

Processing and Quality Control of Herbal Drugs and Their Derivatives

José Otávio Carréra Silva Júnior, Roseane Maria Ribeiro Costa,
Francisco Martins Teixeira and Wagner Luiz Ramos Barbosa
*Universidade Federal do Pará, Instituto de Ciências da Saúde,
Faculdade de Farmácia Rua Augusto Corrêa n. 1,
Cidade Universitária Professor José da Silveira Neto, CEP: 66.075-110, Belém-Pa,
Brazil*

1. Introduction

For thousands of years mankind has used the resources of flora in the treatment of various diseases. There are reports, for example, of the use of plants for therapeutic purposes dating back to 3000 BC in the Chinese work *Pen Ts'ao* by Shen Nung (Tyler, 1996; Ko, 1999). In 78 AD, the Greek botanist Pedanios Dioscorides described about 600 medicinal plants and this treaty remained as a reference source for more than fourteen centuries (Robbers et al., 1996; Tyler, 1996). The therapeutic properties of certain plants were discovered and propagated from generation to generation as a part of the popular culture through observation and experimentation by primitive people (Turolla & Nascimento, 2006).

In the sixteenth century, the Swiss physician Philippus Aureolus Theophrastus Bombastus von Hohenheim, known as Paracelsus (1493 - 1541), formulated the "Theory of Signatures" based on the latin proverb *similia similibus curantur*, which means "alike cures alike". According to this theory it was believed that the shape, color, flavor and odor of the plants were related to their therapeutical properties, giving clues to their clinical use. Some of these plants have become part of the allopathic and homeopathic pharmacopoeias since the nineteenth century, when their therapeutic basis started to be investigated (Elvin-Lewis, 2001). The isolation of morphine from *Papaver somniferum* in 1803 by the pharmacist Friedrich Wilhelm Adam Sertürmer marked the beginning of the extraction process of active ingredients from plants. Since then, other substances have been isolated, such as quinine and quinidine obtained from *Cinchona* sp in 1819, and atropine from *Atropa belladonna*, in 1831, which started to be used instead of plant extracts (Tyler 1996; Schulz, 2001).

The production of drugs by chemical synthesis, the growing of the economic power of pharmaceutical industries and the lack of scientific evidence on the efficacy of substances from plant origin combined with the difficulty of chemical, physico-chemical, pharmacological and toxicological control of plant extracts used so far, boosted their replacement by synthetic drugs (Rates, 2001). After the 1960s, there was a lack of interest from pharmaceutical companies and research institutes in the search for new substances from plant origin. This fact was due to the belief that the main active ingredients from known herbal drugs had already been isolated and all possible chemical modifications in these substances had

already been performed (Schenkel et al., 2000). However, since the 1980s, technical advances and the development of new methods for isolation of active compounds from natural sources have enabled faster identification of substances in complex samples such as plant extracts. And so, there was a resurgence of interest in the research of these substances as prototypes for the development of new drugs from medicinal plants. According to Carvalho (2004), these medicinal plants are the ones which are selected and popularly used as medicine in treating diseases. They are defined by World Health Organization (WHO) 1978, as being any plant, which applied in a particular form and by any means to the human being, is capable of causing a pharmacological effect.

So, even after the development of large pharmaceutical laboratories and synthetic drugs, medicinal plants remained as an alternative form of treatment in various parts of the world. In the last decades, a reevaluation of the use of herbal preparations was observed. These preparations are defined as any medication obtained and prepared, using only vegetable raw material with prophylactic, curative or diagnostic purposes, causing benefit to the user (Carvalho, 2004). Thus, some pharmaceutical companies began to make efforts toward the improvement of herbal medicines and their production on an industrial scale. This new development of herbal medicines, far from returning to the past, is characterized by the pursuit of industrial scale production, differently from the handmade forms which characterized its early stages (Turolla & Nascimento, 2006).

Currently, preparations derived from medicinal plants are widely used in many countries. In Africa, for example, 80% of the population depends on the use of these preparations, which represent alternatives to the high costs of synthetic drugs (Turolla & Nascimento, 2006).

The consumption of medicinal plants and herbal products has been somewhat surprising in recent years, constituting a promising pharmaceutical segment. This fact is primarily noted as a result of easy access, low cost and its popular or cultural compatibility (Tyler, 1996).

Medicinal plants are an important source of substances with biological activities. Many of them have become prototypes for the synthesis of a large number of bioactive compounds. Research indicates that Brazil is the world leader in plant diversity with about 55,000 described species and 350,000 to 550,000 estimated ones. From the cataloged ones, 10,000 species can be medicinal, aromatic or have other utilities (Borges et al., 2003).

Therefore there's a need to combine empirical knowledge about the popular use of medicinal plants with scientific research based on detailed studies of the plant species, as well as botanical, phytochemical, pharmacological, toxicological aspects, and the development of scientific and technological methodologies (Petrovick et al., 1997). The result of this scientific breakthrough is the development of a differential therapeutic herbal product, as unlike the traditional preparations obtained from plants in folk medicine, the scientific and technological grounding provide the development of herbal medicine (Wagner & Wiesenau, 2006; Leite, 2008).

So, the plant species that will be used in the development of phytotherapeutics deserves cares in its acquisition, like a previous unequivocal botanical identification, as popular names of the plants are very often regional, varying according to the region where they grow, so, it is very common that the same plant species has different vernacular denominations. On the other hand, different botanical species may have the same vernacular denomination, Furthermore, for a few different species, with some morphological similarity, the unequivocal distinction between them becomes very difficult; being not rare the cases of poisoning due to incorrect identification of the plant species (Farias, 1985; Mengue, 2001).

Therefore it is indispensable the standardization of the starting material and intermediates in order to assure the quality of the product derived from them.

Standardization of material derived from plants represents a major challenge, especially for reproduction of biological effects. Botanical derivatives, when used for therapeutic purpose, are more complicated concerning the requirements of composition, constancy, stability, microbiological counts, etc. (Yunes et al., 2001).

The chemical standardization of derivatives of medicinal plants, like extracts and fractions, demands the application of methods and techniques that aim at detecting and characterizing substances that guarantee the quality of the plant material used, either by the aspect of the metabolic composition, chemical marker; or by the pharmacological activity, substances related to the activity of the plant, able to be quantified. However, studies prove that the plant secondary metabolism can vary considerably according to air temperature, water availability, ultraviolet radiation, nutrients, soil altitude and air quality in the place where the vegetable grows. Even mechanical stimulations or pathogens' attack can induce such variability, leading to the seasonal production of those potential markers (Gobbo-Neto, 2007).

The method used in the standardization of the starting material and derivatives must be validated considering the following parameters: specificity/selectivity, linearity, interval, precision, limit of detection, limit of quantification, accuracy and robustness, which must be determined and verified (Brazil, 2003).

The concern of regulatory agents about the standardization of herbal medicines provides the evaluation of important aspects such as efficacy and safety of these medications. The traditional use of various medicinal herbs based on folk knowledge and the belief that, being natural does not provoke adverse reactions, caused just a few of them to be evaluated through preclinical and clinical studies to prove their efficacy and safety (Turolla et al., 2006).

Vegetable raw material such as plants, pharmacogens or derivatives (extracts, tinctures, essential oils or dried products) has been widely used in drugstores and pharmaceutical industries. What worries most the scientific community is the use of new plant species as medicines without proven data about their biological action, non-toxicity (assessed in humans), side effects, safety, efficacy and others. Although these herbal medicines have natural active constituents, they should be assessed as drugs and therefore subjected to strict controls at every stage of their idealization, evaluation and development.

2. Definitions

2.1 Phytotherapy (Gaedcke & Steinhoff, 2003)

The term "phytotherapy" means the "prevention and treatment of human diseases using plants, parts of plants or preparations of plants".

The phytotherapy is a form of medicine that has grown noticeably over the years to the point that currently the world market for herbal medicines is around 44 billion dollars.

For Weiss and Fintelman, it is a decisive aspect that the plant or parts of a plant which are used for pharmaceutical purposes are regarded as an active substance as a whole. Herbal medicinal products, in this regard, are always mixtures of a number of substances.

Phytotherapy has to be distinguished from other acknowledged therapeutic disciplines which also utilize plants such as homeopathy, invented by Samuel Hahnemann, and anthroposophy, whose background is on the Rudolf Steiner philosophy. The main difference

is that homeopathy is based on the principle "similia similibus curentur" - similar symptoms are healed by similar medicines. Besides that, homeopathy mostly uses very low doses. Efficacy is increased with the degree of dilution (potentiation).

Similar to homeopathy, anthroposophic medicine is based on a global approach to diseases. It focuses on the strong connection between human being and nature. Besides that, anthroposophy takes into account cosmological aspects when selecting the right medicine for individual cases of illness. In the same way as homeopathic medicines, anthroposophic medicines are mostly obtained from natural origin. They are partly manufactured according to the principles of homeopathy, sometimes also using heat (e. g. roasting, carbonization).

In contrast to homeopathy and anthroposophy, phytotherapy should definitely be understood as an allopathic discipline because the effects which are expected from herbal medicinal products are directed against the causes and the symptoms of a disease. Because of these important differences, phytotherapy, in contrast to actual interpretation, in Germany for example, should not belong to the so-called "special therapeutic disciplines" or to "alternative medicine". It should, moreover, be classified as a regular discipline of natural orthodox science-oriented medicine, because the respective medicinal products have to comply with the same scientific requirements as those of the chemically defined substances in terms of quality, safety and efficacy.

2.2 Herbal drugs

For the term "herbal drug", the following definitions can be found in literature:

Drugs are herbal or animal organisms or organs transformed into a storable condition by drying. Furthermore, the definition comprises other matter extracted from plants or animals without organic structure such as resins, gums, fats, essential oils (Gaedcke & Steinhoff, 2003).

Drugs are complex active substances which are either used by patients as original herbal drugs or which serve as industrial starting materials for the manufacture of finished medicinal products or for the isolation of purified active substances or mixtures of substances. Drugs are either organized on a cellular basis, i. e. microorganisms, plants, animals or parts of these which have been dried or submitted to otherwise preservation or they are mixtures of substances which have been extracted from microorganisms, plants, animals or parts of these which do no longer have cellular structures, such as essential oils, resins, starch, fats, waxes, isolated mucous substances or animal toxins (Teuscher, 1997).

Herbal drugs are plants or parts of plants which have been transformed into storable conditions by drying, such as herba-, folium-, flos-, semen-, and radix drug (Hänsel, 1991).

According to pharmaceutical linguistic rules, **herbal drugs** are dried or processed plants or parts of plants used for the manufacture of pharmaceutical preparations (Saller et al., 1995).

According to pharmaceutical linguistic rules, **herbal drugs** are dried herbal or animal starting materials (Bauer et al., 2002).

Herbal drugs are herbal matter used for pharmaceutical purposes. A "herbal drug" or a produced preparation therefore, is regarded as one active substance irrespective of the question of whether the active constituents of the herbal drug are known or not (Gaedcke & Steinhoff, 2003).

The definition for **herbal drug** in the European Pharmacopoeia is mainly whole, fragmented or cut, plants, parts of plants, algae, fungi, lichen in an unprocessed state, usually in a dried form, but sometimes fresh. Certain exudates that have not been subjected to a specific treatment are also considered to be herbal drugs. Herbal drugs are precisely defined by a

botanical scientific name according to the binomial system (genus, species, variety and author).

According to the definition of the European Pharmacopoeia, the expression "herbal drug" shall apply to herbal materials in dried as well as in fresh conditions, which from a specific point of view makes no sense. As a consequence, two kinds of herbal drugs would have to be distinguished, first, the so-called "fresh herbal drug", which is a contradiction in itself (oxymoron) because the term "drug" implies that a material has been submitted to some kind of drying process, and second the so-called "dried herbal drugs" which due to the same reason, is linguistic nonsense.

Therefore, the starting materials used for the manufacture of herbal medicinal products should preferably be named "herbal drug" or "fresh part of plant".

2.3 Herbal medicinal products

Herbal medicinal products (Phytopharmaka) are regarding the active substance(s), more or less enriched preparations of plants or herbal drugs which, besides that, contain other concomitant substances exerting or not exerting therapeutic activity (Vogel, 1982).

Herbal medicinal products (Phytopharmaka) are those medicinal products which, as active substances, solely contain herbal drug preparations, such as comminuted parts of plants, extracts, pressed juices or distillates of plants. Isolated plant constituents such as digitoxin or menthol, as well as homeopathic medicinal products, are not regarded as herbal medicinal products.

Herbal medicinal products (Phytopharmaka) are complex preparations of herbal origin, i.e. galenic preparations of plants used within the sense of allopathic therapy. Isolated biogenic pure substances and complex preparations obtained from plants intended for use within alternative therapies such as homeopathy or anthroposophy do not fall within this definition (Teuscher, 1997).

2.4 Herbal drug preparations

According to the definition given in the European Pharmacopoeia Supplement 2000, herbal drug preparations are obtained by subjecting herbal drug to treatments such as extraction, distillation, expression, fractionation, purification, concentration and fermentation. These include comminuted or powdered herbal drug, tinctures, extracts, essential oils, fatty oils, expressed juice and processed exudates.

Extracts obtained from herbal drugs comply with the monograph on Extracts. Tinctures obtained from herbal drugs comply with the monograph on Tinctures.

Herbal teas comply with the monograph on Herbal teas.

Instant herbal teas consist of powder or granules of one or more herbal drug preparation(s) intended for the preparation of an oral solution immediately before use.

Extracts are preparations of liquid (liquid extracts and tinctures), semi-solid (soft extracts) or solid (dry extracts) consistency, obtained from herbal drugs or animal matter usually in a dry state.

Different types of extracts may be distinguished. Standardized extracts are adjusted within an acceptable tolerance to a given content of constituents with known therapeutic activity; Standardization is achieved by adjustment of the extract with inert material or by blending batches of extracts. Quantified extracts are adjusted to a defined range of constituents; adjustments are made by blending batches of extracts. Other extracts are essentially defined

by the production process (state of the herbal drug or animal matter to be extracted, solvent, extraction conditions) and their specifications.

Soft extracts are semi-solid preparations obtained by evaporation or partial evaporation of the solvent used for preparation.

Dry extracts are solid preparations obtained by evaporation of the solvent used for their production. Dry extracts usually have a loss on drying or water content not greater than 5 per cent m/m.

Liquid extracts are liquid preparations of which, in general, 1 part by mass or volume is equivalent to 1 part by mass of the herbal drug or animal matter. These preparations are adjusted, if necessary, so that they satisfy the requirements for the content of the solvent and, where applicable, for the constituents.

Tinctures are liquid preparations which are usually obtained using either 1 part of herbal drug or animal matter and 10 parts of extraction solvent or 1 part of herbal drug or animal matter and 5 parts of extraction solvent.

Different types of extracts may be distinguished. Standardized extracts are adjusted within an acceptable tolerance to a given content of constituents with known therapeutic activity; Standardization is achieved by adjustment of the extract with inert material or by blending batches of extracts. Quantified extracts are adjusted to a defined range of constituents; adjustments are made by blending batches of extracts. Other extracts are essentially defined by the production process (state of the herbal drug or animal matter to be extracted, solvent, extraction conditions) and their specifications (Gaedcke & Steinhoff, 2003).

2.5 Classification of extracts in terms of their physical state

Depending on the physical state of an extract the following types can be distinguished:

- Liquid preparation: liquid extracts (fluid extract, tincture), oily macerates,
- Semi-solid preparations: soft extracts,
- Dry extracts: dry extracts.

The difference between fluid extract and tincture is that tinctures are not concentrated but diluted preparations.

The comminuted, mostly dried parts of the plant and the extraction solvent are combined in the extractor. This mixture is allowed to stand for several hours depending on the extraction process. Generally, extraction with additional extraction solvent is repeated until no more constituents are transferred to the extraction solution. In the next step, the extraction solution (miscella, eluate) is separated from the herbal drug residue. Since it contains the extractable herbal matter, the eluate represents an important intermediate product during the total manufacturing process.

If the extraction solvent is ethanol or water or a mixture of both, the preparations resulting after filtration are liquid extracts (fluid or tincture, depending on the grade of concentration). If the extraction is carried out with vegetable oils (e. g. rape oil or olive oil) oily macerates are obtained which are usually filled in soft gelatin capsules.

If the resulting preparation is a dry extract, processing of the eluate continues with cautious evaporation of the majority of the extraction solvent in the vacuum-evaporator. The resulting aqueous soft extract is evaporated to dryness using suitable drying aggregates, e. g. spray dryer, belt dryer or spray belt dryer. In most cases, suitable technical excipients such as maltodextrin, lactose or silicium dioxide have to be added. After grinding and sieving, flowable powder is obtained which can be further processed to tablets or coated tablets or filled in soft or hard gelatin capsules (Gaedcke & Steinhoff, 2003).

2.6 Ratio of the herbal drug to the extraction solvent

Maceration (extraction under steady-state conditions)

In this procedure, the ratio of the herbal drug to the extraction solvent is a decisive factor for the effectiveness (= extent) of the extraction. The quantity of extractable matter is increased with the quantity (mass, volume) of extraction solvent, as using higher amounts of extraction solvent, steady-state conditions are reached later.

The ratio of the herbal drug to the extraction solvent is, therefore, a relevant parameter for the quality of the extract and has to be exactly defined to ensure batch-to-batch conformity. It has to be specified as a fixed ratio, e.g. of 1:5 or 1:10.

Percolation (= exhaustive extraction)

In this extraction procedure, a fixed quantity of herbal drug (mass) is treated with a variable quantity (mass/volume) of extraction solvent until the extractable matter is completely transferred from the herbal drug matrix to the eluate (percolate). The ratio of the herbal drug to the extraction solvent may therefore vary from batch to batch within a certain range. It depends on the characteristics of the herbal drug (content of extractable matter, loss on drying, etc.). Herbal drug preparations manufactured by percolation procedures are described by the average quantity (mass/volume) of extraction solvent with a defined range, e.g. 1:12 (1:10 to 1:14).

This means that in case of tinctures the relevant factor for the quality of the preparation is the ratio of the herbal drug to the extraction solvent, and not the ratio of the herbal drug to the final tincture which is determined after the expression of the extraction solvent. Depending on the effectiveness of the expression process, the resulting ratio can be as follows:

- For tinctures 1:10 - a ratio of the herbal drug to the tincture of 1:7 to 1:9,
- For tinctures 1:5 - a ratio of the herbal drug to the tincture of 1:4 to 1:4, 5.

Subsequent filling-up to the initial ratio of the herbal drug to the extraction solvent of 1:10 or 1:5 is not in compliance with pharmacopoeial standards unless it is necessary for the adjustment of the preparation to a defined content of constituents or a group of constituents with known therapeutic activity (Gaedcke & Steinhoff, 2003).

3. Harvest and postharvest processing

Botanic is an essential area in the study of medicinal plants, which constitutes a very heterogeneous set of plants distributed in different taxonomic groups, with very different morpho-anatomic characteristics and whose unifying factor is their richness of pharmacologically active compounds. As a considerable proportion of medicinal plants are wild, the correct identification of plant material must be made by literature and consultation with experts and herbarium. The lack of scientific identification, or misidentification, will compromise the rest of the scientific work on the plant. So, for medicinal plant research, we should perform a harvesting for botanical identification and another one for phytochemical and pharmacological studies, each one with specific recommendations. From an anatomical standpoint, medicinal plants have aroused interest due to their abundance of secretory structures such as idioblasts, cavities, channels, laticifers and glandular trichomes, among others (Fahn, 1979; Evert, 2006), and the chemical diversity of their secretions which are rich in terpenes, alkaloids and phenolic compounds (Harbone, 1997). These secretions can be identified by conventional chemical analysis or by histochemical methods, which identify

the compounds *in situ*, such as tests for lipids and terpenic substances, tests for phenolic compounds, tests for proteins, tests for alkaloids, tests for carbohydrates and tests for crystals.

3.1 Harvest

For each medicinal plant there is an appropriate time for harvesting the crop. The phytochemical monitoring of the active compounds allows the establishment of the right time to harvest. However, for plants whose active principles are not known yet, there are some general rules which can be applied. The bark should be harvested in spring, early summer and autumn. The humid environment facilitates the detachment of the bark. The bark should be carefully removed by cutting vertical segments. Care must be taken not to "roulette" the trunk, that is, to remove peels in horizontal cotes surrounding the trunk. This procedure prevents the circulation of the sap, resulting in the plant's death.

The underground organs: roots, rhizomes, tubers, bulbs of perennial plants should be harvested during winter, the dormant period, when the concentration of active principles reaches its maximum in these organs. The herbaceous plants and leaves are harvested when they start flowering. Some plants allow more than one cut. Sometimes, when there are well defined rainy and dry periods, the harvesting of the leaves is done during the dry season, allowing the plant to regenerate them during the rainy season. The shoot tips are harvested in full bloom and before seed formation. The flowers are cut before opening up completely, but sometimes the open inflorescences are harvested. The fruits are harvest "at once", i.e. before reaching the mature state. The seeds are harvested when ripe. If they are from fleshy fruits, the leftover pulp which surrounds them should be removed and the seeds dried. Periods of drought and rainfall influence the content of active principles. Thus, the alkaloid content decreases after the rain whereas the content of essential oils increases. The content of essential oils decreases after the drought. The concentration of active principles varies according to the time of the day. In general, glycosides reach their highest concentration in the afternoon, while essential oils reach their maximum level around noon. The exception is chamomile (*Matricaria recutita*), which reaches higher levels and better quality of essential oil during the night (Sharapin, 2000).

3.2 Post-harvest processing

The post-harvest processing aims at the conservation of the physical, chemical, organoleptic and pharmacological characteristics of the plant drug. The inadequate post-harvest processing results in low quality raw material, with loss of active ingredients, increased microbial load and bad commercial presentation.

The loss of active ingredients involve: degradation by metabolic processes; active principles hydrolysis; decomposition by light; enzymatic decomposition; degradation of thermolabile substances by heat; volatilization of essential oils and contamination by bacteria and fungi.

The first stage of post-harvest processing involves examination and manual separation of parts which are damaged, stained and with signs of attacks by insects and / or fungi. The next recommended step, is to wash the drug in running water.

The most important stage of post-harvest processing is undoubtedly drying. The industry uses dried plants, enabling their preservation for prolonged periods of time. The exceptions are plants used to obtain essential oils, homeopathic tinctures and some extracts like artichoke (*Cynara scolymus*), which are processed fresh.

The moisture content in fresh plants varies from 60% to 80%. The drying process reduces the moisture content to 5% - 12%. According to the plant organ, the weight losses during drying are:

- Leaves: 20% to 75%
- Bark: 40% to 65%
- Wood: 30% to 70%
- Roots: 25% to 80%
- Flowers: 15% to 80%

The drying process stops the degradation processes caused by yeasts or enzymes, prevents the development of microorganisms and oxidation and hydrolysis reactions. However, as it involves heat, there are losses of essential oils and volatile substances, as well as the risk of degradation of thermolabile substances during the process. Most medicinal plants can be dried at temperatures between 30°C and 60°C. Plants containing essential oils or volatile substances should be dried at temperatures below 40°C. In all cases good air circulation should be ensured, favoring the process.

The method of drying must be experimentally determined for each plant drug. The slow drying may cause harmful changes, by the action of enzymes, fungi and bacteria, before the process is completed. A very quick drying hardens the superficial layer of the cells and prevents the evaporation of water inside the organ, which also results in the action of enzymes (Sharapin, 2000).

3.2.1 Storage

However great care has been taken during harvesting and drying, the plants lose active ingredients by degradation during storage. Although the recommended time limit for storing leaves and shoot tips is from 12 to 18 months, and for bark and roots from 12 to 36 months, some plants lose active ingredients soon. The conservation of vegetable raw material for a longer period of time depends on storage conditions. Appropriate conditions prevent the product from having contact with the sun, dust, rodents and insects and other degradation factors, thereby, impeding the loss of volatile principles. The material can be wrapped in burlap sacks or bales. The use of plastic bags should be avoided as it does not allow adequate ventilation. The bags should be labeled with the scientific name of the plant and the part used entry date, supplier name origin and approval of quality control.

3.2.2 Grinding

The grinding is intended to reduce the particle size of the plant drug, making it suitable for the next step of the process which is extraction. The extraction of a drug as a whole or divided into coarse fragments will be incomplete due to poor penetration of the solvent in the plant tissue. It will also be very slow, since the cell membranes act as real barriers hindering the extraction procedure. In the case of previously divided drugs, such membranes are partially destroyed, making the dissolution of the cellular components in the outer liquid easier and more efficient. However, the excessive division and the formation of very fine dust may cause problems during the extraction process. In the percolation process there is a powder compaction, which impedes the passage of the solvent, resulting in an incomplete extraction of the drug. In the maceration process the very fine particles might pass to the extract and bring it to a cloudy appearance. The milling process is preceded by screening to remove impurities. In this operation strange material, such as large pieces of wood, other types of metal are removed manually.

4. Microbiological quality control of drug plants and their derivatives

4.1 Raw material

The microbiological quality assurance of herbal medicines must necessarily involve a proper stance regarding the Good Agricultural Practice. Researchers have even recommended the cultivation indoors, with full control over the climate and the presence of pathogens, and the possibility of genetic enhancement combined with biotechnological studies, indicating a tendency to domestication instead of harvesting from the wild (Calixto, 2000, Blumenthal et al., 1998; Bauer & Tittel, 1996). Yet the benefits that the cultivation of medicinal plants may bring to the achievement of desirable objectives related to the eight Millennium Development Goals proposed by the United Nations (UN) and addressed by the World Health Organization (WHO, 2009) (See still missing basic needs - Table 1) indicate the strategic importance of these products to the various traditional communities. These communities include family farmers, indigenous communities, Maroons, and other groupings in a vulnerable socioeconomic position, which have a historical knowledge of the properties and use of various medicinal plants, as it has been noted by several academic papers focused on the ethnopharmacy (Pieroni & Vandebroek, 2007; Yoney et al., 2010; Leonti, 2011). For the success of this strategy it is necessary to ensure these communities with the access to this information and know-how, since they supply raw materials. This connection apparently seems to be unattainable due to social, anthropological and cultural incompatibilities. But what we have observed in practice is the gradual development of market relations between the major urban centers and the more reserved communities, which generally seek to avoid contact with the outside elements. This fact demonstrates the tendency of many of these communities to open channels for knowledge and values exchange in order to facilitate the development and quality of life without their identity loss.

4.2 Regulatory aspects

Heterogeneity exists not only between countries but even within the same country in relation to the maximum microbial limits which are acceptable for herbal products. Moreover, the treatment or application of plant material also involves different microbial limits and surveillance strictness. In some countries, herbal drugs for oral, topical or episodic use, produced by infusions, decoctions and maceration, have different laws when compared to plant drugs presented as capsules, tinctures, tablets, extracts and syrups. However, in general, the tests used to verify the presence of microorganisms in plant drugs show no significant variation and follow the recommendations used for non-sterile pharmaceutical products (Table 1).

4.3 Tests

4.3.1 Sampling and sample preparation

Initially 10 ml or 10 g of sample are needed for the performance of the assays. In case of raw materials, an interesting strategy to homogenize the sample and facilitate the interaction with the culture medium used, is the previous hand-grinding with a properly sterilized mortar and pestle in a sterile environment such as a laminar flow biological safety cabinet. So, when our analysis objects are leaves, stems or other plant constituents in their raw state, the sample preparation becomes important. On the other hand, thinner powders interact well with the solutions used in the homogenization and in the adjustment to near-neutral pH (pH = 7.0), favoring the growth of microorganisms present in the sample. Therefore, the

pharmaceutical form may provide different strategies for sample preparation. In some cases it may be necessary to use other previous procedures, such as the use of inactivating agents, if evidenced the presence of preservatives in cosmetics based on herbal medicines, for example. When there is doubt about the presence of substances with inhibitory microbial growth a preparatory test for assessing the inhibitory capacity can be used. Carry out a transfer of each of the viable strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella*, *Escherichia coli* and *Candida albicans* to 5 ml of soyabean casein broth and incubate at 30°C-35°C for 24 hours for bacteria and at 20°C- 25°C for 24 hours in the case of *Candida albicans*, making decimal dilutions in buffered peptone solution to obtain a suspension containing 50 to 100 viable cells. Use 1 ml of the microorganisms' inoculum separately in the presence or absence of the sample and incubate again as described for bacteria and fungi. If there is massive growth of microorganisms, microbiological assays may be undertaken; in case of little or no growth of viable strains it is necessary to use inactivating agents, for example, 0.5% soy lecithin, 4% polysorbate 20, 0.1% polysorbate 80, among others.

| | Japanese Pharmac. ^{a, b} | | United States Pharmac. ^c | | | British Pharmac. ^b | | Latin Amer. Pharmac. | |
|---|-----------------------------------|-----------------|-------------------------------------|-----------------|-----------------|-------------------------------|-----------------|----------------------------------|---|
| | Category 1 | Category 2 | Source 1 | Source 2 | Source 3 | Category 1 | Category 2 | Argentina ^d | Brazil ^e |
| Aerobic bacteria | 10 ⁷ | 10 ⁵ | 10 ⁵ | 10 ⁴ | 10 ² | 10 ⁷ | 10 ⁵ | 10 ⁷ /10 ⁴ | 10 ⁷ /10 ⁵ /10 ⁴ |
| Molds and yeasts | 10 ⁴ | 10 ³ | 10 ³ | 10 ³ | 10 | 10 ⁵ | 10 ⁴ | 10 ⁴ /10 ² | 10 ⁴ /10 ³ /10 ² |
| Enterobacteria and other Gram negative bacteria | * | 10 ³ | 10 ³ | * | * | * | 10 ² | 10 ⁴ /10 ² | 10 ⁴ /10 ³ /10 ² |
| <i>Escherichia coli</i> | 10 ² | absence | absence | absence | * | 10 ² | absence | absence | 10 ² /10/absence |
| <i>Salmonella</i> | absence | absence | absence | absence | * | * | absence | absence | absence |
| <i>Staphylococcus</i> | * | * | * | * | * | * | * | absence | */*/absence |
| <i>Pseudomonas</i> | * | * | * | * | * | * | * | absence | * |

^a Certain species of *Bacillus cereus*, *clostridia*, *Burkholderia*, *Aspergillus* and *Enterobacter* are also necessary to be tested depending on the origin of the herbal drug raw materials.

^b Category 1: Herbal drugs to which boiling water is added before use and Category 2: Other herbal drugs and their preparations.

^c Source 1: Dried or powdered Botanicals and Botanicals to be treated with boiling water before use.

Source 2: Tinctures, Powdered Botanicals Extracts, Fluidextracts and Nutritional Supplements with Botanicals.

Source 3: Infusions/Decoctions.

^d The first value represents raw materials and final products for infusion preparations and the second value represents final products for topical and oral use.

^e The first value represents Herbal drugs to which boiling water is added before use, the second value represents Herbal drugs to which the extractive process made in cold temperature and the third value represents final products for oral use.

*The limits are not specified.

Table 1. Recommended microbial limits for herbal drugs and their products or botanical ingredients and products

4.3.2 Viable microorganisms count

After preliminary tests, pour 10 ml or 10 g sample into a container containing 90 ml of soyabean casein broth, nutrient broth, buffered peptone broth or other appropriate diluents. It is important to attempt to the fact that the pH should be near-neutral and the characteristics of the sample, such as raw materials from forest origin, may contain higher amounts of fungi and therefore prone to high levels of aflatoxins. So, an appropriate medium would be one that favors the growth of fungi capable of producing these substances. From this first dilution, transfer 1 ml to tubes containing 9 ml of the used diluent, for example, soyabean casein broth, so as to obtain serial dilutions of 10⁻¹, 10⁻² and 10⁻³. In some cases the number of dilutions can be increased to improve the counting (Figure 1). From each dilution, transfer volumes of 0.1 to 0.5 ml to Petri dishes in duplicate

containing Soyabean Casein Agar or Sabouraud-Dextrose Agar for counting bacteria and fungi, respectively. The spreading is accomplished with the aid of Drigalski spatula or a sterile glass rod. In case of bacteria, the incubation should be at 30°C-35 °C for 48 to 72 hours; for fungi, temperatures from 20°C to 25 °C for 5-7 days at least. Antibiotics are commonly used in the culture medium in order to inhibit the growth of undesirable microorganisms. Therefore, amphotericin B is added to the culture medium for bacterial growth (Soyabean Casein Agar or Nutrient Agar) and chloramphenicol to the culture medium for fungi growth (Sabouraud-Dextrose Agar or Potato Agar).

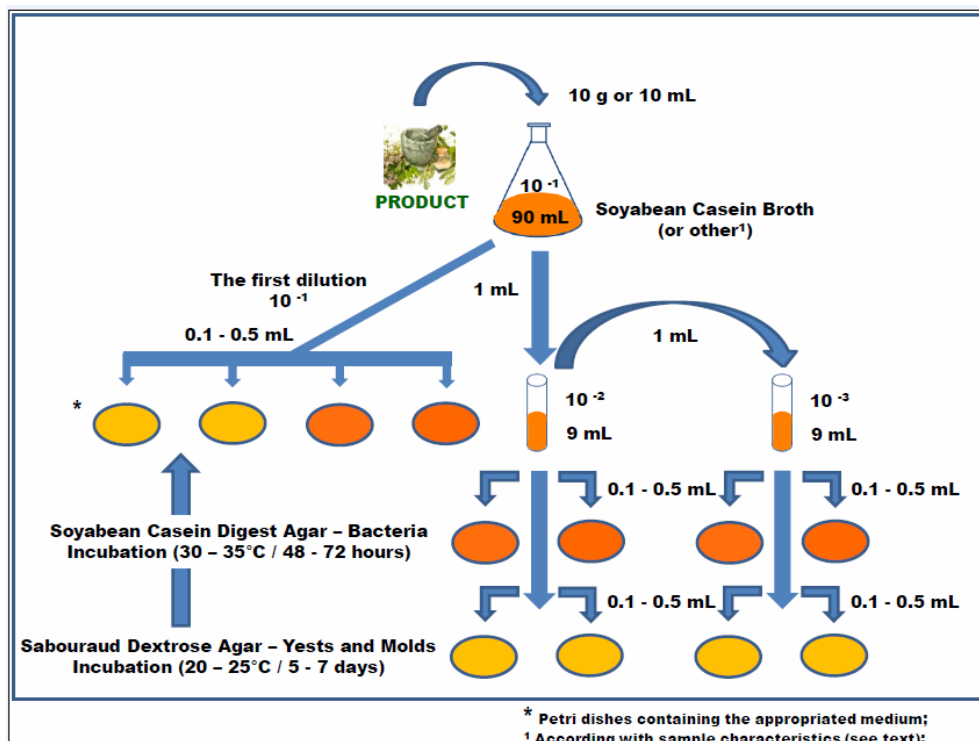


Fig. 1. Viable microorganism count

4.3.3 Search for undesirable microorganisms

Using appropriate enrichment medium, the same amount of sample used for the enumeration of microorganisms (10ml or 10 g of sample in a container containing 90 ml of medium) is used for the detection of pathogens. For detection of *Escherichia coli* and *Salmonella* sp., lactose broth is used and for the latter, after enrichment in lactose broth, the procedure is incubation in tetrathionate broth or selenite cystine broth for 24 hours at 30°C - 35 °C. In the case of *Staphylococcus aureus* and *Pseudomonas aeruginosa*, soybean casein broth is used for enrichment, which should also occur for 24 hours at 30°C - 35 °C. Then, aliquots are transferred to culture medium for isolation and differentiation and subsequent use for conventional biochemical assessments (Figure 2 and 3).

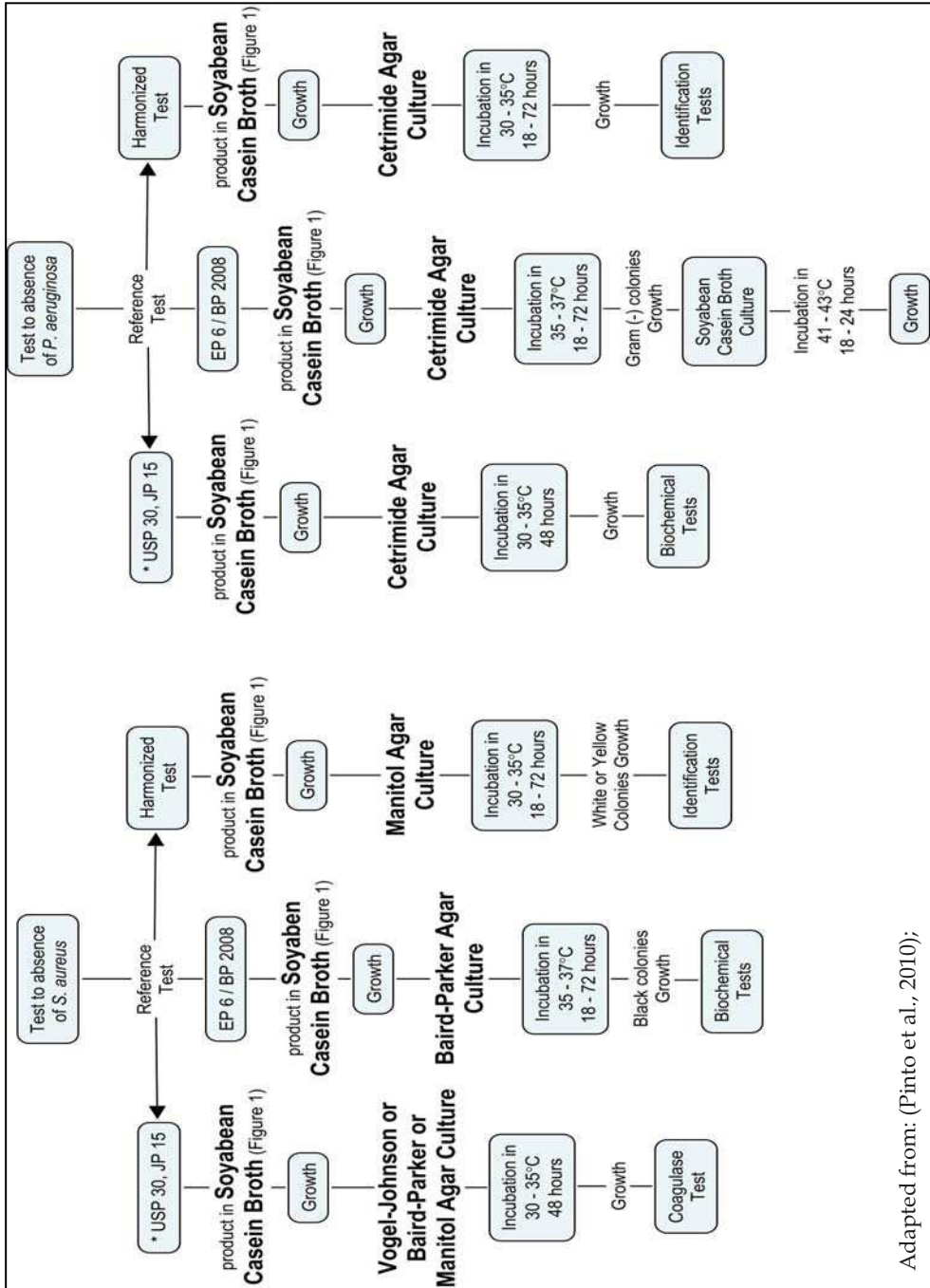
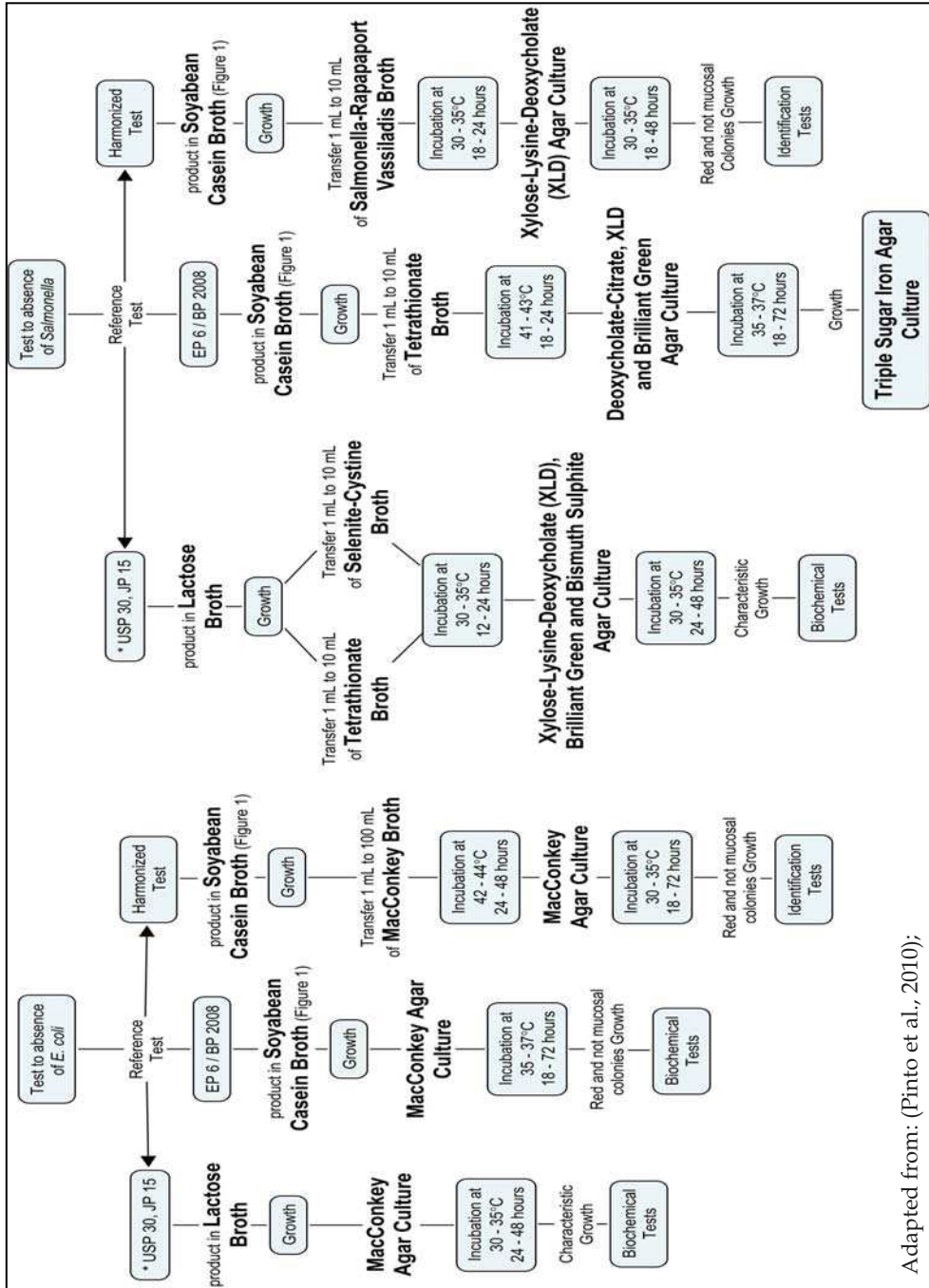


Fig. 2. Enrichment and selective medium to *S. aureus* and *P. aeruginosa*



Adapted from: (Pinto et al., 2010);

Fig. 3. Enrichment and selective medium to *E. coli* and *Salmonella*

5. Physical, chemical and physico-chemical controls of quality of herbal drugs and/or their derivatives: Tinctures and extracts

To ensure quality for vegetable raw material, the WHO, 1992 recommends that the pharmacopoeia specifications for the plant material must include:

- Botanical name, with reference to the authors;
- Specification of the part used;
- Morphological description, macro and microscopic examination;
- Determination of particle size distribution;
- Determination of total ash or sulfated ash (residue on ignition) and acid insoluble ash;
- Determination of extractable matter;
- Determination of water and loss on drying;
- Determination of essential oils;
- Identification by thin layer chromatography;
- Quantitative determination of active constituents;
- Limit tests for heavy metals;
- Determination of pesticide residues.

5.1 Botanical name

The botanical identification is essential to characterize the plant species. This identification cannot be made through popular names since the same species may have different common names and different species may be designated by the same common name. The identification of the plant is given by its "scientific name". The scientific name is always a Latin binomial, a term identifying the gender and both of them together identifying the species. The Latin binomial is followed by the name of the author of the botanical description, usually abbreviated. Finally, the identification is completed with the name of the botanical family to which the plant belongs.

5.2 Specification of the used part

The drug, i.e., used part of the plant, must be specified, for example, inflorescence, leaves, roots, wood or seeds. The specification is described in the language of the country and in Latin. The European Pharmacopoeia adopts as the title of the drug monograph, its name in Latin and as the subtitle, its name in English (or French, depending on the edition). The Brazilian Pharmacopoeia adopts as a title the popular name of the plant and as the subtitle, the specification of the used part, in Latin. Both pharmacopoeias complete the identification with the botanical name of the plant and specifications related to the content of active principles. The European Pharmacopoeia includes the scientific name of the plant when describing the drug, while the Brazilian Pharmacopoeia emphasizes it before the description.

5.3 Morphological description, macro and microscopic examination

A monograph of a plant drug describes the macroscopic, microscopic and organoleptic characteristics. The characteristics described should be compared to the drug sample, as a first step to establish its identity and purity. Whenever possible, authentic samples of the drug should be used as reference samples. The organoleptic characteristics (odor and flavor) often represent a practical guidance regarding the identity and purity of the drug. If the odor and taste vary considerably from the drug prescribed, the drug may be considered out of specification.

The macroscopic characteristics include the shape, size, color, texture, fracture aspects and characteristics of the cut surface. These features are useful for determining the identity and purity of the drug in question. However, since the judgment of these characteristics is very subjective, comparisons to authentic samples should be taken to avoid doubts.

The microscopic characteristics are viewed under a microscope, with or without the addition of chemical reagents. Microscopic analysis is indispensable for powdered drugs. This analysis helps to identify the drug and may be crucial in the identification of adulterants. However to ensure the identification of the drug, the microscopic analysis should be supplemented with data from chemical and physico-chemical analysis, Figures 4 and 5 (Alves, 2008; Alves et al. 2010).



Fig. 4. *Arrabidaea chica* (Humb. & Bonpl.) B. Verlt. (Bignoniaceae). Cross section of the petiole. Detail of the vascular system and sclerenchyma; external phloem; xylem; internal phloem; sclerenchyma; parenchyma cells

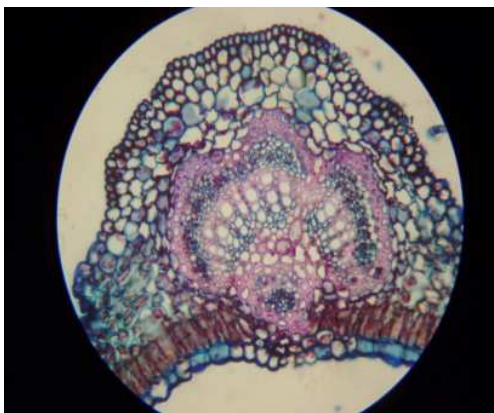


Fig. 5. *Arrabidaea chica* (Humb. & Bonpl.) B. Verlt. (Bignoniaceae). Cross section of the basal lamina. Upper epidermis; lower epidermis; angular collenchyma; fundamental parenchyma; sclerenchyma; phloem; xylem; palisade parenchyma e cuticule

5.4 Determination of particle size distribution

The particle size distribution of herbal drugs determines the contact surface available for interaction with the solvent used to obtain the plant derivative. It is a preliminary and important parameter for choosing the appropriate extraction process, as it has a direct influence on its efficiency (Santos et al., 2000).

Procedure for determination of particle size distribution: an electromagnetic sieve shaker is used. About 10 g of powdered plant material are subjected to a series of sieves with mesh size opening ranging from 2.00 mm to 125 μm , and agitated for 30 minutes. The particle size is analyzed in triplicate and evaluated by quantifying the percentage of powder retention in each sieve (Brazilian Pharmacopoeia V, 2010)

5.5 Determination of ash

The determination of ash includes the determination of total ash, sulfated ash, also called residue on ignition and determination of acid-insoluble ash. The total ash involves determining both the physiological and the non-physiological ash and consists of measuring the amount of non volatilized residue after the drug calcination. Sulfated ash is represented by non volatilized residue after calcination with concentrated sulfuric acid. The metals contained in the drug are converted to sulfates, as these are more stable to heat, and allow more accurate results than those obtained by simple calcination. The acid-insoluble ash is the residue obtained after boiling the residue obtained in the determination of total ash and sulfated ash with dilute hydrochloric acid, filtrating to remove the soluble and igniting the remaining insoluble matter. This procedure determines the level of silica, especially sand and siliceous earth present in the drug.

Procedure for determination of total ash: Accurately 3 grams of the powder is transferred to porcelain crucibles which were previously calcined, cooled and weighed. The samples are charred in a muffle furnace at 450 $^{\circ}\text{C}$ for 2 hours. After cooling in a desiccator, they are weighed on an analytical balance. This procedure is repeated until obtaining constant weight. The ash percentage obtained in triplicate, is calculated in relation to the dried drug (Brazilian Pharmacopoeia V, 2010).

5.6 Determination of extractable matter

The determination of extractable matter is carried out when there are no suitable methods to determine the active constituents of the drug by chemical or physico-chemical processes. Substances extracted with water, with ethanol in various dilutions and, more rarely, with ether are usually determined. The method is based on the solubility of active substances in a given solvent, and when these are not known, on the pharmacological activity of the extract obtained as a solvent.

5.7 Determination of water and loss on drying

The presence of excessive amounts of water in plant drugs is responsible for the growth of bacteria and fungi as well as the hydrolysis of constituents. The pharmacopoeial monographs limit the water content, especially in drugs that have the facility to absorb it, or in which the excessive amounts of water cause deterioration. With few exceptions, the water content in vegetable drugs should vary between 8% and 14%.

The water content can be determined by the gravimetric method, where the drug is desiccated to constant weight in an oven. The heating also causes the loss of volatile

substances. For this reason it cannot be applied to drugs that contain such substances. In this case the azeotropic method may be applied, which consists of distilling the drug sample with toluene or xylene. This method requires special equipment and it is more difficult to carry on than the gravimetric method.

Procedure for determination of water loss on drying and gravimetric method: Exactly 3 grams of powdered plant are transferred to a weighing bottle. The sample is subjected to a temperature of 105° C for two hours followed by cooling in desiccators and weighted (Costa, 1982; Brazilian Pharmacopoeia V, 2010). The operation is repeated until obtaining constant weight. The results of three determinations are evaluated in terms of weight percentage on the sample amount using the equation (Brazilian Pharmacopoeia V, 2010):

$$\% \text{ loss} = \frac{W_b - W_a}{W_s} \times 100 \quad (1)$$

Where:

W_s = sample weight (g)

W_b = weight of the weighing bottle containing the sample before drying (g)

W_a = weight of the weighing bottle containing the sample after drying (g)

Another method that can be applied is the Karl Fischer Method, which is based on the quantitative reaction between water and anhydrous solution of iodine and sulfur dioxide dissolved in pyridine and methanol (Karl Fischer reagents). Usually the excess of reagent is incorporated to the sample, and after waiting for the time required to the quantitative reaction, the reagent excess is titrated with a standard solution of water in methanol. This technique (of unrestricted use) is especially recommended for samples which slowly release their content in water.

5.8 Determination of essential oils

Essential oils are volatile constituents found in many plants and characterized the mixture of terpenes, sesquiterpenes and their oxygenated derivatives and, sometimes, aromatic compounds, which volatilize at room temperature and are oily. Essential oils usually contain pharmacologically active substances. The determination of essential oils is carried out by hydrodistillation collecting the distillate in a graduated tube, in which the aqueous phase is automatically separated from the oily phase and returns to the distillation flask. When the essential oil has a density near to that of water or when the phase separation is difficult, a previously measured quantity of a solvent with low mass density and a suitable boiling point (e.g. xylene) is added in the graduated tube to dissolve the essential oil and facilitate the separation.

5.9 Identification by thin layer chromatography

The TLC is a simple, efficient method and requires no sophisticated devices for its implementation. The method is used for the characterization of plant drugs, their extracts and tinctures, and indicates the presence of the drug or its extracts in a pharmaceutical formulation. When the active principles of the drug are not known, the identification should be made using the substances which are characteristics of the plant, even without pharmacological activity.

These substances, called markers (or positive markers), should be selected among the substances that are characteristic of the plant. Its use, however, should be limited to the

identification of plant material, extracts and tinctures. The markers may also serve to identify the presence of the drug in a pharmaceutical formulation. However, when the markers are not the substances responsible for the pharmacological action of the drug they cannot be used for quantitative measurements. The existence of stained spots or bands with the same R_f of the reference substances, in the chromatogram of the sample is not sufficient to identify the drug.

The existence of other stained spots or bands and their position in relation to the position of the reference substances used must also be described. The use of reference substances that are not the plant constituents is useful in determining the occurrence of counterfeits. These substances are called negative markers. So, *Arnica montana* extracts are analyzed by comparison with rutin solutions, a substance that does not occur in this plant. The appearance of a spot in the chromatogram of the extract with the same R_f and color of rutin indicates a possible forgery with *Calendula officinalis* flowers.



Fig. 6. TLC analysis of the tincture of *Calendula officinalis* L. Eluent I: ethyl acetate; formic acid; acetic acid; water (100:11:11:26); developer: NP/PEG 4000. Observation under light UV 365 nm; P- rutin standard ; T- Calendula tincture (Nunes, 2008; Nunes et al., 2009)

5.10 Quantitative determination of active principles

The selection of a method for quantitative determination of active principles in phytotherapeutics depends on the monograph of the drug in matter, which must to be considered as a whole designed to assure a satisfactory quality to the product. Many times the selection of a quantification method falls on a less specific technique, which despite the ease in performing, shows high precision, as for example, the acid-base volumetric determination of alkaloids, or titration in non aqueous solutions. If analytical equipments are available, among other more elaborated methods, the ultraviolet spectroscopy, a fast and simple technique with relative low cost, can be employed to quantify markers which present at least a chromophore, a functional group that absorbs light between 200nm 400nm. However, some molecules do not present such group and in this case it is worth, to introduce a chromophore in the molecule or to adopt another quantification technique.

Sometime it is not possible to isolate or get the marker in adequate conditions to be used in the method, and it is possible to characterize and quantify a group of substances of the same metabolic class to which the pharmacological activity of the plant can be associated. This is the case of the alkaloids of a species from *Himatanthus* genus, to which antispasmodic activity is alleged, and that were characterized and quantified as total alkaloid fraction in relation to the yoimbine (Barros, 2010). The method was developed in laboratory and validated according to both Brazilian and International regulation (ANVISA, 2003; IHC, 2005), taking into account the following parameters: selectivity, verified by analysis of the reference substance in comparison with the sample and their solvent (Methanol), in the same wavelength, where the absorption of the sample and the substance and the transparency of the solvent can be observed; the linearity, characterized by the determination (R^2) and correlation (r) coefficients of the reference substance, and also, by the statistical analysis of the residue of the concentration values used to evaluate this parameter. Using five from the seven yoimbine concentration values employed to determine the linearity of the method, a calibration curve was built to, in fact, quantify the marker in the sample. The repeatability, the intermediate precision and the accuracy of the method must also be verified. In Brazil, ANVISA determines a 5% limit to the variation of the accuracy of a quantification method, which should not be lower than 95%. Other parameters that validate the developed method are detection and quantification limits. The following figures illustrate the selectivity (Figure 7), the linearity (Figure 8) e the analysis of residue (Figure 9) extracted from *Himatanthus* sp. (Barros, 2010).

Currently, in Brazil the regulation agency recommends for the registration of phytotherapeutic the use of more elaborated methods, which demand techniques like High Performance Liquid Chromatography (HPLC) or Gas Chromatography (GC), for the quantification of markers.

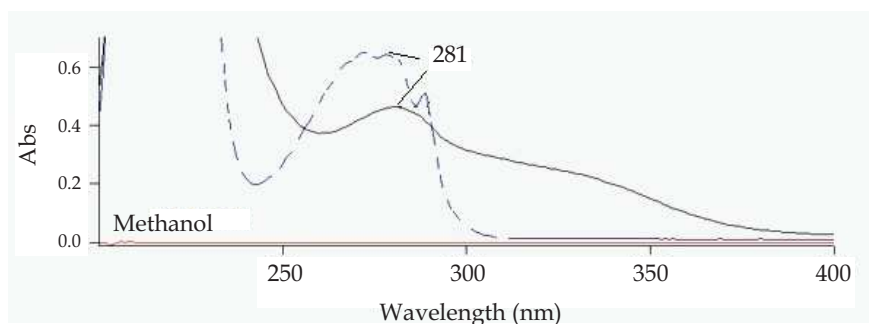


Fig. 7. Selectivity of the method developed with 22 µg/mL Yoimbine as reference, (- - -), in relation to the alkaloid fraction of the aqueous extract of *Himatanthus* sp at 30 µg/mL (-) and to solvent methanol, observed from 200nm to 400nm (absorbance vs wavelength)

5.11 Limit tests for heavy metals

The limit test for heavy metals consists in verifying if the content of metallic impurities that react colorimetrically with sulfide ion does not exceed the limit specified in the monographs in terms of micrograms of lead per gram of test sample. Similarly, the reaction with thioacetamide may be employed for determining the limit of heavy metals in terms of lead.

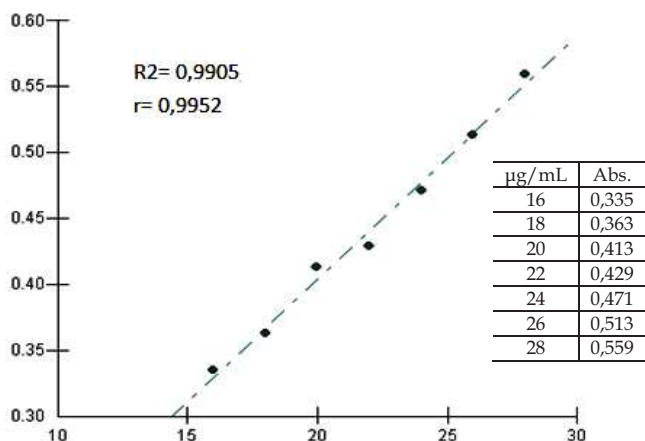


Fig. 8. Graphical representation of the linearity of the method using Yoimbine in methanol at 281nm, the detail concentrations and its respective absorbance (absorbance vs concentration)

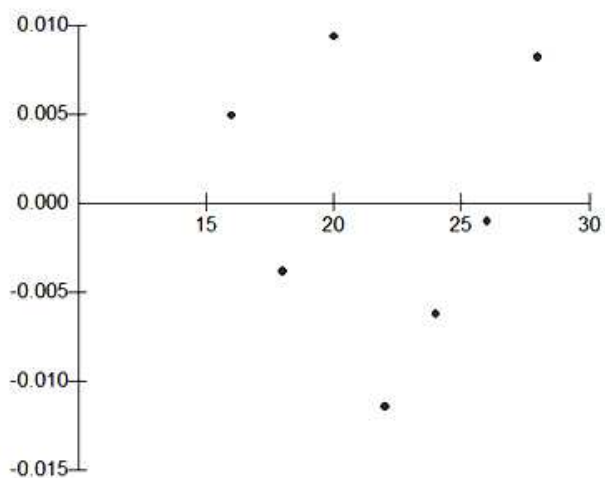


Fig. 9. Graphical representation of the residues analysis of the concentration values of Yoimbine solutions at 16, 18, 20, 22, 24, 26 and 28 µg/mL used in the calibration curve and determination of linearity

Procedure: The tests are conducted in transparent flat-bottomed glass tubes, with a capacity of approximately 70 mL and the external mark corresponding to a volume of 45-50 mL with an internal diameter of 23 mm. The tubes used should be equal in both the inner diameter and in other aspects, since the comparison is direct. The tubes should be observed from above, against a white background. The standard volume used varies according to the specified in the monograph under analysis.

5.12 Determination of pesticide residues

The use of pesticides to protect growing plants used as food from destruction by insects has grown enormously. The perception of the serious danger that the indiscriminate use of pesticides represented has led the WHO to establish limits to their residues. Since then, numerous methods for pesticide residue analysis have been published in the literature. Medicinal plants as well as food plants are subjected to attack by insects and fungi. Weeds are responsible for the reduced yields. Plant drugs accumulate pesticide just as food plants from agricultural practices such as spray application, the capture of the treated soils or administration of fumigants during storage.

6. Other methods that can be used for quality control of herbal drugs and/or their derivatives, tinctures and extracts

6.1 The infrared (IR) and ultraviolet (UV) spectroscopy

Infrared radiation corresponds approximately to the portion of the electromagnetic spectrum lying between the regions of the visible and microwave. The most useful portion to organic chemistry is situated between 4000 cm^{-1} and 400 cm^{-1} . This band when absorbed by an organic molecule, converts into a molecular vibration energy. The process is quantized, like a series of bands, as each change of vibrational energy level corresponds to a series of changes in rotational energy levels (Silverstein et al., 2007). It is possible to obtain spectra of gases, liquids and solids in the infrared. Liquids can be examined in their pure state or in solution. The solvent should be fairly transparent in the region of interest so as not to interfere in the result. Volatile liquids are examined in closed cells with very thin spacers. Solid samples are examined in the form of airborne dust, pressed disk (KBr, ZnSe, etc.), or glassy film deposited on a transparent plate (Silverstein et al., 2007).

The infrared spectrum of a chemical compound is considered one of its most characteristic physic-chemical features and, because of this the infrared spectroscopy is extensively used to identify compounds (Silverstein et al., 2007).

Another type of spectroscopy is performed in the ultraviolet (UV) region involving photons spectroscopy, and called spectrophotometry. Using a range between 200 to 400 nm the molecules undergo electronic transitions of molecular orbitals (Silverstein et al., 2007). The UV spectrophotometry is one of the most used methods in analytical determinations in various fields and may be applied to determinations of organic and inorganic compounds, for example, in the identification of the active principle of the drug. The molecular absorption spectroscopy is valuable for the identification of functional groups in the molecule. More important, however, are the applications of ultraviolet absorption spectroscopy for the quantitative determination of compounds containing chromophoric groups (Vinade & Vinade, 2005). Spectrophotometry in the UV region of the electromagnetic spectrum is one of the most used analytical techniques in terms of robustness, relatively low cost and large number of developed applications. It is based on the Beer-Lambert law, which is the mathematical basis for measuring radiation absorption by samples in solid, liquid or gaseous state in ultraviolet regions, visible and infrared electromagnetic spectrum (Perkampus, 1992). In determining the parameters of quality control of herbal medicines, which are composed of a complex mixture of components and due to the limitations on sensitivity in this method it is often necessary to use preliminary steps for separation and concentration of the desired elements, with a consequent increase of sensitivity. Among these techniques, we can mention the liquid-liquid extraction, precipitation and solid-liquid extraction (Cheng and Bray, 1955).

6.2 Thermal analysis

Thermal analysis is a term used to describe the analytical techniques that measure the physical and chemical properties of a sample as a function of temperature, while the substance is subjected to a controlled temperature program. The sample is subjected to a temperature schedule that consists of a series of preselected segments in which the sample is heated or cooled at a constant rate or kept at a constant temperature (Giolito & Ionashiro, 1988).

In recent decades, the thermo analytical techniques have gained increasing importance in all areas of knowledge in basic and applied chemistry. This development in the use of this methodology really endowed with great potential, was favored by the availability of instruments controlled by microprocessors, capable of providing accurate information about the thermal behavior of materials in a relatively short time. These methods are widely used in quality control of natural and synthetic drugs, because they can quickly provide data on the stability of the analyzed material, in the presence of its thermal behavior (Giolito & Ionashiro, 1988). Other methodologies which are importantly increasing in Brazil are: thermogravimetry (TG), differential thermal analysis (DTA) and differential scanning calorimetry (DSC) which are used to study pre-formulation or drug excipient compatibility. Among several papers published in this area, the most outstanding ones are: application of thermogravimetry (TG) in quality control of Milon (*Cissampelos sympodialis* Eichi.) Minispermaceae (Aragão et al., 2002), thermal analysis and compatibility study of zidovudine with excipients (Araujo et al., 2003); study of thermal stability of metronidazole tablets (Souza et al., 2002); Thermal analysis study of captopril coated tablets by thermogravimetry (TG) and differential scanning calorimetry (DSC) (Bazzo & SILVA, 2005); thermoanalytical study of glibenclamide and excipients (Oliveira et al., 2004); thermoanalytical study (TG, DTG and DSC) of *in natura* and processed coffee (Schnitzler et al., 2005); study, characterization and determination of the purity of commercial samples of saccharin (Schnitzler et al., 2005); determination of moisture content and ash content of commercial samples of guarana using conventional methods and thermal analysis (ARAÚJO et al., 2006); physicochemical characterization of fluid and spray dried extracts of *Symphytum officinale* L. (Silva Júnior, 2006; Silva Júnior et al., 2006), assessing the compatibility between fluoxetine and excipients used in the manufacture of capsules (Stulz & Tagliari, 2006); thermoanalytical study and drug-excipient compatibility of rifampicin and some medications used in the treatment of Tuberculosis (Alves, 2007), thermal stability and compatibility of hydroquinone (Tagliari et al., 2008).

7. Conclusion

The development of herbal medicines, as a rule, involves planning and obtaining intermediate preparations as required steps for processing vegetable raw material into a finished product, which will provide the desired pharmaceutical presentation. Among the available intermediate preparations, Pharmacopoeias recommend the tincture or fluid extract. These liquid presentations can be utilized to obtain standardized dried extracts, which can be used in the production of various pharmaceutical forms. It is important that the starting material is stabilized in order to ensure the reproducibility of the process. It also has to be sufficiently pulverized so as to achieve optimum performance in the process of extracting the chemical constituents of pharmaceutical interest.

To obtain a derivative of any medicinal plant, being it herbal medicine in any form, quality control is required from the cultivation, management and harvesting of the plant species; passing through the production of intermediate product up to the final formulation. Following a set of criteria it is possible to characterize the raw plant, its derivative and the formulation, designating an adequate planning to be followed as to establish the parameters of quality control. When the approach is applied to the analysis of natural extracts, the study of pre-formulation becomes more difficult because often the available markers are altered as a function of pH, solvent polarity, temperature and interactions (excipient and vehicle). When formulating products containing natural ingredients it is essential to identify standardized markers or to develop methods for the quantification of purified chemical groups before, during and after the production process. Therefore, for the technological development of herbal medicines according to the requirements and specifications of the countries' laws, it is essential to establish protocols that ensure quality control of raw materials, vegetable products and ingredients used in their production, as well as validation of qualitative and quantitative techniques appropriate to the chemical markers chosen. Only then, we can get a finished product with assured quality.

8. Acknowledgment

The authors acknowledge the Federal University of Pará (UFPA), Foundation to Support Research and Development (FADESP), National Council for Scientific and Technological Development (CNPq), Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES) and Pará Research Foundation (FAPESPA) by financial support to works realized.

9. References

- Alves, M. S. M. , Mendes, P. C., Vieira, J. G. P., Ozela, E. F., Barbosa, W. L. R., Silva Júnior, J. O. C. Análise farmacognóstica das folhas de *Arrabidaea chica* (Humb. & Bonpl.) B. Verlt., Bignoniaceae. *Rev. bras. farmacogn.* [online]. 2010, vol.20, n.2, pp. 215-221.
- Alves, M.S.M. Caracterização farmacognóstica, química, físico-química e estudos preliminares de pré-formulação da *Arrabidaea chica* (Humb. & Bonpl.) B. Verlt. 2008. Dissertação (Mestrado em Ciências Farmacêuticas), Faculdade de Farmácia, Universidade Federal do Pará, Belém.
- Alves, R. Estufa termoanalítica e de compatibilidade fármaco-excipiente de refampicina e alguns medicamentos utilizados na terapêutica da tuberculose. Dissertação de mestrado - Universidade de São Paulo, São Paulo 2007.
- Aragão, C.F.S.; Souza, F.S.; Barros, A.C.S.; Veras, J.W.E.; Barbosa Filho, J.M.; Macedo, R.O. aplicação da termogravimetria (TG) no controle de qualidade da milona (*Cissampelos sympodialis* Eichl.) Menispermaceae. *Revista Brasileira de Farmacognosia*, v. 12, supl., p.60 - 61, 2002.
- Araújo, A. A.; S. Mercuri, L. P.; Seixas, S. R. S.; Storpitis, S.; Matos, J. R. Determinação dos teores de umidade e cinzas de amostras comerciais de guaraná utilizando métodos convencionais e análise térmica. *Brazilian Journal of Pharmaceutical Sciences*, v. 42, n.2, abr/jun., 2006.

- Araújo, A.A.S. Análise térmica e determinação dos parâmetros cinéticos de preparações farmacêuticas e novas especialidades de zidovudina. 225f. Tese (Doutorado em Ciências Farmacêuticas) – Faculdade de Ciências Farmacêuticas – USP, São Paulo, 2003.
- Bauer, K. H., Frömring, K. H., Führer, C.: *Lehrbuch der Pharmazeutischen Technologie*, 7th ed. Stuttgart, 2002.
- Bauer, R., Tittel, G. Quality assessment of herbal preparations as a precondition of pharmacological and clinical studies. *Phytomedicine* 2: 193-198. 1996.
- Bazzo, G.C.; Silva, M.A.S. Estudo termoanalítico de comprimidos contendo captopril através de termogravimetria e calorimetria exploratória diferencial. *Revista Brasileira de Ciências Farmacêuticas*, vol. 41, n.3, 2005.
- Blumenthal, M., Brusse, W. R., Goldberg, A., Gruenwald, J., Hall, T., Riggins, C. W., Rister, R. S. *The Complete German Commission E Monographs. Therapeutic Guide to Herbal Medicines*. The American Botanical Council, Austin, TX, USA. 1998
- Borges, L.; Sciliar, M.; Almeida, S. Biodiversidade brasileira e produtos industriais, Belo Horizonte: UFMG, 2003. Disponível em <http://www.icb.ifmg.br/~ibem/aulas/grad/tge/biodiv/2002/prodindust/>. Acesso em 12 nov.2006.
- Brasil. RE nº899 de 29 de maio de 2003. Dispõe sobre o Guia para validação de métodos analíticos e bioanalíticos. Ministério da Saúde. Agência Nacional de Vigilância Sanitária. Disponível em: [HTTP://e-legis.anvisa.gov.br](http://e-legis.anvisa.gov.br).
- Calixto, J. B. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). *Braz. J. Med. Biol. Res.* 33(2): 179-189. 2000.
- Carvalho, J.C.T. Fitoterápicos anti-inflamatórios: aspectos químicos, farmacológicos e aplicações terapêuticas. Tecmed. São Paulo, 2004.
- Cheng, K.L.; Bray, R.H. 1-(2-pyridylazo)-2- naphthol as a possible analytical reagent. *Analytical Chemistry*, v. 27, p. 782, 1955.
- Costa, A.F. *Farmacognosia*. 3 Ed. v. 3. Fundação Calouste Gulbenkian: Lisboa, 2000.
- Elvin-Lewis, Memory. Should we be concerned about herbal medicines? *J. Ethnopharmacol.*, Amsterdam, v.75, p.141-164, 2001.
- Evert, R. F. *Esau's plant anatomy*, Hoboken, New Jersey: J. Wiley, 2006.
- Fahn A. *Secretory tissues in plant*. London: Academic Press, 1979.
- Farias, M. R. et al, P.R. O problema da qualidade dos fitoterápicos. *Caderno de farmácia*, Vol. 1, nº 2, 73-82, 1985.
- Farmacopéia Brasileira*, V ed. São Paulo: Editora Atheneu, 2010.
- Ferreira, L.A.; Leite, J.P.V. Desenvolvimento de formulações fitoterápicas. In: LEITE, J.P.V. *Fitoterapia: bases científicas e tecnológicas*. São Paulo: Editora Atheneu, p. 206-251, 2008.
- Gaedcke, F. W. Steinhöf, K. B. (2003). *Herbal Medicinal Products*. Stuttgart, Germany: CRC Press, ISBN: 0-8493-1023-7. p. 1-30.
- Giolito, I.; Ionashiro, M. *Nomenclatura em análise térmica: Parte II*. Cerâmica, São Paulo, v. 34, p 163-164, 1988.
- Gobbo-Neto, L. Lopes, N. Plantas medicinais: Fatores de influência no conteúdo de metabólitos secundários. *Química Nova*, Vol. 30, nº2, 374-381, 2007.

- Hänsel, R.: *Phytopharmaka. Grundlagen und Praxis*, 2nd ed. Berlin, 1991.
- Harbone, J. B. Plant secondary metabolism. In: Crawley M. J. (ed.) *Plant ecology*. 2 ed. Berlin: Blackwell Publishing, 1997, p. 132-155.
- International Conference on Harmonization (2005), in: *Proceedings of the Conference on Harmonization (ICH)*, Commission of the Euro Communities.
- Leite, J.P.V. (2009). *Fitoterapia: Bases Científicas e Tecnológicas*. São Paulo: ATHENEU, ISBN: 978-85-7379-237-9, p. 3-119.
- Leonti, M. The future is written: Impact of scripts on the cognition, selection, knowledge and transmission of medicinal plant use and its implications for ethnobotany and ethnopharmacology. *J. Ethnopharmacol.* 19 [Epub ahead of print]. 2011.
- Nunes, K. M. Caracterização química e físico-química e estudos preliminares de planejamento da formulação fitoterápica semi-sólida contendo *Calendula officinalis* L. 2008. 141 f. Dissertação (Mestrado em Ciências Farmacêuticas) - Universidade Federal do Pará, Belém 2008.
- Nunes, K.M. et al. Padronização da Tintura de *Calendula officinalis* L. para seu Emprego em Formulações Semi-sólidas Fitoterápicas. *Latin American Journal of Pharmacy*, n. 28, v. 3, p. 344-350, 2009.
- Oliveira, G.G.G.; Ferraz, H.G.; Matos, J.D.R. Estudo termoanalítico de glibenclamida e alguns excipientes utilizados em formas farmacêuticas sólidas. In: 2º Congresso Panamericano de Análise térmica e Calorimetria. Poços de Caldas, 2004.
- Perkampus, H. H.; *UV-VIS Spectroscopy and its Applications*, Springer-Verlag: Berlin, 1992.
- Petrovick, P.R.; Gonzalez Ortega, G.; Bassani, V.L. From a medicinal plant to a pharmaceutical dosage form. A (still) long way for the Brazilian medicinal plants. *Ciência e Cultura*. v. 49, n.5/6, p. 364-369, 1997.
- Pieroni, A. & Vandebroek, I. *Traveling cultures and plants: the ethnobiology and ethnopharmacy of migrations*. New York, Berghahn Books. 2007.
- Pinto, M. T. J.; Kaneko, T. M.; Pinto, A. F. *Controle Biológico de Qualidade de Produtos Farmacêuticos, correlatos e Cosméticos*. Terceira Edição. 2010. Editora Atheneu.
- Rates, S.M.K. Plants as source of drugs. *Toxicon*, Amsterdam, v.39, p.603- 613, 2001.
- Robbers, J.E.; Speedie, M.K.; Tyler, V.E. *Pharmacognosy and pharmacobiotechnology*. Baltimore: Willians & Wilkins, 1996. p.1-14.
- Santos, E.V.M. Extração de matérias-primas vegetais. In: SHARAPIN, N. *Fundamentos de tecnologia de Produtos Fitoterápicos*. Cytel, Santafé de Bogotá, 2000. p. 27-60.
- Schenkel, E.P.; Osmann, G.M.; Mello, J.C.P.; Mentz, L.A.; Petrovick, P.R., orgs. *Farmacognosia: da planta ao medicamento*. 2.ed. Florianópolis. UFSC; Porto Alegre: UFRGS, 2000. cap.15, p.291-320.
- Schnitzler, E.; Beninca, C.; Kobelnik, M.; Sotelo, G.F.C.; Carneiro, P.I.B. Estudo, caracterização e determinação do grau de pureza de amostras comerciais de sacarina. Publicatio UEPG. Ciências exatas e da terra, ciências agrárias e engenharias. Ponta Grossa, 2005.
- Schnitzler, E.; Pereira, C.A.; Carvalho Filho, M.A.S. Estudo termoanalítico (TG, DTG e DSC) dos cafés *in natura* e processados. Publicatio UEPG. Ciências exatas e da terra, ciências agrárias e engenharias. Ponta Grossa, v. 11, n. 1, p. 61-66, 2005.

- Schulz, V.; Hansel, R.; Tyler, V.E. Medicinal plants, phytomedicines, and phytotherapy. In: *Rational phytotherapy: a physician's guide to herbal medicine*. 4.ed. New York, Berlin: Springer, 2001. cap.1, p.1-39.
- Sharapin, N. Fundamentos de tecnologia de Produtos Fitoterápicos. Cyted, Santafé de Bogotá, 2000. p. 145-157.
- Silva Júnior, J.O.C.; Vieira, J.L.F.; Barbosa, W.L.R.; Pereira, N.L. Caracterização físico-química do extrato fluido e seco por nebulização de *Symphytum officinale* L. Revista Brasileira de Farmacognosia, v.16 (supl.): Dez. 2006.
- Silva Júnior, José Otávio Carrêra. Obtenção e avaliação de forma farmacêutica semi-sólida fitoterápica contendo extrato seco por nebulização de *Shymphytum officinale* L. (confrei). Tese de doutorado / FCFRP- USP. 2006.
- Silverstein, R.M.; Webster, F.X.; Kiemle, D.J. Identificação espectrométrica de compostos orgânicos. 7. Ed. Tradução: Ricardo bicca de Alencastro - Rio de Janeiro: LTC, 2007. p. 70-122.
- Souza, F.S.; Macedo, R.O.; Veraz, J.W.E. Estudios of cimetidine préformulated and tablets for TG and DSC coupled to the photovisual system. Thermochim. Acta, v. 392-393, p. 99-106, 2002.
- Stulzer, H.K.; Tagliari, M.P. Avaliação da compatibilidade entre a fluoxetina e excipientes usados na fabricação de cápsulas pela farmácia magistral. Visão Acadêmica. Curitiba, v.7, p. 15-23, 2006.
- Tagliari, M.P.; Stulzer, H.K.; Kelmann, R.G.; Kuminek, G.; Silva, M.A.S. Estabilidade térmica e compatibilidade da hidroquinona. Revista Cosmetics & Toiletries. Vol.20, n°2, 2008.
- Tuescher, E. : Biogene Arzneimittel, 5th Ed. Stuttgart: Wissenschaftliche Verlagsgesellschaft mbH; 1997.
- Turolla, M.S.R.; Nascimento, E. Inforomações toxicológicas de alguns fitoterápicos utilizados no Brasil. Brazilian Journal of Pharmaceutical Sciences, v. 42, n.2, abr/jun.,2006.
- Tyler, V.E. Natural products and medicine: an overview. In: Balick, M.J.; Elisabetsky, E.; Laird, S.A., eds. *Medicinal resources of the tropical forest, biodiversity and its importance to human health*. New York: Columbia University Press, 1996. p.3-10. (Biology and resource management series).
- United States Pharmacopoeia. 30 ed. Rockville: The United States Pharmacopeia Convention, 2006.
- Vinadé, M.E.doC.; Vinadé, E.R.doC., Métodos espectroscópicos de análise quantitativa, editora UFSM, 2005.
- Vogel, G.: Pflanzliche Arzneimittel aus medizinischer und pharmakologischer Sicht. In: Qualität pflanzlicher Arzneimittel, Stuttgart: Wissenschaftliche Verlagsgesellschaft mbH; 1982. p. 19-30.
- Wagner, H.; Wiesenauer, M. Fitoterapia: fitofármacos, farmacologia e aplicações clínicas. 2ª edição. São Paulo: Pharmabooks, 2006.
- WHO World Health Organization. Quality Control Methods for Medicinal Plant Materials. Geneva: Organização Mundial de Saúde, 1992.
- WHO. Health in the millennium development goals. (World Health Organization) (<http://www.who.int/mdg/goals/en/>, acessado em 25 de Julho de 2009).

- Yoney, A., Prieto, J. M., Lardos, A., Heinrich, M. Ethnopharmacy of Turkish-speaking Cypriots in Greater London. *Phytother. Res.* 24(5): 731-740. 2010.
- Yunes, A. R.; Pedrosa, C.R.; Filho, C. V. Fármacos e fitoterápicos: a necessidade do desenvolvimento da indústria de fitoterápicos e fitofármacos no Brasil. *Química Nova.* v. 24, n.1, p. 147 -152, 2001.



Quality Control of Herbal Medicines and Related Areas

Edited by Prof. Yukihiro Shoyama

ISBN 978-953-307-682-9

Hard cover, 282 pages

Publisher InTech

Published online 07, July, 2011

Published in print edition July, 2011

The authors of this thematic issue provide a comprehensive summary of most recent knowledge and references on quality control in wide fields. Quality control is essential for natural products like natural medicine and related food products. In this issue fifteen chapters have been included, discussing in detail various aspects of quality control. It will certainly prove useful not only for phytochemical researchers, but also many scientists working in numerous fields. Much effort has been invested by the contributors to share current information. Without their efforts and input 'Quality Control of Herbal Medicine and Related Areas' could not exist.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

José Otávio Carréra Silva Júnior, Roseane Maria Ribeiro Costa, Francisco Martins Teixeira and Wagner Luiz Ramos Barbosa (2011). Processing and Quality Control of Herbal Drugs and Their Derivatives, Quality Control of Herbal Medicines and Related Areas, Prof. Yukihiro Shoyama (Ed.), ISBN: 978-953-307-682-9, InTech, Available from: <http://www.intechopen.com/books/quality-control-of-herbal-medicines-and-related-areas/processing-and-quality-control-of-herbal-drugs-and-their-derivatives>

INTECH
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.