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## Initial characterization of crude extracts from *Phyllanthus amarus* Schum. and Thonn. and *Quassia amara* L. using normal phase thin layer chromatography

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**INITIAL CHARACTERIZATION OF CRUDE EXTRACTS FROM  
*PHYLLANTHUS AMARUS* SCHUM. AND THONN. AND *QUASSIA AMARA* L.  
USING NORMAL PHASE THIN LAYER CHROMATOGRAPHY**

**A Thesis**

**Submitted to the Graduate Faculty of the  
Louisiana State University and  
Agricultural and Mechanical College  
in partial fulfillment of the  
requirements for the degree of  
Master of Science**

**in**

**The School of Renewable Natural Resources**

**by  
Vivian Esther Fernand  
B.S., University of Suriname, 1998  
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## ABSTRACT

The extracts of many plants used in traditional medicine contain curative agents that are used in many modern medicines. As part of the quest for potentially valuable plants of medicinal value, the plant species *Phyllanthus amarus* Schum. and Thonn. and *Quassia amara* L. were chosen based on ethno-pharmacological knowledge from Suriname, South America. *Phyllanthus amarus* (whole plant) was collected in the city Paramaribo and in the country, and *Quassia amara* (wood) was collected in the countryside of Suriname.

The aim of this study was to optimize extraction methods in order to maximize the recovery of secondary metabolites in the crude extracts of *P. amarus* and *Q. amara*. This was accomplished by examining the influence of different extraction solvents on the presence of secondary metabolites in the extracts by thin layer chromatography (TLC), determining the most suitable mobile phase for the plant extracts, and determining the most suitable detection method.

Ten grams of each species were extracted (w/v 1:10) with 50% methanol in water, 99% methanol, and 50% methanol in chloroform. Thin layer chromatography (TLC) was used to analyze the compounds in the plant extracts. In order to detect the most compounds, it was necessary to determine the optimal mobile phase (chloroform/methanol 9:1; 95:5; or 98:2) and most suitable detection method (I: UV-254 nm and Phosphomolybdic acid reagent; II: UV-365 nm and Dragendorff reagent; III: ethanolic sulfuric acid reagent; or IV: ethanolic sulfuric acid and UV-365 nm).

For both plant species, crude extracts from methanol and chloroform-methanol yielded the highest number of fractions. Mobile phase chloroform/methanol 95:5 eluted the most fractions and had the best separation. Detection method I detected a wide variety

of fractions/compounds. In the *P. amarus* extracts the following secondary metabolites were visualized: alkaloids, flavonoids, lignans, phenols and indole derivatives. In *Q. amara* extracts, alkaloids (e.g.  $\beta$ -carbolines, canthin-6-ones) and quassinoids were detected.

Methanol as an extraction solvent gave the best recovery (extraction rate) of secondary metabolites in both plants, and it can be concluded that different extraction solvents influence the extraction rate. Optimized powder extracts were produced as determined by TLC analysis for future bioassay tests.

## CHAPTER 1. INTRODUCTION

According to the World Health Organization (De Silva, 1997), about 80% of the population in many third world countries still use traditional medicine (e.g., medicinal plants) for their primary health care, due to poverty and lack of access to modern medicine. Since about 80 % of the 6.1 billion people of the world live in less developed countries, this means that more than 3.9 billion people will likely use medicinal plants on a frequent basis. Therefore, there is a need to study medicinal plants for their efficacy, safety and quality, and also to search for potentially valuable medicinal material from which novel curative agents may be created for the benefit of all humankind.

The investigation of plants as potential sources of new drugs to treat cancer, AIDS and malaria requires the search of as many resources as possible. The discovery of phytochemical compounds with, for example, cytotoxic and/or anti-tumor activity could lead to the production of new drugs for the treatment of cancer. Therefore, the development of appropriate extraction methods in order to obtain plant extracts with as many phytochemical compounds as possible is important.

The criteria used for selecting plants from Suriname for investigation were based on: (1) traditional medicinal information (ethno-pharmacological knowledge); (2) chemical composition of the plant species; and (3) literature reports on plant extracts' pharmacology and ethnomedical claims. From seven medicinal plants collected in July 2001 in Suriname, two were chosen to be investigated: *Phyllanthus amarus* Schum. and Thonn. (Euphorbiaceae) and *Quassia amara* L. (Simaroubaceae).

In order to extract as many phytochemical compounds as possible, it is important to optimize extraction methods for secondary metabolites, and this can be realized by calculating the extraction rate. The extraction rate (the ratio of how much powder is



produced from a given amount of raw plant material) gives an indication of the strength of the solvent used in the recovery of phytochemicals . Qualitative analysis of the extracts was done by thin layer chromatography (TLC). TLC serves as one of the many methods in providing a chromatographic plant extract fingerprint (Wagner and Bladt, 1996).

### **1.1. Objectives**

1. To optimize extraction methods in order to get the highest extraction rate for crude plant extracts of *Phyllanthus amarus* Schum. and Thonn. and *Quassia amara* L., by:
  - Examining the influence of different extraction solvents on the presence of secondary metabolites in the extracts by Thin Layer Chromatography (TLC);
  - Determining the most suitable mobile phase for the plant extracts and;
  - Determining the most suitable TLC detection method.
2. To produce optimized powder extracts based on the extraction methods developed for future bio-assay tests.

### **1.2. Research Hypothesis**

Different extraction solvents influence the composition and extraction rate of secondary metabolites in extracts of *Phyllanthus amarus* Schum. and Thonn. and *Quassia amara* L.

## CHAPTER 2. LITERATURE REVIEW

### 2.1. Background

Suriname is the middle of the three Guyana's and lies in the northeastern part of South America (Figure 2.1) on the geologically stable structure called the Guyana Shield. Geographically, Suriname is part of the Amazonian area. The total land area is 163,820 km<sup>2</sup>, of which three-quarters is covered with tropical rainforest. In Suriname there are about 430,000 people, the official language is Dutch, and a general dialect known as Surinamese ("Sranang Tongo") is frequently spoken. The majority of the population lives along the coastal area, especially in and around the capital city of Paramaribo. Suriname is culturally more related to the Caribbean region than to Latin America (Bruining, 1977).



**Figure 2.1: Suriname as part of Guyana shield and the Amazonian area**

Of the Caribbean region, Suriname has the most ethnically heterogeneous society, including Amerindians (original inhabitants), Maroons, Creoles, Europeans, Jews, Indians, Javanese, Chinese, and Lebanese. Because of the diverse composition of the population, there is an extensive knowledge of medicinal plants in this country. Since

Suriname has a multicultural society, not all plants that are used as medicinal plants are native to Suriname; some of them came along with the various ethnic groups. As in many other developing countries, the use of traditional medicine forms an integral part of the cultural patterns of the population.

The history of natural product use in ancient times and in folk medicine around the world is the basis for the use of many therapeutic drugs in modern-day medicine. Primitive cultures used plants as a source not only of medicines but also for toxic substances for killing animals, and for stimulants and hallucinogens used in religious rites. Traditionally, natural plant products have been the source in the search for new drugs by pharmaceutical companies (Dillard and German, 2000). Currently, 25% of all modern medicines are directly or indirectly derived from higher plants.

Ethnopharmacological information can be used to provide three levels of resolution in the search for new drugs: (1) as a general indicator of non-specific bioactivity suitable for a panel of broad screens; (2) as an indicator of specific bioactivity suitable for particular high-resolution bioassays; and (3) as an indicator of pharmacological activity for which mechanism-based bioassays have yet to be developed (Cox, 1994).

Historically, ethnobotanical leads have resulted in three types of drug discovery: (1) unmodified natural products where ethnomedical use suggested clinical efficacy (e.g. digitalis); (2) unmodified natural products of which the therapeutic efficacy was only remotely suggested by indigenous plant use (e.g. vincristine); and (3) modified natural or synthetic substances based on a natural product used in folk medicine (aspirin) (Cox, 1994).

In drug discovery, the major secondary metabolites (terpenoids, phenolics, and alkaloids) are of potential medicinal interest. Secondary metabolites are synthesized by the plant during development and are time, tissue and organ specific. They can be induced by biotic and abiotic factors. In contrast to primary metabolites, they are not present in all plant cells and not essential to sustain growth. Functions of secondary metabolites are: deterrence against predators and pathogens, attraction and deterrence against pollinators, allelopathic action, attraction of symbionts, food for pollinators, symbionts, herbivores, pathogens and decomposers and UV protectants (Morton, 1981).

Several ethnobotanical and phytochemical research projects have been conducted on medicinal plants from Suriname over the past three decades. Some of the research fields are the use of medicinal plants by Creoles, Indians, and Javanese (respectively, Titjari, 1985; Raghoenandan, 1994; Tjong Ajong, 1989), anti-microbial activity (Verpoorte, 1982; 1987), arrow poisons (Plotkin, 1990) and receptor binding activity (Hasrat, 1997).

In 1991, the National Herbarium of Suriname (BBS) started with a database of plants which are used in traditional medicine in Suriname. This database has been named after Dr. Kwassi and *Quassia amara*. The “Kwassi” database contains information on 946 plant species, out of the 5,100 known in Suriname. This means that about 18% of the plants are used for ethno-medicinal purposes. However, this percentage of plants used in traditional medicine is higher, since there is no written information on the traditional healing of the different tribes from the Amerindians and Maroons (Werkhoven and Malone, 2000).

*Phyllanthus amarus* (Euphorbiaceae) and *Quassia amara* (Simaroubaceae) were chosen based on their popularity in Suriname and the World in using in the treatment of a

broad range of diseases and the secondary metabolites present. Some examples of commonly used medicinal plants in Suriname are *Anacardium occidentale* (Anacardiaceae), *Annona montana* (Anonaceae), *Annona muricata* (Anonaceae), *Citrus aurantium* (Rutaceae), *Euphorbia thymifolia* (Euphorbiaceae), and *Melia azadirachta* (Meliaceae).

## **2.2. *Phyllanthus amarus* Schum. and Thonn.**

### **Biogeography and Ecology**

*Phyllanthus amarus* is widely distributed in all tropical regions of the planet. Paleobotanical studies have not found the exact geographic origin of this plant. This plant may be indigenous to the tropical Americas (Cabieses, 1993; Morton, 1981; Tirimana, 1987), the Philippines or India (Cabieses, 1993, Chevallier, 2000).

*Phyllanthus amarus* is a common pantropical weed that grows well in moist, shady and sunny places (Cabieses, 1993; Nanden, 1998). Some common names of *Phyllanthus amarus* in North, Central and South America are black catnip, carry-me-seed, chanca piedra, djari-bitá, egg woman, fini-bitá, flor escondida, gale-of-(the)-wind, hurricane weed, quebra-pedra, quinine creole, quinine weed, seed-under-leaf, stone breaker and yerba de la nina (Morton, 1981).

*Phyllanthus amarus* is a member of the Euphorbiaceae family (Spurge family), which groups over 6500 species in 300 genera. Euphorbiaceae is a large family of upright or prostrate herbs or shrubs, often with milky acrid juice (Lewis, 1977) and is mainly a pan-tropical family with some species either more or less temperate. Numerous species of this family are native to North, Central and South America (Unander, 1995). The plants are monoecious or homogamous; leaves are simple, alternate or opposite, some are leathery; flowers are very small and diclinous, they cluster in cup-shaped structures,

greenish, often with glands. The fruit is a three-lobed capsule extending from the cup and commonly the long stalk pendant (Lewis, 1977; Wessels Boer, 1976). The name ‘Phyllanthus’ means “leaf and flower” because the flower, as well as the fruit, seems to become one with the leaf (Cabieses, 1993).



**Figure 2.2: *Phyllanthus amarus* (Quinine weed)**

*Phyllanthus amarus* is an erect annual herb, 10 to 50 cm high, with a smooth cylindrical stem 1.5 to 2 mm thick and deciduous horizontal branchlets 4 to 12 cm long and about 0.5 cm thick, with 15 to 30 leaves (Figure 2.2). The leaves are alternate, on petioles 0.3 to 0.5 mm long, elliptic, oblong or obovate, 5 to 11 mm long and 3 to 6 mm wide, rounded to slightly pointed at the tip, scarcely oblique on one side at the base. The flowers are alone or usually one male and one (larger) female are in each leaf axil together. The seed capsules on stalks are 1 to 2 mm long, round, smooth, 2 mm wide, with 6 seeds. When the fruits burst open the seeds are hurled away. Seeds are triangular (like an orange segment), light brown, 1 mm long, with 5 to 6 ribs on the back (Morton, 1981, Wessels Boer, 1976).

## Origin and Distribution

Plants in the genus *Phyllanthus* can be found around all tropical regions of the world: from Africa to Asia, South America and the West Indies. *Phyllanthus* contains about 550 to 750 species in 10-11 subgenera (Unander, 1995).

*P. amarus* can be found in all the tropical regions of the world: through the roads, valleys, on the riverbanks and near lakes. This plant is a common arable weed of disturbed ground in southern Florida, the Bahamas, the West Indies and tropical America and is naturalized in the Old World tropics. *Phyllanthus amarus* is usually misidentified with the closely related *Phyllanthus niruri* L. in appearance, phytochemical structure and history of use. *Phyllanthus niruri* reaches a length of 60 cm, the fruits are larger, and the seeds are dark brown and warty (Morton, 1981).

## Use

Many Euphorbiaceae species are of economical importance. For example, the cooked root of *Manihot esculenta* (cassava) is used as a major carbohydrate source in the tropics, and many forms of bread and puddings are made of the starch. From the oily seeds of *Ricinus communis* (krapata), miracle oil is produced. Many species are more or less poisonous (Morton, 1981; Wessels Boer, 1976). According to Lewis (1977) the milky sap of many cultivated species, such as *E. pulcherrima* (pointsettia) and *E. tirucallii* (pencil tree) contains toxic compounds that will cause severe poisoning if ingested in large quantities.

*E. pulcherrima* is commercially grown as an ornamental in the Americas. *E. tirucallii* is widely grown in the warm climates of both hemispheres as an ornamental plant and massive barrier hedge. The latex of this plant is a source of hydrocarbons and convertible to fuel. *Alchornea latifolia* is planted as shade for coffee in Mexico. The

wood is soft and used for boxes, kites toys and other articles, as well for fuel. In Mexico *Jatropha curcas* has been long grown as host for insects. The lac is used as varnish on guitars and wood products. The seeds of *Omphalea diandra* are of good flavor. In Suriname two or three seeds are commonly eaten as tidbits (Morton, 1981). The Atlas of Medicinal Plants of Middle America (Morton, 1981) reports that *Phyllanthus amarus* is used in Polynesia as a fish poison.

### **Ethnobotanical Use**

In many countries around the world plants in the genus *Phyllanthus* are used in folk remedies; therefore this genus is of great importance in traditional medicine (Foo, 1993). The genus *Phyllanthus* has a long history of use in the treatment of liver, kidney and bladder problems, diabetes and intestinal parasites. Some related species in this region with medicinal significance are *P. epiphyllanthus*, *P. niruri*, *P. urinaria*, *P. acuminatus* and *P. emblica* (Tirimana, 1987). *P. amarus*, *P. niruri* and *P. urinaria* are used in the treatment for kidney/gallstones, other kidney related problems, appendix inflammation, and prostate problems (Heyde, 1990).

According to Foo and Wong (1992), in a number of countries, the aerial part of *Phyllanthus amarus* is highly valued in traditional medicine for its healing properties. This plant is traditionally used around the world in the treatment of liver ailments and kidney stones. The Spanish name ‘chanca piedra’ means “stone breaker or shatter stone.” In South America, ‘chanca piedra’ has been used to eliminate gall bladder and kidney stones, and to treat gall bladder infections.

*Phyllanthus amarus* has also shown to work as an antifungal, antibacterial and antiviral agent (Houghton et al., 1996). Foo and Wong (1992) report that in India this plant is used in traditional medicine to treat liver diseases, asthma and bronchial



infections. Chevallier (2000) notes that *P. amarus* is also used traditionally in India to treat cardiovascular problems. This popular medicinal herb is also a remedy around the world for influenza, dropsy, diabetes and jaundice (Foo, 1993). Table 2.1 gives a summary of the ethnobotanical use of *Phyllanthus amarus* around the world.

**Table 2.1 Ethnobotanical use of *Phyllanthus amarus* (whole herb; only roots\*) around the world**

Location	Ailment treated / Properties and Actions
Aruba	Blood purifier
Bahamas	Appetizer, ♦ Colds, Fever, ♦ Flatulence*, Flu, Stomachache, ♦ Vermifuge
Barbados	♦ Arbortifacient
Cuba	Edema and Malaria
India	Appetizer, Asthma, Bronchial infections, Diuretic, Dyspepsia, Fever, Jaundice, Liver diseases, Itchiness, Skin ulcers, sores, swellings
Indonesia	Colic, Cough, Diuretic, Eye diseases (external), Kidney diseases, Stomachache*, Toothache*, Venereal diseases
Island of North Caicos	♦ Fever, Prevention of intestinal worms
Trinidad	Diuretic, Venereal diseases
Jamaica	Diabetes, Dysentery, Diuretic, Edema, ♦ Gonorrhea, Jaundice, Stomachache

Morton, 1981; Tjong Ayoung, 1989; Raghoenandan, 1994

♦ = Combined with other plants

In Suriname, *P. amarus* is always sold as fresh and dry plant material in the herb markets. Heyde (1990), Sedoc (1992) and Nanden (1998) note that in traditional medicine an herbal decoction is taken to treat bladder and kidney disorders, cramps and uterus complaints (with other herbs). This plant decoction works also as an appetizer.

*P. amarus* is a restoration herb and can be used as a tonic. In Suriname decoctions are used in herbal baths and after labor (May, 1982; Titjari, 1985; Sedoc, 1992). It is also

used for colic (Wessels Boer, 1976; Heyde, 1990). According to Heyde (1990) plant extracts of *P. amarus* can be used as blood purifiers, for light malaria fevers and anaemia. *P. amarus* helps to release phlegm (Heyde, 1990), and is used to combat fever, flu (Nanden, 1998) and asthma, in combination with other herbs (Titjari, 1985). The plant, when boiled with the leaves, is considered to be a diuretic and can be used in treating diabetes, dysentery, hepatitis, menstrual disorders, and skin disorders (Heyde, 1968; Tirimana, 1987; Heyde, 1990). Sedoc (1992) notes, that in Suriname a decoction of *P. amarus* is taken along with other herbs to treat stomachache. This herb can also be used for constipation (Tjong Ayoung, 1989).

Extracts from the roots can be used for jaundice. Three roots are boiled in ½ liter water and two cups are drunk daily. Decoctions for the other described ailments can be prepared by extracting two fresh plants or five dried plants in ¾ liter water drinking one cup of tea, four times per day (Heyde, 1990).

### **Current Research on Phytochemicals**

The secondary metabolites present in *Phyllanthus amarus* are alkaloids, flavanoids, hydrolysable tannins, major lignans and polyphenols. Table 2.2 lists the secondary metabolites with their respectively phytochemicals that can be found in *Phyllanthus amarus*.

Several chemical investigations have been conducted where the structures of most of these phytochemicals were determined by UV, IR, Mass and NMR spectroscopy (Foo and Wong, 1992; Foo, 1993; Foo, 1995). Houghton et al. (1996) isolated securinega type alkaloids by Column Chromatography (CC) and preparative Thin Layer Chromatography (TLC). This group did qualitative analysis by using TLC, and spots were detected by UV

radiation (254 nm and 365 nm). The unknown compounds were determined by means of UV, IR, mass and NMR spectroscopy.

**Table 2.2**      **Phytochemicals in *Phyllanthus amarus***

Secondary metabolites	Phytochemicals
Alkaloids	Securinine, Norsecurinine, Epibubbialine and Isobubbialine (Houghton et al., 1996)
Flavonoids	Catechin, Gallocatechin, Quercetin, Quercitoside and Rutin (Morton, 1981; Foo, 1993)
Hydrolysable tannins (ellagitannins)	Amariin, Amariinic acid, Amarulone, Corilagin, Elaeocarpusin, Furosin, Geraniin, Geraniinic acid B, Glucopyranose and Glucopyranoside derivatives, Phyllanthusiin D and Repandusinic acid (Foo and Wong, 1992; Foo, 1993; Foo, 1995)
Major lignans	Phyllanthin and Hypophyllanthin (Morton, 1981; Chevallier, 2000)
Phenolics	Gallic acid (Foo, 1993)
Polyphenols	Ellagic acid, Phenazine and Phenazine derivatives (Foo, 1993)

Bratati and Datta (1990) report in an evaluation study of *Phyllanthus amarus* that plant extracts have shown *in vivo* antifungal, anticancer, antispasmodic and hypoglycaemic activity. According to Thyagarajan (1988) plant extracts from this species have beneficial effects on liver functions. Mehratra et al. (1991), and Unander and Blumberg (1991) showed, using *in vitro* studies, that the *Phyllanthus amarus* extracts (polar fractions) also have antiviral activity and are a potential remedy for hepatitis B viral infection.

Since the extracts of *Phyllanthus amarus* have a long history of use in tropical countries in indigenous medicine for the treatment of liver ailments, they were examined during this research. The extracts were optimized, in order to extract as many

phytochemical compounds as possible from the plant. The extracts (50% methanol in water, 99% methanol and 50% methanol in chloroform) were analyzed by thin layer chromatography based upon the presence of secondary metabolites.

### **2.3. *Quassia amara* L.**

#### **Biogeography and Ecology**

The medicinal property of *Quassia amara*, traditionally used for malaria, was discovered in Suriname in 1730 by a slave named Kwasi, who was a well known medicine man. In recognition of his discovery, this plant has been given the scientific name of *Quassia amara* (which means bitter Quassia) by the botanist Linnaeus. In 1730, Kwasi received a medal laced coat with trousers, a sword of honor, and a decorated three-cornered hat from the Prince of Orange, the Stadhouder Willem V (Werkhoven and Malone, 2000). In the mid-eighteenth century this shrub became popular in Europe.

*Quassia amara* is well known in many countries and is recorded in the medical books of Europe, South and Central America, and India as “Suriname quassia” (Tirimana, 1987). Other common names are amargo, bitterhout, bitter wood, guavo, hombre grande, hombron, kwasi-bitá, murupa, palo de causia, pau amarelo, quassia, quassia amarga, quassia Suriname, quinine de Cayenne, Suriname wood (Morton, 1981).

*Quassia amara* is a member of the Simaroubaceae family, which has about 150 species (primarily tropical and subtropical) in approximately 25 genera. Simaroubaceae is a small family with bitter components in the wood, seeds and bark. The leaves in this family are spread and feathered and the flowers usually have scales at the feet of the fumitory (Wessels Boer, 1976).

*Quassia amara* is a shrub or small tree growing mainly on sandy soils in lowland and highland forests and along the riverbanks. This shrub grows 4 to 6 m in height. All

parts are exceedingly bitter. The leaves are alternate, compound, with 3 to 5 (mainly opposite) leaflets, oblong or obovate, 5 to 16 cm long, pointed at the apex, narrowed toward the base.

The leaf midrib (rachis) is conspicuously winged and the stem, stalks and nerves are often red. The flowers are bright red, with five lanceolate petals, which never fully expand but remain mostly closed together forming a spirally twisted cylinder, 2.5 to 4.5 cm long, from which ten stamens protrude, borne in showy racemes 10 to 30 cm long. The fruit is an aggregate of five black, elliptic or obovate drupes 8 to 15 mm long, attached to a fleshly red receptacle, each containing a small seed. The wood is yellow-white (Morton, 1981; Wessels Boer, 1976).

### **Origin and Distribution**

Simaroubaceae, a pantropical family, consists of six subfamilies with 32 genera and more than 170 arboreous or shrubby species in tropical America, Africa, Asia, Malaysia and Northeastern Australia (Simão et al., 1991; Fernanda and Quinn, 1995). Plants in the genus *Quassia* are native to northern South America.

*Quassia amara* is indigenous to Northern Brazil and the Guyanas and it also grows in Venezuela, Columbia, Argentina, Panama and Mexico. This small tree is used and marketed interchangeably with *Picrasma excelsa* that is also called Quassia. *P. excelsa* contains many of the same constituents as *Q. amara* and is used mostly for the same treatments. *P. excelsa* is much taller (up to 25 meters) than *Q. amara* and occurs in the tropics of Jamaica, the Caribbean and the West Indies (Morton, 1981).

### **Use**

The family Simaroubaceae has economic value as a cultivated ornamental. The wood of *Alvaradoa amorphoides* is very strong and is used in Guatemala for carpentry

and fuel. In Puerto Rico the wood of *Picramnia pentandra* is used for house construction (Morton, 1981). The extract of the wood of *Picrasma excelsa* is used commercially in commercial flavoring of aperitives, liqueurs, soft drinks and baked goods (Furia, 1971). According to Morton (1981), brewers have used it in the past as wood chips as a substitute for hops.

The oil extracted from the seed kernels of *Simarouba glauca*, is used for cooking and making soap in El Salvador and has been used commercially to manufacture margarine since 1946. The fruit flesh is edible but not popular. The wood burns readily when green. The dark red heartwood of *Suriana maritima* is hard and heavy. This wood polishes well and is used for small articles. The bark of *Quassia amara* is universally employed as a bitter in digestive tonics, liqueurs, ale, and beer (Morton, 1981).

### **Ethnobotanical Use**

The family Simaroubaceae is the source of New World anti-malarial medicines from the bitter bark. Species in the genus *Quassia* are well known in traditional medicine to treat fever, malaria, and diarrhea (Dou, 1996). Some species of Simaroubaceae with medicinal significance are *Picramnia pentandra*, *Picrasma excelsa*, *Simarouba versicolor*, *Quassia indica*, *Quassia cedron*, *Ailanthus altissima* and *Eurycema longifolia* (Tirimana, 1987).

In Brazil *Simarouba versicolor* bark is used to treat snake bites. The powdered bark of this plant is also used as an insecticide (Lewis, 1977). The wood of *Quassia indica* is used as a bitter tonic and a stomachic in India. In Indonesia, the seeds are used as an emetic, purgative, and in the treatment of fevers. The oil of the seed is used externally in the treatment of rheumatism, and the crushed leaves are used externally in the treatment of bacterial infections (Tirimana, 1987).

*Quassia amara* wood is reputed in traditional medicine to have good stomachic, eupeptic, antiamoebic, antihelmintic, antimalarial, and antianemic properties (Barbetti, 1986). *Quassia* has prophylactic activity against lice and is effective in chronic diseases of the liver (Tirimana, 1987). According to Lewis (1977) the bitter component from the powdered stem is a useful remedy when appetite fails, and it aids in digestion. *Quassia* bark stimulates the secretion of gastric juices. Duke reports (1992) that *quassia* is a folk remedy for liver diseases, high blood pressure, malaria and spasms.

The wood, bark and root segments of *Quassia amara* are being used against fever, as a health tonic and insecticide. By boiling or soaking *Quassia* chips in water, an effective spray can be made against small insects. An insecticidal extract of the bark was first used in the USA in 1850 and in Europe in 1880 (Morton, 1981).

*Quassia* wood was an important export article of Suriname in the early 1900s. The 'lignum quassiae surinamensis' was used in medicine as an antipyretic. In Suriname, bitter wood is still used against fever: a stick 5 cm long and 1 cm wide is steeped in 1 l water or a large bottle of vermouth, and a small cupful is taken three times a day to prevent fever (Heyde, 1968). Drink goblets of *quassia* wood can be used in which alcohol drinks are poured (Wessels Boer, 1976). The bark and stem are used in the treatment of abdominal ache, hemorrhoids, and malaria fevers (Tirimana, 1987).

According to Heyde (1990), a piece of dry wood of 2 cm length put in  $\frac{1}{4}$  glass of water for one night and drunk in the morning on an empty stomach (tonic) is excellent for good health. In Suriname's traditional medicine, a piece of fresh bark from the *Q. amara* tree (1cm long and  $\frac{1}{2}$  cm wide) is drawn in a full glass of water; three times daily a teaspoon of the extract helps against spleen complaints, milt and liver disorders, and stomach disorders with gas and acid digestion. The same results can be obtained with

extracts from the dry quassia wood (1 cm length and ½ cm thick) drawn in 1 l water, which must not be boiled (Heyde, 1990).

**Table 2.3      Ethnobotanical use of *Quassia amara* (bark/wood) around the world**

Location	Ailment treated / Properties and Actions
Brazil	Diarrhea, Digestive, Dysentery, Dyspepsia, Flatulence, Gonorrhea
Caribbean region	Fever, Digestive complaints, Tonic
Chile	Appetizing, Blood purifier, Digestive, Fever, Intestinal parasites, Tonic, Tuberculosis
Costa Rica	Diabetes mellitus, Diarrhea, Fever
Elsewhere	Bite (snake), Cancer, Carcinoma, Fever, Intestinal parasites
Guatemala	Constipation, Diabetes mellitus, Hypertension, Nervousness
Mexico	Dyspepsia, Enema, Fever, Gallbladder disorders, Intestinal parasites, Liver disease, Stomachic, Tonic, Vermicide
Nicaragua	Anemia, Astringent, Bites, Intestinal parasites, Malaria, Stings, Tonic, Worms
Nigeria	Antianemic, Antibiotic, Malaria, Stomachic
Panama	Cure-all, Febrifuge, Fever, Hyperglycemia, Liver, Liver disorders, Malaria, Snakebite
Thailand	Antipyretic
Turkey	Astringent, Diarrhea, Digestive, Diuretic, Dysentery, Fever, Malaria, Tonic
Venezuela	Diuretic, Dysentery, Fever, Laxative, Tonic, Vermifuge

Morton, 1981

*Quassia amara* is also used in Suriname as an aphidicide, insecticide, worm despisser, laxative, and appetite stimulant. Surinamese Maroons use the bark for fever and parasites. In Brazil, a leaf decoction is used in bathing for measles and as mouth rinse after tooth extraction (Duke, 1994). A summary of the ethnobotanical use of *Quassia amara* around the world is given in table 2.3.



### Current Research on Phytochemicals

The wood/bark of *Quassia* contains many phytochemicals and is 50 times more bitter than quinine (Tirimana, 1987; Duke, 1992). The bitter principles of quassia wood are quassinoids (terpenoid compounds), present in amounts of ~0.25% from which 0.1-0.15% are quassin, neoquassin and 18-hydroxy-quassin. The bitterness index (BI) of quassin/neoquassin is  $17 \times 10^6$  (Wagner and Bladt, 1996). Other quassinoids present in the wood are: isoquassin, parain, quassimarín, quassinol, and quassol (Tirimana, 1987; Duke, 1992). Chemically, quassinoids are seco-triterpene- $\delta$ -lactones mostly found in the family Simaroubaceae (Vitányi et al., 1997). Quassinoids are secondary metabolites with phytotoxic and allelopathic activities (Dayan et al., 1999).

Quassinoids have been identified as the major class of compounds responsible for biological activity in this family (Dou, 1996). So far, 170 quassinoids have been isolated and characterized. These compounds have a wide spectrum of biological activity: antileukemic, anti-inflammatory cytotoxic effects, and antifertility activity. Quassinoids are also important as an acaricide and insecticide (Vitányi et al., 1997). Fukamiya et al. (1990) and Polonsky (1985) note the antimalarial and amoebicidal biological activities of quassinoids.

Quassinoids are the active constituents of the *Quassia* wood and have been investigated as anti-tumor agents (Barbetti, 1986). Several of the quassinoids found in the bark have been documented with antineoplastic actions and as anti-ulcer agents. Analytical studies of quassinoids have been conducted using High Performance Liquid Chromatography (HPLC). In 1984, Robins and Rhodes reported HPLC methods for the analysis and purification of quassinoids (i.e. quassin, neoquassin) from *Quassia amara*.

Quassin has important pharmaceutical and insecticidal properties, and its intensely bitter nature makes it ideal as a bittering agent for beverages and foodstuffs. According to Dr. Duke's Databases (2002), the biological activities of quassin are: ambicide, aperitif, emetic, and pesticide. Dou et al. (1996) did qualitative and quantitative HPLC analysis of quassinoids in Simaroubaceae plants. Vitanyi et al. (1997) did the application of HPLC/Mass Spectrometry with Thermospray Ionization to the detection of quassinoids extracted from *Q. amara*.

Kupchan (1976) reports of the quassinoid, quassimarín to have antileukemic and antitumorous properties. Potassium acetate, a chemical present in the wood, is responsible for the flavor of *Quassia amara* (Duke Database, 2002). The phytochemical beta-sitosterol has anti-hypercholesterolemic, antiprostatic, antiprostataadenomic, and estrogenic activity (Tirimana, 1987). Beta-sitostenone is also found in *Quassia amara* wood, but no activity is reported of this chemical. Ajaiyeoba et al. reported in 1999 that plant extracts from *Quassia amara* showed *in vivo* significant antimalarial activity.

Derivatives of the alkaloids canthin-6-one and  $\beta$ -carboline (2,500 ppm) are present in *Q. amara* wood. Some of these alkaloids have shown antibiotic, anti-amoebic and cytotoxic properties (Barbetti, 1990). From the Simaroubaceae family several indole alkaloids, mainly the derivatives of  $\beta$ -carboline and canthin-6-one, have been isolated by column chromatography and preparative thin layer chromatography (TLC). Structures were determined by UV, IR and NMR spectra (Barbetti et al., 1986; Barbetti et al., 1990; Njar et al., 1992).

Barbetti et al. (1986) examined the alkaline fraction from the *Q. amara* wood extract. Three  $\beta$ -carboline alkaloids were isolated. Analytical samples of the alkaloids were obtained by preparative TLC chromatography. The monitoring was done by TLC,

using Dragendorff reagent, phosphomolybdic reagent as spray reagents and UV lamp. In 1990, Barbetti et al. investigated the presence of canthin-6-one alkaloids in the methanol extract of the wood. Identification of alkaloids was done by spectral analysis and was confirmed by direct comparison on TLC.

Chan et al. (1998) and Tan et al. (2002) conducted HPLC analysis of  $\beta$ -carboline and canthin-6-one alkaloids from different Simaroubaceae family members. Tsuchiya et al. (1999) did quantitative analysis of all types of  $\beta$ -carboline alkaloids in medicinal plants and dried edible plants by HPLC with selective Fluorometric detection.

*Quassia amara* wood grown in Suriname was examined during this research. The 50% methanol in water, 99% methanol and 50% methanol in chloroform extracts were examined on the presence of secondary metabolites by using thin layer chromatography as analysis method. This study describes the optimization of extraction methods in order to get the highest extraction rate for crude extracts of *Phyllanthus amarus* and *Quassia amara*.

#### **2.4. Thin Layer Chromatography (TLC)**

Chromatography is an analytical method that is widely used for the separation, isolation, identification, and quantification of components in a mixture. Components of the mixture are carried through the stationary phase by the flow of a mobile phase. Separations are based on differences in migration rates among the sample components (Fried and Sherma, 1994).

Thin layer chromatography (TLC) was chosen over other chromatography methods because it is a simple, quick and inexpensive procedure that can be used for the analysis of mixtures. TLC is a mode of liquid chromatography in which the sample is applied as a small spot or streak to the origin of a thin sorbent layer such as silica gel,

alumina, cellulose powder, polyamides, ion exchangers or chemically bonded silica gel supported on a glass, plastic, or metal plate. This layer consists of finely divided particles and constitutes the stationary phase. The eluent or mobile phase is a solvent or a mixture of organic and/or aqueous solvents in which the spotted plate is placed. The mobile phase moves through the stationary phase by capillary action, sometimes assisted by gravity or pressure (Skoog West Holler, 1988; Fried and Sherma, 1994).

TLC separations take place in the “open” layer, with each component having the same total migration time but different migration distances. Plates can be visualized, depending on the chemical structure of the compounds at visible light, UV-254 nm and 365 nm or by using spray reagents (Wagner, 1984). The effectiveness of the separation depends on the mixture to be separated, the choice of the mobile phase and the adsorption layer (Fritz and Schenk, 1987).

The term retention factor  $R_f$ , is commonly used to describe the chromatographic behavior of sample solutes. The  $R_f$  value for each substance is the distance it has moved divided by the distance the solvent front has moved. Usually, the center of each spot is the point taken for measurement. Comparison of  $R_f$  values makes it possible to research complex mixtures qualitatively. The extent of the surface of the spot is a measure for the quantity of the material present (Fritz and Schenk, 1987).

The selection of a solvent for application of the sample can be a critical factor in achieving reproducible chromatography with distortion free zones. In general, the application solvent should be a good solvent for the sample and should be as volatile as possible and more nonpolar (Fried and Sherma, 1994). The solvents chosen during this research for extraction were therefore methanol-water (A), methanol (B) and chloroform-methanol (C). Silica gel was chosen as stationary phase, since it is an efficient adsorbent

for the TLC separation of most of the plant extracts and plant drug extracts (Barbetti et al., 1986; Houghton et al.1996; Wagner and Bladt, 1996).

When using a complex mixture such as a biological sample, the concentration of the solute of interest is often not known. Trial and error is needed when applying samples to a TLC plate in order to determine the optimal concentration for spotting, which is usually governed by the method of detection employed (Fried and Sherma, 1994).

## CHAPTER 3 MATERIALS AND METHODS

### 3.1. Collection of Plant Material

*Phyllanthus amarus* Schum. and Thonn. and *Quassia amara* L. were collected in Suriname. Since there are no cultivated fields of these plants in Suriname, the plant material had to be collected from different geographical locations. The plants were (haphazardly) sampled from areas where they grow naturally. The environmental conditions under which the plants grow (pasture land, woods), the soil composition, and pH of the soil were recorded (Appendix A).

Plants of *P. amarus* were collected (June; July 2001) in the city (Paramaribo-North (PN) and Paramaribo-South (PZ)) as well in the country (Saramacca-Damboentong (PD)). *Q. amara* (wood) was collected (July 2001) in the countryside (Saramacca-Dirkshoop). In August 2001 the dried plant species were shipped to Baton Rouge.

### 3.2. Sample Preparation and Extraction

*P. amarus* material was air dried under shade for two weeks and *Q. amara* (wood) was oven dried one week at 40°C. The dried plant material was ground (October; November 2001) with a Wiley Mill grinder (Standard Model No. 3). *P. amarus* was ground to 2 mm or smaller particle size. The wood of *Q. amara* was ground to 6 mm or smaller particle size. The ground plant species were stored in a refrigerator at 4°C.

#### Crude Extracts

To investigate if there is a difference in the number of compounds recovered in the samples of *P. amarus* from the different geographical locations (PD, PN, PZ), each sample was extracted separately. From each location 1 gram of plant material was extracted (in May 2002) with 10 ml (1:10, w/v) of either solvent A, 50% methanol in deionized and distilled (D.D) water; B, 99% methanol; C, 50% methanol in chloroform.

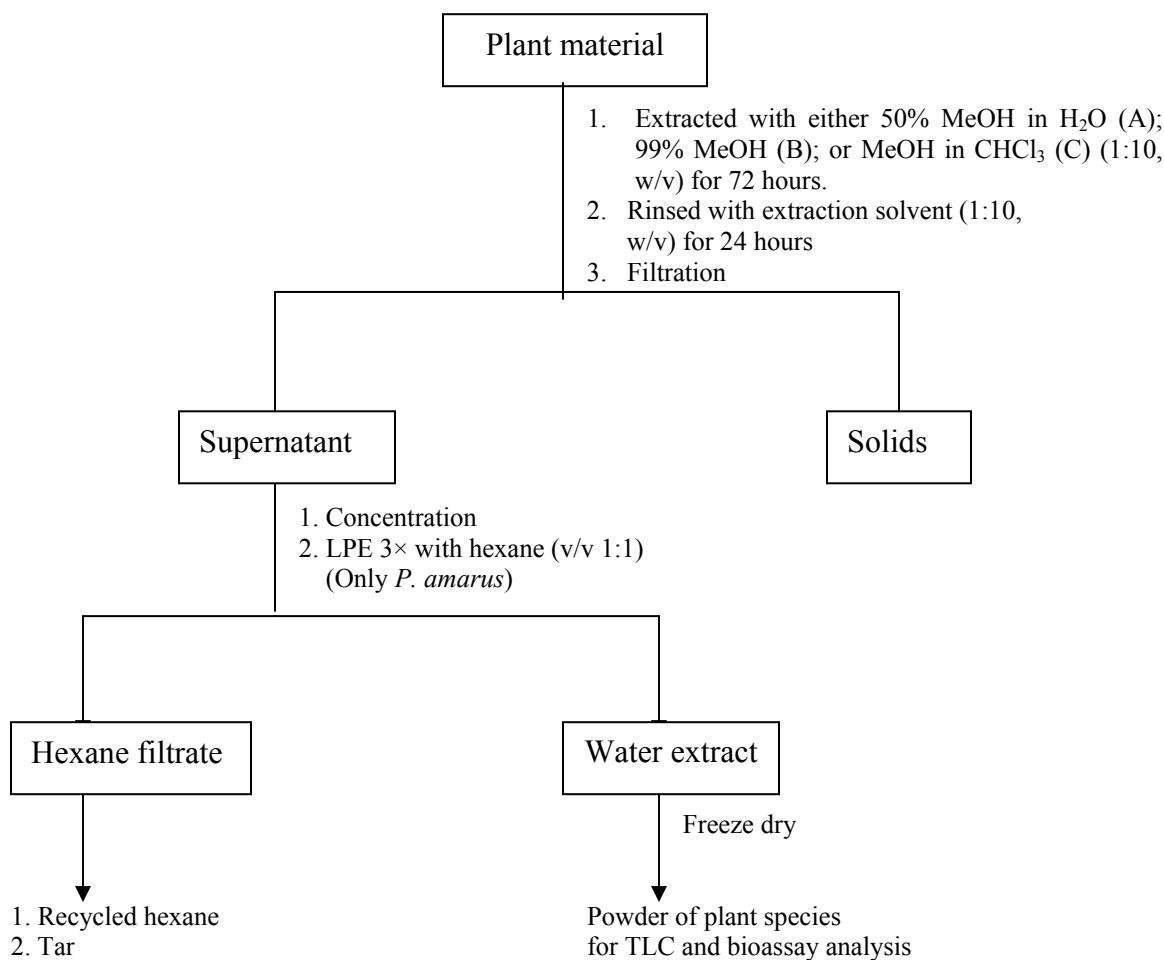
Plant material and solvent were agitated with a laboratory rotator at 30 rpm for three days at room temperature, i.e. 18-23°C. The supernatant was filtered with Whatman filter paper No. 4 and concentrated with blown air to 1ml (Muanza, 1995; Rugutt, 1996). The concentrated liquid extracts were stored at 4°C (May 2002) until TLC analysis (May-July, 2002).

### **Extraction Method Development**

In order to determine the extraction rate, 10 grams of ground plant material of both *P. amarus* and *Q. amara* was extracted with 100 ml (1:10, w/v) of the following solvents: A, 50% methanol in D.D. water; B, 99% methanol; or C, 50% methanol in chloroform. All solvents used were HPLC grade from Fisher Scientific Chemicals. Plant material and solvent were agitated with an orbital shaker at 115 rpm for three days at room temperature, i.e. 18-23°C. The supernatant was drained and the residue was rinsed with 100 ml extraction solvent A, B, or C for 24 hours (Muanza, 1995; Vitányi et al., 1997). The pooled extracts were filtered through Whatman No. 4 filter paper and concentrated at 60 °C using a rotary evaporator. The weight of the powders of both *P. amarus* and *Q. amara* were recorded. A summary of the extraction steps is given in Figure 3.1.

### **Powder Production**

Ground material (1.4 kg each) of *P. amarus* and *Q. amara* was extracted with 14 l (1:10, w/v) methanol (B) by agitation with an orbital shaker at a speed of 115 rpm for 72 hours at room temperature, i.e. 18-23°C. The supernatant was drained and the residue was rinsed with 14 l methanol for 24 hours. The pooled extracts were filtered through Whatman No. 4 filter paper and concentrated at 60 °C using a rotary evaporator (Muanza, 1995; Vitányi et al., 1997).



**Figure 3.1: Block diagram of extraction procedure**

Chlorophyll was removed from the *P. amarus* crude water extract by Liquid-Phase-Extraction (LPE) with hexane (1:1, v/v) three times. After LPE, the water-fraction was further concentrated. The crude water extract was freeze dried (Figure 3.1). The powders of both *P. amarus* and *Q. amara* were weighed (Appendix B, Table B.2) and stored in a refrigerator at 4°C until TLC analysis and bio-assays.

### 3.3. Phytochemical Analysis by using TLC

#### Crude Extracts

Thin layer chromatography (TLC) was employed in this study to analyze the compounds present in the crude plant extracts of the solvents 50% methanol in D.D. water, 99% methanol and 50% methanol in chloroform. Normal phase silica gel GF



precoated TLC (scored 10×20 cm) plates, 250 microns (Analtech, Uniplate No. 02521) were used. The solvent extracts (three per plate) were applied as separate spots to a TLC plate about 1.3 cm from the edge (spotting line), using 20 µl capillary tubes (microcaps disposable pipettes, Drummond Scientific Company).

The mobile phase, chloroform/methanol= 9:1; 95:5; 98:2 (v/v), for each crude extract from *P. amarus* and *Q. amara* was chosen by trial and error. For the powder extracts of *P. amarus* and *Q. amara*, mobile phases with different polarities had to be used, namely, chloroform/methanol= 5:5; 7:3; 9:1 and chloroform/methanol= 9:1; 95:5, respectively (Barbetti et al., 1989; Houghton et al. 1996; Wagner and Bladt, 1996). All TLC separations were performed at room temperature, i.e. 18-23°C.

After sample application of 3 µl for *P. amarus* and 5 µl for *Q. amara*, the plates were placed vertically into a solvent vapor saturated TLC chamber. Three mobile phases were respectively used: chloroform/methanol= 9:1; 95:5; 98:2 (volume ratio). The spotting line was about 0.5 cm from the developing solution. After the mobile phase had moved about 80% from the spotting line, the plate was removed from the developing chamber and dried in a fume hood (Barbetti et al., 1989; Rugutt, 1996; Wagner and Bladt, 1996).

### **Detection Methods**

The eluted spots, representing various fractions/compounds, were visualized by different detection methods:

- I. The plate was visualized at UV–254 nm, spots were circled and the plate was sprayed with 10% ethanolic phosphomolybdic acid reagent (purchased from Sigma Aldrich). The TLC plate was dried 5-10 minutes under a fume hood and heated at 100°C for 3-5 min under observation (Wagner and Bladt, 1996).

- II. At UV-365 nm the spots were circled and the plate was sprayed with Dragendorff reagent (Houghton et al., 1996). This reagent was prepared by dissolving 8 g of potassium iodide (KI) in 20 ml water and by adding it to 0.85 gram of bismuth nitrate ( $\text{BiNO}_3$ ) dissolved in 10 ml 17.4 M glacial acetic-acid to which 40 ml D.D. water was poured (Wagner and Bladt, 1996; Chaves, 1996).
- III. The plate was sprayed with sulfuric acid ( $\text{H}_2\text{SO}_4$ ) reagent (5% ethanolic  $\text{H}_2\text{SO}_4$ ). After drying the plate for 5-10 minutes under a fume hood it was heated at  $100^\circ\text{C}$  for 3 minutes to visualize compounds (Wagner and Bladt, 1996).
- IV. The plate was sprayed with sulfuric acid reagent (5% ethanolic  $\text{H}_2\text{SO}_4$ ), dried under a fume hood for 10 minutes and visualized at UV-365 nm (Wagner and Bladt, 1996).

### **Secondary Metabolites Expected to be Revealed through the Detection Methods**

#### Detection I:

- 🚦 At UV-254 nm quenching zones are detected. Quenching is caused by all compounds with conjugated double bonds, e.g., some alkaloid types (show pronounced quenching) such as indoles, quinolines, isoquinolines, purines, tropines; bitter drugs (e.g. quassin); flavonoids (cause fluorescence quenching); lignans (show prominent quenching); and triterpenes (Barbetti et al., 1986; Wagner and Bladt, 1996).
- 🚦 Phosphomolybdic acid (PMA) reagent: Detection of a large variety of organic compounds, including reducing substances, steroids, bile acids and conjugates, lipids and phospholipids, fatty acids and their methyl esters, substituted phenols,

indole derivatives, prostaglandins, and essential oil components (Barbetti et al., 1986; Fried and Sherma, 1994; Wagner and Bladt, 1996).

#### Detection II:

- At UV-365 nm fluorescent zones are detected. Alkaloids that fluorescence blue, blue green or violet fluorescence can be detected. Triterpenes such as quassin, neoquassin and 18-hydroxy-quassin are also detected. Depending on the structural type, flavonoids show dark yellow, green or blue fluorescence. Phenol carboxylic acids form blue fluorescence zones. Lignans and isoflavones form blue fluorescence zones (Barbetti et al., 1986; Wagner and Bladt, 1996).
- Dragendorff (DRG) reagent: Detection of alkaloids (Barbetti et al., 1986; Wagner and Bladt, 1996).

#### Detection III:

- Sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) reagent → 5% ethanolic H<sub>2</sub>SO<sub>4</sub>: Detection of lignans and general compounds (Fried and Sherma, 1994; Wagner and Bladt, 1996).

#### Detection IV:

- By spraying the plate first with sulfuric acid reagent (5% ethanolic H<sub>2</sub>SO<sub>4</sub>) and then visualizing it at UV-365 nm, the fluorescent zones are made very distinct. For compounds detected here see Detection method II, UV 365 nm (Wagner and Bladt, 1996).

### **Powder Extracts**

Extraction solvent B (absolute methanol) was used to produce powder for bio-assay analysis, because this solvent had a good extraction rate for both *P. amarus* and *Q. amara*. The TLC fingerprint of the produced *P. amarus* and *Q. amara* powders was compared with those of the crude solvent extracts.

Twenty-five milligrams of crude powder from methanol extract (B) of *P. amarus* and *Q. amara* were dissolved in two solvents of different polarities: 1 ml methanol-deionized and distilled water (1); 1 ml absolute methanol (2). The mixture to be analyzed (3  $\mu$ l *P. amarus* and 5  $\mu$ l *Q. amara*) was spotted near the bottom of the plate (1.3 cm). The mobile phases in which the *P. amarus* plates were placed are: CHCl<sub>3</sub>/MeOH = 5:5; 7:3; 9:1. *Q. amara* plates were put in the mobile phases CHCl<sub>3</sub>/MeOH= 9:1; 95:5 (Barbetti et al., 1989; Houghton et al. 1996; Wagner and Bladt, 1996). The eluted spots were visualized at UV 254 nm and spraying with 10% ethanolic phosphomolybdic acid reagent (detection method I).

## CHAPTER 4 RESULTS AND DISCUSSION

### 4.1. Results

#### Fractions Recovered by Various Solvents

The following tables (Tables 4.1- 4.7) give a synopsis of the results of the plates eluted by mobile phase  $\text{CHCl}_3/\text{MeOH}= 95:5$ , from the plant extracts of *P. amarus* and *Q. amara*. All the scanned plates can be seen in Appendix C there is an overview of the scanned plates. The direction in which the fractions (the spots on the TLC plates) are numbered is from the spotting line to the top of the plate. Every plate has its own numbering sequence. The presence or absence of each fraction is noted by a “+” or “-” respectively, in the tables. Comparisons between fractions of different extraction and detection methods were made by calculating the  $R_f$ -value of each fraction (Appendix D).

The results in Table 4.1 (page 31) show that only one fraction was detected in extract A from location PN. Fractions 4 and 8 were detected only in extract C. This indicates that fractions 4 and 8 are less polar. Fraction 5 was detected only in extract B and C from location PD. This fraction/compound is present only in location PD, therefore samples from this location were given a higher priority for producing powder for bioassay analysis.

Table 2.2 (on page 12) lists the phytochemicals present in *P. amarus*. The secondary metabolites present in extracts of *P. amarus* that can be detected with UV-254 nm are: alkaloids; flavonoids; and lignans. Phosphomolybdic acid (PMA) is a general reagent that detects a large variety of organic compounds, in this case phenols and indole derivatives.

The use of mobile phase chloroform/methanol= 9:1 gave a separation that is not equally spread over the plate (Appendix C, Figure C.1). The two last compounds were

still not completely separated. Mobile phase chloroform/methanol= 98:2 is less polar, so the less polar compounds were eluted and the more polar compounds did not travel along with the mobile phase (Appendix C, Figure C.3).

**Table 4.1 Presence/absence of fractions in crude *P. amarus* extracts detected by UV-254 nm and Phosphomolibdic acid (PMA) reagent (detection method I). Cross-reference with Figure C.2 and Table D.1**

<u>Fraction</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	Total
Extraction\ Location									
A1	-	-	-	-	-	-	-	-	0
B1	+	+	+	-	+	+	+	-	6
C1	+	+	+	+	+	+	+	+	8
A2	-	-	-	-	-	-	+	-	1
B2	+	+	+	-	-	+	+	-	5
C2	+	+	+	+	-	+	+	+	7
A3	-	-	-	-	-	-	-	-	0
B3	+	+	+	-	-	+	+	-	5
C3	+	+	+	+	-	+	+	+	7

**Extraction:** A=50% MeOH in H<sub>2</sub>O; B= 99% methanol; C= 50% MeOH in CHCl<sub>3</sub>

**Location:** 1= Saramacca-Damboentong (PD); 2= Paramaribo-North (PN); 3= Paramaribo-South (PZ)

Fraction 1 (Table 4.2 and Figure C.5) becomes visible only in extract C from location PD and PN and in extract B from location PN. Fluorescence alkaloids, flavonoids and lignans were detected in the *P. amarus* extracts using UV-365 nm. Dragendorff (DRG) reagent is specific for alkaloids. Upon spraying with DRG, fraction 2 turned orange, but the color was not stable (it faded away after 5 minutes). Fractions 1, 2, and 3 (Extracts C; Location PD) from this detection method have almost the same R<sub>f</sub>

values (0.13; 0.80; 0.88) as fractions 2, 7, and 8 (0.14; 0.83; 0.91) from detection method I (Appendix D, Tables D.1 and D.2). Therefore these fractions/compounds are most probably the same and detection method II can be eliminated for the determination of samples from location PD.

**Table 4.2** Presence/absence of fractions in crude *P. amarus* extracts detected by UV-365 nm and Dragendorff (DRG) reagent (detection method II). Cross-reference with Figure C.5 and Table D.2

<u>Fraction</u> Extraction\ Location	<u>1</u>	<u>2</u>	<u>3</u>	Total
A1	-	-	-	0
B1	-	+	+	2
C1	+	+	+	3
A2	-	-	-	0
B2	+	+	+	3
C2	+	+	+	3
A3	-	-	-	0
B3	-	+	+	2
C3	-	+	+	2

**Extraction:** A=50% MeOH in H<sub>2</sub>O; B= 99% methanol; C= 50% MeOH in CHCl<sub>3</sub>

**Location:** 1= PD; 2= PN; 3= PZ

The use of mobile phase chloroform/methanol= 9:1 resulted in some compounds that were partially separated. The two last compounds were still blended (Figure C.4). The mobile phase chloroform/methanol= 98:2 (Figure C.6) gave basically the same results as mobile phase 95:5 (Figure C.5).

From extract A of location PD and PN (Table 4.3 and Figure C.8), only one fraction (number 7) became visible. Fraction 3 was detected only in extract C. This is an

indication that fraction 3 is less polar. Fraction 6 was detected only in extracts B and C from location PD. Fraction 6 ( $R_f = 0.74$ ) is not the same fraction as fraction 5 of Detection method I ( $R_f = 0.35$ ), since the  $R_f$ -values are different (Tables D.1 and D.3). Fraction 8 was detected only in extract C from location PD and PN. In location PZ, fraction 8 was detected in extracts B and C. This fraction ( $R_f = 0.93$ ) is the same fraction 8 of Detection method I ( $R_f = 0.91$ ), since the  $R_f$ -values are almost the same (Tables D.1 and D. 3).

**Table 4.3** Presence/absence of fractions in crude *P. amarus* extracts detected by sulfuric acid (5% ethanolic  $H_2SO_4$ ) reagent (detection method III). Cross-reference with Figure C.8 and Table D.3

<u>Fraction</u> Extraction\ Location	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	Total
A1	-	-	-	-	-	-	+	-	1
B1	+	+	-	+	-	+	+	-	5
C1	+	+	+	+	+	+	+	+	8
A2	-	-	-	-	-	-	+	-	1
B2	+	+	-	+	+	-	+	-	5
C2	+	+	+	+	+	-	+	+	7
A3	-	-	-	-	-	-	-	-	0
B3	+	+	-	+	+	-	+	+	6
C3	+	+	+	+	+	-	+	+	7

**Extraction:** A=50% MeOH in  $H_2O$ ; B= 99% methanol; C= 50% MeOH in  $CHCl_3$

**Location:** 1= PD; 2= PN; 3= PZ

Sulfuric acid reagent (5% ethanolic  $H_2SO_4$ ) detects mainly general compounds and specific lignans (Fried and Sharma, 1994; Wagner and Bladt, 1996). The mobile phase chloroform/methanol= 9:1 (Figure C.7) gave an unequally distributed separation of the extracts; most of the fractions/compounds were at the top of the plate. Mobile phase



chloroform/methanol= 98:2 (Figure C.9) is less polar, so the less polar compounds were eluted and the more polar compounds did not move with the solvent mixture.

**Table 4.4** Presence/absence of fractions in crude *Q. amara* extracts detected by UV-254 nm and PMA reagent (detection method I). Cross-reference with Figure C.11 and Table D.4

<u>Fraction</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>	<u>10</u>	<u>11</u>	Total
Extraction												
A	-	-	-	-	-	-	-	+	-	-	-	1
B	+	+	+	+	+	+	-	+	+	+	-	9
C	+	+	+	+	+	+	+	+	+	+	+	11

**Extraction:** A=50% MeOH in H<sub>2</sub>O; B= 99% methanol; C= 50% MeOH in CHCl<sub>3</sub>

The results in Table 4.4, Figure C.11 and Table D.4 indicate that only one fraction/compound (number 8) was detected in extract A and that fractions 7 and 11 were eluted only when extraction solvent C (50% methanol in chloroform) was used. These fractions are probably less polar. On pages 20-22 there is an overview of the phytochemicals present in *Q. amara*. The secondary metabolites in the extracts of *Q. amara* that are detected by UV-254 nm are alkaloids (indole:  $\beta$ -carboline and canthin-6-ones) and triterpenes (quassinoids). PMA spray reagent makes most organic compounds visible. Indole alkaloids and triterpenes (quassinoids) are of medicinal importance.

The mobile phase chloroform/methanol= 9:1 resulted in eluting the fractions more to the top of the plate. The chloroform/methanol= 98:2 mobile phase is less polar, so the less polar compounds eluted first and the more polar compounds stayed at the starting line (Figure C.10).

Fractions 1, 2 and 6 were detected only in extraction A (Table 4.5 and Figure C.12). These fractions are either not present in extract A or they are present but the

concentration of them is too low to be detected by this method. The secondary metabolites that were detected by UV-365 nm in *Q. amara* extract are fluorescence alkaloids and triterpenes (i.e. quassin, neoquassin and 18-hydroxy-quassin). After visualization under UV light the plates were sprayed with DRG reagent to specifically detect alkaloids. Upon spraying with DRG reagent, no fraction turned orange. It must be noted that not all alkaloids become visible with DRG reagent. Quenching alkaloids can be detected with UV-254 nm and fluorescence alkaloids can be detected with UV-365 nm.

**Table 4.5** Presence/absence of fractions in crude *Q. amara* extracts detected by UV-365 nm and Dragendorff (DRG) reagent (detection method II). Cross-reference with Figure C.12 and Table D.5

<u>Fraction</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	Total
Extraction							
<b>A</b>	-	-	+	+	+	-	3
<b>B</b>	+	+	+	+	+	+	6
<b>C</b>	+	+	+	+	+	+	6

**Extraction:** A=50% MeOH in H<sub>2</sub>O; B= 99% methanol; C= 50% MeOH in CHCl<sub>3</sub>

There is not a major difference between the mobile phases chloroform/methanol= 9:1; 95:5; 98:2 (Figure C.12), based on the separation of the fractions on the plates and the number of compounds eluted. The plates from mobile phase 95:5 were chosen to be further analyzed based on the equal spreading of the fractions.

The use of sulfuric acid spray reagent to visualize the fractions/compounds present in extract A (Table 4.6 and Figure C. 13) was not effective, because no compounds were detected. This detection method is suitable to visualize compounds in the less polar extracts B and C. Fraction 6 was detected only in extract C. The compounds

in *Q. amara* extract that were detected by sulfuric acid are the terpenoids and some alkaloids.

**Table 4.6** Presence/absence of fractions in crude *Q. amara* extracts detected by 5% ethanolic H<sub>2</sub>SO<sub>4</sub> reagent (detection method III). Cross-reference with Figure C.13 and Table D.6

<u>Fraction</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	Total
Extraction							
A	-	-	-	-	-	-	0
B	+	+	+	+	+	-	5
C	+	+	+	+	+	+	6

**Extraction:** A=50% MeOH in H<sub>2</sub>O; B= 99% methanol; C= 50% MeOH in CHCl<sub>3</sub>

The mobile phases 9:1 and 98:2 (Figure C.13) eluted fewer compounds than 95:5. Since the mobile phase of chloroform/methanol= 95:5 gave a good separation and eluted more compounds than the other mobile phases, the plates from this mobile phase were used for analysis.

**Table 4.7** Presence/absence of fractions in crude *Q. amara* extracts detected by sulfuric acid (5% ethanolic H<sub>2</sub>SO<sub>4</sub>) reagent and UV-365nm (detection method IV). Cross-reference with Figure C.14 and Table D.7

<u>Fraction</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	Total
Extraction					
A	+	-	+	+	3
B	+	+	+	+	4
C	+	+	+	+	4

**Extraction:** A=50% MeOH in H<sub>2</sub>O; B= 99% methanol; C= 50% MeOH in CHCl<sub>3</sub>

Only fraction 2 from extract A (Table 4.7 and Figure C.14) was not detected by this method. Fewer fractions/compounds become visible with detection method IV than with the other three detection methods. Spraying the plate with sulfuric acid reagent first

and then visualizing the spots at UV-365 nm gives sharp fluorescence zones. The secondary metabolites visualized in *Q. amara* extract are probably fluorescence alkaloids (indole:  $\beta$ -carbolines and canthin-6-ones) and triterpenoids (i.e. quassin, neoquassin and 18-hydroxy-quassin).

There were no major difference between the plates of the mobile phases chloroform/methanol= 9:1; 95:5; 98:2 that were subjected to detection method IV. In general, the plates showed the same separation and number of fractions. The plates from mobile phase 95:5 were chosen to be further analyzed based on the equal spreading of the fractions.

### Extraction Rate

The extraction rate (in percentage) for a plant species is calculated by dividing the weight of the extracted powder by the weight of the extracted plant material. The extraction rate (Table 4.8) was influenced by the extraction solvents used, for both *P. amarus* and *Q. amara*. However, the effect of a solvent on the extraction rate showed a different pattern in the two plant species. In *P. amarus*, methanol yielded the highest extraction rate, whereas 50% methanol in chloroform gave the lowest extraction rate.

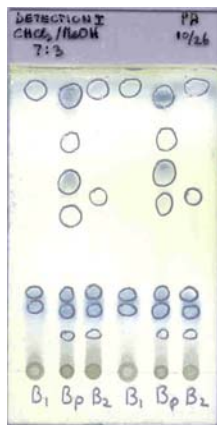
In *Q. amara*, 50% methanol in water yielded a high extraction rate, followed closely by absolute methanol, and 50% methanol in chloroform gave the lowest extraction rate.

**Table 4.8** Extraction rate (%) of *Phyllanthus amarus* and *Quassia amara*

Plant species	50% MeOH in H <sub>2</sub> O	99% MeOH	50% MeOH in CHCl <sub>3</sub>
<i>Phyllanthus amarus</i>	5.8	7.2	2.8
<i>Quassia amara</i>	3.4	3.04	1.56

### Validation of the Optimal Extraction Solvent

The plants from geographical location PD (Saramacca-Damboentong) were used to produce optimized powder extracts of *P. amarus*, since plants from this location had eluted the most and widest variety of fractions/compounds in the crude plant extracts (Tables 4.1- 4.3).



B1= Powder extract dissolved in 50% MeOH in H<sub>2</sub>O  
Bp= *P. amarus* crude extract  
B2= Powder extract dissolved in 99% MeOH

**Figure 4.1:** Fractions eluted from *P. amarus* extract with mobile phase CHCl<sub>3</sub>/MeOH= 7:3 and detected by UV-254 nm and PMA reagent

Although the mobile phase chloroform/methanol 95:5 gave good separation and eluted the most compounds (Tables 4.1-4.7), this mobile phase turned out to be unsuitable for the elution of the fractions/compounds in powder extracts. Therefore, a trial and error was done with mobile phase 5:5; 7:3; and 9:1 in order to find the optimal mobile phase for the powder extract of *P. amarus*. Chloroform/methanol 7:3 turned out to be the most suitable mobile phase, based on the separation and the number of compounds eluted (Figure 4.1 and Table 4.9).

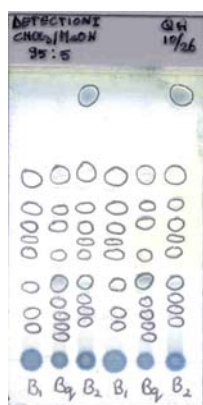
When comparing (Table 4.9) the powder extract of *P. amarus* dissolved in methanol and the crude methanol extract with each other, there were fewer fractions in the powder than in the crude methanol extract. The missing fractions (probably 4, 5 and 6) may have been removed during Liquid Phase Extraction (LPE), since chlorophyll and

other impure non polar fractions/compounds are removed during LPE. Fraction 4\* became visible in the powder extract, which indicates that either a new compound was formed, or the concentration of this fraction was too low to be detected before LPE was done.

**Table 4.9** Rf-values of fractions in crude and powder extracts from *P. amarus*, eluted by CHCl<sub>3</sub>/MeOH= 7:3 and detected by UV-254 nm and PMA reagent

<u>Fraction</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4*</u>	<u>5</u>	<u>6</u>	<u>7</u>
<b>Extraction</b>							
<b>Rf-value of Bp</b>	0.12	0.20	0.25	0.51	0.63	0.75	0.91
<b>Crude extract</b>							
<b>Rf-value of B2</b>	0.13	0.21	0.27	0.58*	0	0	0.93
<b>Powder extract</b>							

The powder extract of *Q. amara* dissolved in MeOH, eluted two fractions/compounds more than the crude extract (fractions 7, 11) but failed to elute compound 1 (Figure 4.2 and Table 4.10). These results indicate that there are more fractions/compounds available in the powder extract (B2) than in the crude extract (Bq).



B1= Powder extract dissolved in 50 % MeOH in H<sub>2</sub>O  
 Bq= *Q. amara* crude extract  
 B2= Powder extract dissolved in 99% MeOH

**Figure 4.2:** Fractions eluted from *Q. amara* extract with mobile phase CHCl<sub>3</sub>/MeOH= 95:5 and detected by UV-254 nm and PMA reagent

The Rf-value for fractions 2-6 and 8-10 in both samples (crude and powder extracts) are approximately equal; this indicates that these fractions are the same. Fraction 7 did not show up in the crude extract spotted on this plate (Figure 4.2 and Table 4.10), but it was visible on the plate in Figure C.11 (Table D.4, fraction 5).

**Table 4.10** Rf-values of fractions in crude and powder extracts from *Q. amara* eluted by CHCl<sub>3</sub>/MeOH= 95:5 and detected by UV-254 nm and PMA reagent

<u>Fraction</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>	<u>10</u>	<u>11</u>
<b>Extraction</b>											
<b>Rf-value Bq</b>	0.08	0.12	0.16	0.20	0.27	0.36	0	0.46	0.51	0.63	0
<b>Crude</b>											
<b>Rf-value B2</b>	0	0.13	0.18	0.21	0.27	0.36	0.40	0.45	0.52	0.64	0.90
<b>Powder</b>											

## 4.2 Discussion

### Extraction Solvents

Although the same extraction ratio (1:10, w/v) was used for both plant species, the extracts of *P. amarus* turned out to be more concentrated than those of *Q. amara*. *P. amarus* extracts may have been more concentrated than *Q. amara* extracts because the particle size of the former was smaller (Φ2 mm) than the latter (Φ6 mm). Therefore, the reaction (contact) surface area between plant particle and extraction solvent was bigger in the case of *P. amarus*, resulting in more concentrated samples for *P. amarus*.

Extraction solvent A (50% methanol in water; is not “weak” (too polar) enough to be used with the stationary phase silica gel. A maximum of only one fraction/compound of *P. amarus* extract and three fractions/compounds of *Q. amara* became visible with four detection methods. This does not mean that extract A produces only one (*P. amarus*)

to three (*Q. amara*) fraction(s)/compound(s) of possible medicinal value, but that only one/three fraction(s)/compound(s) were detected by the analysis method used during this research. Extract A (50% methanol in water) contains the more polar fractions/compounds.

Since extraction solvent B (99% methanol) is less polar than A, it is “weak” and volatile enough to be used with silica gel as stationary phase. Methanol also has proven to be a good solvent (high extraction rate) for *P. amarus* and *Q. amara*. Most of the fractions/compounds that were extracted with methanol became visible during the use of the different detection methods for both species. Extract B from each plant species contains polar and intermediate fractions.

Extraction solvent C (50% methanol in chloroform) was the most non-polar solvent used; therefore, this extraction solvent is the weakest application solvent and also the most volatile solvent applied to the silica gel plate. This can be a reason why most of the fractions/compounds became visible with the different detection methods used, during this research. Extract C from each plant species contains polar, intermediate and some non-polar fractions.

### **Mobile Phase**

The results from the plates put in mobile phases chloroform/methanol= 9:1; 98:2 (Appendix C) were eliminated based on the spreading of separation and the number of fractions eluted. When these mobile phases are eliminated no information is lost, because the phytochemicals that did not come out with 9:1 and 98:2 came out with 95:5 (optimal mobile phase).

Since the solvent mixture (chloroform/methanol) that was used as mobile phase was the same and only the ratio (9:1; 95:5; 98:2) was different, the sort of



fractions/compounds that came out were the same in general. The fractions that were eluted by the different ratios will only differ in polarity; therefore, the more polar fractions will come out when the ratio is more polar and the less polar fractions will come out when the ratio is more to the non-polar side. It must be stated that no generalization is entirely true, including this one.

### **Detection Methods**

The greatest number of fractions/compounds in both plant species *P. amarus* and *Q. amara* were detected with detection method I (UV-254 nm and PMA reagent). *P. amarus* extracts: When comparing the R<sub>f</sub>-values in detection methods I and II (Tables D.1 and D.2), it becomes clear that method II (UV-365 nm and DRG reagent) detects the same fractions as method I. Therefore method II can be eliminated. Detection method III (5% ethanolic sulfuric acid reagent) can not be eliminated, because it detects four additional fractions/compounds (high lighted fractions in Table D.3) more than method I (Table D.1).

*Q. amara* extracts: According to the R<sub>f</sub>-values it is obvious that fractions/compounds detected with methods II and IV (Table D.5 and D.7), are also detected with methods I and III (Table D.4 and D.6). Based on these results, detection methods II and IV can be eliminated. Method III (Table D.6) detects four additional fractions/compounds (high lighted fractions in Table D.6) over method I. Therefore, detection method III cannot be eliminated based on the number of compounds it detects, since these compounds were not detected by Method I.

### **Extraction Rate**

Although the extraction rate of *Q. amara* for 50% methanol in water yielded the highest rate, this solvent was not used to produce powder extracts for bioassay analysis;

the extraction solvent 50% methanol in water did not prove to be suitable (Tables 4.4-4.7). Therefore, for both plant species, methanol was used to produce powder extracts, since the extraction rate for this solvent was the best.

## CHAPTER 5 CONCLUSIONS AND FUTURE WORK

### 5.1. Conclusions

*P. amarus* crude extract B (99% methanol) and C (50% methanol in chloroform) from geographical location PD (Saramacca-Damboentong) recovered the greatest number of fractions/compounds with the use of detection method I (UV-254 nm and PMA reagent). The compounds which were detected with this method in the crude *P. amarus* extracts were probably alkaloids, flavonoids, lignans, phenols and indole derivatives. Some additional compounds (i.e. lignans) were visualized with detection method III. The optimal mobile phase for TLC analysis of *P. amarus* crude extracts was CHCl<sub>3</sub>/MeOH 95:5 and for the powder extracts was CHCl<sub>3</sub>/MeOH 7:3. The powder extracts eluted a smaller number of fractions than the crude extracts (Figure 4.1 and Table 4.9).

Prior work done on *P. amarus* (Table 2.2 page 12) showed the occurrence of alkaloids, flavonoids, hydrolysable tannins, lignans, phenolics and polyphenols in these plant extracts. No hydrolysable tannins were detected during our research, since the extraction procedure used was different. Foo and Wong (1992) and Foo (1993), obtained hydrolysable tannin fractions by column chromatography of the water soluble portion of a 70% aqueous extract on Sephadex LH 20 using aqueous methanol. These tannins were identified by analysis of their <sup>1</sup>H and <sup>13</sup>C NMR spectra and further confirmed by chromatographic comparison with authentic materials.

Houghton et al. (1996) detected with TLC, spots and bands of securinega type alkaloids in *P. amarus* extracts by UV irradiation (254 and 365 nm), with the use of mobile phases CHCl<sub>3</sub>-MeOH (6:1) and Me<sub>2</sub>CO-MeOH (9:1). The spray reagent used was Dragendorff, and showed the presence of alkaloid positive zones. The extraction procedure used during this research was that the air dried leaves were first extracted with

HCl, the filtrate was then basified with  $\text{Na}_2\text{CO}_3$  and extracted with  $\text{CHCl}_3$  which yielded an oily residue.

The crude *Q. amara* extracts B (99% methanol) and C (50% methanol in chloroform) had the greatest number of fractions/compounds eluted when mobile phase  $\text{CHCl}_3/\text{MeOH}= 95:5$  was used. For the visualization of the eluted fractions/compounds, detection method I (UV-254 nm and PMA reagent) was the most suitable, and detection method III (ethanolic sulfuric acid) detected four additional fraction/compounds which most probably were terpenoids and/or alkaloids. The secondary metabolites in *Q. amara* extracts that were detected with method I are probably indole alkaloids (e.g.  $\beta$ -carbolines and canthin-6-ones), and quassinoids (triterpenes). The greatest number of fractions/compounds from *Q. amara* powder extracts was eluted with mobile phase  $\text{CHCl}_3/\text{MeOH } 95:5$ . In the powder extracts there were more fractions/compounds visualized than in the crude extract (Figure 4.2 and Table 4.10).

By repeated chromatography separations, Barbetti et al. (1986), isolated three  $\beta$ -carboline alkaloids from a  $\text{CHCl}_3$ -soluble alkaline *Quassia* wood extract. The identification of the alkaloids was done by  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, NMR, UV, IR and mass spectral data as well as by the chemico-physical properties. In 1990, Barbetti et al. isolated canthin-6-one alkaloids, by repeated chromatography separations of the MeOH-soluble alkaline wood extract. The monitoring was done by TLC (silica gel plates) using the eluents  $\text{CHCl}_3/\text{MeOH}= 90:10$ ,  $\text{CHCl}_3/\text{MeOH}/\text{NH}_4\text{OH}_{\text{aq}}= 85:14:1$ , and benzene/EtOAc/pyridine= 30:60:10. To visualize the compounds a UV lamp was used and the spray detectors Dragendorff and phosphomolybdic acid reagent were used. The identification of these canthin-6-one alkaloids resulted from their  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, NMR, UV, IR and mass spectral data and their structures were confirmed by means of

chemical transformations. In 1987, Vitanyi et al., detected quassinoids in Quassia powder (containing a natural mixture of quassinoids) by HPLC/Mass spectrometry.

Extraction solvent B (99% methanol) had the best extraction rate for *P. amarus* and *Q. amara*. It can be concluded that different extraction solvents influence the extraction rate of plant extracts. The produced powder extracts of *P. amarus* (Figure 4.1. and Table 4.9) and *Q. amara* (Figure 4.2, and Table 4.10) were optimized, since most of the fractions/compounds from the crude extracts were recovered in the powders. The TLC fingerprint of the produced *P. amarus* and *Q. amara* powders were slightly different from those of the crude solvent extract of each species (Figure 4.1 and Figure 4.2). For powdered extracts from *P. amarus*, the mobile phase  $\text{CHCl}_3/\text{MeOH} = 7:3$  was found optimal (compared to  $\text{CHCl}_3/\text{MeOH} = 5:5$  and  $9:1$ ) but it still eluted fewer fractions than from the crude extract. For powdered extracts from *Q. amara*, more fractions were eluted with mobile phase  $\text{CHCl}_3/\text{MeOH} = 95:5$ , but some of the fractions were not the same compounds.

For both plant species extraction solvent A (50% methanol in water) was not appropriate for the analysis method used during this research. It is quite possible that the stationary phase (silica gel), mobile phase (chloroform-methanol) and detection methods (I-IV) are not suitable to detect compounds present in methanol-water extracts (extraction solvent A). In general it can be concluded that the detection of compounds present in plant species depends on the choice of the extraction solvent, stationary phase, mobile phase (sort of solvent) and detection method.

Taking into account all the results from prior works done on *P. amarus* and *Q. amara* extracts, it can be concluded that the analytical methods used during these analysis (i.e. NMR, UV, IR, HPLC/Mass spectrometry) are much more sensitive and accurate

than TLC in analyzing the specific compounds. TLC is a simple, quick and inexpensive analysis method. In order to determine which fraction (spot on the plate) represent a certain secondary metabolite it will be necessary to use specific detector reagents.

## **5.2. Future Work**

The produced powder extracts will be subjected to bioassay analysis concerning anti-cancer activity. In the future, purification of the solvent extracts based on the different polarities of the fractions/compounds will be necessary to produce more purified powders. Purification can be done by: (1) Column chromatography (CC), using solvent mixtures with increasing polarity; (2) Preparative TLC; and (3) HPLC. It also will be necessary to confirm the results of the analysis method used during this study with an analysis method that is more sensitive and accurate, i.e. HPLC-MS. This analysis method would provide more reproducible results (qualitatively and quantitatively).

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## APPENDIX A. PLANT MATERIAL

**Table A.1 Environmental conditions under which the collected plant species grow**

Plant species	Place & Environment collected samples	Soil composition* and pH
<i>Phyllanthus amarus</i>	1. Paramaribo-North (PN):  Pasture land; Disturbed ground	1. Imperfectly drained medium and fine sand to sandy loam, locally sandy clay on medium fine sand → pH 8.0
	2. Saramacca-Damboentong (PD):  Disturbed ground	2. Ridge soils: well (to poorly) drained shells, shell-grit, shell sand, medium and fine sand to sandy loam → pH 7.2
	3. Paramaribo-South (PZ):  Garden	3. (Moderately) well drained medium and fine sand → pH 8.4
<i>Quassia amara</i>	Saramacca-Dirkshoop:  Woods/Forest	Swamp and marsh soils: Poorly and very poorly drained nearly ripe clay with yellow and or red mottles, locally over sand or sandy loam → pH 4.5

\*Van Vuure and Alderlieste, 1977

## APPENDIX B. EXTRACTION LAYOUT

**Table B.1**      Extraction procedure for 10 grams of *P. amarus* crude extracts

Location	Amount of Plant material Extracted	Extraction solvent
PD: Saramacca-Damboentong	10.0 grams	A1= 50% MeOH in H <sub>2</sub> O
	10.0 grams	B1= 99% MeOH
	10.0 grams	C1= 50% MeOH in CHCl <sub>3</sub>
PN: Paramaribo-North	10.0 grams	A2= 50% MeOH in H <sub>2</sub> O
	10.0 grams	B2= 99% MeOH
	10.0 grams	C2= 50% MeOH in CHCl <sub>3</sub>
PZ: Paramaribo-South	10.0 grams	A3= 50% MeOH in H <sub>2</sub> O
	10.0 grams	B3= 99% MeOH
	10.0 grams	C3= 50% MeOH in CHCl <sub>3</sub>

**Table B.2      Inventory of the amount of plant material extracted and produced powder weight for bio-assays**

<b>Amount of Plant material</b>	<b>Extraction Solvent</b>	<b>Powder weight</b>
<i>P. amarus</i> : 10.0 g	50% MeOH in H <sub>2</sub> O 100 ml	1.546 g
100.0 g	50% MeOH in CHCl <sub>3</sub> 1000 ml	2.8 g
1400.0 g	99% MeOH 14 liters	100.8 g
<i>Q. amara</i> : 10.0 g	50% MeOH in H <sub>2</sub> O 100 ml	0.348 g
263.5 g	50% MeOH in CHCl <sub>3</sub> 2.635 l	4.1 g
1400.0 g	99% MeOH 14 liters	42.5 g

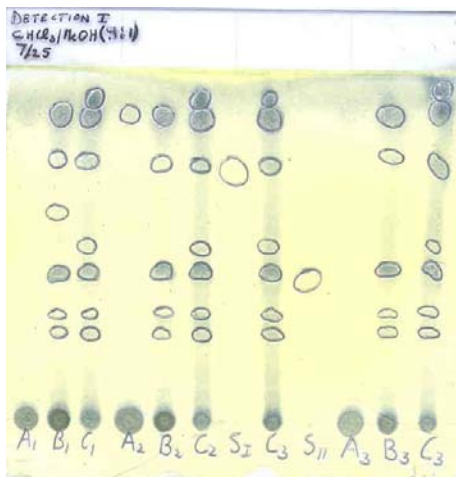
## APPENDIX C. THIN LAYER CHROMATOGRAPHY PLATES

For Figure C.1 - C.9 the following key should be used:

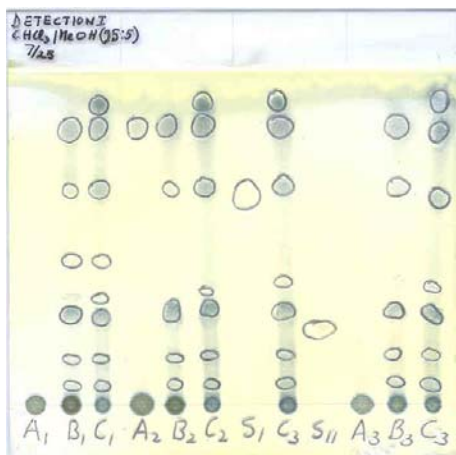
**Extraction:** A=50% MeOH in H<sub>2</sub>O; B= 99% methanol; C= 50% MeOH in CHCl<sub>3</sub>

**Location:** 1= Saramacca-Damboentong (PD); 2= Paramaribo-North (PN); 3=

Paramaribo-South (PZ)

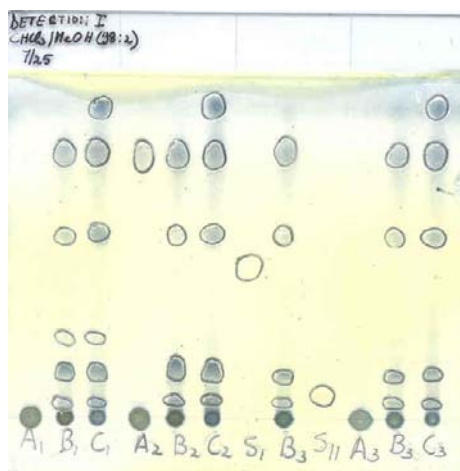


**Figure C.1** Fractions from crude *P. amarus* extracts eluted in mobile phase CHCl<sub>3</sub>/MeOH= 9:1, detected by UV-254 nm and PMA reagent (detection method I)

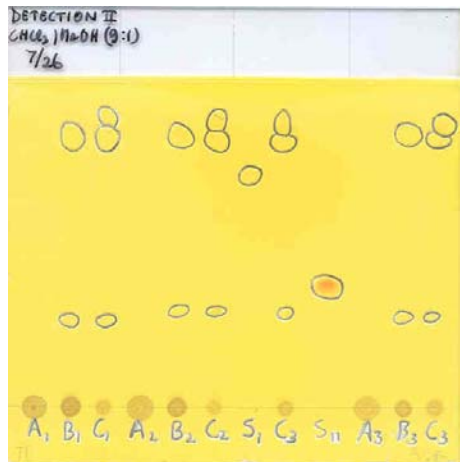


**Figure C.2** Fractions from crude *P. amarus* extracts eluted in mobile phase CHCl<sub>3</sub>/MeOH= 95:5, detected by UV-254 nm and PMA reagent (detection method I)

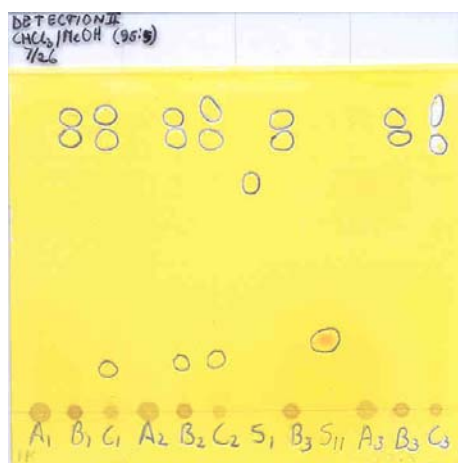




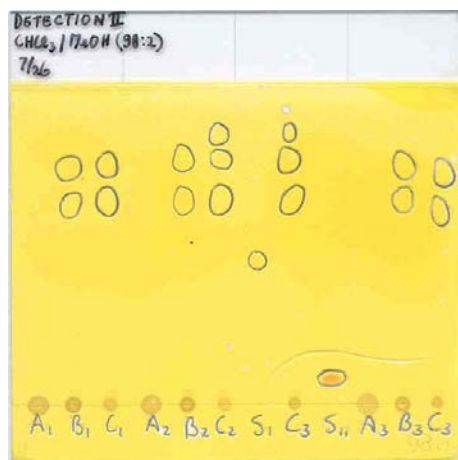
**Figure C.3** Fractions from crude *P. amarus* extracts eluted in mobile phase  $\text{CHCl}_3/\text{MeOH}=98:2$ , detected by UV-254 nm and PMA reagent (detection method I)



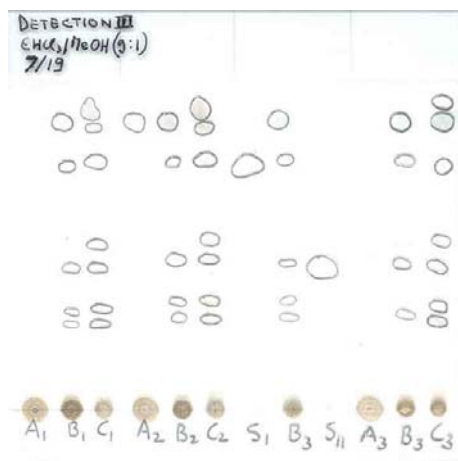
**Figure C.4** Fractions from crude *P. amarus* extracts eluted in mobile phase  $\text{CHCl}_3/\text{MeOH}=9:1$ , detected by UV-365 nm and DRG reagent (detection method II)



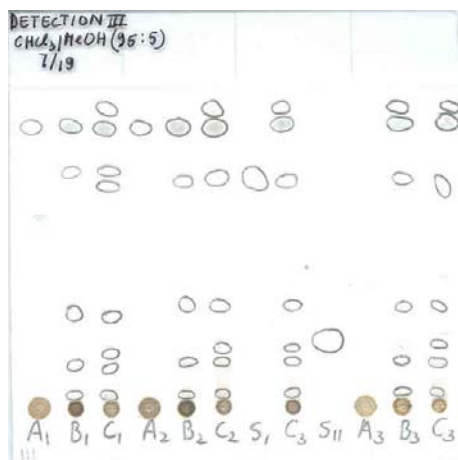
**Figure C.5** Fractions from crude *P. amarus* extracts eluted in mobile phase  $\text{CHCl}_3/\text{MeOH}=95:5$ , detected by UV-365 nm and DRG reagent (detection method II)



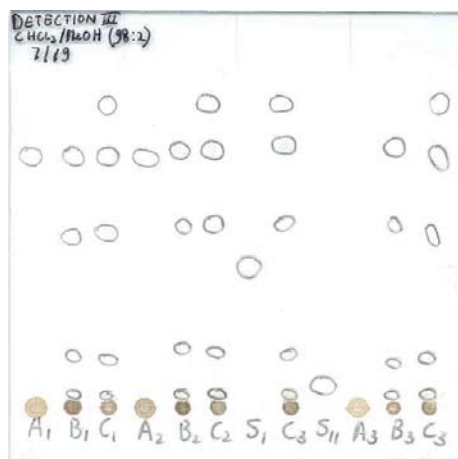
**Figure C.6** Fractions from crude *P. amarus* extracts eluted in mobile phase  $\text{CHCl}_3/\text{MeOH}=98:2$ , detected by UV-365 nm and DRG reagent (detection method II)



**Figure C.7** Fractions from crude *P. amarus* extracts eluted in mobile phase CHCl<sub>3</sub>/MeOH= 9:1 and detected by sulfuric acid reagent (detection method III)



**Figure C.8** Fractions from crude *P. amarus* extracts eluted in mobile phase CHCl<sub>3</sub>/MeOH= 95:5 and detected by sulfuric acid reagent (detection method III)

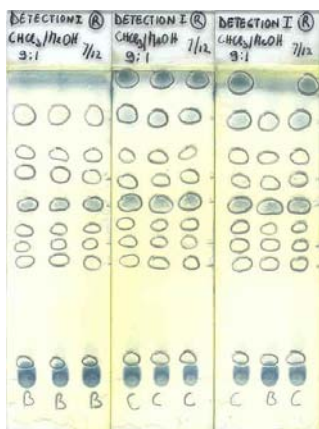


**Figure C.9** Fractions from crude *P. amarus* extracts eluted in mobile phase  $\text{CHCl}_3/\text{MeOH} = 98:2$  and detected by sulfuric acid reagent (detection method III)

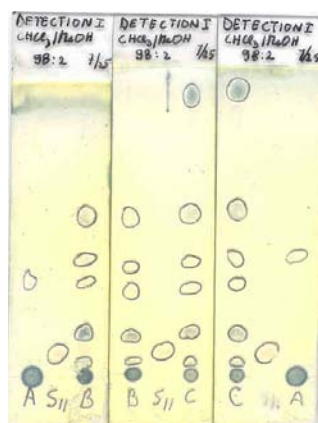
For Figure C.10 – C.14 the following key should be used:

**Extraction:** A=50% MeOH in  $\text{H}_2\text{O}$ ; B= 99% methanol; C= 50% MeOH in  $\text{CHCl}_3$

9:1



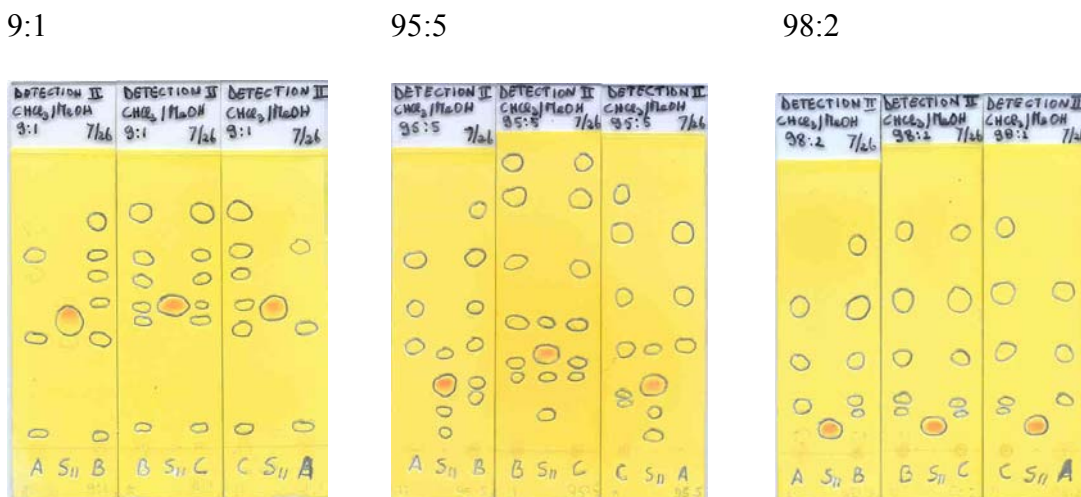
98:2



**Figure C.10** Fractions from crude *Q. amara* extracts eluted in mobile phases  $\text{CHCl}_3/\text{MeOH}= 9:1; 98:2$ , detected by UV-254 nm and PMA reagent (detection method I)



**Figure C.11** Fractions from crude *Q. amara* extracts eluted in mobile phase  $\text{CHCl}_3/\text{MeOH}= 95:5$ , detected by UV-254 nm and PMA reagent (detection method I)

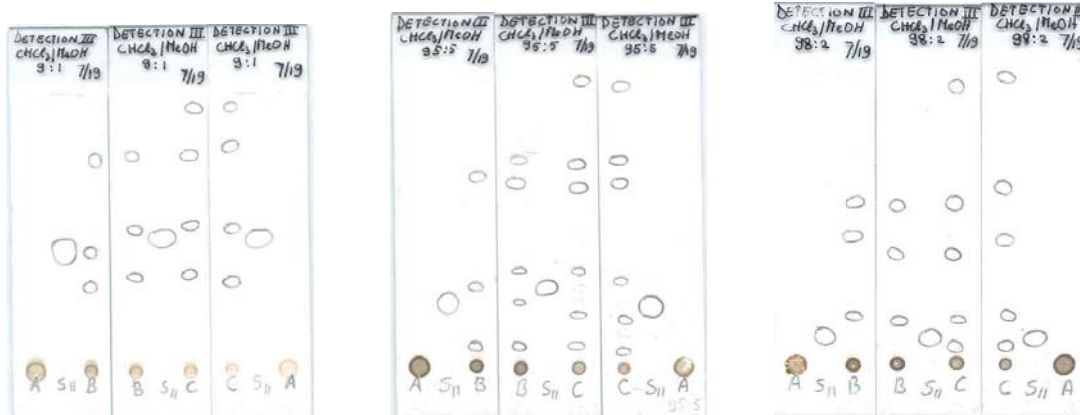


**Figure C.12** Fractions from crude *Q. amara* extracts eluted in mobile phases  $\text{CHCl}_3/\text{MeOH}= 9:1; 95:5; 98:2$ , detected by UV-365 nm and DRG reagent (detection method II)

9:1

95:5

98:2

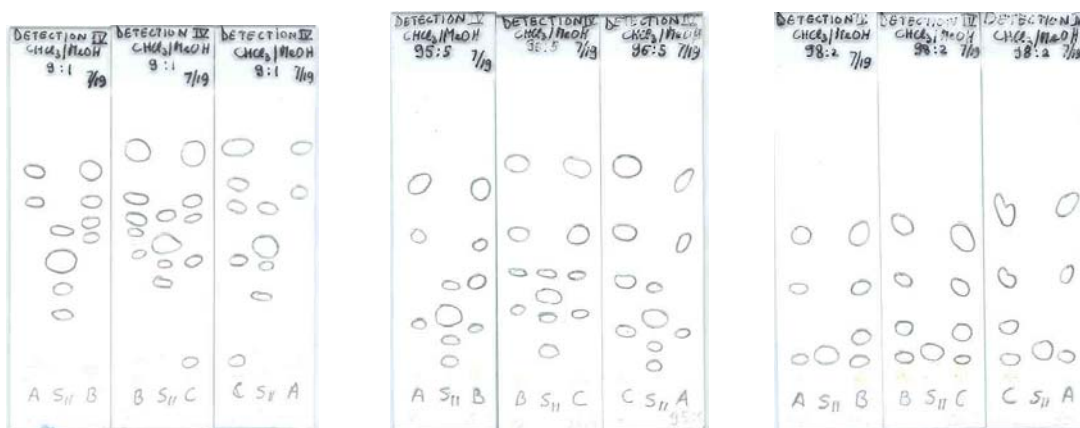


**Figure C.13** Fractions/compounds from crude *Q. amara* extracts eluted in mobile phases CHCl<sub>3</sub>/MeOH= 9:1; 95:5; 98:2, detected by sulfuric acid reagent (detection method III)

9:1

95:5

98:2



**Figure C.14** Fractions from crude *Q. amara* extracts eluted in mobile phases CHCl<sub>3</sub>/MeOH= 9:1; 95:5; 98:2, detected by sulfuric acid reagent and UV-365 nm (detection method IV)

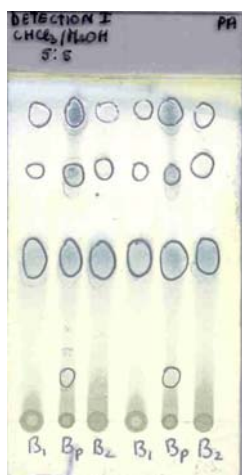
For Figure C.15 and C.16 the following key should be used:

B1= Powder extract dissolved in 50% MeOH in H<sub>2</sub>O

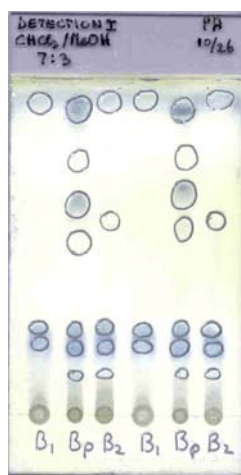
Bp (Bq)= *P. amarus* (*Q. amara*) crude extract

B2= Powder extract dissolved in 99% MeOH

5:5



7:3



9:1

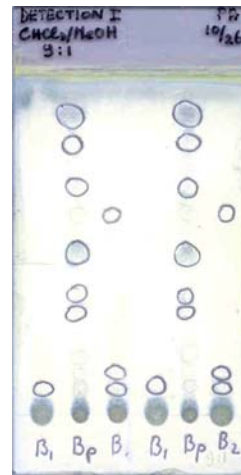
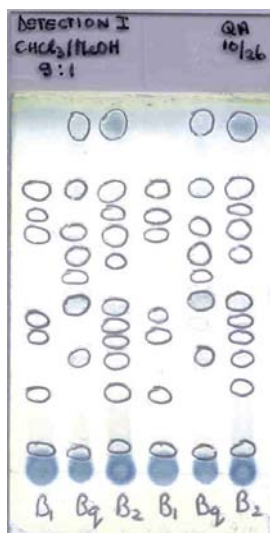


Figure C.15 Fractions from *P. amarus* powder extracts eluted in mobile phases CHCl<sub>3</sub>/MeOH= 5:5; 7:3; 9:1, detected by UV-254 nm PMA reagent

9:1



95:5

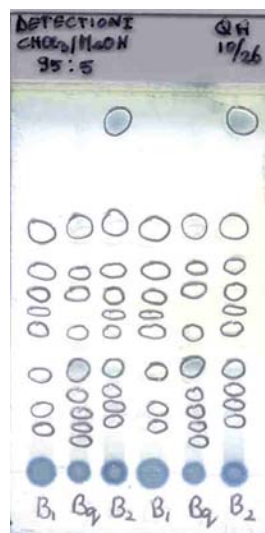


Figure C.16 Fractions from *Q. amara* powder extracts eluted in mobile phases CHCl<sub>3</sub>/MeOH= 9:1; 95:5, detected by UV-254 nm PMA reagent

## APPENDIX D. $R_F$ -VALUES OF FRACTIONS IN PLANT EXTRACTS

In every table the  $R_F$ -value of mobile phase  $\text{CHCl}_3/\text{MeOH} = 95:5$  is reported.

**Table D.1**  $R_F$ -values of fractions in crude *P. amarus* extracts detected by UV-254 nm and PMA reagent (Detection method I)

<u>Fraction</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>
<b>Rf-value: Extraction\ Location</b>								
<b>A1</b>	0	0	0	0	0	0	0	0
<b>B1</b>	0.06	0.14	0.28	0	0.44	0.64	0.83	0
<b>C1</b>	0.06	0.14	0.26	0.33	0.43	0.65	0.83	0.91
<b>A2</b>	0	0	0	0	0	0	0.84	0
<b>B2</b>	0.06	0.15	0.27	0	0	0.65	0.84	0
<b>C2</b>	0.07	0.15	0.29	0.34	0	0.66	0.84	0.91
<b>A3</b>	0	0	0	0	0	0	0	0
<b>B3</b>	0.06	0.15	0.28	0	0	0.66	0.84	0
<b>C3</b>	0.07	0.15	0.29	0.37	0	0.66	0.84	0.92

**Extraction:** A=50% MeOH in  $\text{H}_2\text{O}$ ; B= 99% methanol; C= 50% MeOH in  $\text{CHCl}_3$

**Location:** 1= PD; 2= PN; 3= PZ



**Table D.2** Rf-values of fractions in crude *P. amarus* extracts detected by UV-365 nm and DRG reagent (Detection method II)

<u>Fraction</u>	<u>1</u>	<u>2</u>	<u>3</u>
Rf-value: Extraction\ Location			
A1	0	0	0
B1	0	0.80	0.87
C1	0.13	0.80	0.88
A2	0	0	0
B2	0.15	0.80	0.87
C2	0.16	0.80	0.89
A3	0	0	0
B3	0	0.80	0.85
C3	0	0.78	0.87

**Extraction:** A=50% MeOH in H<sub>2</sub>O; B= 99% methanol; C= 50% MeOH in CHCl<sub>3</sub>

**Location:** 1= PD; 2= PN; 3= PZ

**Table D.3** Rf-values of the fractions in crude *P. amarus* extracts detected by sulfuric acid (5% ethanolic H<sub>2</sub>SO<sub>4</sub>) reagent (Detection method III)

<u>Fraction</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>
Rf-value								
Extraction\								
Location								
<b>A1</b>	0	0	0	0	0	0	0.88	0
<b>B1</b>	0.04	0.13	0	0.29	0	0.74	0.88	0
<b>C1</b>	0.04	0.12	0.17	0.28	0.69	0.74	0.88	0.93
<b>A2</b>	0	0	0	0	0	0	0.88	0
<b>B2</b>	0.04	0.14	0	0.32	0.71	0	0.88	0
<b>C2</b>	0.04	0.14	0.18	0.32	0.72	0	0.88	0.93
<b>A3</b>	0	0	0	0	0	0	0	0
<b>B3</b>	0.04	0.14	0	0.31	0.71	0	0.88	0.93
<b>C3</b>	0.04	0.13	0.19	0.31	0.69	0	0.89	0.94

**Extraction:** A=50% MeOH in H<sub>2</sub>O; B= 99% methanol; C= 50% MeOH in CHCl<sub>3</sub>

**Location:** 1= PD; 2= PN; 3= PZ

**Table D.4** Rf-values of fractions in crude *Q.amara* extracts detected by UV-254 nm and PMA reagent (detection method I)

<u>Fraction</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>	<u>10</u>	<u>11</u>
Rf-value											
Extraction											
A	0	0	0	0	0	0	0	0.58	0	0	0
B	0.11	0.21	0.29	0.35	0.40	0.46	0	0.58	0.73	0.78	0
C	0.11	0.21	0.29	0.34	0.40	0.45	0.54	0.59	0.73	0.78	0.93

**Extraction:** A=50% MeOH in H<sub>2</sub>O; B= 99% methanol; C= 50% MeOH in CHCl<sub>3</sub>

**Table D.5** Rf-values of fractions in crude *Q. amara* extracts detected by UV-365 nm and DRG reagent (detection method II)

<u>Fraction</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>
Rf-value						
Extraction						
A	0	0	0.34	0.49	0.69	0
B	0.18	0.23	0.35	0.48	0.67	0.79
C	0.16	0.21	0.33	0.48	0.68	0.80

**Extraction:** A=50% MeOH in H<sub>2</sub>O; B= 99% methanol; C= 50% MeOH in CHCl<sub>3</sub>

**Table D.6** Rf-value of fractions in crude *Q. amara* extracts detected by 5% ethanolic H<sub>2</sub>SO<sub>4</sub> reagent (detection method III)

<u>Fraction</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>
Rf-value						
Extraction						
A	0	0	0	0	0	0
B	0.06	0.18	0.32	0.61	0.69	0
C	0.07	0.17	0.31	0.60	0.68	0.95

**Extraction:** A=50% MeOH in H<sub>2</sub>O; B= 99% methanol; C= 50% MeOH in CHCl<sub>3</sub>

**Table D.7** Rf-value of fractions in crude *Q. amara* extracts detected by sulfuric acid (5% ethanolic H<sub>2</sub>SO<sub>4</sub>) reagent and UV-365nm (detection method IV)

<u>Fraction</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>
Rf-value				
Extraction				
A	0.21	0	0.46	0.66
B	0.21	0.33	0.45	0.67
C	0.19	0.32	0.44	0.65

**Extraction:** A=50% MeOH in H<sub>2</sub>O; B= 99% methanol; C= 50% MeOH in CHCl<sub>3</sub>

## **VITA**

Vivian Esther Fernand was born in Paramaribo, Suriname, on November 2, 1970, to Mr. Rudi Eugene Fernand and Mrs. Elize Geertruida Fernand-Buyné.

In August 1996, she graduated from the Institute for Advanced Teachers Training, section Chemistry, and in May 1998 she received her Bachelor of Science degree in agronomy from the University of Suriname. She taught chemistry in high school for two years and worked as a research associate for one year in tissue culture at the Center for Agricultural Research in Suriname (CELOS).

In August 2000 she enrolled at Louisiana State University as a research assistant and is currently a candidate for the Master of Science degree in the School of Renewable Natural Resources.