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Secondary Metabolites

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1. Introduction

Secondary metabolites are organic molecules that are not involved in the normal growth and development of an organism. While primary metabolites have a key role in survive of the species, playing an active function in the photosynthesis and respiration, absence of secondary metabolites does not result in immediate death, but rather in long-term impairment of the organism's survivability, often playing an important role in plant defense. These compounds are an extremely diverse group of natural products synthesized by plants, fungi, bacteria, algae, and animals. Most of secondary metabolites, such as terpenes, phenolic compounds and alkaloids are classified based on their biosynthetic origin. Different classes of these compounds are often associated to a narrow set of species within a phylogenetic group and constitute the bioactive compound in several medicinal, aromatic, colorant, and spice plants and/or functional foods.

Secondary metabolites are frequently produced at highest levels during a transition from active growth to stationary phase. The producer organism can grow in the absence of their synthesis, suggesting that secondary metabolism is not essential, at least for short term survival. A second view proposes that the genes involved in secondary metabolism provide a "genetic playing field" that allows mutation and natural selection to fix new beneficial traits via evolution. A third view characterizes secondary metabolism as an integral part of cellular metabolism and biology; it relies on primary metabolism to supply the required enzymes, energy, substrates and cellular machinery and contributes to the long term survival of the producer (Roze et al, 2011).

A simple classification of secondary metabolites includes tree main groups: terpenes (such as plant volatiles, cardiac glycosides, carotenoids and sterols), phenolics (such as phenolic acids, coumarins, lignans, stilbenes, flavonoids, tannins and lignin) and nitrogen containing compounds (such as alkaloids and glucosinolates). A number of traditional separation techniques with various solvent systems and spray reagents, have been described as having the ability to separate and identify secondary metabolites. This chapter proposes to discuss major secondary metabolites classes (terpenoids, phenolic compounds and alkaloids) with different chemical structures and functions being screened, separated, fractionated, purified

or analyzed using various adsorbents and eluents through column chromatography (CC) and thin layer chromatography (TLC).

2. Terpenoids

Terpenoids are the largest and most diverse family of natural products, ranging in structure from linear to polycyclic molecules and in size from the five-carbon hemiterpenes to natural rubber, comprising thousands of isoprene units. All terpenoids are synthesized through the condensation of isoprene units (C_5) and are classified by the number of five-carbon units present in the core structure (Mahmoud et al. 2002). Many flavor and aromatic molecules, such as menthol, linalool, geraniol and caryophyllene are formed by monoterpenes (C_{10}), with two isoprene units, and sesquiterpenes (C_{15}), with three isoprene units. Other bioactive compounds, such as diterpenes (C_{20}), triterpenes (C_{30}) and tetraterpenes (C_{40}) show very special properties and will be also discussed in this chapter.

2.1 Monoterpenes and sesquiterpenes (Plant volatiles)

Plant volatiles are typically lipophilic liquids with high vapor pressures. Non-conjugated plant volatiles can cross membranes freely and evaporate into the atmosphere when there are no barriers to diffusion. The number of identified volatile chemicals synthesized by various plants exceeds 1000 and is likely to grow as more plants are examined with new methods for detecting and analyzing quantities of volatiles that are often minute (Pichersky et al. 2006; Dudareva et al., 2004). Studying the volatile fraction requires analytical methods and technologies that not only evaluate its composition exhaustively but also monitor variations in its profile and detect trace components characterizing the plant being investigated (Bicchi et al., 2011).

The gas chromatography (GC and GC-MS) is a very powerful analytical tool for the identification of essential oil components. However, GC-MS has its limitations. Isomers usually give very similar mass spectra. This is particularly true for terpenes and even more for sesquiterpenes. Therefore, a favorable match factor between mass spectra is not sufficient for identification (Zellner et al., 2010). Retention indices have been used, together with mass spectrometry, for the proper identification of essential oils composition. Misidentification is not rare, however, either if a non-authentic mass spectra library is used, which means a database built with data from the literature, not from the analysis of real standards, or by misuse of retention indices (Joulain & König, 1998). CC has been applied to solve this problem. After isolation, a NMR analysis can be performed for the unknown or suspect compound, so that the correct mass spectrum and retention index can be recorded.

Despite the advances in analytical methods to evaluate the composition of essential oil, CC remains a powerful technology for separation and characterization of specific compounds of interest. Column chromatography has been largely used and reported for searching and identification of new molecules, sometimes associated with their antimicrobial, antibacterial and antifungal activities. Also, this technique has been successfully used to obtain sufficient amounts of a substance for the investigation of its biological properties and allowing the detection of its olfactory properties. Isolation is also applied to and, very important for volatiles, to evaluate its odor.

2.1.1 Compound identification

The major constituents present in the *volatile oils* of different aromatic species may also be isolated or fractionated by silica gel on preparative TLC or CC. For example, the essential oil of *Tanacetum chiliophyllum* was subjected to silica gel CC, using a solvent mixture of *n*-hexane and ethyl acetate, to isolate one of its major components, dihydro- α -cyclogeranyl hexanoate (Salamci et al, 2007).

Djabou et al. (2010) have characterized the essential oils of *Teucrium massiliense* L. from Corsican and Sardinian islands, using a combination of capillary GC/retention indices, GC/MS and ^{13}C NMR spectroscopy after fractionation on CC. A mixture of all Corsican oil samples was submitted to flash chromatography [FC; silica gel, elution with *n*-pentane, then with diethyl ether]. The polar fraction was separated on silica gel and 14 fractions were eluted with a mixture of *n*-pentane and diethyl ether of increasing polarity to give the major components: 6-methyl-3-heptyl acetate, 3-octyl acetate, isobutyl isovalerate, germacrene D and linalool. Successive CC revealed the unknown compound 6-methyl-3-heptyl acetate.

Chemical investigations on the essential oil of *Lippia integrifolia* performed by CC (silica gel using *n*-hexane with increasing amounts of diethyl ether), followed by HPLC, GC-MS, ^1H and ^{13}C -NMR spectroscopy led to the identification of 78 components. A new sesquiterpenic alcohol, *trans*-africanan-1 α -ol, was identified (Coronel et al. 2006).

Sutour et al. (2008) studied the essential oil of *Mentha suaveolens* ssp. *insularis* (Req.) Greuter, finding pulegone to be the major constituent. However, the second most abundant component of the essential oil remained unknown, being isolated by repeated chromatography on silica gel and submitted to a full set of NMR experiments, and identified as *cis-cis-p*-menthenolide. Fractions were eluted with a gradient of solvents of increasing polarity (pentane:diethyl ether 100:0–0:100).

The essential oil from the leaves of *Piper hispidinervum*, a native species from the Amazon, is very rich in safrole (Maia et al., 1987). During an extensive agronomical study with this species, a population produced an essential oil rich in a different phenylpropanoid (up to 78%). The mass spectrum was identical to that of myristicin (Figure 1). However, a difference of near 30 units in retention index between myristicin and the phenylpropanoid was strong evidence that it was another compound, probably a myristicin isomer. No retention indices were available for the two possible isomers, croweacin and sarisan. For identification, the unknown compound was isolated by CC on silica gel and eluted with hexane-ethyl acetate mixtures. After ^1H and ^{13}C NMR studies, it was identified as sarisan (Bizzo et al., 2001).

Fig. 1. Possible isomers of myristicin from the oil of a population of *Piper hispidinervum*; sarisan was the actual compound present.

Evaluating the essential oils from the leaves of 40 individuals of *Croton cajucara* Benth. from a germplasm bank in Manaus (State of Amazonas, Brazil), Quadros et al. (2011) observed some plants, instead of linalool, produced an oil rich in a hydroxylated sesquiterpene. The mass spectrum was very similar to that of 5-hydroxycalamenene. After the last edition of Adams' reference book (Adams, 2007), it was verified that the retention index of the sesquiterpenic alcohol did not correspond to that of 5-hydroxycalamenene. The oil was chromatographed over silica gel with hexane and hexane-ethyl acetate mixtures. The fraction containing the compound of interest was re-chromatographed on a silica gel preparative TLC plate with hexane-ethyl acetate (90:10). The purified compound was extracted from the silica with chloroform. Two clear singlets in the aromatic hydrogens at 6.56 and 6.94 ppm instead of a doublet pointed to a 7-hydroxy-substituted structure (Figure 2). Chemical shifts were in good agreement with published data for *cis*-7-hydroxycalamenene.

Fig. 2. Hydroxylated sesquiterpenes from *Croton cajucara*.

Sometimes, despite all careful efforts and extensive study, wrong structure assignment is published. For example, a new triquinane sesquiterpenic alcohol was isolated from the essential oil of *Anemia tomentosa* var. *anthriscifolia* by CC on silicagel with a hexane-ethyl acetate gradient. A new structural formula, namely (–)-*epi*-presilphiperfolan-1-ol, was initially proposed, after extensive 1D- and 2D-NMR analyses, as well as by GC-MS, chiral bidimensional GC, dehydration reactions, and a comparative (GIAO/DFT) theoretical study of the ^{13}C NMR chemical shifts (Pinto et al., 2009a). Further examination by X-ray diffraction and vibrational circular dichroism studies, however, led to its reassignment to (–)-9-*epi*-presilphiperfolan-1-ol (Figure 3), also a new compound. Its absolute configuration was established as 1*S*,4*S*,7*R*,8*R*,9*S* (Joseph-Nathan et al., 2010).

Fig. 3. Isomers of (–)-presilphiperfolan-1-ol: the 9-*epi*-isomer was isolated from *Anemia tomentosa* var. *anthriscifolia* essential oil.

2.1.2 Olfactory analysis

Aroma concentrate separated from an aqueous solution of Haze honey by adsorptive CC had stronger aroma intensity than that separated by other methods. Fractions separated by preparative GC were sniffed to evaluate the sensory importance of the volatile compounds of Haze honey. Benzeneacetaldehyde, linalool, phenethyl alcohol, *p*-cresol, *p*-anisaldehyde,

methyl-*p*-anisaldehydes, trimethoxybenzene, 5-hydroxy-2-methyl-4*H*-pyran-4-one, and lilac aldehydes seemed to contribute to Haze honey's aroma (Shimoda et al., 1996).

Shimoda et al. (1995) reported that a column extraction method was applied to the separation of volatile compounds from infusions of green tea and black tea, wherein their aromas degrade during distillation even under reduced pressure, and from sake, wherein ethyl alcohol disturbs a quantitative separation of volatile compounds.

Linalool is a very frequent terpenoid in essential oils, usually responsible for the scent of a specific plant, such as mint, basil, and other plant species. In order to evaluate the effect on humans of inhalation of linalool enantiomers (Figure 4), repeated flash chromatography was used to isolate R-(-)-linalool from lavender essential oil, while (S)-(+)-linalool was obtained from coriander oil and commercial linalool (Sugawara et al. 2000).

Fig. 4. Linalool enantiomers.

The Australian finger lime (*Citrus australasica*), an endemic Australian species, is a unique citrus fruit in terms of its size, shape and aroma. The fractionation of finger lime peel oil by chromatography on silica gel (with a gradient of pentane:ether) was able to identify a high number of terpenyl esters enriched in a medium polar fraction. This included citronellyl and geranyl esters, commonly found in lime peel oils, but also several less common (*Z*)-3-hexenyl esters and bornyl esters. In finger limes these compounds, together with isomenthone, menthol, carvone, *cis*-3-hexenol and methyl salicylate, may also contribute to the characteristic fresh green aroma of the fruits (Delort et al., 2009).

L-menthyl-L-lactate [(2*S*, 1'*R*, 2'*S*, 5'*R*)-2-isopropoyl-5-methylcyclohexyl 2-hydroxypropanoate, LM-LL] is widely used as a cooling compound in mint flavors, fruit flavors, oral care products, confections and beverages. L-menthyl-L-lactate was identified in dementholized cornmint oil from India by comparing mass spectrum and retention time with a reference sample on two columns. Separation was achieved by increasing polarity of the hexane-methyl-*tert*-butyl-ether (MTBE) gradient on a flash chromatography column packed with silica gel. It is worth noting that L-menthyl-L-lactate was identified in *M. arvensis* oils from India but not in *M. piperita* oils from the USA. Model experiments proved that LM-LL is formed during water vapor distillation of mint leaves in the presence of lactic acid. Lactic acid is spontaneously formed when mint herb is stored for some days in a humid environment. A prolonged storage of damp mint herb may increase formation of lactic acid and thus give rise to the formation of LM-LL during distillation. The presence of lactic acid will be influenced by the agricultural practice employed (Gassenmeier, 2006).

Gas chromatography-olfactometry (GC-O), gas chromatography-mass spectrometry (GC-MS) and preparative CC were used to identify the key odorants present in laboratory-extracted clementine oil from Spain. Almost 50 odorants were identified using GC-O, many of which were unsaturated aldehydes with high odor spectrum values. α - and β -sinensal, *trans*-4,5-epoxy-(*E*)-2-decanal, (*E,Z*)-2,6-dodecadial and linalool were found to dominate clementine oil aroma. Enrichment of the oxygenates using preparative CC provided further identification of a total of 50 aldehydes, which were originally present in the oil at concentrations not high enough to produce a response using GC-O (Chisholm et al., 2003).

2.1.3 Biological activities

Human enteropathogens (*Campylobacter jejuni*, *Campylobacter coli*, and *Campylobacter lari* strains) were inhibited by the essential oil of wild *Daucus carota* L. DCEO. Major components of the essential oil responsible for the antibacterial activity were isolated on CC and identified as (*E*)-methyl-isoeugenol and elemicin (Rossi et al., 2007).

Confertifolin (6,6,9a-trimethyl-4,5,5a,6,7,8,9,9a-octahydronaphtho[1,2-c] furan-3 (1*H*)-one), isolated from the essential oil of *Polygonum hydropiper* L. (Polygonaceae) leaves using CC, showed activity both against bacteria and fungi. The oil was chromatographed on a column packed with silica gel and eluted with hexane-ethyl acetate gradient solvent system with increasing polarity, yielding 117 fractions (Duraipandian et al., 2010).

The essential oil of *Daucus crinitus* Desf. was submitted to flash chromatography (silica gel, using petroleum ether with increasing amounts of diethyl ether as eluent), and fractions were analyzed by GC-MS. Those containing the isochavicol esters were gathered for the preparation of isochavicol. The antibacterial and antifungal activities of the whole essential oil, of these two esters, and of isochavicol itself, were investigated against a wide range of bacteria and fungi. Additionally, their antimalarial and antiradical properties were also evaluated, showing an interesting antiplasmodial activity of isochavicol (Lanfranchi et al., 2010).

Patchouli oil obtained from *Pogostemon cablin* (Blanco) Benth and its main constituent, patchouli alcohol, were tested for their repellency and toxicity against Formosan subterranean termites (*Coptotermes formosanus* Shiraki). Both were found to be toxic and repellent. The fraction containing patchouli alcohol was purified by silica CC, and its purity was determined by GC-MS. Unusual tissue destruction was noted inside the exoskeleton of the termite after patchouli alcohol was topically applied to the dorsum (Zhu et al., 2003).

A successful breeding program of *Cannabis sativa* cultivars with a low content of the hallucinogenous D-9-tetrahydrocannabinol (THC) is a prerequisite to avoid drug abuse of hemp. Four TLC systems for analyzing cannabinoids were compared, being able to identify the absence of THC in the hemp essential oils from five commercial fiber varieties. The system using HPTLC, RP-18 and acetonitrile-water (9:1) achieved the best separation of hallucinogenous and non-hallucinogenous cannabinoids (Novak et al., 2001).

Nine fractions of essential oil extracted from *Thymus praecox* were separated by silica gel CC and the fractions were demonstrated to have antioxidant activity (Ozen et al, 2011). The essential oil of *Coriandrum sativum* was submitted to silica gel on dry-CC, resulting in six fractions. Essential oil and its fractions presented antimicrobial potential against *Candida* yeast infections (Begnami et al, 2010). Essential oils, distilled from seeds of *Coriander sativum*

and *Carum carvi* and from leaves of *Ocimum basilicum*, were fractionated by silica gel over CC and tested in the laboratory for volatile toxicity against three stored rice pests. Fractions, where combinations of products occurred with or without other minor compounds, were often more toxic than any one compound alone (Lopez et al, 2008).

The main component of the essential oil of *Anemia tomentosa* var. *anthriscifolia*, (-)-9-epi-presilphiperfolan-1-ol (Figure 3) was isolated by CC over silica gel, eluted with hexane and hexane-ethyl acetate (95:5), then tested against *Mycobacterium tuberculosis* (H37Rv) and *M. smegmatis*. A minimum inhibitory concentration of 120 µg/ml was recorded for *M. tuberculosis* (Pinto et al., 2009b).

2.2 Diterpenes and sesterterpenes

The diterpene compounds arise from geranylgeranyl diphosphate, and present 20 carbon units in their basic skeletal type. One of the simplest and most important of the diterpenes is phytol (Figure 5), a reduced form of geranylgeraniol, which forms the lipophilic side-chain of the chlorophylls (Vetter and Schröder, 2011).

Cyclization reactions of geranylgeranyl diphosphate led to many structural types of diterpenoids, presenting a large range of polarity nature, from apolar hydrocarbons such as cembrene (Villanueva and Setzer, 2010), a 14-membered ring, to fully oxidized skeleton of virescenoside (Figure 5), isolated from marine fungus *Acremonium striatisporum* (Ebel, 2010).

Fig. 5. Diterpenes phytol, cembrene and virescenoside W.

Sesterterpenes (C_{25}) may be the least common group of terpenoids. This class of compounds arises from geranylfarnesyl diphosphate (Figure 6), which by cyclization can give rise to various skeletal types, presenting different oxidation levels and several biological activities.

Although many examples of these natural terpenoids are known, they are primarily isolated from fungi and marine organisms.

Considering the large range of polarity nature presented by both diterpene and sesterterpene, the isolation and purification techniques vary and can be classic TLC, preparative thin-layer chromatography (PTLC), CC, flash chromatography (FC), or modern high performance liquid chromatography (HPLC), multflash chromatography, vacuum liquid chromatography (VLC), solid-phase extraction and others (Lanças, 2008). However, the most important factor that has to be considered before designing an isolation protocol is the nature of the target compound, such as solubility (hydrophobicity or hydrophilicity), acid-base properties, charge, stability, molecular size. Taking these factors into account, the choice of chromatographic methods and the stationary phases to be used are important for the design of a purification system.

Silica gel-based material is the most usual stationary phase. However, polyamide, Sephadex® LH-20, alumina (Al_2O_3), florisil and others are also used, mainly for isolation of more polar compounds (Ajit-Simh et al., 2003). The air-dried and ground roots of *Peltodon longipes* were extracted with hexane. The methanol soluble fraction of the hexane crude extract was subjected to open CC on silica gel 60 eluted with hexane/ethyl acetate step gradient. After subsequent purification, several abietane diterpenes were isolated, including royleanone, inuroylleanol, deoxyneocryptotanshinone and 7 α -ethoxyroyleanone (Figure 6) (Fronza et al., 2011).

Fig. 6. Geranylfarnesyl diphosphate and abietane diterpenes from *Peltodon longipes*.

The sesterterpenes 24-*O*-methylmanaolide and 24-*O*-ethylmanaolide (Figure 7), besides others, were isolated from the sponge *Luffariella* cf. *variabilis*. Briefly, the frozen sponge tissue was extracted by methanol:chloroform (1:2). The crude extract was successively partitioned between equal volumes of aqueous methanol and a solvent series of hexane, carbon tetrachloride and chloroform. The hexane fraction was subjected to silica gel columns, using eluents of increasing polarity from hexane: ethyl acetate (Gauvin-Bialecki et al., 2008).

Olefins can form co-ordination products with silver ions, and this ability has been used for chromatographic separation of several terpenes, mainly those with a carbon skeleton higher than C₂₀. The procedure to prepare the modified silica was described as follows: Silver nitrate (AgNO₃) in water (1:2 w:v) is added to silica. After water evaporation, the residue is dried in oven at 130 °C for 15 h, resulting in an almost white powder that should be stored in a dark bottle. Column packing is carried out in the same way as ordinary silica gel column; however, should be wrapped in dark paper or built with amber glass (Norin and Westfelt, 1963).

Methyl ester from kaurenoic acid, iso-kaurenoic acid and grandiflorenic acid (Figure 7) were purified by using argentum silica (Batista et al., 2010). The diterpene acids were isolated from aerial parts of *Wedelia paludosa*. The plant material was extracted by percolation first using hexane, followed by ethanol. The crude ethanol extract was chromatographed over silica gel column. A mixture of these three compounds was obtained and further submitted to isocratic CC on silica gel impregnated with 20% of AgNO₃, eluting with hexane:diethyl ether 97:3 (Batista et al., 2010).

Fig. 7. Sesterterpenes 24-*O*-methylmanaolide and 24-*O*-ethylmanaolide from *Luffariella* cf. *variabilis* and diterpene acids isolated from *Wedelia paludosa*.

Polyamide 6 (ϵ -aminopolycaprolactam) has been used in our laboratory to isolate polar natural products due to its low cost and re-usability. The pre-treatment of polyamide is carried out as follows: before column package, polyamide should be washed with methanol, rinsed with distilled water, and poured into the glass column, using an approximate proportion of 1 kg of polyamide to 10 L of each solvent, in order to eliminate oligomers and other impurities (Ye et al., 2011). After elution with H₂O until column stabilization, elution is performed with H₂O and a miscible polar solvent (usually methanol) gradient. In our experience, the same polyamide column can be re-used to obtain an additional amount of the obtained substance after the followed procedure: the column should be washed starting with 100% methanol to 100% H₂O. We did not observe any substantial reduction in quality of column performance for five consecutive recovery procedures, using the same sample.

A polyamide column was used in the first step to obtain diterpenes from aerial parts of *Euphorbia pannonica*. The plant material was extracted by methanol at room temperature. The obtained crude extract was partitioned by H₂O and dichloromethane and the organic fraction was chromatographed on a polyamide column with mixtures of methanol and H₂O (2:3, 3:2 and 4:1) as eluent. The fraction obtained with methanol-H₂O (3:2) was subjected to silica gel VLC using a gradient system of cyclohexane, chloroform and acetone (3:2:0, 1:1:0, 2:3:0, 3:7:0, 1:4:0, 10:40:1, 4:16:1 and 1:4:1). After purification, two tiglane-type diterpenes, 4,12-dideoxy(4 β)phorbol-20-benzoate-13-isovalerianate and 4,12-dideoxy(4 β)phorbol-20-benzoate-13-isobutyrate (Figure 8), were isolated (Sulyok et al., 2009). Sephadex LH-20 column was used to isolate halisulfate 1 (16) from dark brown sponge. The freeze-dried sponge was extracted by methanol. After the solvent had been filtered and removed, the residue was partitioned between dichloromethane and water. The organic extracts were dried over sodium sulfate and evaporated to obtain a dark brown oil that was chromatographed on Sephadex LH-20 with 1:1 methanol-dichloromethane as eluant to obtain two antimicrobial fractions. Fraction B gave crystals of halisulfate 1 (Figure 8) from dichloromethane (Kernan and Faulkner, 1988).

2.3 Triterpenes

Triterpenes (C₃₀) are a large class of compounds presenting a number of important biological activities; they arise from squalene, a coupling of two farnesil diphosphate units (Abe, 2007).

Cyclization of squalene, or squalene oxide leads to a large number of diverse structural triterpene skeletal types (with 30 carbons), such as lupane, oleanane, ursane types (Tantillo, 2011).

Steroids are modified triterpenoids, lacking the three methyl groups at C-4 and C-14. Sterols are characteristic of eukaryotes. In bacteria they are sparsely distributed with limited array of products (Summons et al., 2006). Skeletal modifications, especially to the side-chain, originate a wide range of biologically important natural products, e.g. sterols, steroidal saponins, cardioactive glycosides, bile acids, corticosteroids, and mammalian sex hormones (Abe, 2007).

Saponins are a group of natural compounds presenting triterpenoidal or steroidal aglycone, designated genin or sapogenin, covalently linked to one or more sugar moieties (Augustin et al., 2011).

Fig. 8. Tigliane diterpenes from *Euphorbia pannonica* and halisulphate isolated from dark brown sponge.

The most usual technique to isolate triterpenes and steroids is by silica open column. As an example, six triterpenes were isolated from petroleum ether extract of *Azorella trifurcata* whole plant (Areche et al., 2009). The crude extract was chromatographed over silica gel using a petroleum ether–ethyl acetate gradient. The fraction eluted with 20% ethyl acetate was purified by CC on silica gel impregnated with 10% AgNO₃, furnishing four triterpenes: lanost-7-en-3β-ol, lanost-9(11)-en-3β-ol, lanosta-7,24-dien-3β-ol and cycloartenol (Figure 9). The acetylation product from the fraction eluted with 40% ethyl acetate, after purification by argentum silica gel CC, yielded the triterpenes lanosta-7,24-dien-3β-yl acetate and 28-acetoxycycloartenyl acetate (Areche et al., 2009).

Several cycloartane-type saponins were isolated from *Astragalus wiedemannianus* methanol crude extract (Polat et al., 2010). Plant material was extracted under reflux. After filtration and solvent removal, the methanol crude extract was solved in water and successively partitioned with hexane, dichloromethane and butanol saturated with water. The butanol fraction was submitted to VLC on reversed-phase material (Lichropep RP-18), employing a H₂O:methanol gradient. Fractions developed with H₂O:methanol 2:8 were rich in saponins. The first fraction was chromatographed over Lichropep RP-18 (H₂O:methanol gradient). The

obtained sub-fractions were submitted to new chromatographic procedures, depending on the nature of target compounds furnishing the saponins.

Fig. 9. Triterpenes from *Azorella trifurcata*.

2.4 Tetraterpenes (Carotenoids)

More than 650 carotenoids (C_{40}) are found in nature, constituting the largest group of natural dyes. The carotenoids are substances with very special properties possessed by no other group of substances; these form the basis of their many varied functions and actions in all kinds of living organisms (Britton, 1995). Carotenoids are biosynthesized by plants, algae, fungi, yeasts and bacteria.

The carotenoids are isoprenoid compounds, biosynthesized by tail-to-tail linkage of two geranylgeranyl diphosphate molecules. This produces the parent C_{40} carbon skeleton from which all the individual variations are derived. This skeleton can be modified: a) by cyclization at one end or both ends of the molecule to give the seven different end groups; b) by changes in hydrogenation level, and c) by addition of oxygen-containing functional groups. Carotenoids that contain one or more oxygen functions are known as xanthophylls, the parent hydrocarbons as carotenes (Britton, 1995). After being absorbed through human diet, some carotenes, among them β -carotene (Figure 10), are pro-vitamin A; other, such as lycopene (Figure 10), are important due to their antioxidant properties.

Carotenoid extracts have been screened by TLC and separated by CC, involving liquid-solid chromatography (adsorption). Various adsorbents have been applied in carotenoid analysis, including Al_2O_3 , silica, magnesium oxide (MgO), calcium hydroxide $[Ca(OH)_2]$, calcium carbonate $[CaCO_3]$, siliceous earth as hyflosupercell and others. In normal phase CC, the

adsorption affinity depends on the number of conjugated double bonds, cyclization and the presence of oxygen substituents (Rodriguez-Amaya, 1999). CC has been used for separations of mixtures of carotenes and xanthophylls, aiming for mainly analytical determinations, standard purifications, biological evaluations of carotenoids and the purification of synthesized carotenoids, especially by flash chromatography.

Separations on basic adsorbents such as MgO and Ca(OH)₂ are mainly determined by the number and type of double bonds in the carotenoid molecules (Bernhard, 1995). The procedure for isolating and purifying carotenoid standards was established because of the difficulty in obtaining standards commercially. The procedure consists of carotenoid extraction with cold acetone, partition to petroleum ether in a separatory funnel with addition of water, concentration in a rotatory evaporator and chromatographic separation of carotenoids on CC developed with petroleum ether containing increasing percentages of ethyl ether and acetone (Rodriguez-Amaya, 1999). A variety of carotenoid standards (some of them are represented on Figure 10) have been isolated and purified using MgO:Hyflosupercel (1:1) CC developed with 2-8% ethyl ether in petroleum ether and 2-95% acetone in petroleum ether: 98% β -carotene (isolated from carrot), 94% lycopene, 99% β -cryptoxanthin, 91% γ -carotene and 91% rubixanthin (from pitanga) (Porcu e Rodriguez-Amaya, 2008), 91-97% neoxanthin, 95-98% violaxanthin, 97-100% lactucaxanthin, 92-96% lutein, 93% β -cryptoxanthin, 96% zeaxanthin and 90-99% β -carotene (from lettuce, papaya or green corn) (Kimura & Rodriguez-Amaya, 2002; Oliveira & Rodriguez-Amaya, 2007) and 97% *trans*- ζ -carotene, 99% *cis*-violaxanthin, 97% *trans*-violaxanthin, 92% prolycopene and 94% lutein (from passion fruit extracts) (Wondracek et al, 2011). The β -cryptoxanthin elutes before lycopene (Figure 10) in MgO: Hyflosupercel and after lycopene in Al₂O₃ column, indicating that the influence of cyclization is greater than that of the presence of hydroxyl substituents in the MgO:Hyflosupercel column (Rodriguez-Amaya, 1999).

MgO:Hyflosupercel on CC has been also widely used to analysis the main carotenoids of tropical fruits such as *Pouteria campechiana* (Agostini-Costa et al, 2010) and to evaluate the variation in the carotenoid composition of *Malpighia glabra* pulp (Agostini-Costa et al, 2003) and *Eugenia uniflora* fruit (Porcu & Rodriguez-Amaya, 2008). Hydrocarbon carotenes, such as β -carotene, lycopene, α -carotene, ζ -carotene and xanthophylls such as β -cryptoxanthin, violaxanthin and neoxanthin (Figure 10), were extracted from these tropical fruits, separated by MgO: Hyflosupercel on CC developed with ethyl ether and acetone gradient in petroleum ether, identified by chemical tests and quantified by visible spectroscopy. The chromatographic behavior of carotenoids bears a definite relationship with theirs structure. Although these data cannot be used as the sole criteria for identifying carotenoids, they serve as useful complementary information (Rodriguez-Amaya, 1999). Carotenoids of *Lycium barbarum* L. fruits, a traditional Chinese herb that possesses vital biological properties, were isolated by a column containing MgO: diatomaceous earth. The β -carotene was eluted with n-hexane, β -cryptoxanthin and neoxanthin with ethyl acetate and zeaxanthin with ethyl acetate-ethanol (80:20 v/v). The zeaxanthin fraction was the most effective in scavenging hydroxyl-free radicals (Wang, 2010).

Other basic materials, especially Ca(OH)₂, ZnCO₃ and CaCO₃, are particularly useful for separating geometrical isomers (Bernhard, 1995). Five lutein derivatives (four isomers and two epoxides) were isolated and separated from inflorescences of *Solidago canadensis* L. and from flowers of *Chelidonium majus* L., using CaCO₃ CC developed with different compositions of toluene-hexane and acetone-hexane. They were identified as lutein-5,6-

epoxide, (9Z)-lutein-5,6-epoxide, (9Z,9'Z)-lutein (neolutein C), (9Z)- and/or (9'Z)-lutein (neolutein B) and (13Z)- and/or (13'Z)-lutein (neolutein A), in addition to (all-E)-lutein, violaxanthin, (9Z)-violaxanthin, flavoxanthin and/or chrysanthemaxanthin (Horvath et al., 2010). Xanthophylls, such as 5,6-diepikarpoanthin, mutatoxanthin, anteraxanthin, zeaxanthin, β -cryptoxanthin and apo-carotenoids, such as capsorubin, capsochrome, capsanthin 5,6-epoxide and capsanthone, were also isolated from *Asparagus falcatus* using CaCO_3 as adsorbent on CC (Deli et al, 2000). The isomers of β -carotene present in acetone extracts from *Malpighia glabra* pulp (Agostini-Costa et al, 2003) and from *Annona coriaceae* fruit (Agostini et al., 1996) were separated on $\text{Ca}(\text{OH})_2$ CC eluted with 2% ethyl ether in petroleum ether.

Separations on silica and Al_2O_3 are mainly determined by carotenoid polarity. The hydrocarbon carotenes are weakly adsorbed, whereas xanthophylls, containing polar substituents, especially hydroxyl groups, are adsorbed more strongly (Bernhard, 1995). A novel carotenoid (xanthophyll) derivative, lutein-3-acetate, extracted from senescing leaves of rice, was isolated and purified using silica gel TLC (Kusaba, 2009). Phytofluene, β -carotene, ζ -carotene, lycopene, zeinoxanthin, cryptoxanthin and lutein (Figure 10) were separated from ripe and partly ripe *Mormodica charantia* seeds and tomatoes through Al_2O_3 and MgO CC (Rodriguez et al., 1975). The main carotenoid (xanthophyll) in the orange muscle of Yesso scallop was isolated and purified by acetone extraction and silica gel CC. Its structure was identified as that of pectenolone (3,3'-dihydroxy- β,β -caroten-4-one) (Figure 10) (Li et al, 2010).

Fig. 10. Main carotenoids discussed in this chapter.

Carotenoid extracts of eight species of lichens were separated by Al_2O_3 column and divided into fractions on silica gel TLC and analyzed by ion-pairing in reverse-phase HPLC. Fourteen carotenoids, such as β -carotene, α -carotene, β -cryptoxanthin, lutein, zeaxanthin, canthaxanthin, astaxanthin, violaxanthin and neoxanthin (Figure 10) were separated and identified (Czeczuga et al, 2010). Stereoisomers and epoxy/carbonyl derivatives of β -carotene were separated through Al_2O_3 CC developed with 10-60% diethyl ether in *n*-hexane (Marty and Berset, 1990).

CC on silica gel 60 was also used for partial separation of carotenoids and DEAE-Toyopearl 650 M to remove B Chlorophyll and polar lipids during carotenoid isolation from a thermophilic filamentous photosynthetic bacterium *Roseiflexus castenholzii*. Purified fractions provided keto-myxocoxanthin and methoxy-keto-myxocoxanthin (Figure 10), besides keto-myxocoxanthin glucoside, keto-myxocoxanthin glucoside ester, myxocoxanthin, myxocoxanthin glucoside ester and others (Takaichi et al., 2001). The major polar carotenoids, myxoxanthophyll and ketomyxoxanthophyll, produced by two cyanobacterias (*Anabaena* sp. and *Nostoc punctiforme*), were also isolated and purified first on a column of silica gel 60. The β -carotene was eluted with hexane, non-polar carotenoids were eluted with acetone/hexane (2:8) and polar carotenoids were eluted with acetone methanol (9:1, v/v). The polar carotenoids (last fraction) were then loaded on a column of DEAE-Toyopearl 650 M and the carotenoids were eluted with acetone/hexane (1:1 v/v) and finally purified on silica gel TLC developed with dichloromethane/ethyl acetate/acetone/methanol (2 : 4 : 2 : 1, v/v) to give myxoxanthophyll and ketomyxoxanthophyll (Takaichi et al., 2005).

3. Phenolic compounds

Phenolic compounds are widely distributed in nature. Their chemical structures may vary greatly, including simple phenols (C_6), such as hydrobenzoic acid derivatives and catechols, as well as long chain polymers with high molecular weight, such as catechol melanins (C_6)₆, lignins ($\text{C}_6\text{-C}_3$)_n and condensed tannins ($\text{C}_6\text{-C}_3\text{-C}_6$)_n. Stilbenes ($\text{C}_6\text{-C}_2\text{-C}_6$) and flavonoids ($\text{C}_6\text{-C}_3\text{-C}_6$) are phenolic compounds with intermediate molecular weight that present many pharmacological and biological activities. Flavonoids, including anthocyanins, flavonols (such as quercetin and myricetin), isoflavones (such as daidzein and genistein) and others are formed by multiple biosynthetic branches that originate from chalcone.

Phenolic compounds have been widely fractionated in medicinal, aromatic and food plants using CC. Repeated silica gel, sephadex-LH20, RP-18, RP-8, MCI-gel, diaion and toyopearl chromatography columns have been used to fractionate simple phenolics, flavonoids and tannins from kernels and nuts (Zhang et al, 2009; Karamac, 2009), fruits such as apples, *Morus nigra*, *Punica granatum* (Lee et al, 2010; Pawlowska et al, 2008); olive oil (Khanal et al, 2011), tea (Gao et al, 2010; Liu et al, 2009), seeds such as lentils (Amarowicz & Karamac 2003), medicinal species, including *Ulmus davidiana* and *Tridax procumbens* (Jung et al, 2008; Agrawal, 2011); and aromatic plants, including mint and sage (She et al, 2010; Wang et al, 1998).

Separations on silica are mainly determined by polarity, where phenolic compounds containing more hydroxyl groups are adsorbed more strongly. Separations on Sephadex LH-20, a crosslinked dextran-based resin for gel permeation, are mainly determined by molecular sizing of the phenolic compound, outside of adsorption and partition mode.

Phenolic acids (such as ferulic acid and gallic acid), flavonoids (such as flavonol, catechins and anthocyanidins derivatives) and procyanidins (Figure 11) from fruits of wild black

berry *Aristotelia chilensis* were obtained using flash and open CC on silica gel and Sephadex LH-20 eluted with hexane, hexane-ethyl acetate (1:1); ethyl acetate-methanol (1:1) and methanol (Cespedes et al., 2010).

Pyrogallol and phenolic acids were purified from defatted extract of *Juglans regia* kernels by repeated CC on Sephadex LH-20 eluted with methanol, followed by silica gel CC developed with chloroform-acetone 40:1, v/v. Ethyl gallate was obtained by recrystallization from methanol. Protocatechuic acid was obtained after CC on Sephadex LH-20 eluted with CH₂Cl₂-EtOH, 1:1, v/v and further purification with silica gel CC developed with Chloroform-methanol, 20:1. Gallic acid and 3,4,8,9,10-pentahydroxydibenzo[b,d]pyran-6-one (Figure 11) were separated by silica gel CC and eluted with a mixture of chloroform-methanol (Zhang, 2009). High-molecular-weight tannins (Figure 11) of defatted walnut, hazelnut and almond kernels were also isolated by CC on Sephadex LH-20 gel eluted with 50% (v/v) acetone (Karamac, 2009). The ethanol fraction of *Dipteryx lacunifera* kernels was found to exhibit high radical scavenging activity and was subjected to further fractionation. CC over silica gel with gradient from chloroform to methanol and Sephadex LH-20 eluted with methanol afforded (-)-eriodictyol, (-)-butin, luteolin, 3',4',7-trihydroxyflavone, butein and sulfuretin (Junior et al., 2008).

The total polyphenols from red wine isolated during vinification and storage were isolated by CC and their contribution to wine sensory properties and antioxidant activity was evaluated. Wine samples were evaporated and loaded onto an open CC packed with LiChroprep RP-18; the column was washed with distilled water followed by methanol to recover total polyphenols extract, further separated by HPLC (Sun et al., 2011).

The alkyl esters of protocatechuic acid (Figure 11) were synthesized and the crude product was then purified by CC using petroleum ether/ethyl ether (7:3 to 5:5) as eluent. The increase in the length of ester alkyl chain attached to the catecholic ring had influenced the stabilization of the radicals formed in the oxidation process. Alkyl protocatechuate compounds demonstrated a higher radical-scavenging activity than the natural antioxidant protocatechuic acid. Moreover, the introduction of alkyl groups in the carboxylic acid led to a significant increase in lipophilicity influenced by the antioxidant activity of protocatechuic acid derivatives (Reis et al., 2010).

The virgin olive oil phenol was partitioned successively with hexane and ethyl acetate and fractionated by CC on silica gel and Sephadex LH-20 to give a dialdehydic form of decarboxymethyl ligstroside aglycone (p-HPEA-EDA) (Figure 11), a phenolic compound that activates AMP-activated protein kinase to inhibit carcinogenesis (Khanal et al., 2011). Phenols and tocopherols were also removed from olive oil by CC over Al₂O₃ during to evaluate changes in the phenolic composition of virgin oil during frying. The concentration of hydroxytyrosol (3,4-DHPEA) and its secoiridoid derivatives (3,4-DHPEA-EDA and 3,4-DHPEA-EA) in virgin olive oil decreased rapidly when the oil was repeatedly used for preparing fried food. However, tyrosol (p-HPEA) and its derivatives (p-HPEA-EDA and p-HPEA-EA) in the oil were much more stable during frying operations (Gomez-Alonso et al., 2003).

Phytoalexins are secondary metabolites that plants synthesize for self-defense, and they have shown great promise in chronic disease prevention. The best known example is resveratrol (Figure 11), an induced phytoalexin found in yeast-infected grape skin (Wu et al., 2011). Peanuts also contain several active components including flavonoids, phenolic acids, phytosterols, alkaloids and stilbenes. The latter are characterized by a 1,2-diphenylethylene

backbone usually derived from the basic unit of trans-resveratrol (3,5,4'-trihydroxy-stilbene) (Lopes et al, 2011). Peanut seeds, when affected by injuries, pathogenic infections, fungal contamination, insect damage and UV light, could produce phytoalexins such as stilbenoid derivatives (resveratrol, arachidins, 3'-isopentadienyl-3,5,4'-trihydroxystilbene, SB-1, chiricanine A, and arahypins), and pterocarpanoid derivatives (e.g., aracarpene-1 and aracarpene-2). Silica gel column eluted with hexane and hexane ethyl acetate (7:3) was used for fractionation the phytoalexin extract from peanut before analysis by HPLC (Wu et al., 2011). Neutral Al₂O₃ - silica gel 60 C18 (1:1 w/w) eluted with 80% ethanol was used as a clean-up column to separate interfering co-eluting with resveratrol from peanut extracts (Potrebko & Resurreccion, 2009).

Fig. 11. Main phenolic compounds discussed in this chapter.

A highly efficient column chromatographic extraction of curcumin from *Curcuma longa* was proposed by Zhan et al., 2011. Curcumin (Figure 11) was extracted with minimum use of solvent, minimum volume and high concentration of extraction solution by CC. Turmeric material was loaded into a column with 2-fold of 80% ethanol. After dissolving target compounds, the column was eluted with 80% ethanol. For non-cyclic CCE procedure, 8-fold of eluent was collected, while for cyclic CCE procedure, only the first 2-fold of eluent was collected as extraction solution. A more than 99% extraction rate for curcumin was obtained in both procedures, compared to a 59% extraction rate through the ultrasonic-assisted extraction with 10-fold of 80% ethanol.

A lipid- and essential oil- free infusion of *Cymbopogon citratus* leaves was fractionated on Lichroprep RP-18 column eluted with water and with aqueous methanol solutions. Dry residue was recovered in 50% aqueous ethanol and was fractionated by gel chromatography on a Sephadex LH-20 using ethanol as mobile phase. All the fractionation process provided three major fractions: a tannin rich fraction; a flavonoid rich fraction and two phenolic acid rich fractions. Tannin and phenolic acid were the fractions responsible for the anti-inflammatory effect through inhibition of transcription factor NF- κ B, inducible nitric oxide synthase expression and nitric oxide production. These fractions probably had a synergistic effect (Francisco et al., 2011).

Oligomeric procyanidins (Figure 11) in apples were extracted with boiled water and purified on an ADS-17 macroporous resin column to obtain a procyanidin extract. The extract was fractionated according to its degree of polymerization on a Toyopearl TSK HW-40 column eluted with methanol to give procyanidins B2 (epicatechin-(4 β -8)-epicatechin) and C1 (epicatechin-(4 β -8)-epicatechin-(4 β -8)-epicatechin). This method was suitable for the preparation of procyanidin oligomers (from dimers through tetramers in one run) for laboratory research, and is potentially applicable to large-scale production in industry (Xiao et al., 2008).

Thea (Theaceae) is traditionally used for producing tea, one of the most popular beverages consumed in the world due to its polyphenol-rich content, which are reported to have various bioactivities, such as antioxidative, antimicrobial, antitumor and antimutagenesis (Liu et al, 2009). *Camellia sinensis* and some other species from the genus *Camellia* have also been used for making tea and are consumed widely (Gao et al, 2010). More than 96 phenolic compounds were identified in *C. sinensis* tea. The hydrolyzable tannin epigallocatechin gallate was the major phenolic component of green tea and partially fermented teas, while fully fermented black teas had traces of epigallocatechin gallate but contained theaflavins. Glycosylated flavonoids, catechins, proanthocyanidins (condensed tannins) and phenolic acid derivatives were found too (Lin et al., 2008). Aqueous acetonic extract of green tea (*C. crassicaule*) further partitioned with ethyl acetate and purified by CC led to the identification of various fractions obtained by CC over Sephadex LH-20 eluted with ethanol. Further repeated CC on MCI-gel CHP20P, Sephadex LH-20, and Toyopearl HW-40F, eluted with methanol/H₂O (0:1-1:0) gave five flavan-3-ols, five flavonol glycosides, three hydrolyzable tannins, two chlorogenic acid derivatives and three simple phenolic compounds (Liu et al, 2009). Phenolic compounds were also isolated from the leaves of *C. pachysandra*. The ethyl acetate and aqueous fractions were separately subjected to repeated CC over Diaion HP-20SS, Sephadex LH-20, MCI-gel CHP20P, and Toyopearl HW-40F to give 22 phenolic compounds, including nine hydrolyzable tannins, 11 flavonol glycosides and two simple phenols, without caffeine or catechin (Gao et al., 2010).

A new phenolic compound was isolated and purified from butanol fraction of Chinese olive (*Canarium album* L.) fruit through AB-8 adsorption resin CC washed with water to remove impurities and eluted with 90% (v/v) aqueous ethanol to get the phenolic eluents. The phenolic were further separated on a polyamide CC eluted with aqueous ethanol to give several fractions, the ethanol concentration being increased from 0 to 100% in increments of 20%. Fraction obtained from 20% aqueous ethanol was further purified on TSK Toyopearl HW-40 (S) CC developed with aqueous ethanol 0 to 20% to get the purified new compound established as 3-o-galloyl quinic acid butyl ester (Figure 11) (He et al., 2009). Polyamide CC eluted with water and 50%, 70% and 100% aqueous methanol further facilitated GC-MS and HPLC separation of phenolic compounds in *Euphrasia rostkoviana* (Blazics et al., 2008).

Lycium barbarum L., a traditional Chinese herb, possesses vital biological properties, such as prevention of cancer and age related macular degeneration. Flavonoids and phenolic acids extracted from fruits of this plant were separated using a Cosmosil 140 C18 OPN column, with phenolic acids being eluted with deionized water and neutral flavonoids with methanol. The flavonoid fraction showed the most pronounced effect in scavenging free radicals, chelating metal ions and reducing power (Wang et al., 2010).

Eight polyphenolic acids were isolated from the aerial part of *Mentha haplocalix* extracted with aqueous acetone 70% at room temperature. Repeated CC on silica gel (developed with chloroform-methanol-H₂O, 9:1:0.1 to 7:3:0.5), Sephadex LH-20 and MCI-gel CHP20P and ODS-A, eluted with H₂O-Methanol (1:0 to 0:1) afforded rosmarinic acid, cis-salvianolic acid, lithospermic acid (Figure 11), propanoic acid, sodium lithospermate B, magnesium lithospermate B, and lithospermic acid B. Lithospermic acid B, sodium lithospermate B and magnesium lithospermate B displayed stronger activities than the other compounds (She et al., 2010). Ten phenolic compounds were also isolated from a butanol fraction of sage extracts, using repeated CC on silica gel, Lichroprep RP-18 and Sephadex LH20. Among them, the most active antioxidants were found to be rosmarinic acid and luteolin-7-O- β -glucopyranoside (Wang et al, 1998).

4. Alkaloids

Alkaloids are defined as basic compounds synthesized by living organisms containing one or more heterocyclic nitrogen atoms, derived from amino acids (with some exceptions) and pharmacologically active. The class name is directly related to the fact that nearly all alkaloids are basic (alkaline) compounds. Alkaloids constitute a very large group of secondary metabolites, with more than 12,000 substances isolated. A huge variety of structural formulas, coming from different biosynthetic pathways and presenting very diverse pharmacological activities are characteristic of the group (Briemann et al., 2006).

Archeological evidence has demonstrated the use of alkaloids (plant parts or extracts) since 4000 B.C., and they continue to be very important today (Roberts & Wink, 1998). Poppy (*Papaver somniferum*) and opium have been known and used since antiquity by Sumerians, Arabs, Persians, Egyptians and Greeks. Morphine, obtained from the poppy latex, was the first crude drug isolated. It was named after Morpheus, the god of dreams, one of the sons of Hypnos, the god of sleep, in Greek mythology (Wink, 1998). Morphine is legally used nowadays as an analgesic for severe pain.

Alkaloids are associated with a wide range of pharmacological activities. Many are toxic and can cause death, even in small quantities. Some have antibiotic activities and others interfere with behavior patterns, such as antidepressants (reserpine) and hallucinogens (mescaline). It seems alkaloid function in plants and animals is linked to defense mechanisms. Toxicity is a good weapon to inhibit the action of predators, like herbivores.

4.1 Alkaloids from plants

CC is a very important and much used technique in alkaloid isolation and analysis. Usually, after CC, the sample is re-chromatographed either by preparative TLC or HPLC, in order to obtain a pure sample for spectroscopic studies.

Phytochemical investigation in plants from the genus *Kopsia* (Apocynaceae) led to the discovery of different classes of alkaloids, presenting antileishmanial, antimitotic and antitumor activities. From *Kopsia hainanensis*, a native medicinal plant from Hainan, China, two new pentacyclic indole alkaloids have been isolated (Chen et al., 2011). The acidic partition of the methanol extract was neutralized and extracted with chloroform. Alkaloids, as basic substances, can be protonated in acidic media and therefore remain in the aqueous phase. This strategy has been very useful for the isolation of this class of compounds. The pH of the aqueous phase is further adjusted and alkaloids are extracted with a solvent. The concentrated extract was repeatedly submitted to CC over silica gel and eluted with a gradient of chloroform-methanol, alkalized with a small quantity of triethylamine to give kopsahainanine B. One fraction was re-chromatographed over reversed-phase silica gel and Sephadex LH-20, using methanol-water and pure methanol as eluents, respectively, to give kopsahainanine A (Figure 12).

Fig. 12. Pentacyclic indole alkaloids from *Kopsia hainanensis*.

The butanolic fraction of *Lobelia chinensis* Lour. was chromatographed on reversed phase Diaion HP 20 gel, eluted with water-methanol in to six fractions. Fraction 6 was re-chromatographed on Sephadex LH-20 followed by preparative silica gel TLC and 7.3 mg of lobachine (Figure 13), a new alkaloid, were isolated (Kuo et al., 2011).

From *Campylospermum flavum*, a medicinal plant from Cameroon, flavonoids, flavones, chalcones and alkaloids have been isolated. Among them, a new indole alkaloid, flavumindole (Figure 13), was obtained from the methanol extract after partition with ethyl acetate-water and purification on silica gel, Sephadex LH-20 and RP₁₈ silica gel, eluted with dichloromethane-methanol mixtures, pure methanol and methanol-water mixtures, respectively (Ndongo et al., 2011). The isolated alkaloid presented a cytotoxicity of 90% according to by brine shrimp (*Artemia salina*) test.

Fig. 13. Lobachine, a new alkaloid from *Lobelia chinensis* and flavumindole, from *Campylospermum flavum*.

Nine indole alkaloids were isolated from the roots, bark and leaves of *Tabernaemontana salzmannii* (Apocynaceae). The alkaloids were fully identified by spectroscopic methods and mass spectra by comparison with literature data (Figueiredo et al., 2010). The dichloromethane extract was repeatedly chromatographed over silica gel eluted with dichloromethane-methanol mixtures. Coronaridine, 3-oxo-coronaridine, (19S)-heyneanine, voacangine, isovoacangine, hydroxyisovoacangine, isovoachristine and olivacine (Figure 14) were isolated. For voachalotine, a further chromatographic step was necessary on preparative TLC (silica gel) eluted with dichloromethane-methanol (99:1). After an *in vitro* screening, it was observed that isovoacangine and voacangine alkaloids were able to induce apoptosis cell death in human leukemic cells, line THP-1.

Fig. 14. Alkaloids from *Tabernaemontana salzmannii*.

Already having an extremely diverse variety in structural formulas, new and (therefore) unusual carbon skeletons have continued to be found in alkaloids research. That was the case reported by Hitotsuyanagi et al. (2010), in a study with *Stemona sessilifolia*. Plants of the genus *Stemona* are rich sources of alkaloids, and two new ones were isolated by CC of the acidic partition from the methanolic extract of the roots. Sessilifoliamide K and sessilifoliamide L were structurally related, presenting an unusual pyrido[1,2-*a*]azonine

skeleton (Figure 15), which can be derived from tuberosteminol-type alkaloids, previously described occurring in *Stemona* genus.

Fig. 15. New unusual pyrido[1,2-*a*]azinone alkaloids from *Stemona sessilifolia*.

4.2 Alkaloids from fungi

Probably the most famous (and infamous) alkaloid producing fungi are the ergots (family Hypocreaceae). Ergotism (holy fire) has killed thousands due to contaminated rye, and even in the last century many cases were reported (Wink, 1998). However, there is a plethora of alkaloid producing-fungi, with different structures and biological activities. Three new alkaloids, lyconadin D, lyconadin E and complanadine E (Figure 16) were isolated from *Lycopodium complanatum* (Lycopodiaceae) by Ishiuchi et al. (2011). The acidified methanolic extract was partitioned with ethyl acetate. The pH of the aqueous phase was adjusted and extracted with chloroform. After solvent evaporation, the chloroform-soluble materials were chromatographed on an amino silica gel column and eluted with hexane-ethyl acetate mixtures, then with a chloroform-methanol-water system. The fractions eluting with pure chloroform were re-chromatographed over silica gel, and lyconadin D and lyconadin E were isolated. Part of the chloroform soluble fraction was eluted with chloroform-methanol over a Sephadex LH-20 column. The fractions were further purified by HPLC (C_{18} column) to yield complanadine E, a dimeric unsymmetrical alkaloid.

Fig. 16. New alkaloids from moss *Lycopodium complanatum*.

The mosses of the genus *Lycopodium* in particular are very prone to produce complex polycyclic alkaloids, with important biological activities, such as inhibition of acetylcholine esterase. From three different species of *Lycopodium*, ten new alkaloids were isolated and characterized (Katakawa et al., 2011). Dihydrolycopoclavamine (Figure 17) was isolated from the extract of *L. serratum* after chromatography on amino silica gel and elution with

hexane-chloroform. For *L. clavatum*, after neutralization of the acidic fraction from the methanolic extract, it was submitted to flash chromatography over silica gel and eluted with a chloroform-methanol gradient, then chloroform-methanol-aqueous ammonium hydroxide and pure methanol. One fraction was re-chromatographed on amino silica gel with hexane-chloroform mixtures leading to lycopoclavamine A. Another fraction was re-chromatographed on silica gel to give lycopoclavamine B (Figure 17). A similar procedure was applied to *L. squarrosus*, from which lycoposquarrosamine A, acetyllycoposerramine-U, 8- α -hydroxyfawcettimine, 8- β -hydroxyfawcettimine, 8- α -acetoxyfawcettimine, 8- β -acetoxyfawcettimine and lycoflexine N-oxide were isolated. It is quite remarkable that all these compounds were separated solely by means of CC using silica gel, amino silica gel or Al_2O_3 , and solvent mixtures of hexane, chloroform, methanol and ethyl acetate.

Fig. 17. Alkaloids from *Lycopodium clavatum*, *L. serratum* and *L. squarrosus*.

4.3 Marine alkaloids

The marine environment has emerged as a promising source of new substances with medicinal application. Considering only sponges, for example, more than 15,000 compounds have been isolated and characterized, including terpenoids, nucleosides, alkaloids and steroids.

Guanidine alkaloids were isolated from *Monanchora arbuscula* after CC over Sephadex LH-20 with methanol then repeatedly over silica gel and eluted with chloroform-ethyl acetate-methanol mixtures. The compounds (Figure 18) were identified by ^1H and ^{13}C NMR, IR and MS (Ferreira et al., 2011).

Fig. 18. Guanidin alkaloids from sponge *Monanchora arbuscula*.

Two new brominated arginine-derived alkaloids were isolated from the Red Sea sponge *Suberea mollis* (Aplysinellidae), namely subereamine A and subereamine B (Figure 19). Halogenated compounds are common in marine natural products. The subereamines were isolated after CC on Sephadex LH-20 of the ethyl acetate extract, followed by chromatography on silica gel with dichloromethane-methanol mixtures and preparative HPLC on C_{18} column (Shaala et al., 2011).

Fig. 19. Brominated alkaloids from sponge *Suberea molis*.

The first natural products containing a 1H-oxazolo [40,50:4,5] benzo[1,2,3-de] [1,6] naphthyridine ring system were isolated from a *Suberites* sp. sponge collected in Okinawa, Japan. Named respectively, nakijinamines C and E (Figure 20), they constitute a group of heteroaromatic alkaloids, hybrids of aaptamine-type and bromoindole alkaloids (Takahashi et al., 2011). Other 23 aaptamine-type alkaloids have been isolated so far, but none contain the hybrid ring system. Another new alkaloid was isolated, namely nakijinamine D, which does not contain the oxazolo ring. The sponges were extracted with methanol and the residue partitioned with ethyl ether and water. The aqueous phase was extracted with butanol and subjected to reversed phase CC (C_{18}) followed by repeated HPLC also on a C_{18} column. The alkaloids were isolated initially as racemates. Antifungal activity was recorded for nakijinamines C and E against *Aspergillus niger*.

Fig. 20. New heteroaromatic alkaloids from *Suberites* sp.

Marine sponges of the genus *Asteropus* have been reported to contain a variety of compounds, such as saponines, sterols and pteridine derivatives. The concentrated methanolic extract was partitioned between water and dichloromethane. The water phase showed toxicity to brine shrimp larvae and was partitioned with butanol. The butanolic extract was submitted to medium pressure liquid chromatography over ODS-A with a methanol gradient. A fraction selected according to the toxicity test result was chromatographed over Sephadex LH-20. The sub-fractions were purified by reversed phase HPLC. The pyroglutamyl alkaloids were new compounds (Figure 21), and therefore fully characterized by spectroscopic methods (Li et al., 2011).

Fig. 21. Pyroglutamyl dipeptides and tetrahydro- β -carboline alkaloids from *Asteropus* sp.

Carboline alkaloids were also found in tunicates of the genus *Eudistoma*. After purification on Sephadex LH-20, the methanolic extract of *Eudistoma glaucus* was chromatographed on a silica gel column with hexane-ethyl acetate mixtures, and then on another column with amino silica gel, also eluted with the same solvents. Normal phase HPLC led to the isolation of eudistomidin H (Figure 22) and eudistomidin I. Eudistomidins H and I were new compounds containing a unique fused-tetracyclic ring system consisting of a tetrahydro β -carboline ring and a hexahydropyrimidine ring. Using the same stationary phases, but changing the solvent system to chloroform-methanol, eudistomidin K was obtained. For eudistomidin J, the last solvent system was also applied, but without the need for a HPLC separation step (Suzuki et al., 2011).

Fig. 22. Carboline alkaloids from tunicate *Eudistoma glaucus*.

Pyridoacridine alkaloids are responsible for the bright colors observed in sponges and ascidians. Two new pyridoacridine alkaloids, namely 13-didemethylaminocycloshermilamine D and dimethyl-deoxyamphimedine (Figure 23), were isolated from the purple chromatotype of the Western Mediterranean morph *Cystodytes dellechiaiei* together with other six known alkaloids of the same kind (Bry et al., 2011). The ascidians were extracted with methanol-dichloromethane-trifluoroacetic acid mixture followed by reverse phase CC. The purple fraction obtained was chromatographed three successive times on reverse phase columns eluted with a methanol-water gradient. After reverse phase semi-preparative HPLC, the two new alkaloids were isolated.

Fig. 23. New pyridoacridine alkaloids from morph *Cystodytes dellechiaiei*.

In this way, the phytochemical investigation of alkaloids has been deeply dependent on CC techniques. Careful and patient repeated CC has led to the isolation of some structurally varied and fascinating compounds, even without the use of modern HPLC methods. To be more efficient and to enhance productivity, of course, combined approaches are the common practice.

It is worth emphasizing the great opportunity that marine organisms offer to researchers looking for biologically active compounds and synthetic models. The real treasures at the bottom of the sea may not come from shipwrecks, but are those which have been lying (or, better, living) there since long before human beings set foot on earth.

5. Conclusion

Even in laboratories with good HPLC and GC-MS facilities, the traditional and classical methods of TLC and CC are still widely used, for rapid preliminary screening of extracts, for isolating and purifying bioactive compounds of secondary metabolism for further study, for comparison of samples with standards and for monitoring chemical synthesis or the course of reactions.

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7. References

- Abe, I. (2007). Enzymatic synthesis of cyclic triterpenes. *Natural Product Reports*, Vol. 24, pp. 1311-1331.
- Adams, R.P. (2007). *Identification of Essential Oil Components by Gas Chromatography / Mass Spectrometry* (4th ed.), Allured Publishing Co., ISBN 978-1-932633-21-4, Carol Stream (IL).
- Agostini, T.S., Cecchi, H.M. & Godoy, H.T. (1996). Composição de carotenóides no marolo e em produtos de preparo caseiro. *Ciência e Tecnologia de Alimentos*, Vol. 16, pp. 67-71.
- Agostini-Costa, T.S., Abreu, L.N. & Rossetti, A.G. (2003). Efeito do congelamento e do tempo de estocagem da polpa de acerola sobre o teor de carotenóides. *Revista Brasileira de Fruticultura*, Vol. 25, pp. 56-58.
- Agostini-Costa, T.S., Wondracek, D.C., Lopes, R.M., Vieira, R.F. & Ferreira, F.R. (2010). Composição de carotenoides em canistel (*pouteria campechiana* (kunth) baehni). *Revista Brasileira de Fruticultura*, Vol. 32, pp. 903-906.
- Agrawal, S.S. & Talele, G.S. (2011). Bioactivity guided isolation and characterization of the phytoconstituents from the *Tridax procumbens* *Brazilian Journal of Pharmacognosy*, Vol. 21, pp. 58-62.
- Ajit-Simh K.A., Bemberis I., Brown S., De-Mill, B., Deorkar, N., Grund, E., Hays, E., Levine, H.L., Levy, R., Mazzeo, J., Natarajan, V., Nevill, J., Noyes, A., O'Neil, P., Seely, R., Sofer, G. & Wheelwright, S.M. (2003). Column packing for process-scale chromatography: guidelines for reproductibility. *Biopharmaceutical International*, Supp (June), pp. 23-30.
- Amarowicz, R. & Karamac, M. (2003). Antioxidant activity of phenolic fractions of lentil (*Lens culznaris*). *Journal of Food Lipids*, Vol. 10, pp. 1-10.
- Areche, C., Cejas, P., Thomas, P., San-Martin, A., Astudillo, L., Gutierrez, M. & Loyola, L.A. (2009). Triterpenoids from *Azorella trifurcata* (Gaertn.) Pers and their effect against the enzyme acetylcholinesterase. *Quimica Nova*, Vol. 32, pp. 2023-2025.
- Arroyo, J., Bonilla, P., Ráez, E., Barreda, A. & Huamán, O. Efecto quimioprotector de *Bidens pilosa* en el cáncer de mama inducido en ratas. (2010). *Anales de la Facultad de Medicina*, Vol. 71, pp. 153-159.
- Augustin, J.M., Kuzina, V., Andersen, S.B. & Bak, S. (2011). Molecular activities, biosynthesis and evolution of triterpenoid saponins. *Phytochemistry*, Vol. 72, pp. 435-457.

- Batista, R., García, P.A., Castro, M.A., Del Corral, J.M.M., Feliciano, A.S. & Oliveira, A.B. (2010). Iso-Kaurenoic acid from *Wedelia paludosa* DC. *Anais da Academia Brasileira de Ciências*, Vol. 82, pp. 823-831.
- Begnami, A.F., Duarte, M.C.T., Furletti, V. & Rehder, V.L.G. (2010). Antimicrobial potential of *Coriandrum sativum* L. against different *Candida* species in vitro. *Food Chemistry*, Vol. 118, pp. 74-77.
- Bernhard, K. (1995). Chromatography: Part II Column Chromatography. In: Britton, G; Liaaen-Jensen; Pfander, H. (Eds.) *Carotenoids isolation and analysis*. Basel: Birkhauser Verlag, pp. 117-130.
- Bicchi, C., Cagliero, C. & Rubiolo, P. (2011). New trends in the analysis of the volatile fraction of matrices of vegetable origin: a short overview. A review. *Flavour and Fragrance Journal*, Vol. 26, pp. 321-325.
- Bizzo, H.R., Lopes, D. Abdala, R.V., Pimentel, F.A., Souza, J.A., Pereira, M.V.G., Bergter, L. & Guimarães, E.F. (2001). Sarisan from Leaves of *Piper affinis hispidinervum* C. DC (long pepper). *Flavour and Fragrance Journal*, Vol. 16: 113-115.
- Blazics, B., Ludanyi, K., Szarka, S. & Kery, A. (2008). Investigation of *Euphrasia rostkoviana* Hayne Using GC-MS and LC-MS. *Chromatographia Supplement*, Vol. 68, pp. S119-S124.
- Briellmann, H.R., Setzer, W.N., Kaufman, P.B., Kirakosyan, A. & Cseke, L.J. (2006). Phytochemicals: The Chemical Components of Plants, In: *Natural Products from Plants* (2nd ed), L.J. Cseke, A. Kirakosyan, P.B. Kaufman, S.L. Warber, J.A. Dule & H.R. Briellmann, pp.1-50, CRC Press, ISBN 978-0-8493-2976-0, Boca Raton.
- Britton, G. (1995). Structure and properties of carotenoids in relation to function. *The FASEB Journal*, Vol. 9, pp. 1551-1558.
- Bry, D., Banaigs, B., Long, C. & Bontemps, N. (2011). New pyridoacridine alkaloids from the purple morph of the ascidian *Cystodytes dellechiaiei*. *Tetrahedron Letters*, Vol. 52, pp. 3041-3044.
- Cespedes, C.L., Valdez-Morales, M.V., Avila, J.G., El-Hafidi, M., Alarcon, J. & Paredes-Lopez, O. (2010). Phytochemical profile and the antioxidant activity of Chilean wild black-berry fruits, *Aristotelia chilensis* (Mol) Stuntz (Elaeocarpaceae). *Food Chemistry*, Vol. 119, pp. 886-895.
- Chen, J., Chen, J.J., Yao, X. & Gao, K. (2011). Kopsihainanines A and B, two unusual alkaloids from *Kopsia hainanensis*. *Organic and Biomolecular Chemistry*, Vol. 9, pp. 5334-5336.
- Chisholm, M.G., Jell, J.A. & Cass, Jr., D.M. (2003). Characterization of the major odorants found in the peel oil of *Citrus reticulata* Blanco cv. Clementine using gas chromatography-olfactometry. *Flavour and Fragrance Journal*, Vol. 18, pp. 275-281.
- Coronel, A.C., Cerda-García-Rojas, C.M., Joseph-Nathan, P. & Catalán, C.A.N. (2006). Chemical composition, seasonal variation and a new sesquiterpene alcohol from the essential oil of *Lippia integrifolia*. *Flavour and Fragrance Journal*, Vol. 21, pp. 839-847.
- Czeczuga, B., Czeczuga-Semeniuk, E. & Semeniuk, A. Chromatic adaptation in lichen phyco- and photobionts. (2010). *Biologia*, Vol. 65, pp. 587-594.
- Deli, J., Molnir, E., Osz, E. & Tdth, G. (2000). Analysis of carotenoids in the fruits of *Asparagus falcatus*: isolation of 5,6-diepikarpoxanthin. *Chromatographia Supplement*, Vol. 51, pp. S-183-S-187.

- Delort, E. & Jaquier, A. (2009). Novel terpenyl esters from Australian finger lime (*Citrus australasica*) peel extract. *Flavour and Fragrance Journal*, Vol. 24, pp. 123-132.
- Djabou, N., Paolini, J., Desjobert, J.M., Allali, H., Baldovini, N., Costa, J. & Muselli, A. (2010). Qualitative and quantitative analysis of volatile components of *Teucrium massiliense* L. - identification of 6-methyl-3-heptyl acetate as a new natural product. *Flavour and Fragrance Journal*, Vol. 25, pp. 475-487.
- Dudareva, N., Pichersky, E. & Gershenzon, J. (2004). Biochemistry of Plant Volatiles. *Plant Physiology*, Vol. 135, pp. 1893-1902.
- Duraipandiyan, V., Indwar, F. & Ignacimuthu, S. Antimicrobial activity of confertifolin from *Polygonum hydropiper*. (2010). *Pharmaceutical Biology*, Vol. 48, pp. 187-190.
- Ebel, R. (2010). Terpenes from Marine-Derived Fungi. *Marine Drugs*, Vol. 8, pp. 2340-2368.
- Ferreira, E.G., Wilke, D.V., Jimenez, P.C., Oliveira, J.R., Pessoa, O.D., Silveira, E.R., Viana, F.A., Pessoa, C., Moraes, M.O., Hadju, E. & Costa-Lotufo, L.V. (2011). Guanidine Alkaloids from *Monanchora arbuscula*: Chemistry and Antitumor Potential. *Chemistry and Biodiversity*, Vol. 8, pp. 1433-1445.
- Figueiredo, E.R., Vieira, I.J.C., Souza, J., Braz-Filho, R., Mathias, L., Kanashiro, M.M. & Côrtes, F.H. (2010). Isolamento, identificação e avaliação da atividade antileucêmica de alcaloides indólicos monoterpênicos de *Tabernaemontana salzmannii* (A. DC.), Apocynaceae. *Brazilian Journal of Pharmacognosy*, Vol. 20, pp. 675-681.
- Fronza, M., Murillo, R., Slusarczyk, S., Adams, M., Hamburger, M., Heinzmann, B., Laufer, S. & Merfort, I. (2011). In vitro cytotoxic activity of abietane diterpenes from *Peltodon longipes* as well as *Salvia miltiorrhiza* and *S. sahendica*. *Bioorganic & Medicinal Chemistry*, Vol. 19, pp. 4876-4881.
- Gao, D.F., Xu, M., Yang, C.R., Xu, M & Zhang, Y.J. (2010). Phenolic Antioxidants from the leaves of *Camellia pachyandra* Hu. *Journal of Agricultural and Food Chemistry*, Vol. 58, pp. 8820-8824.
- Gassenmeier, K. (2006). Identification and quantification of L-menthyl lactate in essential oils from *Mentha arvensis* L. from India and model studies on the formation of L-menthyl lactate during essential oil production. *Flavour and Fragrance Journal*, Vol. 21, pp. 725-730.
- Gauvin-Bialecki, A., Aknin, M. & Smadja, J. (2008). 24-O-Ethylmanoalide, a manoalide-related sesterterpene from the marine sponge *Luffariella* cf. *variabilis*. *Molecules*, Vol. 13, pp. 3184-3191.
- Gomez-Alonso, S., Giuseppe Fregapane, G., Salvador, M.D. & Gordon, M.H. (2003). Changes in phenolic composition and antioxidant activity of virgin olive oil during frying. *Journal of Agricultural and Food Chemistry*, Vol. 51, pp. 667-672.
- He, Z., Xia, W., Liu, Q. & Chen, J. Identification of a new phenolic compound from Chinese olive (*Canarium album* L.) fruit. (2009). *European Food Research and Technology*, Vol. 228, pp. 339-343.
- Hitotsuyanagi, Y., Uemura, G. & Takeya, K. (2010). Sessilifoliamides K and L: new alkaloids from *Stemona sessilifolia*. *Tetrahedron Letters*, Vol. 51, pp. 5694-5696.
- Ishiuchi, K., Kubota, T., Ishiyama, H., Hayashi, S., Shibata, T., Mori, K., Obara, Y., Nakanhata, N. & Kobayashi, J. (2011). Lyconadins D and E, and complanadine E, new Lycopodium alkaloids from *Lycopodium complanatum*. *Bioorganic and Medicinal Chemistry*, Vol. 19, pp. 749-753.

- Joseph-Nathan, P., Leitão, S.G., Pinto, S.C., Leitão, G.G., Bizzo, H.R., Costa, F.L.P., Amorim, M.B., Martinez, N. Dellacassa, E. & Hernández-Barragán, A. (2010). Structure reassignment and absolute configuration of 9-*epi*-presilphiperfolan-1-ol. *Tetrahedron Letters*, Vol. 51, pp.1963-1965.
- Joulain, D. & König, W. (1998). *The Atlas of Spectral Data of Sesquiterpenes Hydrocarbons* (1st ed.), EB-Verlag, ISBN 978-3930826483, Hamburg.
- Jung, M.J; Heo, S. & Wang, M.H. (2008). *Food Chemistry*, Vol. 108, pp. 482-487.
- Junior, G.M.V., Sousa, C.M.M., Cavaleiro, A.J., Lago, J.H.G. & Chaves, M.H. (2008). Phenolic Derivatives from fruits of *Dipteryx lacunifera* Ducke and evaluation of their antiradical activities. *Helvetica Chimica Acta*, Vol. 91, pp. 2159-2167.
- Karamac, M. (2009). *In-vitro* study on the efficacy of tannin fractions of edible nuts as antioxidants. *European Journal of Lipid Science and Technology*, Vol. 111, pp.1063-1071.
- Katakawa, K., Mito, H., Kogure, N., Kitajima, M., Wongseripipatana, S., Arisawa, M. & Takayama, H. (2011). Ten new fawcettimine-related alkaloids from three species of *Lycopodium*. *Tetrahedron*, Vol. 67, pp. 6561-6567.
- Kernan, M.R. & Faulkner, D.J. (1988). Sesterterpene sulfates from a sponge of the family Halichondriidae. *The Journal of Organic Chemistry*, Vol. 53, pp. 4574-4578.
- Khanal, P., Oh, W. K., Yun, H. J., Namgoong, G. M., Ahn, S. G., Kwon, S. M., Choi, H. K. & Choi, H. S. (2011). *p*-HPEA-EDA, a phenolic compound of virgin olive oil, activates AMP-activated protein kinase to inhibit carcinogenesis. *Carcinogenesis*, Vol. 32, pp.545-553.
- Kimura, M. & Rodriguez-Amaya, D. B. (2002). A scheme for obtaining standards and HPLC quantification of leafy vegetable carotenoids. *Food Chemistry*, Vol. 78, pp. 389-398.
- Kuo, P.C., Hwang, T.L., Lin, Y.T., Kuo, Y.C. & Leu, Y.L. (2011). Chemical Constituents from *Lobelia chinensis* and Their Anti-virus and Anti-inflammatory Bioactivities. *Archives of Pharmaceutical Research*, Vol. 34, pp. 715-722.
- Kusaba, M., Maoka, T., Morita, R. & Takaichi, S. (2009). A novel carotenoid derivative, lutein 3-acetate, accumulates in senescent leaves of rice. *Plant Cell Physiology*, Vol. 50, p. 1573-1577.
- Lanças, FM. (2008). Avanços recentes e tendências futuras das técnicas de separação: uma visão pessoal. *Scientia Chromatographica*, Vol. 0, pp. 17-44.
- Lanfranchi, D.A., Laouer, H., Kolli, M.E., Prado, S., Maulay-Bailly, C. & Baldovini, N. (2010). Bioactive Phenylpropanoids from *Daucus crinitus* Desf. from Algeria. *Journal of Agricultural and Food Chemistry*, Vol. 58, pp. 2174-2179.
- Lee, C.J., Chen, L.G., Liang, W. & Wang, A.C.C. (2010). Anti-inflammatory effects of *Punica granatum* Linne in vitro and in vivo. *Food Chemistry*, Vol. 118, pp.315-322.
- Li, H., Dant, H.T., Li, J., Sim, C.J., Hong, J., Kim, D.K. & Jung, J.H. (2011). Pyroglutamyl dipeptides and tetrahydro- β -carboline alkaloids from a marine sponge *Asteropus* sp. *Biochemical Systematics and Ecology*, Vol. 38, pp. 1049-1051.
- Li, N., Hua, J., Wang, S., Cheng, J., Hu, X., Lu, Z., Lin, Z., Zhu, W. & Bao, Z. (2010). Isolation and identification of the main carotenoid pigment from the rare orange muscle of the Yesso scallop. *Food Chemistry*, Vol. 118, pp. 616-619.
- Lin, L., Chen, P. & Harnly, J. M. (2008). New phenolic components and chromatographic profiles of green and fermented teas. *Journal of Agricultural and Food Chemistry*, Vol. 56, pp. 8130-8140.

- Liu, Q., Zhang, Y.J., Yang, C.R. & Xu, M. (2009). Phenolic antioxidants from green tea produced from *Camellia crassicolumna* Var. *multiplex*. *Journal of Agricultural and Food Chemistry*, Vol. 57, pp. 586–590.
- Lopes, R. M., Agostini-Costa, T. S., Gimenes, M. A. & Silveira, D. (2011). Chemical composition and biological activities of *Arachis* species. *Journal of Agricultural and Food Chemistry*, Vol. 59, pp. 4321–4330.
- Lopez, M.D., Jordan, M.J. & Pascual-Villalobos, M.J. (2008). Toxic compounds in essential oils of coriander, caraway and basil active against stored rice pests. *Journal of Stored Products Research*, Vol. 44, pp. 273–278.
- Mahmoud, S.S. & Croteau, R.B. (2002). Strategies for transgenic manipulation of monoterpene biosynthesis in plants. *Trends in Plant Science*, Vol. 7, pp. 366–373.
- Maia, J.G.S., Silva, M.L., Luz, A.I.R., Zoghbi, M.G.B. & Ramos, L.S. (1987). Espécies de *Piper* da Amazônia ricas em safrol. *Química Nova*, Vol.10, pp.200–204.
- Marty, C. & Berset, C. Factors affecting the thermal degradation of all- trans- β -Carotene. (1990). *Journal of Agricultural and Food Chemistry*, Vol. 38, pp. 1063–1067.
- Ndongo, J.T., Shaaban, M., Mbing, J.N., Bikobo, D.N., Atchadé, A.T., Pengyemb, D.E. & Laatsch, H. (2010). Phenolic dimers and an indole alkaloid from *Campylospermum flavum* (Ochnaceae). *Phytochemistry*, Vol. 71, pp. 1872–1878.
- Norin, T. & Westfelt, L. (1963). Thin layer, column, and gas liquid chromatography of resin acid esters and related terpenes. *Acta Chemica Scandinavia*, Vol. 17, pp. 1828–1830.
- Novak, J., Zitterl-Eglseer, K., Deans, S.G. & Franz, C.M. (2001). Essential oils of different cultivars of *Cannabis sativa* L. and their antimicrobial activity. *Flavour and Fragrance Journal*, Vol. 16, pp. 259–262.
- Oliveira, G.P.R. & Rodriguez-Amaya, D.B. (2007). Processed and prepared corn products as sources of lutein and zeaxanthin: compositional variation in the food chain. *Journal of Food Science*, Vol. 72, pp. S79.
- Ozen, T., Demirtas, I. & Aksit, H. (2011). Determination of antioxidant activities of various extracts and essential oil compositions of *Thymus praecox* subsp. *skorpilii* var. *skorpilii*. *Food Chemistry*, Vol. 124, pp. 58–64.
- Pawlowska, A.M., Oleszek, W. & Braca, A. (2008). Quali-quantitative Analyses of Flavonoids of *Morus nigra* L. and *Morus alba* L. (Moraceae) Fruits. *Journal of Agricultural and Food Chemistry*, Vol. 56, pp. 3377–3380.
- Pichersky, E., Noel, J.P. & Dudareva, N. (2006). Biosynthesis of Plant Volatiles: Nature's Diversity and Ingenuity. *Science*, Vol. 311, pp. 808.
- Pinto, S.C., Leitão, G.G., Bizzo, H.R., Martinez, N., Dellacassa, E., Santos, F., Costa, F.L.P., Amorim, M.B. & Leitão, S.G. (2009a). (-)-*epi*-Presilphiperfolan-1-ol, A New Triquinane Sesquiterpene from the Essential Oil of *Anemia tomentosa* var. *anthriscifolia*. *Tetrahedron Letters*, Vol. 50, pp.785–787.
- Pinto, S.C., Leitão, G.G., Oliveira, D.R., Bizzo, H.R., Ramos, D.F., Coelho, T.S., Silva, P.E.A., Lourenço, M.C.S. & Leitão, S.G. (2009b). Chemical Composition and Antimycobacterial Activity from the Essential Oil from *Anemia tomentosa* var. *anthriscifolia*. *Natural Product Communications*, Vol. 4, pp.1675–1678.
- Polat, E., Bedir, E., Perrone, A., Piacente, S. & Alankus-Caliskan, O. (2010). Triterpenoid saponins from *Astragalus wiedemannianus* Fischer. *Phytochemistry*, Vol. 71, pp. 658–662.

- Porcu, O.M. & Rodriguez-Amaya, D.B. (2008). Variation in the carotenoid composition of the lycopene-rich Brazilian fruit *Eugenia uniflora* L. *Plant Foods for Human Nutrition*, Vol. 63, pp. 195–199.
- Potrebko, I. & Resurreccion, A.V.A. (2009). Effect of Ultraviolet Doses in Combined Ultraviolet- Ultrasound Treatments on trans-Resveratrol and trans-Piceid Contents in Sliced Peanut Kernels. *Journal of Agricultural and Food Chemistry*, vol.57, pp. 7750-7756.
- Quadros, A.P., Chaves, F.C.M., Pinto, S.C., Leitão, S.G. & Bizzo, H.R. (2011). Isolation and Identification of *cis*-7-hydroxycalamenene From the Essential Oil of *Croton cajucara* Benth. *Journal of Essential Oil Research*, Vol. 23: 20-23.
- Reis, B., Martins, M., Barreto, B., Milhazes, N., Garrido, E.M., Silva, P., Garrido, J. & Borges, F. (2010). Structure-property-activity relationship of phenolic acids and derivatives. Protocatechuic acid alkyl esters. *Journal of Agricultural and Food Chemistry*, Vol. 58: 6986–6993.
- Roberts, M.F. & Wink, M. (1998). Introduction, In: *Alkaloids: Biochemistry, Ecology and Medicinal Applications*, M.F.Roberts & M. Wink, pp. 1-6, Plenum Press, ISBN 0-306-45465-3, New York.
- Rodriguez, D.B., Lee, T.C. & Chichester, C.O. (1975). Comparative Study of the Carotenoid Composition of the Seeds of Ripening *Momordica charantia* and Tomatoes. *Plant Physiology*, Vol. 56: 626-629.
- Rodriguez-Amaya, D.B. *A guide to carotenoid analysis*. (1999). Washington: OMINI-ILSI Press, 64p.
- Rossi, P.G., Bao, L. Luciani, A., Panighi, J., Desjobert, J.M., Costa, J., Casanova, J., Bolla, J.M. & Berti, L. (2007). (E)-methylisoeugenol and elemicin: antibacterial components of *Daucus carota* L. essential oil against *Campylobacter jejuni*. *Journal of Agricultural and Food Chemistry*, Vol. 55, pp. 7332-7336.
- Roze, L.V., Chanda, A. & Linz, J.E. (2011). Compartmentalization and molecular traffic in secondary metabolism: a new understanding of established cellular processes. *Fungal Genetics and Biology*, Vol. 48, p. 35-48.
- Salamci, E., Kordali, S., Kotan, R., Cakir, A. & Kaya, Y. (2007). Chemical compositions, antimicrobial and herbicidal effects of essential oils isolated from Turkish *Tanacetum aucheranum* and *Tanacetum chiliophyllum* var. *chiliophyllum*. *Biochemical Systematics and Ecology*, Vol. 35, pp. 569-581.
- Sarr, M., Ngom, S., Kane, M. O., Wele, A., Diop, D., Sarr, B., Gueye, L., Andriantsitohaina, R. & Diallo, A. S. (2009). *In vitro* vasorelaxation mechanisms of bioactive compounds extracted from *Hibiscus sabdariffa* on rat thoracic aorta. *Nutrition & Metabolism*, Vol. 6, n. 45, p. 1-12.
- Shaala, L.A., Bamane, F.H., Badr, J.M. & Youssef, D.T.A. (2011). Brominated Arginine-Derived Alkaloids from the Red Sea Sponge *Suberea mollis*. *Journal of Natural Products*, Vol. 74, pp. 1517-1520.
- She, G.M., Xu, C., Liu, B. & Shi, R.B. (2010). Polyphenolic acids from mint (the aerial of *Mentha haplocalyx* Briq.) with DPPH radical scavenging activity. *Journal of Food Science*, Vol. 75, pp. C359-C362.
- Shimoda, M., Shigematsu, H., Shiratsuchi, H. & Osajima, Y. (1995). Comparison of the odor concentrates by SDE and adsorptive column method from green tea infusion. *Journal of Agricultural and Food Chemistry*, Vol. 43, pp. 1616-1620.

- Shimoda, M., Wu, Y. & Osajima, Y. (1996). Aroma compounds from aqueous solution of haze (*Rhus succedanea*) honey determined by adsorptive column chromatography. *Journal of Agricultural and Food Chemistry*, Vol. 44, pp. 3913-3918.
- Sugawara, Y., Hara, C., Aoki, T., Sugimoto, N. & Masujima, T. (2000). Odor distinctiveness between enantiomers of Linalool: difference in perception and responses elicited by sensory test and forehead surface potential wave measurement. *Chemical Senses*, Vol. 25, pp. 77-84.
- Sulyok, E., Vasas, A., Rédei, D., Dombi, G. & Hohmann, J. (2009). Isolation and structure determination of new 4, 12-dideoxyphorbol esters from *Euphorbia pannonica* Host. *Tetrahedron*, Vol. 65, pp. 4013-4016.
- Summons, R.E., Bradley, A.S., Jahnke, L.L. & Waldbauer, J.R. (2006). Steroids, triterpenoids and molecular oxygen. *Philosophical Transactions of the Royal Society B: Biological Sciences*, Vol. 361, pp. 951-968.
- Sun, B., Neves, A.C., Fernandes, T., Fernandes, A., Mateus, M., Freitas, V., Leandro, M. C. & Spranger, M.I. (2011). Evolution of phenolic composition of red wine during vinification and storage and its contribution to wine sensory properties and antioxidant activity. *Journal of Agricultural and Food Chemistry*, Vol. 59, pp. 6550-6557.
- Sutour, S., Bradesi, P., Rocca-Serra, D., Casanova, J. & Tomi, F. (2008). *Chemical composition and antibacterial activity of the essential oil from Mentha suaveolens ssp. insularis* (Req.) Greuter. *Flavour and Fragrance Journal*, Vol. 23, pp. 107-114.
- Suzuki, T., Kubota, T. & Kobayashi, J. (2011). Eudistomidins H-K, new β -carboline alkaloids from the Okinawan marine tunicate *Eudistoma glaucus*. *Bioorganic and Medicinal Chemistry Letters*, Vol. 21, pp. 4220-4223.
- Takahashi, Y., Kubota, T., Shibasaki, A., Gono, T., Fromont, J. & Kobayashi, J. (2011). Nakijinamines C-E, New Heteroaromatic Alkaloids from the Sponge *Suberites* Species. *Organic Letters*, Vol. 13, pp. 3016-3019.
- Takaichi, S., Maoka, T., Yamada, M., Matsuura, K., Haikawa, Y. & Hanada, S. (2001). Absence of carotenes and a presence of a tertiary methoxy group in a carotenoid from a thermophilic filamentous photosynthetic bacterium *Roseiflexus castanholzii*. *Plant Cell Physiology*, Vol. 42, pp. 1355-1362.
- Takaichi, S., Mochimaru, M., Maoka, T. & Katoh, H. (2005). 4, 6 Myxol and 4-Ketomyxol 2'-Fucosides, not Rhamnosides, from *Anabaena* sp. PCC 7120 and *Nostoc punctiforme* PCC 73102, and Proposal for the Biosynthetic Pathway of Carotenoids. *Plant Cell Physiology*, Vol. 46, pp. 497-504.
- Tantillo, D.J. (2011). Biosynthesis via carbocations: Theoretical studies on terpene formation. *Natural Product Reports*, vol. 28, pp. 1035-1053.
- Francisco, V., Figueirinha, A., Neves, B.M., Rodríguez, C.G., Lopes, M.C.; Cruz, M.T. & Batista, M.T. *Cymbopogon citratus* as source of new and safe anti-inflammatory drugs: Bio-guided assay using lipopolysaccharide-stimulated macrophages. (2011). *Journal of Ethnopharmacology*, Vol. 133, pp. 818-827.
- Vetter, W. & Schröder, M. (2011). Phytanic acid—a tetramethyl branched fatty acid in food. *Lipid Technology*, Vol. 23, pp. 175-178.
- Villanueva, H.E. & Setzer, W.N. (2010). Cembrene diterpenoids: conformational studies and molecular docking to tubulin. *Records of Natural Products*, Vol. 4, pp. 115-123.

- Wang, C.C., Chang, S.C., Inbaraj, S.B. & Chen, B.H. (2010). Isolation of carotenoids, flavonoids and polysaccharides from *Lycium barbarum* L. and evaluation of antioxidant activity. *Food Chemistry*, Vol. 120, pp. 184–192.
- Wang, M., Li, J., Rangarajan, M., Shao, Y., Voie, E.J., Huang, T.C. & Ho, C.T. (1998). Antioxidative Phenolic Compounds from Sage (*Salvia officinalis*). *Journal of Agricultural and Food Chemistry*, Vol. 46, pp. 4869–4873.
- Wink, M. (1998). A Short History of Alkaloids, In: *Alkaloids: Biochemistry, Ecology and Medicinal Applications*, M.F. Roberts & M. Wink, pp. 11–44, Plenum Press, ISBN 0-306-45465-3, New York.
- Wondracek, D.C., Vieira, R. F., Silva, D.B. & Agostini-Costa, T.S. (2012). Saponification influence in carotenoid determination in cerrado passion fruit. *Química Nova*, in prelo.
- Wu, Z., Song, L. & Huang, D. (2011). Food grade fungal stress on germinating peanut seeds induced phytoalexins and enhanced polyphenolic antioxidants. *Journal of Agricultural and Food Chemistry*, Vol. 59, pp. 5993–6003.
- Xiao, J.S., Liu, L., Wu, H., Xie, B.J., Yang, E.N. & Sun, Z.D. (2008). Rapid preparation of procyanidins B2 and C1 from granny smith apples by using low pressure CC and identification of their oligomeric procyanidins. *Journal of Agricultural and Food Chemistry*, Vol. 56, pp. 2096–2101.
- Ye, J.H., Wang L.X., Chen H., Dong J.J., Lu, J.L., Zheng, X.Q., Wu, M.Y. & Liang, Y.R. (2011). Preparation of tea catechins using polyamide. *Journal of bioscience and bioengineering*, Vol. 111, pp. 232–236.
- Zellner, B.D., Dugo, P., Dugo, G. & Mondello, L. (2010). Analysis of Essential Oils, In: *Handbook of Essential Oils*, K.H.C. Baser, G. Buchbauer, pp. 151–184, CRC Press, ISBN 978-1420063158, Boca Raton.
- Zhan, P.Y., Zeng, X.H., Zhang, H.M. & Li, H.H. (2011). High-efficient column chromatographic extraction of curcumin from *Curcuma longa*. *Food Chemistry*, Vol. 129, pp. 700–703.
- Zhang, Z., Liao, L., Moore, J., Wua, T. & Wang, Z. (2009). Antioxidant phenolic compounds from walnut kernels (*Juglans regia* L.). *Food Chemistry*, Vol. 113, pp. 160–165.
- Zhu, B.C.R., Henderson, G., Yu, Y. & Laine, R.A. (2003). Toxicity and Repellency of Patchouli Oil and Patchouli Alcohol against Formosan Subterranean Termites *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae). *Journal of Agricultural and Food Chemistry*, Vol. 51, pp. 4585–4588.

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Chromatography is a powerful separation tool that is used in all branches of science, and is often the only means of separating components from complex mixtures. The Russian botanist Mikhail Tswett coined the term chromatography in 1906. The first analytical use of chromatography was described by James and Martin in 1952, for the use of gas chromatography for the analysis of fatty acid mixtures. A wide range of chromatographic procedures makes use of differences in size, binding affinities, charge, and other properties. Many types of chromatography have been developed. These include Column chromatography, High performance liquid chromatography (HPLC), Gas chromatography, Size exclusion chromatography, Ion exchange chromatography etc. In this book contains more details about the applications of chromatography by various research findings. Each and every topics of this book have included lists of references at the end to provide students and researchers with starting points for independent chromatography explorations. I welcome comments, criticisms, and suggestions from students, faculty and researchers.

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