yields.

In addition, statements on the amount of added echinacea do not take into account the plant part used in the product. Perry *et al.* (1997) and Stuart & Wills (2000) have found that the alkylamide content is highest in the root, which contains about 75 % of total plant alkylamides. Whilst the flower has a reasonable concentration of alkylamides, low levels were found in stem and leaf suggesting lower medicinal benefit. The concentration of cichoric acid in mature plants, in contrast, was found to be highest in the flower, then in the root and leaf, but was quite low in the stem.

The demonstrated vulnerability of active constituents to loss during the transition to manufactured products underlines the need for better quality management by the industry. It also highlights the need for improved labelling of products. Products which contain high levels of active constituents cannot now be identified by purchasers. Following the mandatory practice of the processed food industry, it is suggested that product labels should contain a more standardized format on echinacea content and include the concentration of nominated active constituents or marker compounds. This, however, requires industry and regulatory authority agreement on which are the key active constituents or marker compounds, a point of still considerable contention.

The use of marker compounds is only relevant in multi-component extracts. They should not be used for those extracts which are manufactured to optimize the concentration of one or two compounds. Marker compounds need to be well chosen. One current practice with many standardized extracts is to use echinacoside as the marker compound but there is evidence to indicate it has minimal medicinal value and the level of other active constituents is not known. In the USA, products are often sold standardized to a level of 'total phenolics', usually at 40 g/kg, as measured by spectrophotometry. However, the methods used to quantify total phenolics do not identify the key components with immune activity (e.g. cichoric acid) and the results of the analysis can vary depending on the method and standard used.

Valerian

Valeriana officinalis (European valerian), a member of the Valerianaceae family, is the major species of this genus used as a medicinal herb but a range of subspecies and varieties has been

utilized. The plant grows under a wide range of ecological conditions which, through polymorphism, has led to the development of forms with marked regional differences. In addition, the genus exhibits polyploidy leading to diploid, tetraploid and octoploid forms. This has resulted in some taxonomic confusion, with some workers considering the group as one species with a number of subspecies and others dividing it into smaller species. Other species of valerian that also find phytotherapeutic application include *V. wallichii* DC. (Indian valerian), *V. fauriei* Briq. (Japanese valerian) and *V. edulis* Nutt. (Mexican valerian) (Evstatieva *et al.* 1993; Dweck, 1997). The root and rhizome are the plant parts utilized in phytotherapy.

Historical background and usage

Valerian is a plant native to Europe and Asia but is now naturalized in north-eastern America (Grieve, 1980). Valerian has been used in traditional medicine since Dioscorides and Galen. It was considered to have a strong influence on the cerebrospinal system, in particular as a sedative in conditions of nervous unrest, stress and neuralgia. Both *V. officinalis* and *V. wallichii* were used in Ayurvedic traditional medicine for hysteria, neurosis and epilepsy. Reference has been made to extensive use of valerian to treat shell-shock after World War I and it was used to promote sleep, particularly by the civilian population, in Britain during World War II. The Eclectics referred to it as a cerebral stimulant used in chorea, hysteria, despondency and low-grade forms of fever where a nervous stimulant was required (Felter & Lloyd, 1983).

Current usage of valerian is mainly as a mild sedative. This property has found application for insomnia, restlessness and nervous tension. It may be useful for the treatment of depression or anxiety, especially in combination with other herbs, specifically St John's wort. Valerian and its oil have also been utilized in perfumery, because of the strong and characteristic odour of the root.

Active constituents

The active constituents of valerian are a complex mixture of related compounds, but most phytotherapeutic interest centres on the sesquiterpenoids and the valepotriates.

The essential oil is stored in the root hypoderm and is a mixture of mono- and sesquiterpenoids. The sesquiterpenes of phytotherapeutic interest are bicyclic and consist of the valerane, kessane and elemene ring systems. The most important constituents are valerenal and valerenic acid, which are based on the valerane ring structure (Fig. 2) (Houghton, 1997).

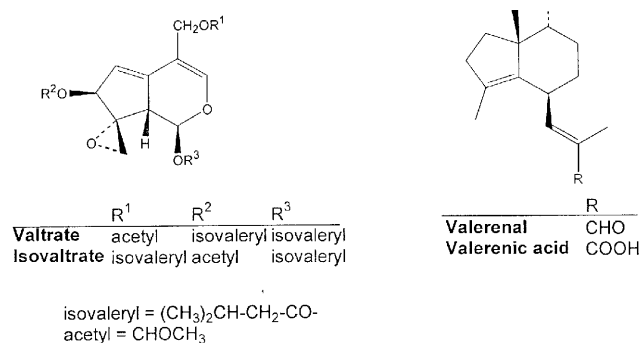


Fig. 2. Structure of valeranes and valepotriates in valerian.

Sesquiterpene acids such as valerenic acid are important for activity but are water soluble and are therefore not components of the essential oil.

Iridoids. Valepotriates are structurally monoterpene iridoids without the sugar moiety (Houghton, 1997). They are thermolabile and decompose under acid or alkaline conditions or in alcoholic solutions (De Smet *et al.* 1997). A wide range of valepotriates have been identified but they can be classed as monoenes (a single double-bond in the ring structure) or the more important dienes (two double-bonds) which include valtrate and isovaltrate (Fig. 2).

The ester linkages of the valepotriates hydrolyse readily to produce baldrinals. This reaction can occur in the gastrointestinal tract or in valerian root or its extracts and further decomposition into inactive products can occur (Schneider & Willems, 1982; Houghton, 1997).

Species differences. Valerenic acid and acetoxyvalerenic acid are found only in *V. officinalis* (Hansel & Schulz, 1982). *V. officinalis* has been reported to contain 4–20 g essential oil/kg. *V. edulis* (Mexican valerian) contains 30–80 g iridoids/kg (including a substantial valtrate and isovaltrate content) and *V. wallichii* (Indian valerian) 30–60 g iridoids/kg (Hansel & Schulz, 1982; Bos *et al.* 1993; Wagner & Bladt, 1996). *V. officinalis* contains a lower level of valepotriates at 5–20 g/kg (Wagner & Bladt, 1996).

Constituent reactivity. There is little information on the pharmacokinetics of valerian or its constituents. The main decomposition products of valtrate and isovaltrate include the metabolites baldrinal and homobaldrinal, whereas the decomposition products of dihydrovaltrate do not include baldrinal-like metabolites (von der Hude *et al.* 1986). Human digestion has the same decomposing effect. This is not an inherent problem since the initial decomposition products of valepotriates are medicinally active (Wagner *et al.* 1980). Oral, intravenous and intraduodenal administration of radiolabelled didrovaltrate in mice demonstrated that it is absorbed to a small extent in the unchanged form (Wagner & Jurcic, 1980).

Laboratory studies

Sedative activity. *Valeriana officinalis* contains several groups of compounds which are responsible for sedative activity. While much early research concentrated on the essential oil (Hendriks *et al.* 1981), it is now believed to contribute only about one-third of sedative activity (Wagner & Bladt, 1996). Valerenic acid and its derivatives have been identified as important sedative components (Hansel & Schulz, 1982). Intraperitoneal administration of valerenic acid showed aspecific central depressant properties in tests on mice. Other isolated sesquiterpenoid compounds did not cause impairment of performance. The activity resembled central nervous depression rather than muscle relaxation or a neuroleptic effect (Hendriks *et al.* 1985).

The valepotriates and their decomposition products also have sedative activity (Wagner *et al.* 1980). Comparison of the action of valepotriates with that of chlorpromazine (an anti-psychotic drug) showed that their sedative effect was weaker, but unlike chlorpromazine they actually improved coordination. Tests of valepotriates on cats showed decrease in anxiety and aggression (Eickstedt & Rahmann, 1969).

Administration of valerian reversed the anxiogenic effect of acute diazepam withdrawal in dependent rats (Andreatini & Leite, 1994) and showed pronounced sedative properties in mice

by reducing motility and increasing sleeping time. Direct comparison with diazepam and chlorpromazine revealed a moderate sedative activity for valerian (Leuschner *et al.* 1993).

Interaction with neurological receptors. Much scientific research on agents with sedative activity examines potential interaction with receptors mediating sedation. Such studies with valerian or its constituents have generated conflicting results.

Aqueous and hydroalcoholic extracts of valerian displaced bound muscimol from synaptic membranes; however, the effect is attributed to the presence of γ -aminobutyric acid (GABA) in the extract (Santos *et al.* 1994; Cavadas *et al.* 1995). The release of GABA in rat brain synaptosomes was found to be stimulated by aqueous extracts but not ethanolic extracts; GABA was present in the aqueous extracts but not in the ethanolic extracts (Ferreira *et al.* 1996). Mennini *et al.* (1993), in an *in vitro* study on receptors in rat brain, claimed that while valepotriates may contribute to the sedative effect it is primarily generated by constituents other than sesquiterpenes and valepotriates.

The ability of extracts of valerian or valerenic acid to displace radiolabelled melatonin from its receptor sites in the human cerebellum has been assessed. Valerenic acid and aqueous preparations of valerian were ineffective but the ethanolic extracts displaced melatonin completely in a dose-dependent manner. Valerian in this study showed no significant affinity for the GABA-A receptor (Fauteck, 1996). The significance of this finding will only become clear with further studies. The active component has not yet been identified and it is not known whether it acts as an agonist or antagonist for the melatonin receptor. While valerenic acid was inactive, valerenic acid derivatives are possibly active.

Other activity. Intraperitoneal administration of valerian root extract or valerenic acid demonstrated anticonvulsant activity against picrotoxin (but not pentetrazol and harman) (Hiller & Zetler, 1996). The effect of an undefined valerian extract on activation, performance and mood of healthy subjects under social stress conditions was found to influence subjective feelings of somatic arousal, despite high physiological activation during a task-oriented environment. No sedative effects were demonstrated which suggested that valerian has thymoleptic activity (Kohnen & Oswald, 1988).

Clinical trials

Sleep quality. In a large uncontrolled multicentre trial involving >11 000 patients, treatment with aqueous valerian extract was rated as successful in treating difficulty in falling asleep (72%), discontinuous sleep (76%) and restlessness and tension (72%) (Schmidt-Voigt, 1986). Similar improvements in sleep patterns have been reported in a number of studies (e.g. Jansen, 1977; Kamm-Kohl *et al.* 1984; Lindahl & Lindwall, 1989; Donath & Roots, 1995).

Subjective assessment by patients of an aqueous extract of valerian that did not contain valepotriates or essential oil, but presumably contained valepotriate decomposition products and valerenic acid, reported improved sleep latency and sleep quality without increasing sleepiness the next morning. The group of subjects who rated themselves as good sleepers were largely unaffected by valerian, but the poor or irregular sleepers reported a significant improvement (Leathwood *et al.* 1982). The greater benefit of valerian for poor sleepers has been confirmed in other studies. Leathwood & Chauffard (1985) found that poor sleepers had a reduced time to fall asleep from 16 to 9 min, with no significant decrease in time for the

predominantly good sleepers. Dressing *et al.* (1992) found that use of a herbal preparation of valerian (with no valepotriates detected) and lemon balm increased sleep efficiency in stages 3 and 4 with poor sleepers showing greater benefit than normal sleepers. A similar trial by Dressing *et al.* (1996) on subjects with light insomnia improved primary sleep quality and accompanying indicators such as time to fall asleep, total duration of sleep, concentration and performance ability.

Gerhard *et al.* (1996) reported that a valerian syrup improved subjective perception of sleep quality but there was a slight but significant impairment of vigilance and a retardation in the processing of complex information. The impairments were, however, less than for subjects taking benzodiazepines.

Depression and anxiety. A number of clinical trials have investigated the combined use of a valerian and St John's wort preparation for the treatment of depression or anxiety. The findings of such trials include a combination of valerian root and St John's wort exhibiting benefits equivalent to the drug amitriptyline (Tryptanol) but without the high frequency of side-effects caused by this drug such as dry mouth and lethargy (Hiller & Rahlfs, 1995). Steger (1985) found this herbal combination achieved significant improvement compared with the antidepressant desipramine as assessed by physicians and Panijel (1985) found it to be more effective than diazepam (Valium) for patients with moderate anxiety but was accompanied by fewer side-effects. A comparable reduction in symptoms of fear and depressive mood were observed in comparison with amitriptyline in patients diagnosed with fear and depression (Kniebel & Burchard, 1988). The lack of side-effects was confirmed in a drug monitoring study of 5682 patients (Quandt, 1994) and Herberg (1994) found that there was no impairment in respect to safety-related performance and well-being and it did not produce any exaggeration of behaviour with simultaneous intake of alcohol.

A summary of the effects of clinical trials is given in Table 2.

Safety issues

Toxicity. Some valepotriates have shown pronounced cytotoxicity *in vitro* (Bounthanh *et al.* 1981) but subsequent research indicated that the valepotriates were not cytotoxic when given orally as they do not survive the acidity of the stomach and formed safe decomposition products (Braun *et al.* 1984). No acute toxicity was found for valtrate, didrovaltrate and acevaltrate after oral administration to mice (Eickstedt & Rahmann, 1969). A study of prolonged administration (30 d) of valepotriates observed that oral doses were innocuous to pregnant rats and their offspring but some toxic effects were observed when valepotriates were administered by the intraperitoneal route (Tufik *et al.* 1994).

Valepotriates developed mutagenic activity only in the presence of S9 mix in the Salmonella/microsome test and the SOS-chromotest while baldrinal and homobaldrinal showed mutagenic effects in both tests and without metabolic activation (von der Hude *et al.* 1986). Valepotriates of the diene type (valtrate, isovaltrate, acevaltrate) showed cytotoxic potential in two human *in vitro* cancer cell lines while valepotriates of the monoene type were 2–3-fold less toxic and the decomposition products, baldrinal and homobaldrinal, were 10–30 times less toxic. Valerenic acids also showed low toxicity.

Overdose. The reported side-effects of excessive intake of valerian are blurred vision, change in heartbeat, excitability, headache, nausea, restlessness and uneasiness (USP Drug

Table 2. Summary of clinical trials with valerian preparations

| Study design and herbal preparation | Results | Reference |
|--|---|--------------------------------|
| Uncontrolled, multicentre (aqueous valerian extract (5–6:1, w/v)) | Successful treatment of difficulty in falling asleep, discontinuous sleep and restlessness in over 70% in each case | Schmidt-Voigt (1986) |
| Double-blind, placebo-controlled (aqueous valerian extract (5–6:1, w/v)) | Improvement in sleep disturbance | Jansen (1977) |
| Randomized, double-blind, placebo-controlled (aqueous valerian extract (5–6:1, w/v)) | Improvement in sleep latency time and sleep quality | Kamm-Kohl <i>et al.</i> (1984) |
| Double-blind, comparative, crossover (valerian, hops, lemon balm, extracts; hops and lemon balm extracts) | Significant improvement in poor sleep for combination containing valerian | Lindahl & Lindwall (1989) |
| Double-blind, placebo-controlled, crossover (dried aqueous ethanolic extract of valerian (3–7:1, w/v)) | Normalized sleep profile, lowered periods of wakefulness and increased the efficiency of the sleep period | Donath & Roots (1995) |
| Placebo-controlled, comparative (freeze-dried aqueous valerian extract (3:1, w/v); proprietary OTC valerian preparation) | Improved sleep latency and sleep quality without increased sleepiness the next morning | Leathwood <i>et al.</i> (1982) |
| Double-blind, placebo-controlled (dried aqueous valerian extract) | Reduction in average time taken to fall asleep, improvement in sleep quality and depth without increased sleepiness the next morning | Leathwood & Chauffard (1985) |
| Placebo-controlled (dried aqueous ethanol extracts of valerian (4.5:1, w/v) and lemon balm) | Significant increase in sleep efficiency in stages 3 and 4; significant increase in delta sleep (which was of benefit to poor sleepers) | Dressing <i>et al.</i> (1992) |
| Randomized, double-blind, placebo-controlled, multicentre (dried aqueous ethanol extracts of valerian (4.5:1, w/v) and lemon balm) | Improvement in sleep quality, daily condition, initiative, change in condition, time to fall asleep, total duration of sleep, concentration and performance ability | Dressing <i>et al.</i> (1996) |
| Placebo-controlled (valerian syrup, valerian and hops tablet) | Improvement in subjective perception of sleep quality | Gerhard <i>et al.</i> (1996) |
| Randomized, double-blind, controlled (valerian and St John's wort concentrate) | Equivalent benefit to the drug amitriptyline for treatment of depression | Hiller & Rahlfs (1995) |
| Double-blind, controlled (valerian and St John's wort concentrate) | Significant improvement in depressive symptoms compared with the drug desipramine | Steger (1985) |
| Double-blind, controlled (valerian and St John's wort concentrate) | Significantly more effective than diazepam for the treatment of moderate anxiety | Panijel (1985) |
| Double-blind, multicentre (valerian–St John's wort combination) | Comparable reduction in symptoms of fear and depressive mood compared with amitriptyline | Kniebel & Burchard (1988) |
| Drug monitoring study (valerian–St John's wort combination) | Low percentage of reported side-effects | Quandt (1994) |
| Double-blind, placebo-controlled, three-way crossover (St John's wort extract; valerian–St John's wort combination) | Both herbal products were shown to be harmless and comparable to placebo with respect to safety regarding performance and well-being | Herberg (1994) |

OTC, over-the-counter.

Information, 1988). Traditional texts also refer to large doses causing headache, stupor, mental excitement, visual illusions, giddiness, restlessness, agitation and even spasmodic movements (Grieve, 1971; Felter & Lloyd, 1983). In the first reported case of valerian overdose, the patient who had consumed twenty times the recommended therapeutic dose presented with mild symptoms, all of which resolved within 24 h (Willey *et al.* 1995).

Quality considerations

Establishment of definite quality standards awaits further work on identifying the exact role of the valerenes and valepotriates and their decomposition products in generating the beneficial effects with human subjects. The current trading of valerian is based on species, especially of *V. officinalis*. Quality standards for *V. officinalis* generally relate to the level of essential oil and the European Pharmacopoeia defines valerian root as containing not less than 5 ml essential oil/kg (European Pharmacopoeia, 1996). Higher quality is considered to be associated with roots containing higher concentrations of essential oil and considerable plant breeding efforts, are being undertaken to increase the level of essential oil. Studies in the Netherlands indicate seasonal variation in the composition of valerian roots, with the highest level of essential oil in autumn-harvested crops while maximum valepotriate concentrations are present in spring-harvested plants (Bos *et al.* 1993). Valerenic acid and acetoxyvalerenic acid are only present in *V. officinalis* (Hansel & Schulz, 1982) and can therefore be used for species identification. Dry extracts of *V. officinalis* standardized to levels of valerenic acid and its derivatives are now widely traded and are almost regarded as definitive preparations by the industry. However, these are the components most likely to survive extraction and drying. As mentioned earlier, the level of valepotriates is generally lower in *V. officinalis* than other species.

The crop is invariably traded in the dried form and the temperature during drying affects the level of active constituents. Valepotriates decompose above 40° to yield valeric and iso-valeric acids; the latter has a characteristic undesirable odour that can be used to indicate poorly dried or stored valerian (Woerdenbag *et al.* 1997). Douglas (1993) reports that maximum retention of valepotriates is achieved by drying without a forced-air flow at 35° and at 60° in a forced-air flow. For essential oil retention, forced-air drying at 40° was recommended.

Few reports have been published on the storage and processing of valerian products. Woerdenbag *et al.* (1997) reported that optimum retention of active constituents during storage of dried root requires low humidity and low temperature conditions. Valerenic acid was found to be stable but the valepotriates decomposed to form baldrinals. Preparation of extracts usually involves dissolution of the active constituents in aqueous ethanol, and Woerdenbag *et al.* (1997) found that valerenic acid and its derivatives were not extracted in < 300 ml ethanol/l and maximum extraction was achieved at >500 ml ethanol/l. Valepotriates were only extracted with >700 ml ethanol/l. Bos *et al.* (1996) reported considerable variation in the levels of valerenic acid and derivatives in thirty-one manufactured tinctures, tablets and capsules available in the Netherlands with values ranging from < 1 to 25 mg/ml (or g). Valepotriates and baldrinals were found only in some capsule products.

St John's wort

St John's wort is a member of the Guttiferae (Clusiaceae) family. The dried aerial parts, usually gathered during the flowering period or shortly before, are used medicinally. St John's wort is

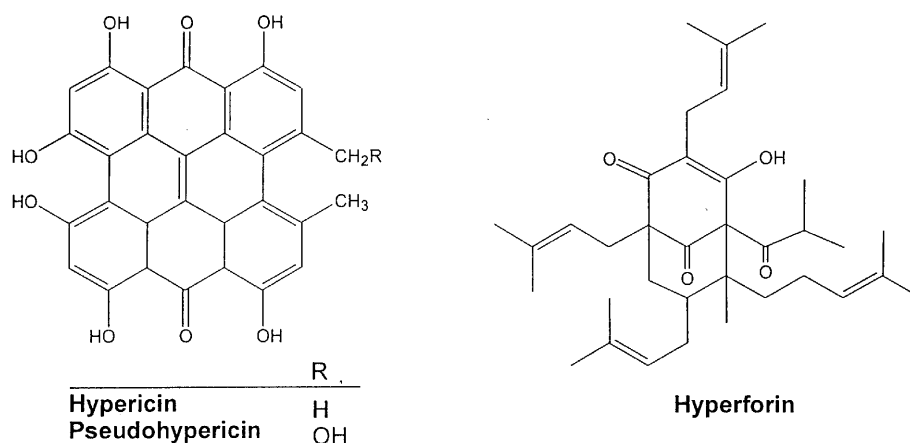


Fig. 3. Structure of hypericin, pseudohypericin and hyperforin.

not a weed in its native regions in Europe, Asia and North Africa, but it has become a weed in most temperate regions of the world.

Historical background

Use of St John's wort as a medicinal plant has been documented since the Middle Ages. In the 19th century it was considered primarily for the nervous system, particularly for nervous afflictions (excitability, menopausal neurosis, hysteria) and disorders of the spine, spinal injuries, neuralgia and sciatica. The ointment and infused oil were used topically on a wide range of wounds including ulcers, swellings, bruises and even on tumours (British Herbal Medicine Association's Scientific Committee, 1983; Felter & Lloyd, 1983).

Since the 1950s, the use of St John's wort as a treatment for depression has become prominent, largely owing to clinical trials conducted in Germany. Initially, liquid preparations standardized to their hypericin and pseudohypericin content were used in trials. Then solid dosage forms standardized to these marker compounds were tested against placebo or conventional antidepressant medications. Recently, the value of hypericin and pseudohypericin as quality marker compounds has been questioned and hyperforin, which was hitherto regarded as unstable, has been proposed as an important constituent with antidepressant activity. Given the rapid increase in the use of St John's wort as an antidepressant, this review will be limited to this context.

Active constituents

*Naphthodianthrone*s. The main naphthodianthrone, hypericin and pseudohypericin (Fig. 3), are present at 0.5–6.0 g/kg, although levels are rarely higher than 2 g/kg in commercial samples (Wagner & Bladt, 1996). These compounds, which consist of two anthrone units joined by three bonds in an eight-ringed structure, are highly conjugated and consequently bright red in colour. Collectively the naphthodianthrone, hypericin and pseudohypericin are called 'total hypericin'.

The naphthodianthrones show a restricted solubility in almost all solvents, but more than 40 % of the amount present is extractable from the crude herb when preparing a tea with water at 60–80° (Niesel & Schilcher, 1990). This increase in solubility suggests the possible presence of factors in the herb which modify the solubility of the naphthodianthrones. Accordingly, K salts of hypericin and pseudohypericin have been identified as 'soluble' pigments in *Hypericum* species (Falk & Schmitzberger, 1992).

Acylphloroglucinols. These compounds are characteristic of species of the Guttiferae. In St John's wort, hyperforin (Fig. 3) is the major of the two compounds reported (the other is adhyperforin which is present at much lower levels) (Erdelmeier, 1998). The acylphloroglucinols are light- and O₂-sensitive and are particularly unstable in lipophilic solvents such as hexane (Erdelmeier, 1998). Both hyperforin and adhyperforin occur exclusively in the reproductive parts of the plant and their levels are highest in the fruits, which can contain up to 50 g hyperforin/kg (Erdelmeier, 1998).

Other constituents. Other constituents which may have activity, or contribute to or modify the activity of the above components, include flavonoids, procyanidins (Melzer *et al.* 1991) and essential oil (Franchomme & Penoel, 1990). These possibilities are illustrated by the study cited in the introduction, which found that procyanidins increased the bioavailability of hypericin (Butterweck *et al.* 1989).

Laboratory studies

Information from *in vitro* studies on active constituents is inconclusive. Hypericum extract and hypericin inhibited dopamine- β -hydroxylase *in vitro* (Obry, 1996). Hypericin also potentiated neurotransmitter binding at the GABA-A, benzodiazepine and serotonin receptors (Curle *et al.* 1996). The non-hypericin fraction of hypericum inhibited monoamine oxidase (EC 1.4.3.4)-A *in vitro*, unlike hypericin and the flavanols (Demisch *et al.* 1989; Holzl *et al.* 1989). Amentoflavone demonstrated binding activity at the benzodiazepine receptor *in vitro* (Nielsen *et al.* 1988). One group of researchers, on the basis of their extensive *in vitro* and *in vivo* studies, has suggested that hyperforin significantly contributes to the antidepressant activity (Chatterjee *et al.* 1998; Dimpfel *et al.* 1998).

Studies on the whole extract of hypericum have revealed the following results which may reflect on antidepressant activity, although the current understanding of the relative importance of these mechanisms is uncertain: (1) inhibition of synaptic uptake of noradrenaline, serotonin and dopamine *in vitro* and inhibition of GABA re-uptake (Perovic & Muller, 1995; Muller *et al.* 1997, 1998). It is unusual to find this action on all three uptake systems (Muller *et al.* 1997). Hyperforin was identified as the compound conferring this activity (Muller *et al.* 1998); (2) downregulation of β -adrenoceptor and serotonin receptor density in the cortex after subchronic administration *in vivo* (Muller *et al.* 1998). The downregulation of these receptors *in vivo* is expected on subchronic administration of antidepressants and is not incompatible with inhibition of uptake observed *in vitro*. A hyperforin-enriched fraction led to significant β -receptor downregulation after subchronic treatment (Muller *et al.* 1998); (3) upregulation of central serotonergic receptors from cerebral tissue *in vivo*, which is consistent with effects caused by some synthetic antidepressants (Muller *et al.* 1997; Teufel-Mayer & Gleitz, 1997); (4) inhibition of catechol-O-methyltransferase (EC 2.1.1.6) *in vitro* (Thiede & Walper, 1993), and inhibition of dopamine- β -hydroxylase (EC 1.14.17.1) *in vitro* (Kleber *et al.* 1999); (5)

suppression of interleukin 6 in blood samples *ex vivo* (Thiele *et al.* 1993). This suppression may assist in deactivating the hypothalamic–pituitary–adrenal axis, leading to inhibition of elevated corticotropin releasing factor and other adrenal regulatory hormones. These changes could be linked to antidepressant activity (Nemeroff, 1998); (6) inhibition of monoamine oxidase-A and monoamine oxidase-B activity *in vitro*, although this inhibition was found to be weak (Muller *et al.* 1997).

Clinical trials

Hypericin, pseudohypericin (Staffeldt *et al.* 1993; Kerb *et al.* 1996) and hyperforin (Biber *et al.* 1998) have been shown to have good bioavailability from oral doses of St John's wort extract in tablet form.

Many clinical trials have been conducted over the past 17 years, mainly using some form of standardized hypericum extract (equivalent to 0.4–2.7 mg total hypericin/d). There has been a tendency in more recent years to use higher doses of total hypericin (2.7 mg/d, about 5 g of herb and recently even 5.4 mg/d).

Criticisms levelled at the trials conducted from 1979 to 1995 include: few trials conducted on severely depressed subjects, relapses occurring within 1 year after cessation of the studies were not registered, dose–response studies were not performed with patients and, in trials comparing standard antidepressant medications, the doses were too low and the number of patients too small (Linde *et al.* 1996; Reuter, 1997).

Table 3. Summary of clinical trials with St John's wort preparations

| Study design and herbal preparation | Results | Reference |
|---|--|-------------------------------|
| Randomized, double-blind, controlled, multicentre (St John's wort standardized extract) | Equivalent efficacy as the drug fluoxetine in reducing mild and moderate depressive episodes in elderly patients | Harrer <i>et al.</i> (1999) |
| Randomized, double-blind, placebo-controlled, crossover, multicentre (St John's wort standardized extract) | Significant improvement in depressive symptoms | Hansgen <i>et al.</i> (1994)* |
| Randomized, double-blind, placebo-controlled (St John's wort standardized extract) | Significant improvement in depressive symptoms | Halama (1991)* |
| Randomized, double-blind, placebo-controlled (St John's wort standardized extract) | Significant improvement in depressive and somatic symptoms (such as disturbed sleep) | Hubner <i>et al.</i> (1994)* |
| Randomized, double-blind, placebo-controlled, multicentre (St John's wort standardized extract) | Significant improvement in depressive and somatic symptoms (such as disturbed sleep) | Quandt <i>et al.</i> (1993)* |
| Randomized, double-blind, placebo-controlled (St John's wort standardized extract) | Significant improvement in depressive symptoms | Sommer & Harrer (1994)* |
| Randomized, double-blind, controlled, multicentre (St John's wort standardized extract) | Similar benefit as the drug maprotiline in reducing depressive symptoms, but with longer time of onset and fewer side-effects | Harrer <i>et al.</i> (1994)* |
| Randomized, double-blind, placebo-controlled (St John's wort standardized extract (5 g hyperforin/l); St. John's wort standardized extract (50 g hyperforin/l)) | Significant reduction for the Hamilton depression score compared with placebo for the group taking the 50 g hyperforin/l extract | Laakmann <i>et al.</i> (1998) |

* Trials which scored $\geq 80\%$ for methodology in a meta-analysis of randomized clinical trials of St John's wort for depression (Linde *et al.* 1996).

A critical analysis of twenty-three randomized clinical trials including a total of 1757 out-patients has shown that hypericum extracts are more effective than placebo for the treatment of mild to moderately severe depressive disorders (Linde *et al.* 1996). Fifteen trials with 1008 patients were placebo-controlled and eight with 749 patients compared hypericum with standard antidepressant drugs (maprotiline, imipramine, bromazepam, amitriptyline, desipramine). Of the eight trials comparing hypericum with antidepressant drugs, six used single preparations and two used a combination of hypericum and valerian. Three of the trials used hypericum in combination with other plant extracts, one trial was single-blind, two were open and the remainder were double-blind.

Most trials had reasonably good methodology, with ten trials scoring 80% or more of the possible points in both assessment systems used. The daily dose of total hypericin varied considerably between 0.4 and 2.7 mg, as did the duration of treatment (2 to 12 weeks). Hypericum extracts were significantly superior to placebo, with mean scores on the Hamilton depression scale 4.4 points better for patients treated with hypericum (in the nine trials providing data for analysis). Results from comparative trials suggest that hypericum may work as well as standard antidepressants, as indicated by the scores on the Hamilton depression scale after treatment. In these trials with standard antidepressants, however, the evidence was insufficient to form definite conclusions owing to the limited number of patients in the trials.

In the six trials comparing single hypericum preparations with standard antidepressants, side-effects occurred in 20% of patients taking hypericum extracts compared with 36% of patients on standard antidepressants. The authors concluded that further studies are required, with the type of depression amongst subjects better delineated. They also suggested that comparison of studies using different preparations of hypericum is problematic, even when standardized for total hypericin, as the preparations may vary in other substances contributing to the antidepressant effect (Linde *et al.* 1996).

The safety and efficacy of two hypericum extracts (standardized to 5 and 50 g hyperforin/l respectively) were compared in 147 patients with mild to moderate depression in a randomized, double-blind placebo-controlled trial (Laakmann *et al.* 1998). After 6 weeks of treatment, only the group taking the 50 g hyperforin/l extract showed a significant reduction for the Hamilton depression score when compared with placebo. However, an analysis of two different clinical trials suggested that St John's wort extracts with low amounts of hyperforin demonstrated a clinical efficacy which was comparable to that of an extract containing a much higher quantity of this compound (Friede *et al.* 1998).

A summary of the results of clinical trials is given in Table 3.

Quality issues

Until quite recently, research has focused on extracts which have been characterized by their total hypericin content. The currently adopted pharmacopoeial method for measuring total hypericin is the spectrophotometric method published in the German Pharmaceutical Codex in 1991 (DAC 91). This method employs the natural absorption of light at 590 nm by hypericin and pseudohypericin and was adapted from an earlier method published in the 1986 edition of the codex (DAC 86). The extinction coefficients used to calculate the level of total hypericin vary by about 20% between the two methods: the DAC 91 method gives a result which is 20% lower than that of the DAC 86 method. However, compared with results by HPLC, even the DAC 91 method applied to extracts overestimates the level of hypericin plus pseudohypericin by 30% on average (Gaedcke, 1997).

These discrepancies between test methods could in part explain the inconsistent levels of total hypericin found in a survey of ten St John's wort products on the US market analysed by the DAC 91 method (Monmaney, 1998). Five products contained levels of about 80 % of that claimed on the label, suggesting perhaps some had been benchmarked to the DAC 86 method. Another two products were found to contain 130–140 % of that claimed, suggesting they had been standardized using good HPLC techniques. However, three products, which included leading brands, contained only 20–50 % of the total hypericin claimed on the labels.

The danger of focusing on only one group of active components as the determinant of quality has been highlighted in the case of St John's wort. Dry extracts from China are available on the market which are standardized to the accepted 30 g total hypericin/kg. However, TLC fingerprint analysis shows that some of these products are not manufactured from St John's wort. Presumably they are sourced from other species of hypericum with unknown antidepressant activity.

The recent information that hyperforin is important for antidepressant activity is further evidence of such over-reliance on one chemical group. Hyperforin is an ideal quality marker because it is unstable in light and sensitive to oxidation (Erdelmeier, 1998). Only carefully prepared extracts of properly shade-dried plant material will quantitatively retain this compound. However, the same error should not be made for hyperforin as for hypericin. Selection of this compound in preference to other components by late harvesting and selective extraction is not justified on current evidence. State-of-the-art knowledge would suggest that St John's wort extracts quantified to deliver optimum quantities of hypericin, pseudohypericin and hyperforin as determined by HPLC, represent the preferred material for delivering the antidepressant activity of this herb.

Several of the trials which scored 80 % or more for methodology in the meta-analysis of randomized clinical trials of St John's wort for depression (Linde *et al.* 1996) are cited in Table 3.

Future issues and conclusions

Use of herbal medicines in the Western world is now a significant factor in modern health care. The rapid and continued growth in the herbal market has been stimulated in part by a greater scientific understanding of how herbs work and clinical proof of their efficacy. Although current information from clinical trials is not perfect, for example only some of the many commonly used herbs have been studied and most studies involved small subject numbers, it has justified a growing interest in herbal therapy by the more adventurous conventional health care providers. Better and more clinical trials, in particular of herbal products which are phytochemically well-defined, should accelerate this trend to herbal treatments.

Development of the herbal industry has also been supported by changing community attitudes. The desire of urbanized populations for a more natural lifestyle and greater personal control over health care has heightened interest in and usage of alternate therapies including medicinal herbs. Modern consumers are, however, demanding in their expectations of product safety, efficacy and reliability. Long-term satisfaction of these expectations will require a greater understanding of the complex quality issues and how they impinge on the production of good herbal medicinal preparations. Modern analytical techniques can now provide a wealth of phytochemical information about individual compounds in herbal raw materials and products. This information needs to be interpreted in terms of how they confer pharmacological activity,

their pharmacokinetics and how they interact with each other. This particularly applies to herbs which are in widespread use.

Stability and consistency of processed and manufactured products are issues which are often neglected in the herbal arena with relatively few studies published on these topics. Again, modern analytical techniques, in conjunction with the above information, should enable the development of meaningful criteria for the shelf-life of herbal products and ensure processing techniques retain herbal efficacy in endproducts. Some information will be reassuring to the consumer. A key issue in consumer reassurance is product labelling. Processed foods and pharmaceutical products have highly regulated compliance regimens for ingredient labelling. Medicinal herbs traditionally traded as a raw plant product were exempt from product labelling but, with the trend to processed products and standardized extracts, consumer pressure is leading to serious scrutiny in many countries to have labelling based on active-ingredient concentrations. A barrier to such labelling is the lack of definitive studies on the key active ingredients in many medicinal herbs.

Future research to overcome the current deficiencies would seem to require a more collaborative approach between chemists and pharmacologists with studies also taking into account the effects of manufacturing processes. Such collaboration would also seem to extend to plant breeders and agronomists as herbs become more widely cultivated. Maintaining medical efficacy in plants must be considered even more important than increasing crop yield.

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