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Medicinal plants popularly used for gastrointestinal disorders in Costa Rican Central Valley

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Abstract : A survey was conducted of medicinal plants for gastrointestinal disorders used by inhabitants of the Central Valley in Costa Rica by means of interviews with medicinal plant vendors of the central markets of the cities of Alajuela, Cartago, Heredia and San José. Fifteen medicinal plants and one plant mixture were recorded; the last one was prepared *in situ* by the vendor upon request and its components varied according to the availability of plants. A qualitative chromatographic comparison was made on the patterns of plants from different origin and different times of the year. Differences were noticed with regard to the concentration of major components in many plants.
A quantitative method using HPLC was used to examine *Quassia amara* and *Plantago major*.

Keywords : Medicinal plants, gastrointestinal disorders, survey, *Quassia amara*, *Plantago major*, HPLC analysis.

INTRODUCTION

The purpose of this research was to record medicinal plants currently sold in the markets of the Central Valley in Costa Rica for gastrointestinal disorders. Their toxicity, antimicrobial and antiulcer activity caused by *Helicobacter pylori* were also tested.

Compared to synthetic drug treatments, information available on the relative safety of herbal remedies is limited. Some of them are considered to be safe because of their long history of usage (Ernst, 1998). Examples of allergic or toxic reactions, adverse and mutagenic effects and mistaken plant identity have also been reported. For example, *Aristolochia clematitis* L. and *A. serpentaria* L. have been traditionally used in Western medicine, but phytochemical and pharmacological studies have shown that aristolochic acid, a compound found in those plants, besides having antineoplasic activity is also one of the most effective carcinogens yet known (De Smet, 1992). Another example is the case for *Teucrium sp.* and *Tussilago farfara*, praised by Hesiod and Hippocrates for their curative effects in respiratory diseases, but few years ago both herbs were implicated in human poisoning (Huxtable, 1999).

In general, the uses for the diverse flora of Central American countries as medicinal plants are documented. This has mainly been through studies conducted by Universities and Institutes at their country of origin and the initiatives from Tramil (Program of applied research to the popular medicine in the Caribbean, http://www.funredes.org/endacaribe/Tramil.html), CYTED (Iberoamerican Program Of Science and Technology for the development, http://www.cyted.org/Nueva.asp), and the Convenio Andres Bello (CAB, http://www.cab.int.co). However, even though information regarding inventories of medicinal plants is available for each country, the interest has not developed uniformly and yet, there is lack of information regarding long term toxicity, safety and use of standard/measured doses in many cases.

In spite of the research carried out by Costa Rican scientists on this subject, local people have been interested in medicinal plants only relatively recently, unlike the habitants of other Central American countries as Guatemala, where even small companies developed medicinal products since a long time ago. In 1994 there were 200 different plant species used under different plant-based preparations: tinctures, capsules, teas or creams (Orellana, et al., 1994).

According to the literature, around 1134 native plant species of 132 families of Central America were catalogued according to their medicinal uses. Most of the reports were related to gastrointestinal disorders, followed by dermatological and respiratory diseases. In the case of Costa Rica, 194 plant species were reported, a 58% for the treatment of gastric illnesses (Romero, 2001).

This report includes preliminary survey of medicinal plant species used by rural and urban communities from Costa

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Rican Central Valley and covers selected markets of the most important cities.

MATERIALS AND METHODS

Research area and data collection

The research program included visits to the central markets of the cities of Alajuela, Cartago, Heredia and San Jose, interviews with sellers, systematic recording and scientific identification of plants. The research team comprised two interviewers, who concentrated on finding the herbs, identifying them and enquiring into the medical uses. At least two visits to each place were made, and it was only during the second visit when plant material was bought and when the sellers knew about the aims and nature of the research. After the first purchase of plant material, three other visits were programmed quarterly for getting fresh plant material for purposes of comparison.

Identification of plants

All samples were initially identified by their external characters by Luis D. Vargas, Bioprospecting Strategic Action Unit of the National Institute of Biodiversity (INBio).

Plant extraction for qualitative analysis

The plant materials used popularly were collected and extracted. The extraction procedures and the analysis were performed four times every three months during a year, utilising fresh samples from the same vendors said to have been collected from the same vicinities. Extracts of each plant species were obtained utilising two different protocols.

Protocol A. Doses : The samples were bought at the San Jose Central Market and the recommended doses per day were obtained according to Table 1.

Protocol B. Qualitative Analysis : Two sets of plants were bought: one at the San Jose Central Market and the other at Finca Narobi. The recommended doses per day were obtained for all according to Table 1 and were lyophilised.

An equivalent weight of 0.2 g of dried extract was extracted first with methylene chloride (3 x 20 ml) followed by ethyl acetate (3 x 20 ml) for each sample (the 0.2 g was dissolved in a volume of distilled water corresponding to the same volume of A). The organic phases were combined and evaporated to dryness under reduced pressure. The organic concentrate was dissolved in 4 ml distilled water and was applied to a RP C18 cartridge eluting successively with 4 ml (8.5:1.5), (6:4) and (0.5:9.5) water: methanol. The elution gave 4 fractions for each sample. All fractions were analysed qualitatively by TLC and HPLC analysis methods. Samples obtained thereafter for the comparative study during the year, were extracted exhaustively using the same system and treated afterwards with the described procedure.

Preparation for quantitative analysis

Quassia amara and *Plantago major* extracts for quantitative HPLC analysis were obtained using two different protocols. The utilised plant materials were bought at the Central Market of San Jose, and from Finca Narobi.

Protocol C. Lyophilisation of doses : The plant material used was extracted (Table 1), and lyophilised. The lyophilised extract was dissolved in 50 % Ethanol and applied to a RP C18 cartridge. The filtrate was evaporated to dryness under reduced pressure, the dried residue was dissolved in 10.00 ml of 50 % EtOH solution and filtered through a 0.22 µm Millipore filter prior to chromatographic analysis.

Protocol D. Hydroalcoholic extraction : *Q. amara* bark cut in small pieces, or *P. major* leaf powder (2 g fresh weight), suspended in 20 ml 50 % ethanol were subjected to ultrasound treatment for one hour at a constant temperature of 25 °C. The samples were filtered and evaporated to dryness under reduced pressure, dissolved in 50 % EtOH solution and applied to a RP C18 cartridge. The filtrates were dried under reduced pressure, dissolved in 10.00 ml 50% EtOH and filtered through a 0.22 µm Millipore filter prior to HPLC analysis.

Apparatus and conditions

TLC was carried out on silica gel $60F_{254}$ plates (0.25 mm Merck, Darmstadt, Germany) using hexane: ethyl acetate (1:9) as eluent. Plates were examined under UV light and sprayed with p-anisaldehyde-sulfuric acid, followed by heating.

HPLC analysis was carried out with a Waters 900E apparatus, equipped with a PDA 986 UV-VIS diode array detector and Millenium 3.2 software. A Prep-Nova-Pak HR-C18 column (7.8 mm x 300 mm, 6 µm I.D.) with a gradient elution system 15% MeOH/ H_2O to 100% MeOH for 70 min was employed. The flow rate was kept constant at 1.5 ml/min with the column at room temperature and the photo diode array detector was monitored from 200-600 nm.

RESULTS

The information obtained is shown in Table 1. Most of

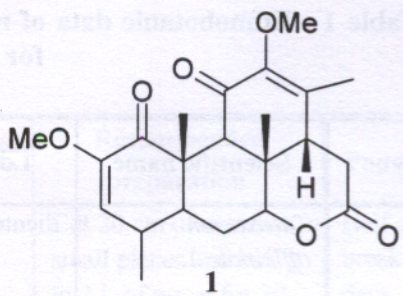
the plants were sold in all the markets we visited with the exception of *Achillea millefolium*, *Ocimum basilicum* and *Mimosa albida*. The special mixtures were only offered in the Central Market at San Jose, and they were prepared *in situ* by the vendor upon request and according to the availability of the plants.

The plants have been used for gastrointestinal disorders, respiratory and dermatological illnesses, and for anti-inflammatory treatments (Table 2).

Besides fresh plants, it was possible to find different products: tinctures, creams, teas, etc all imported; and also some posters with information about medicinal plants, mostly prepared by the vendors. The sellers were all intermediates and buy the plants from farmers or maybe from people that got them from the wild; the quality of the plants was different according to the vendors. We decided to buy the plants in one particular shop at the Central Market of San Jose as this market offered all the species and the quality was fairly uniform.

The four utilised extracts from each plant were obtained as described in Materials and Methods under Protocol B, and its TLC and HPLC chromatographic patterns were obtained. The patterns from plants of different sources were compared qualitatively. There were no significant differences between the chromatographic patterns in plants from different regions and at different times of the year. The fingerprint of major components was observed for each plant, and the only difference was with respect to concentration. One example can be seen for *Quassia amara* in Figure 1. The peaks 1 and 2 correspond to quassin and neoquassin, respectively. Always in samples from Finca Narobi, the amount of quassin was higher, meanwhile for the plants from the San Jose Central Market, the amounts of these compounds were lower.

A qualitative and quantitative method using HPLC with photo-diode array detector was set up to analyse *Quassia amara* and *Plantago major*. We chose these two species due to the accessibility of quality standards of their active components. Referring to authentic compounds previously isolated in our laboratory, we identified quassin (**1**) and neoquassin (**2**) from *Q. amara*, and verbascoside (**3**) from *P. major*. The identification was made on the basis of their ultraviolet absorption spectra, retention time and the spiking peak method (Figs. 2 and 3). Calibration curves (coefficient of correlation >0.996) were prepared for the quantification of the compounds and the results are shown in Tables 3 and 4.



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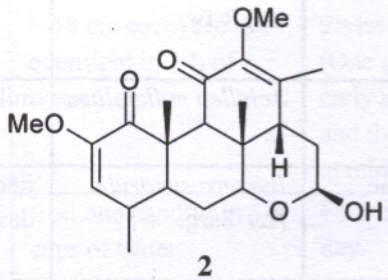

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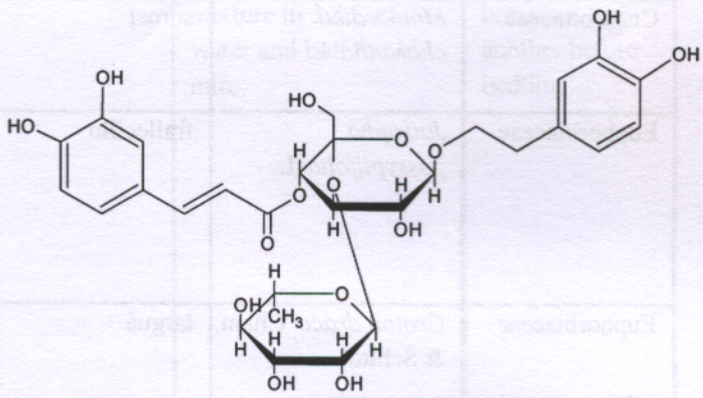

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DISCUSSION

Costa Rican people mainly utilise medicinal plants that they collect from their own gardens or they obtain from family or friends. It is not so common for Costa Ricans to go to places for buying them, and usually the shopping is made when they buy their groceries.

The traditional form of using medicinal plants varied from one place to another, although the raw material used in all the places were the same. This situation was expected. Table 1 shows the recommended preparation and doses given at one shop in the Central Market of San Jose.

From the plants sold in the markets, five are exotic and ten are native to Costa Rica, and as seen in Table 2, all of them with the exception of *Triumffetta bogotensis*, have reports regarding uses as medicinal plants. The

Table 1 : Ethnobotanic data of medicinal plants sold in the central valley of Costa Rica for gastrointestinal disorders

Family	Scientific name	Local name	Parts used	Recommended preparation[1]	Popular doses[1]
Asteraceae	*Taraxacum officinale* L.	diente de león	leaves, roots	Boil for 3 min two tablespoons of pounded leaves and roots in 2 cups of water.	Drink one cup after meals
Asteraceae	*Neurolaena lobata* (L.) R.Br.	gavilana	leaves	Cut 7 leaves and add 1 L of boiling water.	Two cups per day every two days.
Asteraceae	*Achillea millefolium* L.	milenrama	whole plant	1/3 handful (~37 g) in 1 L of boiling water	One cup per day
Burseraceae	*Bursera simaruba (L.)* Sarg.	jinocuabe, indio desnudo	bark	Boil one handful in 1 L of water per 30 min	Two cups per day. The first one before breakfast
Cucurbitaceae	*Momordica charantia* L.	sorosí	whole plant	3 or 4 spoons of cut plant per litre of boiling water	~ 60 mL twice a day.
Euphorbiaceae	*Jatropha gossypiifolia* L.	frailecillo	leaves, aerial parts	Boil 1 or 2 leaves in 3 cups of water. Also blend half of handful (30g) of aerial parts in less than 1 L of water.	Drink during day
Euphorbiaceae	*Croton draco* Cham. & Schltdl.	targuá	latex		Four drops of latex with goat's milk or with water before breakfast every day, for 8 or 10 weeks.
Fabaceae/mim.	*Mimosa albida* Humb. & Bonpl. Ex Willd.	uña de gato	whole plant	10 g in 1 L of boiling water	One cup before breakfast
Lamiaceae	*Satureia viminea* L.	menta	aerial part	One tablespoon of aerial parts per cup of boiling water.	2 or 3 cups of water per day.
Lamiaceae	*Ocimum basilicum* L.	albahaca	whole plant	One hand roll (~72 g) in half a litre of boiling water	One cup per day
Liliaceae	*Aloe vera (L.)* Burm. F.	sábila	leaves	Leaves peel and cut into pieces of 2cm.	One 2 cm piece every two days
Plantaginaceae	*Plantago major* L.	llantén	whole plant	Concoct one handful (approx. 6 leaves) in 1 L of water.	One cup per day

Family	Scientific name	Local name	Parts used	Recommended preparation[1]	Popular doses[1]
Simarubaceae	*Quassia amara* L.	hombre grande	woodies	Cut 20 cm of woodies in small pieces and boil it in 3 L of water for 30 minutes	Half glass before breakfast for 30 days
Tiliaceae	*Triumfetta bogotensis* DC.	mozote	stems	Two pounded stems (~18 cm each) are left overnight in 1 L of water	Two small glasses per day (One glass at early morning and the other one at midday).
Verbenaceae	*Lippia alba* (Mill.) N.E.Br. Ex Britton	juanilama	aerial part	Boil one handful in 3-4 cups of water.	Four cups per day.
	Special mixture A (J. gossypiifolia, N. lobata, Q. amara, L. alba, S. viminea, M. charantia, B. simaruba, Rosmarinus officinalis, Buddleja americana, Artemisia vulgaris, Senna reticulata	frailecillo, gavilana, hombre grande, juanilama, menta, sorosí, jinocuabe, romero, salvia virgen, artemisa, saragundí		500 g of the whole mixture in 3.5 L of water and boil for 15 min.	One glass before breakfast and another before bedtime.
	Special mixture B (N. lobata, R. officinalis, Q. amara, L. alba, B. americana, A. vulgaris)	gavilana, romero, hombre grande, juanilama, salvia virgen, artemisa.		500 g of the whole mixture in 3.5 L of water and boil for 15 min.	One glass before breakfast and another before bedtime.
	Special mixture C (N. lobata, R. officinalis, Bursera simaruba, B. americana, Equisetum sp. P. australis, Hymenaea courbaril, A. vulgaris, Rubus sp. and a non-identified plant).	gavilana, romero, sorosí, salvia virgen, cola de caballo, llantén, guapinol, artemisa, mora.		500 g of the whole mixture in 3.5 L of water and boil for 15 min.	One glass before breakfast and another before bedtime.

[1] Preparation and doses given at the Central Market of San Jose by the vendors.

Table 2 : Information on chemical analysis of medicinal plants sold in the central valley markets - Costa Rica

Family	Scientific name	Reported use	Origin	Authentication	Bibliography
Asteraceae	*Taraxacum officinale* L.	gastrointestinal and dermatological diseases and as anti-inflammatory	Europe	micro- and macroscopically. TLC analysis	Würzburg et al., 2002 House, 1995 Cáceres, 1996
Asteraceae	*Neurolaena lobata* (L.) R.Br.	gastrointestinal and dermatological illnesses	Central America and Mexico		Rodríguez, 2001 House, 1995
Asteraceae	*Achillea millefolium* L.	gastrointestinal and dermatological complaints.	Europe and Asia	micro- and macroscopically. TLC and GC analysis	Würzburg et al., 2002 House, 1995 Cáceres, 1996
Burseraceae	*Bursera simaruba (L.)* Sarg.	gastrointestinal and dermatological diseases.	Central America and Tropical South America		Rodríguez, 2001 Cáceres, 1996
Cucurbitaceae	*Momordica charantia* L.	respiratory, gastrointestinal and dermatological illnesses	Tropics		House, 1995 Cáceres, 1996
Euphorbiaceae	*Jatropha gossypiifolia* L.	gastrointestinal and respiratory complaints, as anti-inflammatory	America		Rodríguez, 2001 House, 1995
Euphorbiaceae	*Croton draco* Cham. & Schltdl.	gastrointestinal, dermatological, diseases and as anti-inflammatory.	America		Rodríguez, 2001 Ocampo and Maffioli, 1985.
Fabaceae/mim.	*Mimosa albida* Humb. & Bonpl. Ex Willd.	dermatological and respiratory complaints.	Central America and Tropical South America		House, et al. 1995 House, 1995
Lamiaceae	*Mentha sp.* (*Satureja viminea* L.)	gastrointestinal illnesses	Neotropic		Ocampo, 1983 Ocampo and Maffioli, 1985
Lamiaceae	*Ocimum basilicum* L.	gastrointestinal and dermatological complaints	Probably Asia.	micro- and macroscopically. TLC analysis	Würzburg et al., 2002Cáceres, 1996
Liliaceae	*Aloe vera (L.)* Burm. F.	gastrointestinal and dermatological illnesses	Africa	micro- and macroscopically. TLC and HPLC analysis	Würzburg et al., 2002 Wagner and Bladt,1996 House, 1995 Cáceres, 1996
Plantaginaceae	*Plantago major L.*	gastrointestinal, respiratory and dermatological diseases and as anti-inflammatory	Europe and Asia	macroscopically.	House, 1995 Cáceres, 1996

Family	Scientific name	Reported use	Origin	Authentication	Bibliography
Simarubaceae	*Quassia amara* L.	gastrointestinal complaints	Latin America	microscopically. TLC analysis	Würzburg et al., 2002 Wagner and Bladt,1996 House, 1995 Cáceres, 1996
Tiliaceae	*Triumfetta calderonii* *T. semitriloba* Jacq. *T. lappula* *T. speciosa* Seem.	gastrointestinal diseases	Tropical America		House, 1995 Navarro, 1970 Nuñez, 1975
Verbenaceae	*Lippia alba* (Mill.) N.E.Br. Ex Britton	gastrointestinal, respiratory, dermatological, neurological illnesses and as anti-inflammatory	America	macroscopically	House, 1995 Cáceres, 1996

Table 3 : Percentage of quassinoids in *Quassia amara* extracts

Extract	% of quassin	% total quassinoids
Market, method C	0.015	0.021
Market, method D	0.034	0.048
Narobi, method D	0.018	0.039

Table 4 : Percentage of verbascoside in *Plantago major* extracts

Extract	% of verbascoside
Market, method C	4.61
Market, method D	3.78
Narobi, method D	3.06

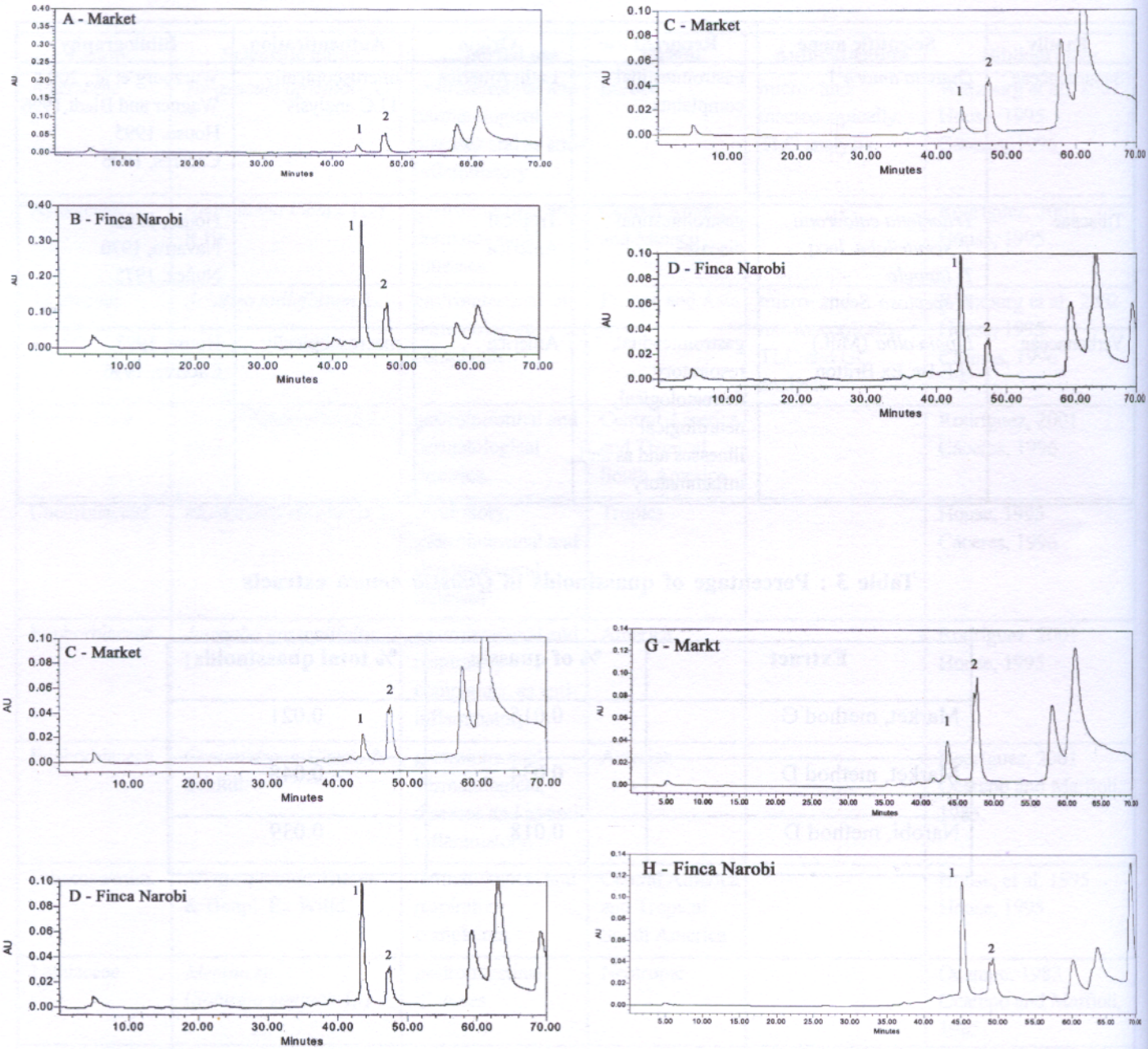

Figure 1 : HPLC chromatograms of Q. amara at 254 nm (Peak labels correspond to quassin **1** and neoquassin **2**. Chromatograms **A** and **B** correspond to extracts month 1, chromatograms **C** and **D** to extracts month 4, chromatograms **E** and **F** to extracts month 7 and chromatograms **G** and **H** to extracts month 10 B.

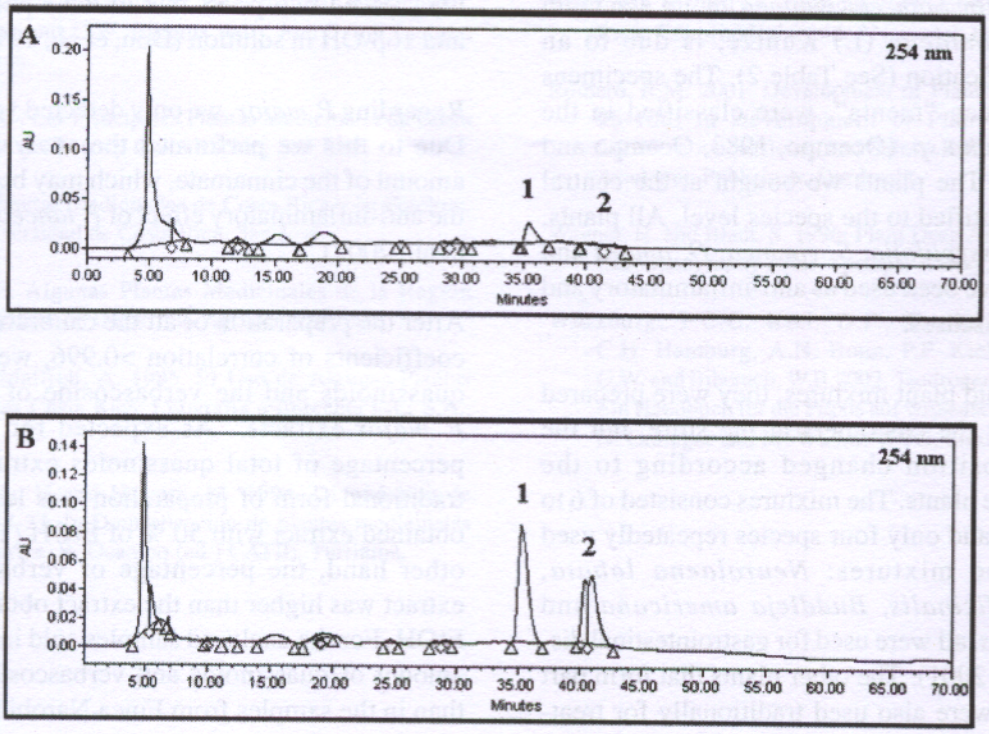

Figure 2 : HPLC chromatograms of *Quassia amara* obtained under protocol C (Peak labels correspond to quassin **1** and neoquassin **2**. Chromatogram **A** corresponds to the extract and **B** to the extract plus addition of standard samples of quassin and neoquassin).

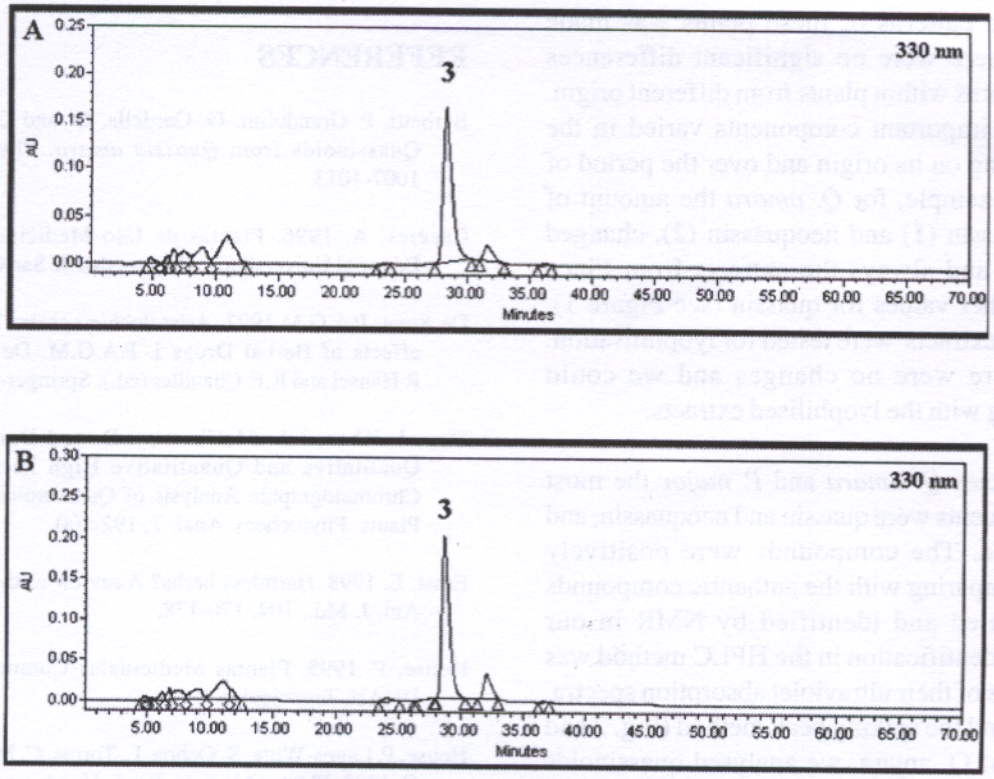

Figure 3 : HPLC chromatograms of *Plantago major* obtained under protocol C (Peak **3** corresponds to verbascoside. Chromatogram **A** corresponds to the extract and **B** to the extract plus addition of standard sample of verbascoside).

lack of reports for *Satureja viminea* or its synonym *Clinopodium vimineun* (L) Kuntze, is due to an erroneous classification (See Table 2). The specimens of the Costa Rican "menta", were classified in the literature as *Mentha sp.* (Ocampo, 1983, Ocampo and Maffioli, 1983). The plants we bought at the central market were identified to the species level. All plants, apart from *J. gossypiifolia*, *S. viminea*, *Q. amara* and *T. bogotensis*, have been used as anti-inflammatory and for respiratory diseases.

Regarding the sold plant mixtures, they were prepared upon request by the customers at the store, but the mixture composition changed according to the availability of the plants. The mixtures consisted of 6 to 11 plant species and only four species repeatedly used in all the three mixtures: *Neurolaena lobata*, *Rosmarinus officinalis, Buddleja americana* and *Artemisa vulgaris*, all were used for gastrointestinal disorders (Romero, 2001). The other plants that form part of the mixtures were also used traditionally for treatment of gastrointestinal problems (House, 1995, Cáceres, 1996, Ocampo, 1983).

TLC and HPLC methods were used to evaluate the quality of herbal remedies. The chromatograms of the four extracts were obtained using Protocol B from each plant bought from the Central Market of San Jose and Finca Narobi. The comparison between the chromatographic patterns of these plants was made qualitatively. There were no significant differences between the patterns within plants from different origin. The quantity of important components varied in the extracts depending on its origin and over the period of collection. For example, for *Q. amara* the amount of quassinoids, quassin (**1**) and neoquassin (**2**), changed over the period and always the extracts from Finca Narobi gave higher values for quassin (see Figure 1). Besides the four extracts were tested for lyophilisation. Fortunately there were no changes and we could continue working with the lyophilised extracts.

For the two species *Q. amara* and *P. major* the most important constituents were quassin and neoquassin, and the verbascoside. The compounds were positively identified by comparing with the authentic compounds previously isolated and identified by NMR in our laboratory. The identification in the HPLC method was made on the basis of their ultraviolet absorption spectra, retention times and the spiking peak method (Fig. 2 and 3). In the case of Q. amara, we analysed quassinoids because they were identified as responsible for biological activity (Barbetti, et al., 1993): peak (**1**) at a retention time of 35.32 min was identified as quassin and the peak (**2**) at 40.72 min as neoquassin. Neoquassin (**2**) showed two peaks due to the equilibrium of 16α- and 16β-OH in solution (Dou, et al., 1996).

Regarding *P. major*, we only detected verbascoside (**3**). Due to this we performed the analysis based in the amount of the cinnamate, which may be responsible for the anti-inflammatory effect of *P. lanceolata* (Würzburg et al., 2002).

After the preparation of all the calibration curves, with coefficients of correlation >0.996, we quantified the quassinoids and the verbaşcoside of *Q. amara* and *P. major* extracts. As expected for *Q. amara*, the percentage of total quassinoids extracted using the traditional form of preparation was less than half the obtained extract with 50 % of EtOH (Table 3). On the other hand, the percentage of verbascoside in the extract was higher than the extract obtained with 50 % EtOH. For the analysed samples sold in the market, the amount of quassinoids and verbascoside were higher than in the samples from Finca Narobi (Table 4).

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