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Pre- and post-harvest processing of medicinal plants

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Abstract

Herbal medicine is used worldwide either as a sole treatment method or as part of a comprehensive treatment plan alongside orthodox methods of diagnosis and treatment. A survey reported that, in the USA, nearly one-sixth of women took at least one herbal product in 2000. Despite their widespread use, numerous reports show that the herbal products available to consumers are of variable quality. This disparity in quality of herbal preparations can be attributed to the fact that their production is complicated. To produce high-quality herbal products, attention must be paid to, among others, phytochemical variations due to plant breed, organ specificity, stages of growth, cultivation parameters, contamination by microbial and chemical agents, substitution, adulteration with synthetic drugs, heavy metal contamination, storage and extraction. This review focuses on organ specificity, seasonal variations, the effect of drying and storage, and the extraction of phytochemical constituents. Special emphasis is placed on the four most frequently used herbal products in the USA: echinacea, *Ginkgo biloba*, ginseng and St John's Wort.

Keywords: drying; echinacea; extracting; *Ginkgo biloba*; ginseng; herbal products; medicinal plants; St John's Wort; storing

Introduction

Complementary and alternative medicine is gradually being integrated into comprehensive treatment plans alongside orthodox methods of diagnosis and treatment (Fong, 2002). As an example, nearly one-sixth of US women took at least one herbal preparation in 2000, with the four most frequently used supplements being echinacea, ginkgo, ginseng and St John's Wort (Yu *et al.*, 2004). Similarly, a survey of University of Illinois' Chicago clinic clients showed that 79% of the survey respondents used or had used herbal preparations, with the commonly used products being soy, green tea, chamomile, ginkgo, ginseng, echinacea and St John's Wort (Mahady *et al.*, 2003). Unfortunately, scientific reports show that botanical preparations are often of variable quality. Harkey *et al.* (2001) tested 25 commercially available ginseng preparations, of which six of the tested products had ginsenoside concentrations below 1%. Gilroy *et al.* (2003) quantitatively assessed the content of 59 echinacea products. The cichoric acid content of the tested samples was between 0% and 1.46%. Of the tested 59 echinacea products, only seven products contained between 0.18% and 0.82% echinacoside (Gilroy *et al.*, 2003). The total hypericin content of commercial St John's Wort preparations, taken as hypericin and pseudohypericin, was between 2.9 and 114% of the claimed

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content (Wang *et al.*, 2004). Although not mentioned in this review, other popular supplements also show inconsistency in their phytochemical composition. Why are the consumer available products so unequal in their quality? To answer this question, one must examine the process of their preparation: from cultivation to extraction. Quality problems in finished products can be due to: use of the wrong species, lack of organ specificity, harvesting at a non-optimum stage of growth, non-optimum cultivation parameters such as soil, light, water, temperature and nutrients, contamination by microbial and chemical agents, non-optimum drying and storage, non-optimum extraction strategy and long-term finished product storage. Quality problems in finished products are further complicated by the fact that in many cases the phytochemical constituents responsible for the claimed activity of the herbal product are often unknown or only partly explained. However, to prepare reproducible phytochemical products successfully, all of the above-described operations need to be conducted according to certain protocols. This review will focus on the operations dealing with specificity of the plant part, the seasonal variations, the effect of drying and storage, and extraction, and how these factors can affect the final phytochemical composition of a preparation.

Pre-harvesting

Harvesting specific plant parts

Phytochemical constituents are not evenly distributed throughout the plant. Kabganian *et al.* (2002a) showed that alkamides and echinacoside were predominantly located in the bark of the *Echinacea angustifolia* root. The alkamide and echinacoside concentrations of root bark were 6.1 ± 0.8 and 12.2 ± 0.8 mg/g dry weight, respectively, while those of the tap root devoid of bark were 1.3 ± 0.1 and 1.6 ± 0.2 mg/g dry weight, respectively. Willis and Stuart (2000) reported that the cichoric acid content of *E. purpurea* roots was 22.1 ± 3.9 mg/g dry weight, while that of the flowers was 26.1 ± 3.8 mg/g dry weight. Assinewe *et al.* (2003) reported that *Panax quinquefolius* roots contained 5.78% total ginsenosides, while leaves contained 3.33%. The higher root ginsenoside concentration was significantly higher ($P < 0.05$). The average total ginsenoside contents in mg/g dry weight of *P. quinquefolius* mature leaves and of 4-year-old roots were 41.8 ± 4.7 and 30.0 ± 5.0 mg/g dry weight, respectively (Li *et al.*, 1996). The percentage hypericin content of *Hypericum perforatum* (St John's Wort) stem, leaves, flower buds and flowers were 0, 0, 21 and 79%, respectively (Seidler-Lożykowska, 2003). Burdock (*Arctium lappa* L.) root, which contains chlorogenic acid and caffeic acid, is cultivated and consumed for its diuretic and antipyretic properties. Chen *et al.* (2004) showed that freeze-dried extracts of the whole and peeled root showed different results in the 2,2-diphenyl-1-picrylhydrazyl (DDPH) free radical scavenging assay. Peeled root showed about half the free radical scavenging activity and 30% less chlorogenic acid content than its whole counterpart. Therefore, in addition to the correct species, the appropriate plant part must be harvested to ensure proper phytochemical preparation.

Influence of the developmental stage on the phytochemical concentration

Gray *et al.* (2003b) documented the hyperforin and hypericin concentrations as a function of the developmental stage of *H. perforatum*. Respective hyperforin and hypericin concentrations of 27.4 and 1.5 mg/g dry weight were obtained for plants during the period of initial flower bloom. *H. perforatum* plants, during the seed production period, yielded hyperforin and hypericin concentrations of 35.0 and 0.9 mg/g dry weight, respectively. Differences in hyperforin and hypericin concentrations between the developmental stages were statistically significantly different (ANOVA, α 0.05) (Gray *et al.*, 2003b). Hevia *et al.* (2002a) reported significantly different hypericin concentrations of 3 and 1 mg/g dry weight, respectively, for *H. perforatum* cultures at the flower bud and dehiscing stages (LSD, α 0.05). The essential oil yields of *H. perforatum* at the vegetative, floral budding, flowering and fruiting stages were 0.070, 0.082, 0.092 and 0.058 (% w/w), respectively (Schwob *et al.*, 2004). In the floral budding, flowering and fruiting stages, sesquiterpene hydrocarbons were the dominant detected compounds (Schwob *et al.*, 2004). Although the fruiting stage yielded less essential oil on a percentage per weight basis, it offered the most qualitatively rich composition.

Although not quantitatively described, O'Reilly (2000) indicated that the flavonol glycosides, ginkgolide A and bilobalide content of *Ginkgo biloba* leaves decreased during the growing season, the ginkgolide B concentrations remained relatively constant, and the concentration of ginkgolide C increased. The age and the sex of the ginkgo affect the phytochemical concentrations. In the Netherlands, three *G. biloba* trees differed in their ginkgolide A concentrations. A young nursery, a 60-year-old female and a 60-year-old male ginkgo showed maximum leaf ginkgolide A concentrations of 0.30 ± 0.03, 0.05 ± 0.00 and 0.01 ± 0.00 mg/g dry weight, respectively (van Beek and Lelyfeld, 1992). Ginsenoside concentrations also vary as a function of plant

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development. Soldati and Tanaka (1984) reported ginsenoside concentrations in *Panax ginseng* roots to be 10, 50 and 90 mg/g dry weight for 3-, 5- and 6-year-old roots, respectively. In British Columbia, leaves from commercially grown 4-year-old *P. quinquefolius* plants contained 18.6 ± 4.3 mg/g dry weight of ginsenosides in June, compared to 41.8 ± 4.7 mg/g dry weight ginsenosides in September (Li *et al.*, 1996).

As with ginkgo and St John's Wort, echinacea also displays a change in phytochemical concentration as a function of the stage of development. Stuart and Willis (2000) reported that the cichoric acid content of pre-flowering, flowering, mature and senescent *E. purpurea* roots was 34.6, 80.4, 230.0 and 375.6 mg/g dry weight (LSD, $P < 0.05$ of 68.1), indicating a preponderance for a fall harvest. Gray *et al.* (2003a) reported cichoric acid concentrations of 5.0 ± 0.27 and 8.7 ± 0.29 mg/g dry weight for 2 and 3 year *E. purpurea* roots, respectively. Seidler-Łożykowska and Dabrowska (2003) reported polyphenolic acid yields of 3.0 and 3.4% in 1- and 2-year-old *E. purpurea* roots, respectively. With respect to flower heads, both Letchamo *et al.* (1999) and Stuart and Willis (2000) reported a decrease in the content of cichoric acid as a function of flower head development. With respect to alkamides, Stuart and Willis (2000) reported a decrease in alkamide as a function of *E. purpurea* root development, with alkamide concentrations of 11.7 mg/g dry weight and 9.0 mg/g dry weight for flowering and senescent stages, respectively (LSD, $P < 0.05$ of 2.2). In the Southern hemisphere, *E. angustifolia* roots harvested in November yielded 9.17 mg/g dry weight, while those harvested in April yielded 7.07 mg/g dry weight (Berti *et al.*, 2002). Gray *et al.* (2003a) studied alkamide content as a function of crop age. The mean total alkamide content of 2-year-old *E. purpurea* root was 6.8 ± 0.46 mg/g dry weight, while that of a 3-year-old root was 2.7 ± 0.21 mg/g dry weight. Although, 2-year-old *Echinacea* roots have higher alkamide concentrations, a higher total alkamide mass will be obtained with the harvesting of a 3-year-old root crop because of the increased root mass (Powell *et al.*, 2001). Flower heads, on the other hand, showed an increase in the content of alkamides as a function of different developmental stages (Letchamo *et al.*, 1999; Stuart and Willis, 2000).

Medicinal plants other than echinacea, St John's Wort, ginkgo or ginseng also show variations of their phytochemical concentrations as a function of stages of development. *Arctostaphylos uva-ursi* was grown in four sites ranging in altitude from 1740 to 2030 m, from which the berries were collected in the autumn and in the spring. In the autumn, the arbutin content in the berries was up to 36%, and 1.5% greater than that of the spring collection (Parejo *et al.*, 2002). The total content of essential oil of chamomile (*Chamomilla recutita*) crops cultivated in southern Italy and harvested 14 days apart was similar; however, their essential oil components were different. Harvesting the Bona cultivar 14 days later resulted in a 6% increase in α-bisabolol and a 433% decrease in α-bisabololoxide (D'Andrea, 2002). Tamura and Nishibe (2002) monitored the content of the iridoid glucosides catalpol and aucubin and of the phenylethanoid glucoside acteoside in plantain (*Plantago lanceolata* L. cv. Grasslands Lancelot) leaves over a period of 126 days. Levels of aucubin and acteoside were at their maximum on days 98 and 126, respectively, while the level of catalpol remained essentially constant over the course of the experiment. Carrier *et al.* (2002) studied flower head development and silymarin changes in milk thistle (*Silybum marianum* (L.) Gaertner) during two growing seasons. Flower heads were arbitrarily divided into four developmental stages. The average plant produced 2.1 ± 0.1, 3.5 ± 0.7 and 2.6 ± 0.6 g dry weight of seeds per flower head during developmental stages two, three and four, respectively. The average silymarin and fixed-oil content of stage two, three and four seeds were 1.4 ± 0.9, 15.7 ± 2.8 and 26.5 ± 4.0 mg of silymarin/g dry weight and 3.6 ± 3.0, 21.6 ± 6.9 and 26.0 ± 1.1% lipid, respectively. In *Cassia podocarpa*, the total anthraquinone concentrations of peak flowering leaves and peak fruiting bark were 2.4 (% w/w) and 0.21 (% w/w), respectively (Abo and Adeyemi, 2002). In conclusion, medicinal plants need to be harvested at specific times, and each plant has its own optimum harvest time.

Drying and storage

Drying

Freshly harvested medicinal plants occupy large volumes and pose difficulty in transportation and storage. For handling and storage purposes, reducing the water content of freshly harvested medicinal plants is imperative. By reducing the water content, the material becomes easier to handle and less prone to microbial degradation. The water content is usually removed through thermal drying. The methods available for drying medicinal plants can be grouped into natural and mechanical drying on the basis of heat source or energy utilization (Cai *et al.*, 2004). In both processes, water present in the interior of the medicinal plant must move to the surface by internal diffusion. A large plant surface area combined with air movement favours quick evaporation of water from the plant's surface. The rate at which water is evaporated from the surface of the plant must be balanced with the rate at which water is removed from the interior. Unbalanced evaporation rates can result in deterioration of the final product. The reasons for

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drying grain crops also apply to medicinal plant drying: the aim is to control the moisture content of the medicinal plant crop to an acceptable level, which permits quality delivery of the herb to the place of ultimate use, and likely ensures long-time storage with little deterioration. However, natural or mechanical drying can be catastrophic to medicinal plants if not properly conducted, as extensive high-temperature drying can cause both physical and chemical changes.

In natural drying, exposure to the sun and/or the desiccating effect of air currents promotes the removal of water from the material. Downs and Compton (1955) noted that natural air-drying is easy to control and seldom damages the crop. Natural drying is a popular method of medicinal plant drying, especially in areas where maturity and harvesting of the plants coincides with the beginning of the dry season, and their phytochemicals are not photosensitive. Cinnamon (*Cinnamonum cassia* Prel.) bark is usually sun-dried after harvesting in July and August when the quality of the bark is high (Cai *et al.*, 2004). However, Downs and Compton (1955) reported that the reliance on favourable weather conditions limits the use of natural drying. Weather dependence is somewhat counteracted by solar dryers. The use of plastic house-type solar dryers was successfully reported in drying of spices and medicinal plants (Muller *et al.*, 1993). Plastic house-type solar dryers were reported to be more economical and efficient than conventional drying systems, provided that supplementary heating was used. Wisniewski (1997) reported that, in Poland, about 60 commercially cultivated medicinal plants crops were dried, mostly, in flat-type solar dryers.

Mechanical drying includes freeze drying, artificial drying, microwave drying, far infrared drying, vacuum drying and spray drying. Freeze drying, also known as lyophilization or sublimation, consists of removing water by ice sublimation without passing through the liquid state. Under a high vacuum, ice is converted into water vapour and removed, leaving the material in a dry state. Freeze drying is an ideal preservation method, but is very expensive and lends itself only to the drying of high-value products. Artificial drying uses fuels such as charcoal, natural gases, firewood or electricity to heat incoming air. With the use of artificial dryers, both labour and drying time of medicinal plants are reduced. Artificial drying systems include, among others, plate chamber and conveyor dryers. In plate chamber dryers, warm air blows across plates on which plants are placed and usually has a low handling capacity, but is particularly useful for drying flowers and leaves. Plate chamber-style dryers usually count on manual labour for biomass handling. In conveyor dryers, fresh plants travel on a conveyor belt through a countercurrent flow of warm air with drying times and drying temperature ranges of 2.5–6 h and 40–80°C, respectively. Conveyer dryers are usually characterized by high throughput with relatively little labour input, but have high capital and energy costs.

The drying of medicinal plants is necessary for preservation purposes, but drying protocols must be designed such that they do not result in a decrease in phytochemical concentrations. *Echinacea* biomass is touted for its alkamide, polysaccharide and caffeic acid content (Bauer and Wagner, 1991). Alkamides seem to be resistant to artificial drying. The alkamide concentration of chopped 33°C oven-dried *E. purpurea* roots was 5.05 ± 0.78 mg/g dry weight, compared to 5.24 ± 0.89 mg/g dry weight for fresh unchopped roots (Perry *et al.*, 2000). Similar results were reported for whole *E. angustifolia* roots, where the alkamide concentrations were 3.8 ± 0.2 and 3.3 ± 0.4 mg/g dry weight when dried at temperatures of 23 and 60°C, respectively (Kabganian *et al.*, 2002b). Stuart and Willis (2003) also reported no effect of drying temperature on root alkamide concentration. However, Hevia *et al.* (2002b) reported mean alkamide concentrations of 0.5 and 0.6 mg/g dry weight for whole *E. purpurea* roots dried at 80 and 40°C (LSD, $P < 0.05$), respectively, indicating that drying at 80°C may be too high, even for alkamides. Echinacoside and chlorogenic acid appear to be heat sensitive. Li and Wardle (2001) reported an 18% decrease in echinacoside concentrations of *E. angustifolia* roots when increasing the drying temperature from 35 to 45°C. Increasing the drying temperature from 23 to 60°C reduced the original echinacoside concentration of *E. angustifolia* roots by 45% (Kabganian *et al.*, 2002b). The chlorogenic acid concentrations of *E. angustifolia* roots were 1.71 and 0.97 mg/g dry weight for 35 and 45°C drying regimes, respectively (Li and Wardle, 2001). The case is not as clear for cichoric acid. The drying of *E. purpurea* roots at 35 and 45°C resulted in increases in the cichoric acid concentrations from 12.72 to 15.25 mg/g dry weight, respectively. The values for cichoric and chlorogenic acids were significantly different (Duncan, $P = 0.05$) (Li and Wardle, 2001). However, Hevia *et al.* (2002b) reported a significant decrease in the mean cichoric acid concentrations from 11.4 to 6.2 mg/g dry weight (LSD, $P < 0.05$) for whole *E. purpurea* roots when increasing the drying temperature from 40 to 80°C, respectively. Li and Wardle (2001) monitored the effect of drying temperature on polysaccharides, and determined that the overall polysaccharide content of *E. angustifolia* roots was reduced by 15% when increasing the drying temperature from 35 to 45°C. On the other hand, the polysaccharide content of *E. purpurea* roots increased by 16% when the drying temperature was increased from 35 to 45°C (Li and Wardle, 2001).

Commercial drying of ginseng usually involves the use of forced air (Davidson *et al.*, 2004). Drying of *P. quinquefolius* at temperatures above 40°C resulted in decreases in the ginsenoside concentrations, texture,

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colour and flavour, and in addition caused severe shrinkage (Van Dalfsen *et al.*, 1995; Reynolds, 1998; Shokhansanj *et al.*, 1999). Li and Morey (1987) recommended ginseng drying at low temperatures when its moisture content was less than 28% (wet basis) to prevent excessive surface and internal colour darkening. Davidson *et al.* (2004) dried ginseng roots using a three-stage (38°C–50°C–38°C) temperature control process. They first dried the ginseng roots at a temperature of 38°C until the moisture content of the roots was in the range of 50–55% (wet basis). The drying air temperature was then increased to 50°C and maintained at that temperature until the moisture content of the roots was in the range of 18–20% (wet basis). Finally, the air temperature was decreased to 38°C and maintained until the moisture content of the roots was 8–10% (wet basis). When compared to a 38°C single-stage process, the use of this three-step process reduced the drying time by 40% with the total ginsenoside concentrations of both drying regimes at about 65 mg/g dry weight (Davidson *et al.*, 2004). However, the drying of ginseng did result in a decrease in the total ginsenoside concentrations. Davidson *et al.* (2004) reported significantly different total ginsenoside concentrations of 87 and 66 mg/g dry weight (Duncan, $P = 0.05$) for fresh and 38°C dried ginseng, respectively.

O'Reilly (2000) reported that fresh ginkgo leaves contain 70–80% moisture and must be dried within hours of harvesting to prevent mould growth. Unfortunately, no details were given as to the temperature regime used for drying and its effect on terpene and flavonoid concentrations. Other plants, in addition to St John's Wort, ginkgo, echinacea and ginseng, can display phytochemical concentration decreases during drying. Plantain (*Plantago lanceolata* L.) phytochemicals were shown to be sensitive to drying treatments. As compared to fresh biomass, the plantain phytochemicals catapol, aucubin and acteoside decreased by 50, 25 and 29%, respectively, when dried for 8 h at 60°C (Tamura and Nishibe, 2002). For flavonoids from *Passiflora alata*, increasing the inlet gas temperature of the spray dryer from 115 to 150°C resulted in a 12% increase in flavonoid degradation (Runha *et al.*, 2001). The artemisinin concentrations of 35°C oven-dried and field-dried *Artemisia annua* L. leaves were 4.5 and 8.7 mg/g dry weight, respectively (Laughlin, 2002). In the case of feverfew (*Tanacetum parthenium*) leaves, Tanko *et al.* (2003) reported that increasing the drying temperature from 30 to 60°C in a thin-layer dryer did not result in a parthenolide concentration decrease. The parthenolide concentrations were 18.3 ± 1.6 and 22.5 ± 3.2 mg/g dry weight when dried with air at temperatures of 30 and 60°C, respectively (Tanko *et al.*, 2003). However, drying at temperatures greater that 60°C may be deleterious to parthenolide. Rushing *et al.* (2004) reported significantly different (LSD, $P < 0.05$) parthenolide concentrations of 42 and 30 mg/g dry weight when dried with air at 40 and 90°C, respectively.

Storage

The main aim of storing medicinal plants is to prevent the deterioration of their quality. This is done indirectly through the control of moisture and air movement and by preventing attack by insects, rodents and microorganisms. During storage, the main objective is to reduce metabolic activity, rendering the medicinal plants less susceptible to deterioration. This can be achieved by either reducing the moisture content to a safe level and cooling the plants or by modifying the atmospheric conditions of the system where the medicinal plants are stored. Khalid *et al.* (1988) studied the preservation of *Ziziphus vulgaris* (Unnab), *Viola odorata* (Banafshah) and *Cordia latifolia* (Sapistan), and reported that hydrogen phosphide (Phosphine) could be used to preserve dried herbs from insect invasion. The phytochemical concentrations of some medicinal plants may be affected by storage. Willis and Stuart (2000) studied the alkamide and cichoric acid content of ground and dried *E. purpurea* roots for a 60-day period. Interestingly, storage for 60 days in the dark at 5°C had no effect on the alkamide concentrations, but caused a 70% decrease in the cichoric acid concentrations. In a reverse fashion, storage of the ground roots in the light at 20°C did not decrease the cichoric acid concentrations, but decreased the alkamide concentrations by 65% (Willis and Stuart, 2000). Perry *et al.* (2000) studied the stability of alkamides in chopped *E. purpurea* roots that were stored for a 64-week period. The total alkamide concentration of chopped *E. purpurea* roots stored at 24°C for 16 weeks was 3.28 ± 0.88 mg/g dry weight, and decreased to 0.66 ± 0.13 mg/g dry weight after 64 weeks of storage. Lower storage temperatures appeared favourable for conserving alkamides. The alkamide concentrations of chopped *E. purpurea* roots stored at 3°C were 5.09 ± 1.20 and 2.82 ± 0.62 mg/g dry weight at 16 and 64 weeks, respectively. Decreasing the storage temperature of chopped *E. purpurea* roots to −18°C best preserved the alkamide concentration, with 16- and 64-week concentrations of 6.28 ± 1.75 and 4.04 ± 0.67 mg/g dry weight, respectively (Perry *et al.*, 2000).

Storage at a temperature in the range of −15 to 24°C had no effect on the parthenolide concentrations of feverfew leaves stored over 120 days; however, the length of the storage period had a significant effect (Tanko *et al.*, 2003). The parthenolide concentration of dried whole feverfew leaves stored for 120 days at −15°C, 6°C and 24°C was 16.6 ± 0.3, 15.6 ± 0.1 and

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17.4 ± 1.5 mg/g dry weight, respectively. Storage of the feverfew leaves for 120 days at 24°C resulted in a parthenolide decrease from 23.6 ± 1.1 to 17.4 ± 1.5 mg/g dry weight (Tanko *et al.*, 2003). Losses in parthenolide during storage were also reported by other groups. Ground feverfew biomass stored unprotected from light at room temperature for 9 months resulted in a 50% decrease in its parthenolide concentration (Heptinstall *et al.*, 1992), and freshly dried feverfew leaves decreased from 0.38 to 0.18% after 2 years of storage at room temperature (Smith and Burford, 1992).

Extraction

Extraction is a key operation in the manufacture of botanical preparations (Shi *et al.*, 2002) and refers to the mass diffusion of target solutes from an insoluble plant solid to its surroundings. In short, extraction can be described by the following steps: transport of solvent into particles; desorption of compounds from plant matrices; solubilization of solutes in the solvent; and diffusion of solute molecules to the bulk liquid. Since target solutes or phytochemicals are of different nature, the choice of the appropriate solvent and extraction technique is critical for botanical preparations. Many techniques are available for phytochemical preparation: liquid–solid, supercritical fluid, pressurized liquid and pressurized hot water extraction.

Conventional solid–liquid extraction methods

Conventional solid–liquid extraction (SLE) methods in industry include infusion, decoction, maceration and percolation, which are all usually carried out at atmospheric pressure with organic solvents (Schwartzberg, 1975). In the USA, phytochemicals are extracted with generally regarded as safe (GRAS) solvents (Sanders, 1993). Due to their differences in polarity, different solvents show different selectivities toward the target compounds. Based on the solubility and polarity of target component, selection of the appropriate solvent is critical in SLE processes. If SLE processing is chosen, all efforts must be made to remove organic solvent residues from the extracts (Ollanketo *et al.*, 2002).

Extracting hyperforin from St John's Wort with 50 and 100% ethanol extracted 21 and 64%, respectively, of the total recoverable amount. No hyperforin was extracted while solely using water as the extraction solvent. Increasing the temperature from 40 to 60°C did not improve the extraction yields (Meier, 2003). Although no yields are given, flavonol glycosides and terpene trilactones are allegedly extracted from dried *Ginkgo biloba* leaves with a mixture of acetone and water, before being concentrated, dried and reconstituted with ethanol (O'Reilly, 2000). The iridoid glycosides, catalpol and aucubin, were extracted from *Veronica longifolia* leaves with methanol, ethanol, 2-propanol and ether. Catalpol yields of 0.8 ± 0.1, 0.4 ± 0.1, 0.2 ± 0.0 and 0 ± 0.0 mg/g dry weight and aucubin yields of 0.6 ± 0.1, 0.3 ± 0.1, 0.2 ± 0.0 and 0 ± 0.0 mg/g dry weight were obtained with methanol, ethanol, 2-propanol and ether extractions, respectively (Suomi *et al.*, 2000). The use of ethanol, methanol, acetonitrile and acetone for extracting silybinin A from milk thistle seeds returned 4.0 ± 0.1, 1.5 ± 0.1, 1.5 ± 0.1 and 2.0 ± 0.1 mg/g dry weight, respectively (Wallace *et al.*, 2004). Gafner *et al.* (2004) reported total saponins recovery from *P. quinquefolius* roots of 61.7 ± 0.1, 59.4 ± 0.5 and 51.5 ± 0.2 mg/g dry weight for extraction with 50% ethanol, ethanol–glycerin–water (20:40:40) and 65% glycerin solvent systems, respectively. Iridoid glycoside, flavanolignan and saponin recoveries illustrate the importance of solvent selection in SLE processing.

Supercritical fluid extraction

Supercritical fluid extraction (SFE) is based on the solvating power of fluids, which are maintained above their critical point. Supercritical fluids have the combination of gas-like mass transfer properties and liquid-like solvating strength. Their very low surface tensions facilitate penetration into microporous materials, such as herb matrices. In SFE, density is related to solvating power (Taylor, 1996). Increases in solvating power can be obtained by manipulating two parameters: temperature and pressure. However, the manipulation of these parameters does not always result in increased yields. For example, *Curcuma zedoaria* is extracted for its 1,8-cineole, camphor, isoborneol, bornaeol, elemene, curzerene, curzerenone, furanodiene, germacrone, curdione, furanogermenone and zederone content. When increasing the pressure of the solvent from 16 to 20 MPa, no increases in phytochemical concentrations were observed in the *C. zedoaria* system (Ma *et al.*, 1995).

In the phytochemical extraction industry, carbon dioxide is often the SFE solvent of choice because of low toxicity, chemical inertness and ease of recovery by venting gaseous CO_2. However, two drawbacks must be stated: the necessary equipment is expensive and the technique is not appropriate for the extraction of all phytochemicals. On the other hand, for certain compounds SFE-CO_2 has found widespread use. Total ω-3 fatty acids extracted from brown seaweed (*Sargassum hemiphyllum*) by SFE-CO_2 at 37.9 MPa and 50°C were 16.2 ± 1.3 mg/g dry weight, compared to 13.1 ± 1.3 mg/g dry weight when

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extracted by Soxhlet with a chloroform–methanol mixture (Cheung *et al.*, 1998). Similar total vitamin E recoveries of about 22 mg/g dry weight were obtained either with SFE-CO_2 at 27.6 MPa and 40°C, or with SLE with a chloroform–methanol mixture (Ge *et al.*, 2002). Sun *et al.* (2002) used SFE-CO_2 to extract alkamides from *E. angustifolia* dried roots and reported yields of 35 mg/g dry weight at 55 MPa and 60°C. SFE-CO_2 processes can also include the use of modifiers, where ethanol can be added to CO_2 to increase its polarity. In a *G. biloba* phytochemical preparation, the combination of primary extraction with 70% ethanol, followed by SFE-CO_2 with 5% ethanol modifier at 300 MPa and 60°C, returned 2.1% of the terpenoids and flavonoids, while SLE with chloroform and acetone resulted in a 1.8% yield (Yang *et al.*, 2002). Catchpole *et al.* (2002) reported that SFE-CO_2 extraction of dry *E. purpurea* aerial parts at 29.5 MPa and 45°C with 12.1% ethanol yielded 3.79% of the alkamides. In St John's Wort, the maximum recoveries of hyperforin were 2.56% (29.5 MPa, 40°C, 10% ethanol; Catchpole *et al.*, 2002) and 1.24% (10 MPa, 40°C; Mannila *et al.*, 2002). However, for certain classes of compounds, the addition of modifier does not increase recovery. For example, hypericin and flavonoids could not be extracted using SFE-CO_2 with modifier from St John's Wort biomass (Catchpole *et al.*, 2002; Mannila *et al.*, 2002). Catchpole *et al.* (2002) also reported that SFE-CO_2 with ethanol mixtures could not solvate chichoric or polyphenolics from *E. purpurea* biomass. Furthermore, the addition of a modifier presents severe drawbacks. The presence of a modifier can result in a higher critical temperature, which may lead to thermal degradation. For example, to remain in the supercritical region of the phase diagram, CO_2 mixed with 10% methanol needs to be maintained at 7.446 MPa and 60°C. However, when increasing the methanol concentration by 5%, the SFE-CO_2 operating parameters need to be increased to 7.476 MPa and 73.4°C (de Lourdes *et al.*, 1997). The increase in temperature may favour the degradation of certain phytochemicals. Furthermore, the modifier may condense upon depressurization and leave organic residue, undermining the advantage of SFE-CO_2 extraction as an environmentally friendly extraction method.

Pressurized liquid extraction

Pressurized liquid extraction (PLE), also known as accelerated solvent extraction, has recently been developed and uses organic solvents at pressures of about 14 MPa, and extraction temperatures above the solvent boiling point. By pressurizing and operating at or above boiling point solvent temperatures, PLE has the advantage of short extraction times, low solvent consumption and high extraction yields. The enhanced performance when using PLE occurs because of the following reasons: the solubility of solutes increases with increasing solvent temperature; higher solvent temperatures result in higher diffusion rates; and greater disruption of the strong solute–matrix interactions (Richter *et al.*, 1996).

The extraction of hypericin from St John's Wort using PLE at 14 MPa, 60°C and methanol generated 3.5 mg/g dry weight, compared to 2.8 mg/g dry weight by Soxhlet extraction (Benthin *et al.*, 1999). Choi *et al.* (2003) compared PLE to ultrasonic-assisted extraction for extracting total ginsenosides from *P. quinquefolius*. A 10-min PLE at 10.4 MPa, 120°C with water supplemented with 1% Triton X-100 resulted in 1.01 total ginsenosides, expressed as normalized peak area, which was similar to what was obtained with a 2-h ultrasonic-assisted extraction process also with 1% Triton X-100 (Choi *et al.*, 2003). A PLE method was developed for the extraction of cocaine from *Erythoroxylum coca* leaves. By using PLE with methanol at a pressure of 20 MPa and a temperature of 80°C, cocaine could be extracted in only 10 min, as compared to several hours by SLE (Brachet *et al.*, 2001).

Pressurized hot water extraction

Pressurized hot water extraction (PHWE) is a novel extraction procedure, consisting of extracting the desired phytochemicals with hot/liquid water. Liquid water at temperatures ranging from 100°C up to 374°C, which is the critical temperature of water, can be reached in pressurized systems (Hawthorne *et al.*, 2000). PHWE is especially useful for extracting polar phytochemicals from plant matrices. Interestingly, the dielectric constant (ϵ) of water at 20°C and ambient pressure is 80; however, ϵ of 250°C water maintained in the liquid state is 27. This latter value is quite close to the dielectric constants of methanol ($\epsilon = 33$) and ethanol ($\epsilon = 24$) at 25°C. In other words, if the temperature can be increased, water can display the properties of certain organic solvents. A drawback to PHWE is the high temperature of the water, exposing the phytochemical to degradation. However, if the desired phytochemical can sustain a high water temperature, PHWE is an environmentally friendly and low-cost technique for botanical preparation.

Miller and Hawthorne (2000) reported that the solubility of liquid organic flavour and fragrance compounds in hot/liquid water was increased by a factor up to 60 when the temperature was increased from 25 to 200°C. In extracting flavour and fragrance compounds from savory and peppermint using water at 100–175°C, polar flavour compounds were extracted (Kubatova *et al.*, 2001). At 125°C all the carvone, puegone, piperitone, eucalyptol, menthone, neomenthol and menthol were extracted from *Mentha*

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piperita biomass. At 100°C, more than 80% of thymol, carvacrol, borneol, linalool and thymoquinone were extracted from *Satureja hortensis* biomass (Kubatova *et al*., 2001). PHWE was used to extract paclitaxel from *Taxus cuspidate*, with extraction yields decreasing with water temperatures above 140°C (Kawamura *et al*., 1999). Paclitaxel yields of 0.2 ± 0.0 and 0.8 ± 0.1 mg/g dried bark were obtained with PHWE at 100 and 140°C, respectively. The values obtained with PHWE were within the range of that obtained with PLE (0.9 ± 0.1 mg/g dried bark, 150°C methanol) (Kawamura *et al*., 1999). Ibañez *et al.* (2003) used water (25, 100, 150 and 200°C) to extract antioxidant compounds from rosemary leaves and reported that PHWE techniques allowed for selective extraction. The antioxidant activity of the extracts obtained by PHWE was comparable to those obtained by supercritical fluid extraction of rosemary leaves. PHWE at 100–140°C was used to extract silymarins from milk thistle seed meal (Duan *et al*., 2004). The rate of extraction increased significantly with temperature (maximum extraction yield in 200 min at 100°C, maximum yield in 55 min at 140°C), but compound degradation became a significant problem at the higher temperatures. In summary, water can be used as an extraction solvent, as it is environmentally friendly, inexpensive and easy to dispose of. It is important to note that the target phytochemical(s) must not be subject to degradation, as the degradation of thermal labile active compounds could have undesirable biological activity.

In conclusion, this review considers the impact of certain critical pre- and post-harvest factors on the phytochemical composition of medicinal plants and the products derived from them. To prepare a high-quality product, the appropriate part of the medicinal plant must be harvested at the optimum stage of development, dried and stored at temperatures and conditions that do not decrease the phytochemical concentrations, and extracted using a technique that maximizes phytochemical recovery. Only if all these steps are respected will a high-quality product showing batch to batch consistency be manufactured.

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