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Antimicrobial activity of Peruvian medicinal plants

Master thesis

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Abstract

In frame of this work, antimicrobial activity of ethanol extracts from 16 plants (belonging to 14 families) traditionally used in Peruvian folk medicine for the treatment of various infections was tested against 9 bacteria and 1 yeast by broth microdilution method. For 8 of which this is the first report of antimicrobial activity. *Abuta grandifolia*, *Maytenus macrocarpa*, *Naucleopsis glabra* and *Pterocarpus rohrii* inhibiting all tested microbial strains at MICs ranging from 0.25 to 8 mg/ml; 0.125 to 0.25 mg/ml; 0.0625 to 4 mg/ml and 0.25 to 16 mg/ml, respectively, were considered as potentially prospective sources of new bioactive substances. In addition, a comparative analysis of five plants known for anti-infective activity was performed. The Peruvian plants showed up to 1000 times higher antimicrobial efficiency.

Key words: Peru, medicinal plants, antimicrobial activity, MIC, broth microdilution method

Abstrakt

V rámci této práce byla studována antimikrobiální aktivita 16 rostlin (náležejících do 14 čeledí), používaných v tradiční peruánské medicíně k léčbě různých infekčních onemocnění. 8 z nich dosud nebylo zkoumáno z hlediska jejich antimikrobiálních účinků. Ethanolové extrakty byly testovány na 9 bakteriích a 1 kvasince bujónovou mikrodiluční metodou. *Abuta grandifolia*, *Maytenus macrocarpa*, *Naucleopsis glabra* a *Pterocarpus rohrii*, inhibující všechny testované kmeny v rozmezí MIC od 0,25 do 8 mg/ml; 0,125 až 0,25 mg/ml; 0,0625 až 4 mg/ml a 0,25 až 16 mg/ml, resp., byly vybrány jako perspektivní zdroje nových biologicky aktivním látek. Z provedené srovnávací analýzy s 5 druhy léčivých rostlin považovaných za vysoce antimikrobiálně účinné vyplynulo, že testované druhy peruánských léčivých rostlin vykazují až 1000 krát vyšší antimikrobiální účinek.

Klíčová slova: Peru, léčivé rostliny, antimikrobiální aktivita, MIC, bujónová mikrodiluční metoda

Prohlášení

Prohlašuji, že jsem tuto diplomovou práci vypracovala samostatně, pod vedením školitele doc. Ing. Stanislava Smrčka, CSc. (Katedra organické a jaderné chemie, Přírodovědecká fakulta Univerzity Karlovy v Praze) a konsultanta Ing. Ladislava Kokošky, PhD. (Institut tropů a subtropů České zemědělské univerzity v Praze), a že jsem všechny použité prameny řádně citovala.

Jsem si vědoma toho, že případné využití výsledků získaných v této práci mimo Univerzitu Karlovu v Praze je možné pouze po písemném souhlasu této univerzity.

V Praze dne 30. 8. 2006

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Podpis

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List of abbreviations

ATCC	American Type Culture Collection
CFU	colony-forming unit
DMSO	dimethyl sulfoxide
G+	Gram-positive (bacteria)
G-	Gram-negative (bacteria)
MIC	minimum inhibitory concentration
TBS	Tris-buffer saline
MDR	multi drug resistance

Contents

1. Foreword

The concept of growing crops for health rather than for food or fiber is slowly changing plant biotechnology and medicine. Rediscovery of the connection between plants and health is responsible for launching a new generation of botanical therapeutics that include plant-derived pharmaceuticals, multicomponent botanical drugs, dietary supplements and functional foods. Many of these products will soon complement conventional pharmaceuticals in the treatment, prevention and diagnosis of diseases, while at the same time adding value to agriculture [1].

There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases. Another big concern is the development of resistance to the antibiotics in current clinical use.

The phytochemical research based on ethnopharmacological information is generally considered as an effective approach in discovery of new anti-infective agents from higher plants [2]. The chemical diversity of plants has made them the source of choice for the isolation of pharmacologically relevant metabolites. Approximately 25 % of the drugs prescribed worldwide come from plants, whereas 11 % of the drugs considered as basic and essential by the World Health Organization (WHO) derive exclusively from plants [3].

In Peru about 20,000 plant species or 8 % of the total number of plants in the world can be found. Most of them are native or grow in the Peruvian Amazonia. However, probably less than 1 % has been studied for their chemical composition and medicinal use [4]. Despite a rich tradition of folk medicinal usage of plants in many parts of Peru and certain ethnobotanical studies published [5-11], there is still a great lack of information concerning chemistry and pharmacology of certain species.

Thus we decided to test 16 medicinal plants used traditionally by local inhabitants of Callería District in Peru for the treatment of various infections, and evaluated them for potential antibacterial activity, in order to confirm their popular use and to compare their efficiency with standard plant sources of antibacterial agents.

2. Objectives

The aim of this work is antimicrobial activity screening of sixteen Peruvian medicinal plants: *Abuta grandifolia, Bertholletia excelsa, Brunfelsia grandiflora, Caesalpinia spinosa, Cordia alliodora, Dipteryx micrantha, Dracontium loretense, Equisetum giganteum, Maytenus macrocarpa, Naucleopsis glabra, Phyllanthus amarus, Piper aduncum, Pterocarpus rohrii, Solanum mammosum, Terminalia catappa* and *Uncaria tomentosa*, which are used in traditional Peruvian folk medicine for treating conditions likely to be associated with microorganisms.

The subsequent steps are:

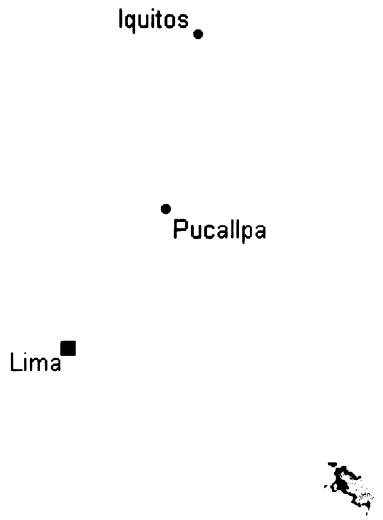
1. Summarization of information on botany, origin and geographical distribution, ethnopharmacological uses, biological activities and chemical composition of these plants.
2. Determination of minimum inhibitory concentration (MIC) of ethanol extracts from different parts of selected plants using the broth microdilution method.
3. Comparative study of selected plants (*Allium sativum, Curcuma longa, Hypericum perforatum, Vicia faba,* and *Vitis vinifera*) with documented antimicrobial properties.
4. Selection of prospective Peruvian medicinal plants as potential source of bioactive constituents for further study.

3. Introduction

3.1. Study area

The study was performed in two villages of Calleria district – Antonio Raimondi and Nueva Belén – located 20 km and 23 km respectively outside the city of Pucallpa in the Amazon basin of Peru (74°W, 8°S, with an average elevation of 150 m a.s.l).

Figure 1. *Location of Pucallpa, the capital of the Ucayali Department (green) in Peru*



Pucallpa (see Figure 1), administrative centre of Calleria district and also the capital of the Ucayali Department, is situated on the banks of the river Ucayali, a principal tributary of the Amazon. Pucallpa is characterized by hot and humid climate and only slight variation throughout the year. The rainfall ranges from 1500 to 2100 mm. The mean annual temperature is 25.7°C and mean annual relative humidity reaching 80 %.

The largest indigenous ethno-linguistic group living in this region is the Shipibo-Conibo group [12].

3.2. Peruvian folk medicine

In Peru, due to its complicated geography and economic factors related to cultural and logistical problems, traditional medicine continues to survive. Like in other developing countries, medicinal plants still represent the main therapeutic tool in folk healing [2].

Traditional medicine was officially prohibited in Peru in 1969, but the prohibition was not enforced. The National Institute of Traditional Medicine, under Peru's Health Ministry, is the official institution working on the regulation of traditional medicine. It seats in Iquitos, the country's largest Amazonian city. The Institute has 17 branches throughout the country and disseminates information and conducts research on folk healing remedies, runs projects that involve growing and studying more than 600 medicinal plants. A germplasm bank can be found at the institute, containing 120 selected species [13].

The recognition and the use of medicinal plants is an untouchable heritage of most preliterate cultures. Over the centuries, every population has developed its knowledge in recognizing, harvesting and using plants to cure infirmities [14]. Folk medicine, one of Peru's oldest cultural traditions, is practiced by shamans or healers. In rural communities of the Andes, the herbalist or *curandero*, the individual who is knowledgeable about all healing and harmful plants, assumes a primary role. He is considered a priest, an intermediate figure between our world and the world of the spiritual forces. At the same time he is also a therapist and an expert on all healing plants, psychotropic plants (used to awake religious spirits or to gain an altered state of mind), and harmful plants. The shamanic culture in the Andean area of Peru is very old. Its origins certainly predate the Columbian eras and, since then, have been enriched by continuous intercultural and interethnic relationships [15].

In the Peruvian Amazonia there is an important ethnobotanical knowledge, preserved by the indigenous inhabitants, who still largely depend on natural resources. Nevertheless, it should be verified and preserved by modern scientific methods [16].

3.3. Ethnobotanical approach

The ethnobotanical approach uses the medical knowledge of traditional societies to select plants for testing bioactivities. The success rates of this approach are substantially higher than those of random screening, with the additional advantage that, to some extent, the continued use of crude preparations are, in fact, comparable to small-scale clinical trials, raising the chances of obtaining something amenable to human use [17].

Previously a great number of studies concerning the use of medicinal plants in several parts of Peru have been carried out. Schultes and Raffaud [5] in their book The Healing forest briefly described some medicinal and toxic plants of the Northwest Amazonia. Bastien [11] summarized facts about some Andean medicinal plants in his book Healers of the Andes. Davidson [10] briefly pointed the survival of traditional medicine in a Peruvian *barriada*. Luna [7] observed the healing practices of Peruvian shamans. De Feo [9] more precisely described some medicinal and magical plants of northern Peruvian Andes. A summary report about project running in Peru in search for new biomedicines was published [18]. Ethnobotanical use of Peruvian medicinal plants of different ethnical groups, like the Aguaruna people, Ese'ejas, Mestizo people, Shipibo–Conibo, etc. was published by several authors [8, 16, 19-21].

3.4. Antimicrobial activity of higher plants

Higher plants produce hundreds to thousands of diverse chemical compounds with different biological activities. It is believed that these compounds have an important ecological role. They can work as pollinator attractants and as chemical defenses against insects, herbivores and microorganisms. In order to adapt to environmental insults, plants produce a vast number of natural products that have antimicrobial potential. These include isoflavonoids, indoles, phytosterols, polysaccharides, sesquiterpenes, alkaloids, glucans, tannins, vitamins, trace minerals, and many other phytochemical substances. Some members of above mentioned groups of plant compounds have previously been found to be active against plant and human pathogenic microorganisms [22].

The Peruvian flora offers great possibilities for the discovery of new compounds with antimicrobial activity. It is estimated that more than 17,000 plant species occur in Peru of

which about 30 % are endemic [2]. Despite of several reports describing antibacterial activity of some Peruvian medicinal plants e.g. [2, 6, 23-29] there is still great lack of scientific information verifying their antimicrobial properties.

3.5. Screening methods of antimicrobial activity

In the crude extract, active principles are generally present at low concentrations only. The test system has, therefore, to be sensitive enough to detect them reliably [30]. Antibacterial assays can be classified into three groups, namely diffusion, dilution and bioautographic methods. For screening of plant extracts in a battery of microorganisms, the broth microdilution method in 96-well microtiter plates is the most suitable technique facilitated to determine MIC values. The method is robust, is not expensive, gives reproducible results, is c. 30 times more sensitive than other methods used in the literature, requires a small quantity of sample, can be used for large number of samples, leaves a permanent record of quantitative results to compare and requires little time. One or two of the series of wells should be used with a known antibiotic to provide reference MIC values for the test organism [31].

3.6. Selected Peruvian plants

Abuta grandifolia (Mart.) Sandwith

Family: Menispermaceae

Synonyms: *Cocculus grandifolius* Mart., *Abuta concolor* Benth.

Vernacular names: Abuta, caimitillo, pancha muca, sanango, trompetero (Shipibo-Conibo).

Origin and geographic distribution: Found throughout the Amazon in Peru, Brazil, Ecuador and Columbia, it is cultivated by many people to beautify their gardens.

Description: Woody liana or small bushes until 3 m high, sarmentose or climber. Trees are until 6 m high. The young stalks are glabrous. Leaves have the consistency of paper or parchment, ovobate or oblanceolate, glabrous, 10-30 x 3-12 cm. The apex is acuminate and the base is cuneate. The pecioles are 2-10 cm long. Flowers without petals and have six sepals and six stamens. The fruit is an oblong drupe with the base slightly attenuate and it has a small peduncle. It is yellow and glabrous. The pedicel is 20-25 mm long [32].

Traditional uses: This species is used in preparation of arrow poisons such as *curare* among several ethnic groups in Amazonia, including the Siona, Karijona, Andoke, Taiwano and Makuna. The Sionas also drink an infusion of the leaves as a treatment for high fevers [33]. Among the Quechua in Ecuador, the leaves are boiled and then applied to infected eyes as a compress. It is valued as an antidote for the bites of the snakes. Abuta has been traditionally used by women who have problems with their menses and to balance female hormones. In Ecuador, it has been known to stop uterine hemorrhages during childbirth. In the Brazilian Amazon also considered effective against malaria, hepatic ailments and gastric ulcers [4]. The Ese´ejas in the Peruvian Amazon take the bark infusion against tuberculosis and lung diseases [19], while the Machiguenga use it as a contraceptive. The bark decoction is mentioned to have antianemic, tonic [34] and diuretic properties and is used to treat diabetes and uterus infections [8]. The decoction of the stems and roots mixed with wild honey is used to treat sterile women and for post-menstrual hemorrhages [35].

Biological activity: *In vitro* studies reported that this plant has antioxidant [34], insecticidal [36], antiplasmodial [37], cytotoxic and antimicrobial [38] properties.

Methanol extract from bark performed antibacterial activity inhibiting *Bacillus subtilis* [8]. Dichloromethane extract from leaves showed 57% inhibition against *Mycobacterium tuberculosis* at concentration 50 μg/ml, but the bark and root extracts were less than 50% effective [23]. A bark decoction partially inhibited the growth of *Pseudomonas aeruginosa* and *Mycobacterium gordonae*, but showed no effect against *Escherichia coli*, *Salmonella gallinarum*, *Klebsiella pneumoniae* and *Candida albicans* in agar plate test [27].

Chemical composition: The bisbenzylisoquinoline alkaloids (krukovine and limacine) and tropolon grandirubrin were isolated from the bark. Isoquinoline alkaloides such as berberine and palmatine were mentioned [35].

Bertholletia excelsa Bonpl.

Family: Lecythidaceae

Synonyms: *Bertholletia nobilis* Miers

Vernacular names: Brazil nut, para nut (English), almendra, juvia (Venezuela), Braziliaansche noot, para noot (Surinam), castaña (Peru), tapa (Bolivia), castanha do Brasil, castanha-do-Pará, castanheira (Brazil).

Origin and geographic distribution: *B. excelsa* is found throughout the Amazon rainforest of Guyana and Amazonian Colombia, Venezuela, Peru, Brazil, and Bolivia. Brazil nut trees are cultivated in botanical gardens far outside their natural range and minor plantations have been established in Surinam, French Guiana, Malaysia, and Ghana.

Description: Trees to 50 m tall, unbuttressed. Bark fissured. Leaves scattered at ends of branches; petioles 20-35 mm long; blades 17-36 x 6-15 cm, oblong, glabrous adaxially, with cuticular papillae abaxially; base rounded; margins entire to slightly crenulate, undulate; apex apiculate. Inflorescences terminal or axillary, spikes or paniculate arrangements of spikes with one, infrequently two, orders of branching; pedicels not well-defined. Flowers zygomorphic; calyx-lobes 2, not imbricate, the margins entire; petals 6,

pale yellow to white. Fruits dehiscent, the opening smaller than seeds, falling to ground with seeds inside at maturity, 10-12 cm, usually globose. Seeds with boney testa [39].

Traditional uses: In the Brazilian Amazon, the nuts are grated with the thorny stilt roots of *Socratea* palms into a white mush known as *leite de castanha* and then stirred into manioc flour. This food is a valuable source of calories, fat, and protein for much of the Amazon's rural and tribal peoples. The oil is extracted from the nuts and used by indigenous and rural people for cooking oil, lamps, soap, and livestock feed. The empty seed pods, often called *monkey's pots* are used to carry around small smoky fires to discourage attacks of black flies. The husks of these seed pods have also been used in Brazilian folk medicine to brew into tea to treat stomachaches, and the tree bark is brewed into tea to treat liver ailments. Its fruit juice is popularly used against hepatitis and the tea prepared with stem bark is indicated for chronic diseases of the liver and also considered an antimalarial agent [40].

Biological activity: Since the Brazil nut has long been a common food, rather than a herbal remedy, it hasn't been the subject of any clinical research outside of that concerning its selenium content [41]. Selenium is an essential trace mineral in the human body with antioxidant, anticancer, and cancer-preventative properties [42]. Acetone and methanol extracts of stem bark showed significant in vitro trypanocidal activity against trypomastigote form of *Trypanosoma cruzi* since in the concentration of 500 µg/ml [40].

Chemical composition: Brazil nut oil contains mainly palmitic, oleic and linolenic acids, small amounts of myristic and stearic acids and phytosterols such as β-sitosterol, campesterol, campestanol, sitostanol, and stigmasterol [43]. In addition to protein and fat, Brazil nuts provide the highest natural source of selenium (3 - 497 ppm in seeds). The proteins found in Brazil nuts are very high in sulfur-containing amino acids like cysteine (8 %) and methionine (18 %) [44, 45] and are also extremely rich in glutamine, glutamic acid, alanine, and arginine. The presence of these amino acids (chiefly methionine) enhances the absorption of selenium and other minerals in the nut. In addition to the chemicals discussed above, Brazil nuts contain antimony, cerium, cesium, europium, lanthanum, lutetium, samarium, scandium, selenoprotein, tantalum, tungsten, and ytterbium [46].

Brunfelsia grandiflora (D.Don)

Family: Solanaceae

Synonyms: *Brunfelsia calycina* Benth.

Vernacular names: Chiricaspi chacruco, chiricaspi picudo, chiricaspi salvaje (Ketchwa), hu-ha-hai, yai-huha-hai (Siona), chi-pi-ri-tsontinba-ka (Kofin), borrachero, chiric sanango, sanango (Columbia, Peru).

Origin and geographic distribution: *Brunfelsia grandiflora* is wide-ranging and polymorphic, occuring in western South America from Venezuela south to Bolivia. Sometimes cultivated as an ornamental or medicinal plant.

Description: Small glabrous tree, the leaves apically crowded or scattered on the flowering branchlets; petioles stout, 5-10 mm long; leaves subcoriaceous, oblong-elliptic, slightly narrowed to acute base, shortly and subacutely cuspidate, 1.5-2 cm long, 5-8 cm wide, the 5-7 slender lateral nerves and laxly reticulate veins somewhat conspicuous only beneath; bracts caducous; pedicels 1 cm long; calyx 1.5-2 cm long, the ovate teeth obtuse or mucronulate; corolla tube 3-4 cm long, the limb 3-5 cm across, the lobes subrotund [47].

Traditional uses: It is employed as additive to the *ayahuasca* hallucinogen. The most effective parts of the plants are considered to be the roots. The most widely employed medicinal uses are those to treat syphilis and rheumatism and for their diuretic and diaphoretic properties [35]. In the Amazon, the root is prepared into a tincture with *aguardiente* (rum) for rheumatism and venereal disease. In Peru Amazonian *curanderos* near Pucallpa apply a decoction of leaves externally for arthritis and rheumatism [9]. They also employ a root decoction for chills. It is highly regarded as agents to treat fevers because of the curious sensation of chills which they produce. It is further used by treating common colds, bronchitis, lung disease and tuberculosis, snakebite, and as an enema for kidney disorders and ulcers [5]. Indigenous tribes in the northwest Amazon utilize *manacá* to increase urination and perspiration in detoxification rituals. They also use it for treating yellow fever [4]. In Brazil herbalists use the root as a laxative and blood cleanser, dermatitis cure, and to promote menstrual flow. The Kofans and the Siona-Secoyas of Ecuador utilize this species as a hallucinogen, the latter group using it also as an

abortifacient Ese'ejas prepare a juice from crushed leaves against colds and bronchitis [19]. Contrary to other parts of plant, the decoction of leaves doesn't induce nausea [48].

Biological activity: The chemistry and medicinal uses of the genus have been reviewed [49]. The root extract demonstrated antioxidative, anti-inflammatory, antipyretic, antimutagenic, CNS depressant, convulsant, cytotoxic, and and insecticidal activity [48].

Species of *Brunfelsia* are rich in psychoactive scopoletin, a furocoumarin which is claimed to be anti-inflammatory [50], cytotoxic and antiproliferative [51]. The bark extract was found to induce convulsions in mice [52].

Chemical composition: The aerial parts of plant are rich in coumarin scopoletin (0.1%), monoterpenes (geraniol, α-terpineol, linalool), diterpenes (neo-hytadiene, phytol), sesquiterpenes (α-ionone, β-ionone, nerolidol, farnesyl acetone, geranyl acetone, β-damascenone, β–bisabolene, elemol, β-eudesmol), alkaloids such as 1-(3-methyl-butyl)-pyrrole-3-carbaldehyde, 1-(3-methyl-butyl)-pyrrole and 1-methyl-pyrrole, sterols (sitosterol, stigmasterol), lipids (linoleic acid, linolenic acid, palmitic acid), and also salicylic acid esters. The bark contains further coumarins esculetin, scopolin and alkaloid manaceine. From roots were isolated also quinolizidine alkaloids hopeanine and manacine; coumarin aesculetin, flavonol glycosides [53]. Other isolated compounds are lignans, saponins, tartaric acid, lactic acid, quinic acid [54] A pyrrole-3-carboxamidine named brunfelsamidine was isolated from root and bark [52].

Caesalpinia spinosa (Molina) Kuntze

Family: Caesalpiniaceae

Synonyms: *Caesalpinia stipulata* (Sandwith) J.F., *Caesalpinia tinctoria* (H. B. K) Bentham ex Reiche, *Caesalpinia pectinata* Cavanilles, *Poinciana spinosa* Molina, *Coulteria tinctoria* HBK, *Tara spinosa* (Molina) Britt & Rose

Vernacular names: Tara, taya (Peru), divi divi de tierra fría, guarango, cuica, serrano, tara (Colombia), vinillo, guarango (Ecuador), tara (Bolivia, Chile, Venezuela), acacia amarilla, dividivi de los Andes (Europe).

Origin and geographic distribution: Native to Cordillera region of Bolivia, Chile, Peru, and occurs in Colombia, Cuba, Ecuador and Venezuela, domesticated in its native range and has a long history of cultivation in North Africa, notably in Morocco and also in tropical East Africa.

Description: Shrub or small tree up to 5 m high with reflexed prickles along its spreading spinose grey-barked densely leafy branches. Leaves bipinnate, smooth or with sparse, short prickles; pinnae 2-3 pairs, often 10 mm long, with about 8 pairs of subsessile, firm, reticulate-veined, oblong-elliptic, glabrous leaflets, oblique at base, rounded at apex, about 2.5 cm long, 1 cm broad. Flowers reddish-yellow, in narrow racemes 8-12 cm long; pedicels puberulent, 5 mm long, auriculate below the short calyx tube; larger calyx segments serrulate, about 6 mm long, the petals less than twice as long, about as long as the stamens. Pods red, flat, 10 cm long, 2.5 cm broad, 4-7 seeded. Seeds large, round and black at maturity [55].

Traditional uses: Traditionally used as eye drops and to treat tonsillitis [55]. The plant produces a resin used to stabilize food, it is used as cure for fever, flu, wound healing, prevent hair lose and is useful against lice and insects [56].

Biological activity: Plant extracts from peel, fruits and leaves of *C. spinosa* have inhibitory effect on the activity of some bacteria. Liu [57] reported that the extracts act selectively in some Gram-positive bacteria, like *Staphylococcus aureus* and *Bacillus subtilis*. The hexane extract of leaflets promoted partial inhibition of the mycelial growth of fungi *Fusarium solani* and *Phoma tarda* [58].

Chemical composition: Pods are high in tannins, contain up to 53 % of gallotannins, 9.5 % of free gallic acid and 6.5 % ellagitannins [59]. Main gallotannins are methyl gallate, ethyl gallate, n-propyl gallate, isobutyl gallate, n-butyl gallate, isoamyl gallate, n-stearyl gallate, pyrogallol, m-hydroxybenzoic acid, p-hydroxybenzoic acid, 4-hydroxybenzoic acid isoamyl ester, 4-hydroxybenzoic acid n-amyl ester, 2,5-dihydroxybenzoic acid and 6-hydroxyisophthalic acid [60].

Cordia alliodora (Ruiz & Pav.) Oken

Family: Boraginaceae

Synonyms: *Cerdana alliodora* Ruiz & Pav., *Cordia gerascanthus* Jacq., *Lithocardium alliodorum* Kuntze

Vernacular names: Cypre, Ecuador laurel, laurel, salmwood, Spanish elm (English), bois de Chypre, pardillo (French), kotia (Samoa), ajo ajo, ajosquiro, chullachaqui caspi, grauana (Peru), alatrique, canalete (Colombia), capá, laurel blanco, laurel negro (Bolivia), kotia (Tonga).

Origin and geographic distribution: Widespread distribution occurring from northern Mexico through Central and South America and as far south as Bolivia, southern Brazil and northern Argentina. It is also found on most of the Caribbean Islands from Cuba to Trinidad.

Description: Tree to 12 m high (to 20 m where indigenous), cultivated at low elevation; petioles 1-3 cm long; leaf blades oblong or lanceolate to elliptic, 10-20 x 3-8 cm, stellate-pilose or glabrate on both surfaces; inflorescences loosely branched, 10-30 cm across; calyx cylindric, 4-6 mm long, densely stellate-tomentose, with 10 prominent ribs; corolla white, drying brown, marcescent, the lobes 5-7 mm long; fruit cylindric, about 5 mm long, enveloped by the persistent corolla and calyx tube [61].

Traditional uses: A decoction of the leaves is used as a tonic for pulmonary diseases in traditional Mexican medicine and is applied on bruises and swellings in Salvador. An ointment made of the plant seeds is employed in the Caribbean Islands to treat skin diseases [62].

Biological activity: Methyl-3-(2,4,5-trimethoxyphenyl)-propionate from the root bark showed high antifungal and larvicidal activities in biological tests [63]. Phenylpropanoid and prenylated hydroquinone from the root bark exhibited antifungal properties against the phytopathogenic mold *Cladosporium cucumerinum*. The phenylpropanoid derivative, whose structure is closely related to β-asarone, also demonstrated a marked activity against

larvae of the yellow-fever-transmitting mosquito *Aedes aegypti* [64]. Six ant-repellant triterpenoids have also been identified in the leaves [65].

The dichloromethane extract from the root bark of *C. alliodora* exhibited activities against the phytopathogenic fungus *Cladosporium cucumerinum* the yeast *Candida albicans* [66].

Chemical composition: Chemical investigations of the heartwood of *C. alliodora* have resulted in the isolation of a prenylated hydroquinone terpenoid alliodorin [67]. In addition, other prenylated quinonoid compounds like alliodorol, allioquinol C, cordiol A, cordiaquinol C, cordallinol, and cordiachromens A-C have been identified [68]. Phenylpropanoid derivative characterized as 1-(3′-methoxypropanoyl)-2,4,5-trimethoxybenzene and a prenylated hydroquinone, 2-(2Z)-(3-hydroxy-3,7-dimethylocta-2,6-dienyl)-1,4-benzenediol have been isolated from the root bark [64]. Six ant-repellant triterpenoids (3α-hydroxyolean-12-en-27-oic acid and five oxidated derivates) have also been isolated from the leaves [65].

Dipteryx micrantha Harms.

Family: Leguminosae

Synonyms: *Dipteryx ferrea* Ducke, *Coumarouna odorata* Wild., *C. ferrea* Ducke, *C. micrantha* (Harms) Ducke

Vernacular names: Cumaru, cumaru amarelo, cumaru roxo, cumaru do Amazonas, kumbaru, paw, muirapaye (Brazil), cumara, cuamara (Guyana), sarrapia (Colombia), guayae, faux, fevetonka, faux gaiac (French Guiana), angustura, serrapia, yape (Venezuela), ebo (Costa Rica, Panama, Honduras), tonka bean (English), shihuahuaco, Charrapilla murcielago (Peru).

Origin and geographic distribution: The cumaru is widely distributed in the Neotropics, extending from the humid forests of Honduras through Central America, to northern South America. It is found in all of the Amazonian countries and along the Caribbean and Atlantic coasts of the Guianas.

Description: Large tree of the primary forest, attaining up to 30 m, although smaller in secondary forests or when cultivated. The trunk is cylindrical, a light brownish-yellow color, with smooth bark and short buttresses (to 1 m). The leaves are compound, alternately pinnate, with elliptical-oblong leaflets which are frequently asymmetric, the whole attaining 20 cm in length by 8 cm in width. The panicle inflorescences are terminal, rusty colored, with hermaphrodite, zygomorphic, aromatic flowers. The fruit is a oblong-oval indehiscent drupe, 5-7 cm long by 3 cm in diameter, yellow-green when mature. The pericarp (pulp) is fleshy, sour, not edible, enveloping woody endocarp. The seed is smooth, hard, 2.5-3 cm in length, dark purple-red in color, furnishing a clear yellow, aromatic (coumarin) oil [69].

Traditional uses: Historically, the extraction of coumarin (orthocoumaric anhydride) from cumaru seed was nearly as important as its principal use. Coumarin was used in the perfume and cosmetic industry and as a flavoring for tobacco. A water extract of bark is popularly used as an antispasmodic and general tonic, acting as an efficient moderator of cardiac action and respiration [69]. The sap of *Dipteryx odorata* was quoted as being administered orally, in the case of a strong attack of furunculosis, in order to purify the blood [70]. The plant is used for burns treating [33].

Biological activity: Polymethoxy flavonoids have shown effects on mammalian cells [71]. Anticarcinogenic [72] and anticoagulant [73] effects of coumarine were described. Seed extract of *D. odorata* exhibits pharmacological activity in the muscular system [74].

Chemical composition:. The presence of coumarins (umbelliferone), isoflavonoids (7-hydroxy-4′,6-dimethoxyisoflavone and 3′,7-dihydroxy-4′,6-dimethoxyisoflavone), triterpenoids, fatty acids and cassane diterpenoids in seeds were described. Additionally, some micromolecules specific of the heartwood as isoflavones (retusin, retusin-8-methyl ether, 3′-hydroxyretusin-8-methyl ether, odoratin, dipteryxin), triterpenes (lupeol and betulin) and methyl esters of fatty acids have been isolated. Examination of the leaf revealed the presence of coumarin, o-coumaric acid, melilotic acid, salicylic acid, ferulic acid, and p-hydroxybenzoic acid [75].

Dracontium loretense K. Krause

Family: Araceae

Synonyms: *Dracontium trianae* Engl., *D. ornatum* K. Krause, *D. spruceanum* (Schott) G.H. Zhu, *Echidnium spruceanum* Schott

Vernacular names: Jergón sacha, fer-de-lance, sacha jergon, hierba del jergon, erva-jararaca, jararaca, jararaca-taia, milho-de-cobra, taja-de-cobr.

Origin and geographic distribution: Widely distributed in the tropical rain forests of Peru, Colombia and Ecuador.

Description: Jergón sacha is a rainforest understory plant that consists of a single, giant, deeply-divided leaf borne from an underground tuber on a long, thick stem which resembles the trunk of a sapling. When fertile, the flower stem emerges from near the base of the plant and rises up to 1–2 m in height. At the end is a large, maroon spathe (a single, petal-like sheath) with bright red-orange, berry-like seeds crowded on a fleshy stalk inside [76].

Traditional uses: Local villagers as well as Indian tribes throughout the Amazon rainforest use the large tuber or rhizome of the plant as an antidote for snake bites, especially bites from the genus *Bothrops*. The tuber is chopped up quickly, immersed in cold water and drunk or applied externally in form of macerate [38] or paste [5]. Indian tribes in Guyana also employ it as an antidote for stingray wounds, spider bites and for poison dart and arrow wounds. In addition to snakebite, the powdered tuberous rhizome is taken internally for asthma, menstrual disorders, chlorosis, and whooping cough in Brazilian herbal medicine. The root powder is used topically for scabies and the juice of the fresh rhizome is applied externally to treat sores caused by blowflies. The whole plant is also decocted and put in baths for gout. In Peruvian natural herbal medicine, it is used as a natural remedy for gastrointestinal problems, hand tremors, HIV/AIDS, cancer, and to enhance general immune strength [48]. The roots are edible [35].

Biological activity: Neutralizing activity was described on *Dracontium croatii* ethanol extract against the edema-forming, defibrinating and coagulant effects of *Bothrops asper*

venom in Swiss Webster mice [77]. Antimycotic activity of *D. loretense* against *Mycobacterium tuberculosis* was studied, but with less than 50% efficiency [23]. Methanol extract of rhizome showed antioxidative properties [34].

Chemical composition: Initial phytochemical screening indicates that the rhizome contains alkaloids, flavonoids, phenols, saponins, sterols, triterpenes, and starch; yet, none of these have been quantified or identified [48].

Equisetum giganteum L.

Family: Equisetaceae

Synonyms: *Equisetum caracasanum* DC.

Vernacular names: Cola de caballo (Peru), árvore de-natal canutillo, carricillo, cauda de cavalo, cauda de eqüina, cauda de rapos, cavalinha, cola de caballo, cola de cavalo, cola de iguana, cola de raton, cola grande de caballo, eqüisseto, erca de canudo, hierba de platero, lija vegetal, limpiaplata, limpia-plata, limpiaplatos, milho de cobra, tembladera, tembladora, yerba del platero (Brazil), caballo chupa (Ecuador), cavalinho gigante, chicote de fraile (Argentina) rabo de cavalo, Rabo de zorro, Taikiji kawayu (Bolivia)

Origin and geographic distribution: Native to South America and Central America, from central Chile east to Brazil and north to southern Mexico.

Description: The stems, growing 2-5 m tall, spring from a creeping rhizome, or root-stock, which produces at its joints a number of roots. Two kinds of stems are produced fertile and barren: they are erect, 1-2 cm diameter, jointed, brittle and grooved, hollow except at the joints and with air-cells in their walls under the grooves. There are no leaves, the joints terminating in toothed sheathes, the teeth corresponding with the ridges and representing leaves. Branches, if present, arise from the sheathbases and are solid. In most cases, the fertile or fruiting stem is unbranched and withers in spring, almost before the barren fronds appear. It bears a terminal cone-like catkin, consisting of numerous closely-packed peltae, upon the under margins of which are the sporanges, containing microscopic spores, attached to elastic threads, which are coiled round the spore when moist and uncoil when dry [32].

Traditional uses: Traditionally known in the Amazonian regions of Bolivia for the treatment of tuberculosis, infections of the gums and oral mucosa, for the treatment of digestive disorders such as diarrhea, enterocolitis, jaundice, liver and spleen imbalances, arteriosclerosis [9]. Besides, being useful in kidney and bladder trouble, a strong decoction acts as an emmenagogue; being cooling and astringent, it is of efficacy for hemorrhage, cystic ulceration and ulcers in the urinary passages [4]. Also used to treat appendicitis [70]. The decoction applied externally will stop the bleeding of wounds and quickly heal them, and will also reduce the swelling of eyelids [78].

Biological activity: Whole plant extracts have diuretic [79] and hypoglycemic [80] effects *in vivo*. Cytotoxicity test with *Artemisia salina* showed 80% mortality at 10 mg/ml [78]. Two varieties of horsetail (*Equisetum* spp.) showed antioxidant activity [81].

Antimycotic activity against *Fusarium oxisporum* a *Penicillium notatum was* described [82]. Antibacterial tests performed using *Staphylococcus aureus*, *Escherichia coli* and *Aspergillus niger* [83] was inactive at concentration of 62.5 mg/ml of plant water extract. Tests with bacteria *Salmonella typhi* and the yeast *Candida albicans* at plant extract concentration of 100 mg/ml didn't show any activity either [84]. Petroleum ether extracts of related species *Equisetum telmateia* have led to MIC values of 39.1 µg/ml against *Staphylococcus epidermidis*, 312.5 µg/ml agains *Staphylococcus aureus* and *Candida albicans*. Ethanol extract showed no inhibition [85].

Chemical composition: The presence of steroids, phenolic compounds, alkaloids, flavonoids and aconitic acid was mentioned [4]. From aerial part, an invertase hysteretic invertase enzyme was isolated [86]. Among the identified components in oleoresin were found alkanes like 3,6-dimethyl decane and *n*-heneicosane, a cuticular hydrocarbon; fatty acids like dodecanoic acid, a glyceride; methyl esters such as 3-nonynoic acid methyl ester; steroid triterpenes such as ergosta-4,7,22-trien-3-one; 26-hydroxicholesterol and gorgost-5-en-3-ol and a naturally-occurring anabolic steroids like methenolone [87].

Maytenus macrocarpa Briq.

Family: Celastraceae

Synonyms: *Maytenus ebenifolia* Reiss., *M. multiflora* (Ruiz & Pav.) Loes., *M. tarapotensis* Briq., *Celastrus macrocarpus* Ruíz & Pav., *Haenkea macrocarpa* (Ruiz Lopez & Pavon) Steud., *H. multiflora* Ruiz & Pav.

Vernacular names: Chucchu huashu, chuchuasi, chuchasha, chuchuhuasha (Ecuador), chocha huasha, chuchuhuasi (Shipibo-Conibo), chuchash, chuchuhuasca, chuchuwasha (Brazil).

Origin and geographic distribution: This tree can be found in Bolivia, Colombia, Ecuador and Peru.

Description: Tree up to 30 m tall, trunk glabrous, subtabular roots frequently. Stalks are generally compressed. Leaf are alternate, elliptic or ovate-elliptic, 7-15 x 2–5 cm. The apex is reduced to sharp or obtuse and the leaf base is obtuse. The margin is entire. The underside is green. Flowers are in fascicles (a contracted cyme). Pedicels are 3-5 mm long. Blossoms are globoses, with sepals slightly adpressed to cernuous. Sepals are 0.5-1 mm long and ciliates. Petals are obovates, style is elongated and the stigma is 2-lobulated. Fruit is ovated, 10-14 x 6-8 mm, with rounded apex [32].

Traditional uses: *M. macrocarpa* is probably the best known of all jungle remedies, in Colombia as well as in Peru used as aphrodisiac, antirheumatic and muscle relaxant medicine. Bark maceration is considered antidiarrheic, antitumor, menstrual regulator, for upset stomach. Its main use is in a cordial or liquor. Its Peruvian name, *chuchuhuasi*, means "trembling back", which refers to its long-standing use for arthritis, rheumatism, and back pain. The bark soaked in the local sugarcane rum (*aguardiente*) is a popular jungle drink. In Peruvian herbal medicine systems, chuchuhuasi alcohol extracts are still used to treat osteoarthritis, bronchitis, diarrhea, hemorrhoids, and menstrual irregularities and pain. In Colombia, the Siona Indians boil a small piece of the bark (5 cm) in 2 liters of water until 1 liter remains, and drink it for arthritis and rheumatism. They also regard the decoction as a stimulant [48]. Luna [7] mentions *M. macrocarpa* as an additivum to *ayahuasca* hallucinogenic drink.

Biological activity: Ethanol extracts of the bark evidenced anti-inflammatory and analgesic activities in various studies with mice. Its anti-inflammatory action was at least partially linked to triterpenes and antioxidant chemicals isolated from the trunk bark [48]. Its antitumoral properties were attributed to tingenone and pristimerin [88]. Anti-inflammatory and anti-arthritic properties determined that alkaloids can effectively inhibit enzyme production of protein kinase C. Among the best studied compounds belong alkaloids maytein and maytansin [89]. β-dihydroagarofuran sesquiterpene polyol esters showed marginal antitumor activity and one also showed low MDR reversing activity on the parasite protozoan *Leishmania tropica* line [90].

Chemical composition:. The main plant chemicals found in this plant include sesquiterpene polyesters [91], triterpenes krukovine A – E [92], macrocarpin A - D, dammarane triterpenes [93], friedelan triterpenes (3-oxo-29-hydroxyfriedelane, 3-oxofriedelan-25-al, canophyllol), chinonoide triterpenes such as tingenon and prismiterin [94], catechin tannins, ebenifoline alkaloids [95], euojaponine alkaloids, laevisine alkaloids, maytansine and mayteine alkaloids (maysin, normaysin, maytansin, maybutin, maytenin, maytanvalin), phenoldienones and proanthocyanidins [48].

Naucleopsis glabra Spruce ex Baillon

Family: Moraceae

Synonyms: *Brosimum acutifolium* subsp. *obovatum* (Ducke) C.C. Berg.

Vernacular names: Tamamuri (Peru), moruré, mururé, congona, mercurio vegetal, puma chaqui, capinurí (Brazil).

Origin and geographic distribution: In Peru in the departments of Loreto, Huánuco, Ucayali and Madre de Dios.

Description: Dioecious tree that grows until 20 m high. It produces a yellowish and translucent sap. Stalks can be yellowish to brown (dark) when are glabrous or yellow to white when are pilose. Leaf arrangement: alternate. Leaves are coriaceous, lanceolates to oblongs, 7–31 x 1.5-10 cm. the apex is acuminate and the base is acute, obtuse or truncate. The bundle is glabrous. The underside is wrinkle and glabrous (but not in the middle vein).

The inflorescence is an emergency, axillary or under the leaves and unisexual. Female flowers are 7-10 mm of diameter, sessile or sub sessile, with 3-7 tepals, with the free part conic or spinose. The pistil is partially jointed to the perianth and has two stigmas. Male flowers are 5-12 mm of diameter. They are in groups of 4 or more, with peduncle (1.5-7 mm long), 3-7 tepals and 2-4 stamens. The fruit is an aggregate of achenes immersed in the succulent receptacle, subglobose, 2-5 cm of diameter and with spinoses or pyramidal pseudo bracts [32].

Traditional uses: *N. glabra* has been used as antirheumatic, antianemic, for the treatment of dyspepsia and gastric ulcers by the Shipibo-Conibo, ethnic group of the Peruvian amazonia in the basin of the river Ucayali [33]. The latex from *Naucleopsis* sp. has been used in South America in Colombia, Ecuador and north-west Brazil as a source of dart poison [96].

Biological activity: There are no reports on biological activity of *N. glabra.*

Chemical composition: Dart poison called *niaará* prepared by the Chocó Indians of western Colombia from latex of *N. glabra* contains cardiac glycosides α-antiarin a β-antiarin, antialloside and convallatoxin. The ethanol extract (1 %) of *Naucleopsis caloneura* led to isolation of an aliphatic ketone, sitosterol and seselin [97]. Betaines were isolated from latex of several *Naucleopsis* species [98].

From *Brosimum acutifolium* (synonym) bark had been isolated flavans: acutifolins A–F [99], brosimine A and brosimine B and three other flavanes [100] and also 40,7-dihydroxy-8-prenylflavan [101], flavonolignans mururins A, B and C [102], further isobavachin, liquiritigenin, 4-hydroxylonchocarpin, 4-hydroxyisocordoin, isoliquiritigenin, coniferaldehyde, syringaldehyde, sitosterol and stigmasterol, flavonoids named brosimacutins A-I [103] and brosimacutins J-M with cytotoxic properties [104].

Phyllanthus amarus Schum. et Torn.

Family: Euphorbiaceae

Synonyms: *Nymphanthus niruri* Lour., *Phyllanthus carolinianus* Blanco, *P. debilis* Willd., *P. humilis* Salisb., *P. kirganelia* Blanco, *P. niruri* Mueel Arg., *P. pentaphyllus* Wright, *P. urinaria* Wall.

Vernacular names: Maigo-lalo (Chamorro), nikémméwúr, walimo, walumo (Chuukese), six o'clock, sleeping plant (English), te kaimatu (I-Kiribati), moemoe, pī (Maori, Cook Islands), tuui (Marquesan), jil jino auo, jil jino awa, jiljino (Marshallese), ukalla ruchel (Palauan), fua lili'i, lau lili'i (Samoan), moemoe, moemoe uouo, tebe (Tahitian), moemoe (Tuamotuan), rurudai (Yapese).

Origin and geographic distribution: It is indigenous to the rainforests of the Amazon and other tropical areas throughout the world, including the Bahamas, southern India, and China. *P. amarus* is quite prevalent in the Amazon and other wet rainforests, growing and spreading freely (much like a weed).

Description: Small herbs, usually under 30 cm tall, with numerous small oblong-elliptic or squarish leaves, glabrous, about 6-12 mm long; flowers very small, in cymules hidden under the leaves; cymules bisexual, of 1 male and 1 female flower; calyx-lobes 5, acute; pedicels 2 mm long; capsule small, depressed-globose; seeds 5-7-ribbed [105].

Traditional uses: The Spanish name of the plant, *chanca piedra*, means "stone breaker" or "shatter stone". It was named for its effective use to generations of Amazonian indigenous peoples in eliminating gallstones and kidney stones. In addition to kidney stones, the plant is employed in the Amazon for numerous other conditions by the indigenous peoples, including colic, diabetes, malaria, dysentery, fever, flu, tumors, jaundice, vaginitis, gonorrhea, and dyspepsia. A standard herb infusion or weak decoction is prepared as the traditional remedy. Depending on what it's employed for, 1-3 cups are taken daily [106].

Biological activity: In clinical research over the years, the plant has demonstrated liver protective, antilithic (expels stones), pain-relieving, hypotensive, antispasmodic, antiviral,

antibacterial, antioxidative, anticarcinogenic, diuretic, antimutagenic, and hypoglycemic activities [48].

Leaf extract in 70% ethanol demonstrated significant *in vitro* antibacterial activity against *Staphylococcus aureus* (MIC 200 µg/ml), *Micrococcus luteus* (MIC 900 µg/ml), but was inactive against *Escherichia coli* (MIC > 50 µg/ml) [107]. On the other hand, methanol extract of leaves inhibited *Staphylococcus aureus* at concentration of 1 mg/ml [108], *Pseudomonas aeruginosa* at 2 mg/ml and *Salmonella typhi* at 4 mg/ml [109]. Chloroform extract of leaves at concentration 1 mg/ml tested against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* was inactive [108]. On the other hand, ethanol root extract was found to be active against *E. coli* [110]. The antimicrobial tests mentioned above were performed by disc diffusion method at agar plates. Antimalarial activity validated with traditional uses [111].

Quercetin, quercitrin, rutin and limonene [54] and phyllanthin [110] are described as responsible for antimicrobial effect.

Chemical composition: The main plant chemicals in chanca piedra include monoterpenes (cymene, (-)-limonene), triterpenes (lupeol, phyllanthenol), coumarines (ellagic acid, brevifolin), steroids (estradiol, 24-iso-propyl cholesterol), tannins (corilagin, geraniin), flavonoids (gallocatechins, (+)-catechin, (-)-epicatechin, quercetin, quercetol, quercitrin, rutin), lignans (hypophyllanthin, hinokinin, lintetralins, lintetralins, niranthin, nirphyllin, phyllanthin, phyltetralin), triterpenes (lupeol), steroids (fraternosterol, phyllantosterol, β-sitosterol), indolizidine alkaloids (nirurin, phyllanthine, phyllochrysine), pyrrolizidine alkaloids norsecurinines and salicylic acid [48].

Piper aduncum L.

Family: Equisetaceae

Synonyms: *Artanthe adunca* (L.) Miq., *A. celtidifolia* (Kunth) Miq., *Piper aduncifolium* Trel., *P. angustifolium* R. & P., *P. anguillaespicum* Trel., *P. celtidifolium* Kunth, *P. disparispicum* Trel., *P. elongatum* Vahl var. *laevifolium* (C.DC.) Trel., *P fatoanum* C.DC., *P. flavescens* (C.DC.) Trel, *P. hebecarpum* C.DC, *P. intersitum* Trel., *P. intersitum* Trel. var. *porcecitense* Trel., *P. martinicense* C.DC., *P. multinervium* M.Martens &

Galeotti, *P. oblanceolatum* Trel. var. *fragilicaule* Trel., *P. pseudovelutinum* C.DC. var. *flavescens* C.DC., *P. stehleorum* Trel., *P. submolle* Trel., *P. subrectinerve* C.DC., *Steffensia adunca* (L.) Kunth, *S. celtidifolia* Kunth

Vernacular names: Aerta ruão, anisillo, bamboo piper (English), cola de caballo (Peru), cordoncillo, cow's foot, false kava, false matico, guayayo, higuillo, higuillo de hoja menuda (Spanish), jaborandi-do-mato (Portuguese-Brazil), jointwood, man anesi wiwiri, matico, pimenta-de-macaco (Portuguese-Brazil), Santa María negra, spiked pepper (English), yanggona ni Onolulu (Fiji), yaqona ni Onolulu (Fiji)

Origin and geographic distribution: *Piper aduncum* is common throughout Central America where it is found between sea level and 2,000 m a.s.l. along roadsides and in forest clearance areas on well-drained soils. It occurs in Mexico, Central America, Surinam, Cuba, Southern Florida, Trinidad and Tobago, and Jamaica and is very common in Costa Rica on open or partly shaded sites.

Description: A shrub or small tree up to 7 m tall and 10 cm or more in stem diameter, with short silt roots and medium-hard, brittle wood; foliage and twigs aromatic. Can grow as individual plants or in thickets. Branches are erect, but with drooping twigs and swollen, purplish nodes. Leaves alternate, distichous, elliptic, 12-22 cm long, shortly petiolate; lamina scabrid above, with sunken nerves, softly hairy beneath. Inflorescence a leaf-opposed, curved spike on a 12-17 cm peduncle, white to pale yellow, turning green with maturity. Flowers crowded in regular transverse ranks. Perianth absent; usually 4 stamens. Fruit a 1-seeded berry, compressed into greyish, wormlike spikes. Seeds brown to black, 0.7–1.25 mm long, compressed, with a reticulate surface [112].

Traditional uses: The leaf infusion is taken orally as a tonic and stomach pain reliever. It is also considered astringent. In the upper Ucayali, the locals use the leaves in baths to relieve rheumatic pains. The plant is considered effective against neuralgia. In Colombia, the tincture is used as a headache reliever and a stimulant. The leaves are applied externally as antiseptic and vulnerally [4]. Essential oils from this species have antibacterial properties and may also be used as an insecticide and a molluscicide. Tea made from the leaves and roots is used to treat diarrhea, dysentery, vomiting, ulcers, and can also be used for the control of bleeding [113].

Biological activity: The methanol a dichloromethane extracts were active in the brine shrimp cytotoxicity bioassay [38]. *Piper aduncum* exhibited attractive antiviral, cytotoxic [114], antigonorrheal [115] and antiprotozoal [116] activities.

Ethanolic extract of *P. aduncum* also showed inhibitory activity in agar-well diffusion tests against the AIDS-related pathogens *Candida albicans*, *Cryptococcus neoformans*, *Mycobacterium intracellularae*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Pseudomonas aeruginosa* and isolated 4-methoxy-3,5-bis-(3′-methylbenzoic acid) inhibited *Bacillus subtilis* at MIC of 12.5 μg/ml [117]. The ethanol extract of leaves performed mediate antimicrobial activity against oral pathogens [118]. Ethanol solution of essential oil from stems of *P. angustifolium* exhibited moderate bacteriostatic and fungistatic activities against *Trichophyton mentagrophytes* (MIC 10 μg/ml), *Pseudomonas aeruginosa* (MIC 30 μg/ml), *Candida albicans* (MIC 50 μg/ml) and *Escherichia coli* (MIC 100 μg/ml) described by Tirillini [119]. The essential oils from fruits of *P. aduncum* showed antifungal activity with the MIC of 10 μg/ml as determined against *Cladosporium cladosponoides* and *C. sphaerospermum* [120].

Dihydrochalcones asebogenin, piperaduncins A, B and C, flavonone sakuranetin, anodendroic acid methyl ester, carotenoid lutein and benzoic acid derivates posessed antimicrobial, cytotoxic [121] and molluscicidal [122] properties.

Chemical composition: Phenylpropanoids, such as myristicin, nerolidol and dillapiol, prenylated benzoic acid derivatives including nervogenic acid, chromenes [123, 124], chalcones, dihydrochalcones asebogenin, piperaduncins A, B and C [117], sesquiterpenes - humulene, caryophyllene epoxide and humulene epoxide, flavonone sakuranetin, anodendroic acid methyl ester, carotenoid lutein, monoquiterpenes (m-cymene, β-pinene), sitosterol and stigmasterol were isolated [125].

Essential oil of *P. angustifolium* (synonym) is rich in camphor (23 %), camphene, isoborneol, α-pinene, β-bisabolol, caryophyllene and myristicine [119].

Pterocarpus rohrii M. Vahl

Family: Leguminosae

Synonyms: *Amphymenium rohrii* (Vahl) Kunth, *A. villosum* Mart. ex Benth. *Apalatoa spicata* Aubl., *Lingoum rohrii* (Vahl) Kuntze, *L. rufescens* (Benth.) Kuntze, *Phellocarpus floridus* Benth., *Piscidia florida* Mart. ex Benth., *Pterocarpus. apalatoa* Rich., *P. floribundus* Pittier, *P. hayesii* Hemsl., *P. magnicarpus* Schery, *P. reticulatus* Standl., *P. rufescens* Benth. *P. rupestris* Pittier, *P. steinbachianus* Harms, *P. villosus* (Mart. ex Benth.) Benth., *P. violaceus* Vogel, *P. zehntneri* Harms

Vernacular names: Chepa (Guatemala), cuajada amarilla (Costa Rica), drago (Nicaragua), jaguar caspi (Peru), jicarillo, llora sangre (Mexico), palisangre; palo de sangre (Colombia, Mexico, Nicaragua, Peru), palo sangre (Honduras, Peru), sangre, sangre blanca, sangre blanco (Honduras), sangre de gallo (Colombia, Panama), sangré de gallo; sangre drago (Honduras, Nicaragua), sangre drago del Pacifico, sangredo (Colombia, Nicaragua), sangrito, supilote (Nicaragua), tablón (Colombia), verdolago blanco (Bolivia), yema de huevo (Honduras).

Origin and geographic distribution: Common in swamp forests of South America (Colombia, Venezuela, Suriname, Ecuador, Peru, Bolivia).

Description: Tree that grows to 30 m high. From the bark exudes red sap. Leaves are imparipinnates with alternate leaflets. The leaflets are oblongs or oblong-elliptics, more or less symmetric and variable size. The apex is acuminate and the base is rounded or truncate to slightly emarginated. Bundle is glabrous or densily pilose. The underside of the leaflets, the leafstalk and the pedicels are pilose and lately glabrescent. Stiples are deciduous and 3-10 cm long. The inflorescence has few or lots of yellow or orange flowers. Calyx is 7-11 mm long. Corolla is formed by three different petals: the banner is 11–19 x 14 mm with or without a violet spot in the middle; keel is equal or shorter than the two wings. The flowers have ten monadelphous or diadelphous stamens. The fruit is an indehiscent samara, rounded, even, until 5 mm thick, with only one seed completely surrounded by a hard and slightly wavy wing [32].

Traditional uses: This tree is very popular for it red timber, commonly used in carpentry. An alcohol extract of bark is used in medicine as a tonic, antimalarial remedy [5] and for wound healing [126] in the Amazon regions. In that case the stem bark is pulverized and directly applied on the skin.

Biological activity: There are no reports on biological activity of *P. rohrii,* but taxonomically closely related species, *P. indicus,* had been tested for antimicrobial activity and showed a wide spectrum of activity against the tested bacteria and protozoan [127]. In a posterior study, extract from leaves of the same plant showed moderate activity against *Candida albicans* and low activity against *Pseudomonas aeruginosa, Escherichia coli* and *Aspergillus niger,* but was found inactive against *Staphylococcus aureus, Bacillus subtilis* and *Trichophyton mentagrophytes* [128]. The ethanol stem bark extract of *P. amazonum* showed good *in vivo* antimalarial activity against *Plasmodium vinckei* [129].

Chemical composition: There are no reports published on chemical composition of *Pterocarpus rohrii.*

Solanum mammosum L.

Family: Solanaceae

Synonyms: *Solanum platanifolium* Hook., *Solanum globiferum* Dunal, *Solanum mamosissimum* Ram. Goyena

Vernacular names: Tintona (Peru), cocona venenosa, torito (Bolivia).

Origin and geographic distribution: Indigenous to South America, commonly planted also in Central America and Caribic. In Europe cultivated as ornamental for the specific shape of fruit.

Description: Annual to shrubby perennial, to 1.5 m tall, stems flexuous, white and brown simple pubescent with a few porrect-stellate hair clusters, with spines 11-14 mm long, flattened, straight or curved. Leaves with petioles 20-45 mm long, blades orbicular in outline, 6-14 x 7-14 cm, base slightly cordate, apex acute, margins sharply angular or dentate, white pilose on both surfaces, acicular spines on midrib and veins below.

Inflorescence congested umbelliform, 1- or 2- to 6-flowered, pedicels 6-10 mm long, villose; calyx campanulate, 5 mm in diameter, lobes linear acuminate 5 mm long, villose; corolla 18-24 mm long, lobes lanceolate-oblong, acuminate, purple; anthers 9-12 mm long. Berry 32-60 mm long, 30-45 mm in diameter, mammose, the basal protuberances 3 or 5, orange, shiny [130].

Traditional uses: *Solanum mammosum* is used in some treatments such as skin ailments that call for poison and scabies, furunculosis and rashes. The fruits are mashed and this paste is rubbed over the affected area. Before soap was common in villages, the juice was used as a detergent to wash clothes [129]. In Guatemala it is used for the treatment of protozoal infections [131].

Biological activity: Steroidal alkaloids and their glycosides occurring in numerous species of *Solanum* are known to possess a variety of biological activities including antifungal, antiviral, molluscicidal, teratogenic, and embryotoxic. Preparations containing solasodine are currently being employed in the treatment of certain skin cancers [132]. Alzerreca [133] demonstrated that a *S. mammosum* fruit methanol crude extract showed a good molluscicidal activity on *Lymnaea cubensis* snails. Cipollini [134] described antifungal properties of *Solanum* fruit glycoalkaloids.

Methanol extracts from *Solanum deflexiflorum* Bitter (MIC 0.31 mg/ml) and the dichloromethane extract from *Solanum leucocarpun* Dunal (MIC 0.31 mg/ml) were bioactive against the Gram-positive bacteria *Bacillus subtilis* in agar-well diffusion assay [135].

Chemical composition: A mixture of steroidal glycoalkaloids obtained from *Solanum mammosum* fruits contains solasonine 1 and solamargine 2 and the stereoisomeric glycosidic alkaloid tomatine 3 and their aglycones solasodine 4 and tomatidine 5 obtained by hydrolysis of the glycosides. Molluscicidal spirosolane and spirostane glycosides also recognized [133].

Terminalia catappa L.

Family: Combretaceae

Synonyms: *Terminalia procera* Roxb., *T. latifolia* Blanco, non Swartz, *T. intermedia* Bertol., *T. moluccana* Lamk, *T. paraensis* M, *T. subcordata* Willd, *Buceras catappa* Hitchc. Rep., *Juglans catappa* Lour.

Vernacular names: Almendra (Peru), amendoeira, badamier (French), castanhola, ketapang, lingkak (Malaysia), talisai, dalinsi, logo (Indonésia), huu kwang (Laos), báng bien, dat mue (Thailand), tropical almond (English).

Origin and geographic distribution: Native to South-East Asia, where it is common throughout the area, commonly planted as ornamental tree in Australia, Polynesia, West Africa and the lowlands of South and Central America.

Description: Tree up to 35 m tall, with an upright, symmetrical crown and horizontal branches. As the tree gets older, its crown becomes more flattened to form a spreading, vase shape. The leaves are large, 15-25 cm long and 10-14 cm broad, ovoid, glossy dark green and leathery. They are dry-season deciduous; before falling, they turn pinkish-reddish or yellow-brown, due to pigments such as violaxanthin, lutein, and zeaxanthin. The flowers are monoecious, with distinct male and female flowers on the same tree. Both are 1 cm diameter, white to greenish, inconspicuous with no petals; they are produced on axillary or terminal spikes. The fruit is a drupe 5-7 cm long and 3-5 cm broad, green at first, then yellow and finally red when ripe, containing a single seed [136].

Traditional uses: Tannin and a black dye can be obtained from the bark, foliage and fruits. Young leaves are taken internally for colic. The astringent bark, leaves and fruits are used to treat diarrhea, dysentery and as a febrifuge. A decoction of the leaves is used to get rid of intestinal parasites (Philippines), treat eye problems, rheumatism, wounds (Samoa), and stop bleeding during teeth extraction (Mexico). The juice of young leaves is taken internally for headache and colic and externally for scabies and other skin problems. Bathing in water with macerated leaves is supposed to be good for the itch and external ulcers. Fallen leaves are used to treat liver diseases in Taiwan folk medicine. Fruits and

bark are believed to help against coughs (Samoa) and asthma (Mexico). In Brazil, the bark decoction is used against fever [137].

Biological activity: For popular use of this species worldwide, numerous studies were published, describing antioxidant [138, 139], hepatoprotective [140], antiHIV [141], antidiarrheal [142], anti-inflammatory [143], antidiabetic [144], immunostimulating, hypocholesterolemic, diuretic, and antitumor activity [54].

Antimicrobial activity of dried root was tested by cup plate agar diffusion method. The methanol extract exhibited MIC of 0.065 mg/ml against *Escherichia coli* and chloroform extract exhibited MIC of 0.4 mg/ml against *Staphylococcus aureus*. The chloroform as well as methanol extracts showed good antimicrobial activity against Gram-positive and Gram negative microorganisms [145]. Methanol extract of leaves was tested at 100 mg/ml and showed antifungal activity against *Pythium ultimum*, *Rhizoctonia solani*, *Sclerotium rolfsii* and *Aspergillus fumigatus*, but was inactive against tested bacteria *Escherichia coli*, *Staphylococcus aureus*, *Xanthomonas campestris*, *Bacillus subtilis* and the yeast *Candida albicans* [146].

Chemical composition: Amino acids, fatty acids, tannins (castamollinin, castalin, catappanin A, chebulagic acid, geraniin, granatin B, punicalin, punicalagin, terflavin A and B, tergallagin, tercatain, (-)-epicatechin, (+)-catechin) and β-carotene were isolated [147]. Tannic acid, phytic acid, polyphenols including corigalin, gallic acids, ellagic acid [148], flavonoids and flavone glycosides – quercetin, isovitexin, vitexin, isoorientin and rutin [149] were also described.

Uncaria tomentosa DC.

Family: Rubiaceae

Synonyms: *Uncaria surinamensis* Miq., *Ourouparia tomentosa* K. Schum., *Nauclea tomentosa* Wild., *Nauclea aculeata* Kunth

Vernacular names: Garabato, rangaya, unganangui, tua juncara, samento, saventaro (Ashánika), kug kukjaqui (Aguaruna, Huambisa, Jibaros), paotati-mosha, uña de gato, uña

huasca, uña de gavilan (Shipibo-Conibo), gatura, gatuna, toront, tambo huasca, ox tooth, willca cora (sacred plant – English).

Origin and geographic distribution: Indigenous to the Amazon rainforest and other tropical areas of South and Central America, including Peru, Colombia, Ecuador, Guyana, Trinidad, Venezuela, Suriname, Costa Rica, Guatemala and Panama.

Description: Large climbing shrub approximately 20 m high. The young branches have a square shape. The branches have strong, 2 cm long by 0.4 cm to 0.6 cm wide, woody thorns that point down, not entwined. The claws are in clusters of three and born in the base of the leaves (bud). Leaves primary reddish, oblong to elliptic, 6 to 12 cm long, simple, of opposite disposition, velvety, opaque, dark yellowish green. Flowers hermaphrodite, fragrant, solitary or grouped in clusters. Fruit of brownish color, fuzzy and dry, 3.5-4 cm long, fusiform, bivalve. The seeds have meaty albumen [150].

Traditional uses: The Asháninka use this plant to treat asthma and inflammations of the urinary tract, to recover from childbirth, as a kidney cleanser, to cure deep wounds, for arthritis, rheumatism, and bone pain, to control inflammation and gastric ulcers, and for cancer. Indigenous tribes in Piura use it to treat tumors, inflammations, rheumatism, and gastric ulcers. Indian tribes in Colombia use the vine to treat gonorrhea and dysentery. Other Peruvian indigenous tribes use it to treat diabetes, urinary tract cancer in women, hemorrhages, menstrual irregularity, cirrhosis, fevers, abscesses, gastritis, rheumatism, inflammations, for internal cleansing and tumors. Reportedly, the plant has also been used as a contraceptive by several different tribes of Peru [48].

Biological activity: Analgesic, antimutagenic, anti-inflammatory, antioxidant, antiviral, cytotoxic, immunostimulant, hypotensive ativity has been described [25]. Epicatechins and cinchonains are responsible for anti-inflammatory and antiviral properties [151]. The cinchonains are also strong antioxidants, what is typical for the entire group of proanthocyanidins. The antitumor activity was described [152].

Antibacterial activity has been studied on *Photobacterium phosphoreum* and *Salmonella typhimurium*. [153]. *U. tomentosa* dichloromethane and methanol extracts showed antimicrobial activity against *Micrococcus flavus* and *Bacillus subtilis* and was inactive

against *Candida albicans* and *Sacharomyces cerevisiae*. Isolated oxindol alkaloid isopteropodine was active only against the Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*, with a MIC value of 150 μg/ml and 250 μg/ml, respectively [154].

Uncaria tomentosa extract protects mice from a lethal dose of *Listeria monocytogenes* [155].

Chemical composition: About 130 substances have been isolated and identified. Roots, bark and leaves are rich in pentacyclic oxindol alkaloids (pteropodine, isopteropodine, uncarine, speciophyline, mytrafylline, hirsutine, hirsuteine) and tetracyclic oxindol alkaloids (isorhynchofylline, rhynchofylline), pentacyclic triterpenoids, carboline alkaloids, proanthocyanidines cinchonain Ia and Ib, quinovic acid glycosides, polyfenols, coumarines, phytosterols (*β*-sitosterol, campesterol, stigmasterol) and others [48].

4. Experimental part

4.1. Materials and methods

4.1.1. Chemicals and instruments

Chemicals

Ethanol 96% pharm.	Lach-Ner, s.r.o., Neratovice, CZ
Dimethyl sulfoxid (DMSO) p.a.	Lach-Ner, s.r.o., Neratovice, CZ
Tris- buffer saline pH 7.6 (TBS)	Sigma-Aldrich, Prague, CZ

Antibiotics

Ciprofloxacin	Sigma-Aldrich, Prague, CZ
Nystatin	Sigma-Aldrich, Prague, CZ

Cultivation media

Mueller-Hinton broth (pH 7.4 ± 0.2) — Oxoid, Basingstoke, UK

Composition: Beef, dehydrated infusion 300.0 g/l
Casein hydrolysate 17.5 g/l
Starch 1.5 g/l

Brain heart infusion broth (pH 7.4 ± 0.2) — Oxoid, Basingstoke, UK

Composition : Brain infusion solids 12.5 g/l
Beef heart infusion solids 5.0 g/l
Proteose peptone 10.0 g/l
Glucose 2.0 g/l
Sodium chloride 5.0 g/l
Di-sodium phosphate 2.5 g/l

Wilkins-Chalgren anaerob broth (pH 7.1 ± 0.2) — Oxoid, Basingstoke, UK

Composition: Tryptone 10.0 g/l
Gelatin peptone 10.0 g/l
Yeast extract 5.0 g/l
Glucose 1.0 g/l
Sodium chloride 5.0 g/l
L-Arginine 1.0 g/l

Sodium pyruvate	1.0 g/l
Menadione	0.0005 g/l
Haemin	0.005 g/l

For each culture broth was prepared in two different concentrations: simple and double, following the product instructions. The required quantity was weighted on the analytical scales KERN 770 and dissolved in distilled water, heated if necessary. Then it was placed into penicillin bottles of 20 ml volume, which were sealed, sterilized by autoclaving and stored in a fridge.

Sterilization

All prepared media, TBS and laboratory material were sterilized in the autoclave at 120°C and 1.2 MPa for 30 minutes or single-use sterile needles, syringes, 96-well microtitration plates U-type and test tubes were used.

Laboratory instrumens

Homogenisator Grindomix GM 100	Retsch GmbH, Haan, GER
Sartorius filtration device	Sartorius AG, Goettingen, GER
Evaporator Büchi Rovapor 205	Büchi Labortechnik AG, Flawil, CH
Anaerobic Jar HP11	Oxoid, Basingstoke, UK
Densitometer Densi-La-Meter	Lachema, a.s., Neratovice, CZ
UV-VIS spectrometer Helios ε	Spectronic Unicam, Cambridge, UK
Analytical balance KERN 770	Kern & Sohn GmbH,Balingen, GER
Pipettes (single-, 12-channel, volume 0.1 – 1 ml)	Eppendorf AG, Hamburg, GER

4.1.2. Plant material

4.1.2.1 Peruvian medicinal plants

Plants were chosen after consultations with five local healers and herbalists from Pucallpa and adjacent villages (San Francisco, Nueva Belen) and their information was compared with literature describing ethnomedicinal uses of plants in Peruvian Amazon [4, 33, 35, 156, 157]. Plant samples were purchased from herbalist on local market in Pucallpa. After his confirmation of species authenticity of whole plants growing in the field, voucher specimens were prepared and authenticated by Dr. Polesny from Institut of Tropics and Subtropics, Czech University of Agriculture Prague. All selected species are frequently used by local healers and commonly occur in the studied area. Voucher specimens are deposited at the Herbarium of Faculty of Forestry Sciences, National University of Ucayali, Pucallpa. Ethnobotanical data of the plant species selected for the study (botanical names, families, common names, voucher specimen numbers and uses of the tested parts in traditional medicine) are summarized in Table 1.

Table 1. *Ethnobotanical data of tested Peruvian medicinal plants*

Species (family) and voucher specimen number	**Common name**	**Part tested**	**Ethnomedicinal uses**	**Preparation**
Abuta grandifolia (Mart.) Sandwith (Menispermaceae) POL 0012	Abuta	Stem bark	Gastric ulcers, hepatic ailments, malaria, snake bites [4], contraceptive, tuberculosis [19], antianemic, menstrual problems, tonic [8], diuretic, diabetes, uterus infections [35], typhoid fever [33]	Decoction
Bertholletia excelsa Bonpl. (Lecythidaceae) POL 0017	Castaña	Seed pods	Hepatitis, stomachaches [40]	Infusion
Brunfelsia grandiflora D. Don (Solanaceae) POL 0001	Chiri sanango	Root	Rheumatism, yellow fever [4], bronchitis, colds, fevers, kidney disorders, lung diseases like tuberculosis, snakebite, scrofula, syphilis, ulcers [5], abortifacient, hallucinogenic, laxative, blood cleanser [40]	Tincture, decoction
Caesalpinia spinosa (Molina) Kuntze (Caesalpiniaceae) POL 0002	Tara	Pods	Eyewash, tonsillitis [55]	Macerate, infusion

Table 1. (Continued) *Ethnobotanical data of tested Peruvian medicinal plants*

Species (family) and voucher specimen number	**Common name**	**Part tested**	**Ethnomedicinal uses**	**Preparation**
Cordia alliodora (Ruiz & Pav.) Oken (Boraginaceae) POL 0014	Ajosquiro	Stem bark	Colds, influenza, fever, rheumatism [33]	Decoction
Dracontium loretense K. Krause (Araceae) POL 0003	Sacha jergón	Rhizome	Asthma, bronchitis, cough, viral infections, venomous insect bites, wounds [48], diarrhea [5]	Macerate, fresh, paste
Dipteryx micrantha Harms (Leguminosae) POL 0013	Shihuahuaco	Stem bark	Antispasmodic, tonic [69], furunculosis [70], burns treating [33]	Decoction
Equisetum giganteum L. (Equisetaceae) POL 0004	Cola de caballo	Aerial part	Arteriosclerosis, diarrhoea, enterocolitis, liver and spleen imbalances, oral infections, tuberculosis [9], astringent, emmenagogue, hemorrhage, ulcers [4], appendicitis [70], wounds [78]	Infusion
Maytenus macrocarpa Briq. (Celastraceae) POL 0005	Chuchuhuasi	Stem bark, root bark	Bronchitis, diarrhea, tumors, menstrual problems, arthritis, rheumatism, osteoarthritis, hemorrhoids [48]	Macerate, decoction
Naucleopsis glabra Spruce ex Pittier (Moraceae) POL 0015	Tamamuri	Stem bark	Rheumatism, anemia, dyspepsia, gastric ulcers [33]	Decoction

Table 1. (Continued) *Ethnobotanical data of tested Peruvian medicinal plants*

Species (family) and voucher specimen number	**Common name**	**Part tested**	**Ethnomedicinal uses**	**Preparation**
Phyllanthus amarus Schum. et Thonn. (Euphorbiaceae) POL 0007	Chanca piedra	Aerial part	Bronchitis, diarrhea, gallstones, hepatitis, kidney problems, kidney stones, urinary infections, worms, to stimulate menstruation [78]	Infusion, decoction
Piper aduncum L. (Piperaceae) POL 0006	Matico	Aerial part	Antiseptic, vulnerally [4], diarrhea, dysentery, vomiting, ulcers [113]	Bath, infusion
Pterocarpus rohrii Vahl (Leguminosae) POL 0016	Palisangre	Stem bark	Antimalarial, tonic [5], wound healing [126]	Pulver, tincture
Solanum mammosum L. (Solanaceae) POL 0018	Tintona	Fruit	Furunculosis, scabies, skin ailments, rashes [129], protozoal infections [131]	Paste
Terminalia catappa L. (Combretaceae) POL 0008	Almendra	Leaves	Colic, eye problems, external ulcers, intestinal parasites, itch, liver diseases, rheumatism, scabies, wounds [20, 137]	Decoction, juice, bath
Uncaria tomentosa DC. (Rubiaceae) POL 0009	Uña de gato	Stem bark	Anticancer, antidiabetic, anti-inflammatory [4]	Infusion, decoction

Extract preparation

Air dried material was finely ground to powder, using a homogenisator (Restsch Grindomix, 5000 turn per minute). The extracts were prepared by maceration of powdered plant material (15.00 g) in 450 ml of 80% ethanol (375 ml of 96% ethanol + 75 ml of distilled water), at room temperature for five days in a dark place. All extracts were subsequently filtered (paper filter 80 g/m^2) under pressure, with a Sartorius filtration device, and evaporated *in vacuo* on vacuum evaporator Rotavapor R-200 at 40°C. The residue for each extract obtained after evaporation is given in Table 2.

Table 2. *Yields of Peruvian plants extracts (in 450 ml of 70% ethanol) after evaporization*

Species	Part tested	Yield (g)	Yield (%)
Abuta grandifolia	Stem bark	0.77	5.13
Bertholletia excelsa	Seed pods	0.60	4.00
Brunfelsia grandiflora	Root	2.03	13.53
Caesalpinia spinosa	Pods	7.30	48.67
Cordia alliodora	Stem bark	0.83	5.53
Dipteryx micrantha	Stem bark	1.19	7.93
Dracontium loretense	Rhizome	1.18	7.87
Equisetum giganteum	Aerial part	1.10	7.33
Maytenus macrocarpa	Stem bark	1.79	11.93
Maytenus macrocarpa	Root bark	1.67	11.13
Naucleopsis glabra	Stem bark	0.66	4.40
Phyllanthus amarus	Aerial part	1.97	13.13
Piper aduncum	Aerial part	0.91	6.07
Pterocarpus rohrii	Stem bark	1.88	12.53
Solanum mammosum	Fruit	0.71	4.73
Terminalia catappa	Leaves	2.18	14.53
Uncaria tomentosa	Stem bark	1.00	6.67

It is advisable to extract the plants and to evaporate the extracts at low temperature in order not to destroy any thermo-labile antimicrobial agents present in extract. Therefore,

sterilization of the extracts by autoclaving or other strenuous methods should be avoided as well. Also sterilization by membrane-filtration has its disadvantage since many antimicrobial agents can be adsorbed on the filter material, rendering the extract inactive [158].

Extract stock solution

On analytical balance (KERN 770) 0.16 mg of each crude evaporated extract was weighed and dissolved in 0.5 ml of DMSO and afterwards 4.5 ml of sterile TBS pH 7.6 was added, to create a stock solution of 32 mg/ml for testing in antimicrobial assay.

4.1.2.2 Common antimicrobial plants

Plants were selected due to their usage in traditional medicine for treating infections and for occurrence in literature as source of strong antimicrobial agents [159-161]. Plant material samples of *Allium sativum* and *Vitis vinifera* were bought in Czech supermarket (Delvita, Prague, CZ). *Hypericum perforatum* was obtained as dried herb from a pharmacy (Natura, Děčín, CZ), *Vicia faba* was bought from Semo s.r.o. (Smržice, CZ) and *Curcuma longa* was obtained from the greenhouses of the Institute of Tropics and Subtropics (ITS) of the Czech University of Agriculture Prague. Ethnobotanical data of the plant species selected for the study (botanical names, families, common names, antimicrobial constituents and uses of the tested parts in traditional medicine) are summarized in Table 3.

Table 3. *Ethnobotanical data of common antimicrobial plants with documented antimicrobial activity*

Species (family)	**Common English name**	**Part tested**	**Active antimicrobial compound**	**Ethnomedicinal uses**
Allium sativum (Liliaceae)	Garlic	Bulb	Allicin, ajoene (thiosulfunates)	Candidosis, meningitis, pneumonia, cholera, dysentery, tuberculosis [161]
Curcuma longa (Zingiberaceae)	Curcuma	Rhizome	Curcumin (polyphenol)	Diarrhea, dysentery, gonorrhea, urogenital infections [161]
Hypericum perforatum (Hypericaceae)	St. John's wort	Flowering tops	Hypericin (polycylic quinone) Hyperforin (phloroglucin derivative)	Gastritis, hemorraghe, pulmonary disorders, hysteria, neuralgia [159]
Vicia faba (Fabaceae)	Broad bean	Seed	Fabatin (protein)	Diuretic, expectorant, tonic [161]
Vitis vinifera (Vitaceae)	European red grapes	Fruit	Resveratrol (polyphenolic phytoalexin)	Cholera, diarrhea, smallpox, skin, kidney and liver infections [160]

Extract preparation

Air dried material (*Hypericum perforatum*, *Vicia faba*) was finely ground to powder, using a homogenisator (Restsch Grindomix, 5000 turn per minute). In case of *Allium sativum*, *Curcuma longa* and *Vitis vinifera,* fresh material was used for homogenisation. The extracts were prepared by maceration of homogenised plant material (15.00 g) in 450 ml of 80% ethanol (375 ml of 96% ethanol + 75 ml of distilled water), at room temperature for five days in a dark place. All extracts were subsequently filtered (paper filter) under pressure, with a Sartorius filtration device, and evaporated *in vacuo* on vacuum evaporator Rotavapor R-200 at 40°C. The residue for each extract obtained after evaporation is given in Table 4. In case of *Curcuma longa* the procedure was repeated, due to low yield (0.36 g), to obtain a sufficient amount of material for testing.

Table 4. *Yields of common antimicrobial plants extracts (in 450 ml of 70% ethanol) after evaporization*

Species	Part tested	Yield (g)	Yield (%)
Allium sativum	Bulb	2.39	15.93
Curcuma longa	Rizome	0.36	2.40
Hypericum perforatum	Flowering tops	3.35	22.33
Vicia faba	Seed	1.05	7.00
Vitis vinifera	Fruit	4.67	15.56

Extract stock solution

On analytical balance (KERN 770) 0.64 mg of each crude evaporated extract was weighed and dissolved in 0.5 ml of DMSO and afterwards 4.5 ml of sterile TBS pH 7.6 was added, to create a stock solution of 128 mg/ml for testing in antimicrobial assay.

4.1.3. Microorganisms

Microbial strains used in assays are known to cause infections indicated by the ethnopharmacological uses of the tested plants, and were selected to represent yeasts and different groups of pathogenic bacteria according to their physical and chemical

characteristics and natural resistance pattern. Microorganisms, their taxonomy insertion and pathogenity description is summarized in Table 5.

Table 5. *Microorganisms used in antimicrobial assay, their description and pathogenity*

Name	**Taxonomy group**	**Pathogenity to humans**	**Clinical syndromes**
Bacillus cereus	G+ spore-forming rods	Pathogen	Food poisoning, diarrhea
Bacillus subtilis	G+ spore-forming rods	Pathogen	Food poisoning, diarrhea
Enterococcus faecalis	G+ cocci	Opportunistic pathogen	Abscesses, meningitis, wounds, nosocominal and urinary infections
Staphylococcus epidermidis	G+ cocci	Opportunistic pathogen	Sepsis, osteomyelitis, nosocominal infections
Staphylococcus aureus	G+ cocci	Opportunistic pathogen	Furunculosis, skin boils, pneumonia, osteomyelitis
Streptococcus pyogenes	G+ cocci	Pathogen	Impetigo, scarlet fever, pharyngitis, tonsillitis, arthritis
Bacteroides fragilis	G- rods	Opportunistic pathogen	Abscesses, diarrhea
Escherichia coli	G- rods	Opportunistic Pathogen	Diarrhea, urinary infections, meningitis, pneumoniaions,
Pseudomonas aeruginosa	G- rods	Opportunistic pathogen	Dermatitis, wound, GIT and respiratory infections
Candida albicans	Yeasts	Opportunistic pathogen	Candidosis, nosocominal infections

Standard microorganisms should be used preferably, so that the screening can be repeated in other research laboratories [158]. All bacteria used were obtained from the American Type Culture Collection (ATCC): *Bacillus cereus* ATCC 11778, *Bacillus subtilis* ATCC 6633, *Bacteroides fragilis* ATCC 25285, *Candida albicans* ATCC 10231, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Streptococcus pyogenes* ATCC 19615.

Streptococcus pyogenes was grown and tested in Brain-heart infusion broth, *Bacteroides fragilis* in Wilkins-Chalgren anaerobe broth under anaerobic conditions using Anaerobic Jar HP11. The other microorganisms were grown and tested in Mueller-Hinton broth.

Susceptibility test

The susceptibility of bacteria to Ciprofloxacin and the yeast to Nystatin was checked as antibiotic controls. The antibiotics were disolved in 5% DMSO in TBS pH 7.6.

4.1.4. Antimicrobial assay

The antimicrobial activity of the Peruvian medicinal plants and comparative group of European antimicrobial plants was determined by the broth microdilution method [162] in 96-well microtitration plates of U type.

Extract dilutions of Peruvian medicinal plants

Two-fold dilutions of each extract were prepared in appropriate broth media in concentrations from 16 to 0.0156 mg/ml. Firstly, each well was filled with 0.1 ml of normal concentrated media, except first rows, which were filled with a double concentrated media. Secondly, 0.1 ml of extract stock solution of concentration 32 mg/ml was added into first wells to obtain a desired concentration of 16 mg/ml. Subsequently, 0.1 ml of such solution was transferred into next row and this step was repeated to get solutions of 16; 8; 4; 2; 1; 0.5; 0.25; 0.125; 0.0625; 0.0313 and 0.0156 mg/ml.

Extract dilutions of Common antimicrobial plants

Two-fold dilutions of each extract were prepared in appropriate broth media in concentrations from 64 to 0.0625 mg/ml. Firstly, each well was filled with 0.1 ml of normal concentrated media, except first rows, which were filled with a double concentrated media. Secondly, 0.1 ml of extract stock solution of concentration 128 mg/ml was added into first wells to obtain a desired concentration of 64 mg/ml. Subsequently, 0.1 ml of such solution was transferred into next row and this step was repeated to get solutions of 64; 32; 16; 8; 4; 2; 1; 0.5; 0.25, 0.125 and 0.0625 mg/ml.

Controls

Finally, one well not containing an antimicrobial agent should be inoculated and used as a growth control and a second un-inoculated well should be used as a broth sterility control. The solution of DMSO (5% v/v) in TBS, for measuring the effect of solvent, was assayed as the negative control, simultaneously. All extract samples were tested in triplicate.

Standard inoculum

Bacterial and yeast culture were inoculated from stored suspensions (using a sterile injection) into fresh appropriate broth medium in test tube and were incubated overnight in Biological Thermostat BT 120 at 37°C. A standardized inoculum of concentration of 10^7 CFU/ml was prepared afterwards by diluting 1:10 a portion of the suspension with a turbidity matching that of a 0.5 McFarland standard (measured on DensiLa densitometer) in sterile broth. The final desired inoculum concentration in wells is $5x10^5$ CFU/ml. For inoculating wells containing 0.1 ml of broth 0.005 ml of standard inoculum is required.

Inoculation and incubation

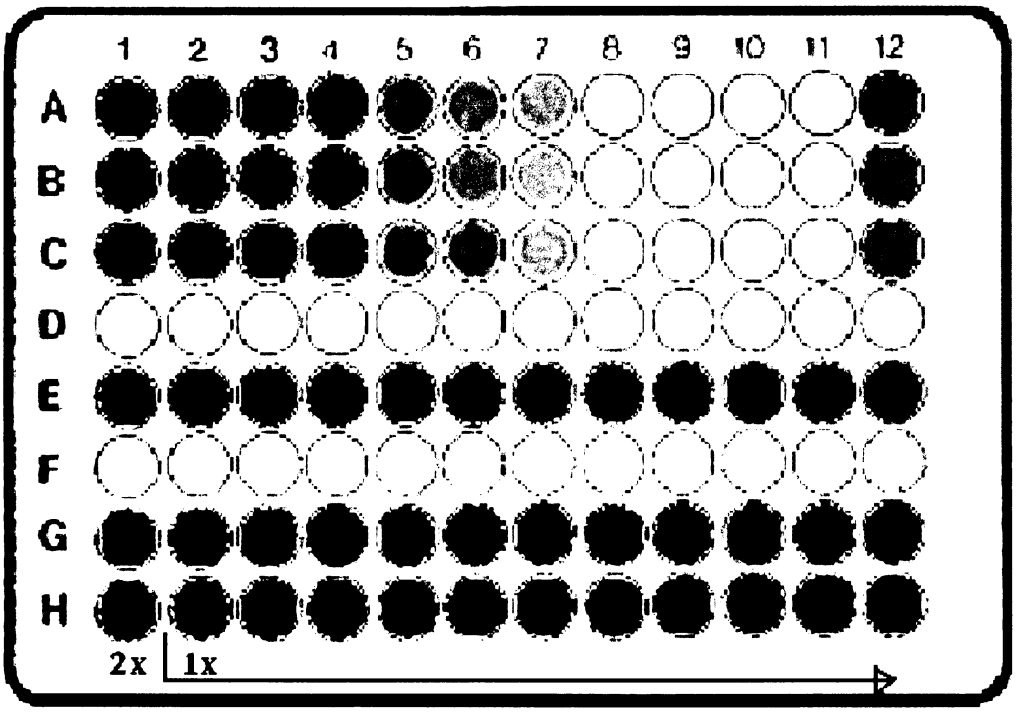
Each well was inoculated with 5 μl of bacterial suspension at a density of 10^7 CFU/ml, which conforms to one drop using a standard needle of 0.70 mm outside diameter [162]. The microtitre plates were incubated in thermostat at 37°C for 24 h (or 48 h for the yeast).

Quantitative interpretation

The growth of microorganisms was observed as turbidity determined by the UV-VIS spectrophotometer Helios ε at 600 nm. Sterile water was used as the background. Each sample was first stirred in the well to suspend the grown microorganism culture by *in and out pippeting* the content using an automatic Eppendorf pipette. Afterwards the sample was transferred into cuvette. Between each sample the cuvette was cleansed with 0.1 ml of ethanol and 0.1 ml of sterile water to avoid misrepresenting next solution sample. The samples should be measured from the lowest concentration rising upwards to minimize the concentration mistakes. Figure 2 shows a schedule of filled test plate.

Minimum inhibitory concentrations (MICs) were calculated based on the density of the growth control and were the lowest extract concentrations that resulted in 80% reduction in growth compared to the extract-free growth control.

Figure 2. *Schedule of filling a microtitre plate for antimicrobial testing of Peruvian medicinal plants*



Extract tested in two-step dilutions ranging from 16 to 0,0156 mg/ml (extract, media, bacteria/yeast)

Growth control (media + bacteria/yeast)

Extract sterility control (media + extract)

Broth sterility control (media)

Negative control of extract solvent (5% DMSO in TBS)

Antibiotic control (media + bacteria/yeast + Ciprofloxacin/Nystatin)

2x Wells with double-concentrated media

1x Wells with simple-concentrated media

5. Results

5.1. Peruvian plants

The summary of the investigated species and the obtained MIC values are given in Table 6. Seventeen ethanol extracts from 16 plants (belonging to 14 families) were tested for antimicrobial activity against nine bacteria (six Gram-positive and three Gram-negative) and one yeast.

With exception of stem bark extract of *Maytenus macrocarpa*, all extracts tested possessed antibacterial activity against at least four bacterial strains (*Bacillus cereus*, *Enterococcus faecalis*, and both staphylococci) at the concentrations of 16 mg/ml or below. The extracts exhibited stronger activity against Gram-positive bacteria (88 %) than Gram-negative bacteria (53 %) and yeast (35 %).

The broadest spectrum of action was performed by *Abuta grandifolia*, *Maytenus macrocarpa, Naucleopsis glabra* and *Pterocarpus rohrii*, which inhibited all tested microorganisms in concentrations ranging from 0.0625 to 16 mg/ml.

Extract from the root bark of *Maytenus macrocarpa* showed the lowest spectrum of action against all the strains with MICs ranging from 0.125 mg/ml against *Bacillus cereus, Bacillus subtilis, Staphylococcus epidermidis, Streptococcus pyogenes* and *Escherichia coli,* to 0.250 mg/ml against *Enterococcus faecalis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans.* The same activity was obtained, independent of the kind of bacteria: Gram-positive, Gram-negative or the yeast.

The lowest MIC was obtained by *Naucleopsis glabra*, which inhibited *Streptococcus pyogenes* at the extract concentration of 0.0625 mg/ml. The extract of *N. glabra* was also significantly more active against Gram-positive bacteria (MICs: 0.0625 mg/ml against *S. pyogenes* mentioned and 0.125 mg/ml against *B. cereus, B. subtilis, E. faecalis* and *S. epidermidis*) than against Gram-negative bacteria and the yeast (4 mg/ml).

A. grandifolia extract showed a lower activity against Gram-positive strains (MIC ranged from 0.25 to 1 mg/ml) than species mentioned above, but the Gram-negative bacteria inhibition (MIC 2 mg/ml) and yeast inhibition (MIC 8 mg/ml) was higher than *N. glabra.*

The ethanol extract of *Pterocarpus rohrii* inhibited the growth of *B. cereus, B. subtilis* and *S. epidermidis* at concentration of 0.25 mg/ml and also of *E. faecalis* at concentration of 0.5 mg/ml. It showed lower antimicrobial activity against the Gram-negative bacteria and the yeast, having the MICs ranging from 4 and 16 mg/ml.

Bertholletia excelsa extract showed good activity against Gram-positive strains (MIC from 0.25 to 1 mg/ml), but from Gram-negative bacteria only anaerobic *Bacteroides fragilis* was inhibited at MIC 8 mg/ml. Yeast did not show growth inhibition. The extract from fruits of *Solanum mammosum* inhibited only Gram-positive bacteria, MIC ranging from 0.5 for *B. cereus, B. subtilis, E. faecalis* to 16 for *S. pyogenes*, and the yeast *C. albicans* at MIC 1 mg/ml. Two extracts (*Terminalia catappa* and *Phyllanthus amarus*) inhibited all bacteria on different concentration levels, but not the yeast. The extract from aerial part of *Piper aduncum* was significantly more active against Gram-positive (MICs ranging from 1 to 2 mg/ml) than against Gram-negative bacteria (MICs >16 mg/ml). Good activity was observed also in the *Uncaria tomentosa* extract, which inhibited six bacteria and five of them with MICs from 0.25 to 1 mg/ml. Other extracts showed only slight inhibition of tested microorganisms.

Candida albicans resulted to be the most resistant microorganism strain used, ihibiting only 36 % of extracts tested. *M. microcarpa*, *S. mammosum* and *C. alliodora* proved the best anticandidal activity with MICs of 0.25; 1 and 2 mg/ml, respectively. In general, the Gram-negative bacteria resulted to be resistant (MIC ≥ 1 mg/ml) to the plant tested, except to *M. macrocarpa* extract. *Escherichia coli* proved to be also difficult to inhibit, with the susceptibility to only 47 % of extracts, among which the most interesting were *M. macrocarpa* (MIC 0.125 mg/ml) and *C. alliodora* (MIC 1 mg/ml).

The most susceptible microorganism was *Enterococcus faecalis*, which was inhibited by all tested species, except the stem bark extract of *M. macrocarpa* with MICs ranging from 0.125 to 8 mg/ml. The lowest MIC (0.0625 mg/ml) was detected at *N. glabra* bark extract.

Table 6. *Minimum inhibitory concentrations (mg/ml) of ethanol extracts of Peruvian medicinal plants tested by broth microdilution method*

Plant species	Part tested	Microorganisms									
		G+ bacteria							G- bacteria		Yeast
		B.c.	***B.s.***	***E.f.***	***S.a.***	***S.e.***	***S.p.***	***B.f.***	***E.c.***	***P.a.***	***C.a.***
Abuta grandifolia	Stem bark	0.25	0.5	0.25	0.5	0.25	1	2	2	2	8
Bertholletia excelsa	Seed pods	0.25	0.25	0.25	1	0.25	0.5	8	NA	NA	NA
Brunfelsia grandiflora	Root	4	16	8	4	4	NA	16	NA	16	NA
Caesalpinia spinosa	Pods	8	16	0.5	16	16	NA	16	NA	NA	NA
Cordia alliodora	Stem bark	1	2	1	2	0.5	NA	NA	1	1	2
Dipteryx micrantha	Stem bark	2	1	0.5	4	1	8	2	NA	NA	NA
Dracontium loretense	Rhizome	8	NA	8	8	16	NA	NA	NA	NA	NA
Equisetum giganteum	Aerial part	8	16	8	8	16	4	8	NA	NA	NA
Maytenus macrocarpa	Stem bark	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Maytenus macrocarpa	Root bark	0.125	0.125	0.25	0.25	0.125	0.125	0.25	0.125	0.25	0.25

Table 6. (Continued) *MICs (mg/ml) of ethanol extracts of Peruvian medicinal plants tested by broth microdilution method*

Plant species	Part tested	Microorganisms									
		G+ bacteria							G- bacteria		Yeast
		B.c.	***B.s.***	***E.f.***	***S.a.***	***S.e.***	***S.p.***	***B.f.***	***E.c.***	***P.a.***	***C.a.***
Naucleopsis glabra	Stem bark	0.125	0.125	0.125	0.125	0.125	0.0625	4	4	4	4
Phyllanthus amarus	Aerial part	16	1	0.25	4	1	4	4	16	8	NA
Piper aduncum	Aerial part	1	1	2	1	2	2	NA	NA	NA	NA
Pterocarpus rohrii	Stem bark	0.25	0.25	0.5	4	0.25	4	16	4	8	16
Solanum mammosum	Fruit	0.5	0.5	0.5	2	1	16	NA	NA	NA	1
Terminalia catappa	Leaves	2	4	8	1	0.25	16	16	8	4	NA
Uncaria tomentosa	Stem bark	1	1	0.25	1	1	NA	NA	8	NA	NA

NA = no activity (MIC > 16 mg/ml), G+ (Gram-positive), G- (Gram-negative) bacteria

B.c., Bacillus cereus; B.s., Bacillus subtilis; B.f., Bacteroides fragilis; E.f., Enterococcus faecalis; E.c., Escherichia coli; P.a., Pseudomonas aeruginosa; S.a., Staphylococcus aureus; S.e., Staphylococcus epidermidis; S.p., Streptococcus pyogenes; C.a., Candida albicans.

5.2. Common antimicrobial plants

Antimicrobial effects of ethanol extracts from various parts of *Curcuma longa* (rhizome), *Hypericum perforatum* (flowering tops), *Vicia faba* (seeds), *Vitis vinifera* (fruits) and *Allium sativum* (bulbs) are presented in Table 7.

The ethanol extract of *H. perforatum* was the most active, inhibiting predominantly Gram-positive bacteria (MICs ranging from 0.25 to 64 mg/ml) of which *Bacillus cereus* was the most susceptible (MIC 0.25 mg/ml). From Gram-negative bacteria only *Escherichia coli* (MIC 64 mg/ml) was inhibited. The ethanol extract didn't show any inhibition at yeast.

The extract of *C. longa* inhibited only Gram-positive bacteria (MICs ranging from 0.25 to 2 mg/ml), of which *B. cereus* was the most susceptible (MIC 0.25 mg/ml). On the other hand, it was the only extract inhibiting *Streptococcus pyogenes* (MIC 1 mg/ml). No positive results were obtained with Gram-negative bacteria and the yeast.

In case of *V. vinifera* the ethanol extract showed inhibition activity only against *Enterococcus faecalis* (MIC 16 mg/ml) and S*taphylococcus aureus* (MIC 32 mg/ml), showing no inhibitory effects for the other microbial strains tested.

V. faba was only active against two Gram-positive bacteria with MICs of 8 mg/ml for *E. faecalis* and 32 mg/ml for *S. aureus*.

Ethanol extract of *A. sativum* showed antimicrobial activity only against two Gram-positive bacteria with MICs of 8 mg/ml (*B. cereus* and *B. subtilis*). It was the only extract inhibiting the yeast *Candida albicans* (MIC 16 mg/ml).

Gram-negative bacteria *Pseudomonas aeruginosa* was the most resistant strain used, it was not inhibited by any of extract tested.

The most susceptible organism was *E. faecalis* which was inhibited by four from five extracts tested, MICs ranging from 1 to 16 mg/ml. Whereas against *B. subtilis* the lowest MIC was obtained (0.25 mg/ml) by extract from *C. longa* and *H. perforatum.*

Table 7. *Minimum inhibitory concentrations (mg/ml) of ethanol extracts of common antimicrobial plants tested by broth microdilution method*

Plant pecies	Part tested	Microorganism								
		G+ bacteria						G- bacteria		Yeast
		B.c.	***B.s.***	***E.f.***	***S.a.***	***S.e.***	***S.p.***	***E.c.***	***P.a.***	***C.a.***
Allium sativum	Bulb	8	8	NA	NA	NA	NA	NA	NA	16
Curcuma longa	Rhizome	0.25	1	1	2	NA	1	NA	NA	NA
Vitis vinifera	Fruit	NA	NA	16	32	NA	NA	NA	NA	NA
Hypericum perforatum	Flowering tops	0.25	1	2	2	64	NA	64	NA	NA
Vicia faba	Seed	NA	NA	8	32	NA	NA	NA	NA	NA

NA = no activity (MIC > 64 mg/ml), G+ (Gram-positive), G- (Gram-negative) bacteria

B.c., Bacillus cereus; B.s., Bacillus subtilis; B.f., Bacteroides fragilis; E.f., Enterococcus faecalis; E.c., Escherichia coli; P.a., Pseudomonas aeruginosa; S.a., Staphylococcus aureus; S.e., Staphylococcus epidermidis; S.p., Streptococcus pyogenes; C.a., Candida albicans.

The solvent (5% DMSO in TBS) used for dissolving the crude extracts and the standard antibiotics always gave negative results, showing that it did not influence in the antimicrobial activities observed for the plant extracts. No growth inhibition was observed in the broth sterility control and extract sterility control as well.

The results of susceptibility test to antibiotics, Ciprofloxacin for bacteria and Nystatin for yeast, are enclosed in Table 8.

Table 8. *Susceptibility test of microorganisms to antibiotics tested by broth microdilution method and obtained minimum inhibitory concentrations MICs (μg/ml)*

Microorganism	**MIC (μg/ml)**
G+ bacteria	Ciprofloxacin
Bacillus cereus	1
Bacillus subtilis	4
Enterococcus faecalis	1
Staphylococcus aureus	1
Staphylococcus epidermidis	1
Streptococcus pyogenes	0.5
G- bacteria	
Bacteroides fragilis	2
Escherichia coli	1
Pseudomonas aeruginosa	1
Yeast	Nystatin
Candida albicans	4

5.3. Comparision of the most effective plants

For comparision the potencial of the Peruvian plants with plants standardly described in literature as antimicrobial, two most active representatives of each group (*M. macrocarpa* and *N. glabra* in contrast to *H. perforatum* and *C. longa*) were selected and the results are summarized in Table 9.

Table 9. *A comparision between two most active ethanol extracts from Peruvian plants (M. macrocarpa and N. glabra) and common antimicrobial plants (C. longa and H. perforatum) tested, with assigned minimum inhibitory concentrations (mg/ml) observed in broth microdilution method*

Plant species	Microorganism								
	G+ bacteria						G- bacteria		Yeast
	B.c.	***B.s.***	***E.f.***	***S.a.***	***S.e.***	***S.p.***	***E.c.***	***P.a.***	***C.a.***
Maytenus macrocarpa	0.125	0.125	0.25	0.25	0.125	0.125	0.125	0.25	0.25
Naucleopsis glabra	0.125	0.125	0.125	0.125	0.125	0.0625	4	4	4
Curcuma longa	0.25	1	1	2	NA	1	NA	NA	NA
Hypericum perforatum	0.25	1	2	2	64	NA	64	NA	NA

NA = no activity (MIC > 64 mg/ml), G+ (Gram-positive), G- (Gram-negative) bacteria
B.c., Bacillus cereus; B.s., Bacillus subtilis; E.f., Enterococcus faecalis; E.c., Escherichia coli; P.a., Pseudomonas aeruginosa; S.a., Staphylococcus aureus; S.e., Staphylococcus epidermidis; S.p., Streptococcus pyogenes; C.a., Candida albicans.

The Peruvian medicinal plants were significantly more effective against organisms tested. While *M. macrocarpa* and *N. glabra* inhibited all strains at MICs ranging from 0.0625 to 4 mg/ml, for *H. perforatum* and *C. longa* extracts tested in even higher concentration scale (0.0625 to 64 mg/ml) MICs only against 67 % and 55 % respectively microorganisms tested were observed ranging from 0.25 to 64 mg/ml.

6. Discussion

6.1. Peruvian plants

The use of tested plants in Peruvian Amazon folk medicinal remedies for treating various health problems has already been reported by several authors [4, 33, 35, 48, 156, 157]. The most frequent medicinal uses of the investigated plants are treating skin infections (7 plants), respiratory infections (7 plants), diarrhea (5 plants) and rheumatism (4 plants). The primary forms of usage are decoction, infusion and macerate; therefore in order to follow the ethnobotanical approach, 70% ethanol extracts were prepared.

However, for eight (50 %) from sixteen plants tested, no scientific information concerning the antibacterial properties has been reported. The results of the antimicrobial screening indicate that bioactivity could be detected in 94 % of the Peruvian medicinal plants tested. This reinforces the concept that the investigation of ethnobotanically used plants will reveal a substantial number of positive responces to *in vitro* screens.

Abuta grandifolia extracts from bark and leaves possessed antibacterial activity in *in vivo* tests previously inhibiting *Bacillus subtilis* [8] and *Pseudomonas aeruginosa* [27], what supports results obtained in this work, where the bark extract demonstrated good inhibition of these bacteria, too (*B. subtilis* MIC 0.5 mg/ml and *P. aeruginosa* MIC of 2 mg/ml). In contrast, no effect against *Escherichia coli*, *Salmonella gallinarum*, *Klebsiella pneumoniae* and *Candida albicans* in agar plate test [27] was performed, whilst our results showed moderate activity against these strains. Since the previous phytochemical studies on *A. grandifolia* confirmed presence of isoquinoline alkaloids berberine and palmatine [35], which were previously described as strong antimicrobial agents [163], the contribution to the strong antimicrobial effect of these constituents is supposed.

Maytenus macrocarpa seems to be the most interesting plant tested. Although the root bark extract was the most active in study, the extract of stem bark did't show any inhibition, which points to the presence of compound responsible for antimicrobial activity selectively in undergroung plant parts. According to results achieved in this study, the stem bark extract showed promising activity inhibiting all microorganisms tested (ranging from 0.125 to 0.25 mg/ml) including a strong inhibition of *P. aeruginosa*, which has in clinical cases

a high degree of resistant forms against different antibacterial drugs [164]. The broad spectrum of activity of extract from this plant against bacteria may well explain its varied uses in Peruvian folk medicine, particularly to its use in treating diarrhea and respiratory infections [48]. The antimicrobial activity of *M. macrocarpa* has not previously been described. Since the antimicrobial studies on taxonomically closely related species, *M. blepharodes*, showing positive results due to the presence of triterpenoids in roots against Gram-positive bacteria and *C. albicans* [165] it is possible to expect that the strong antimicrobial action of extract from root bark of *M. macrocarpa* detected in our study could be caused by presence of related types of compounds and/or by the presence of tannins, which have been previously detected in the plant [48].

Naucleopsis glabra showed antimicrobial action against all strains used in the test, particularly the Gram-positive bacteria. There are no previously reports on antimicrobial activity for this species to compare our results with. The presence of flavonoids next to presence of phytosterols in *Brosimum acutifolium*, (synonym) is reported in literature [103, 116]. Antimicrobial activity of flavonoids [166] and phytosterols [167] has been proved. These two groups of compounds may explain the strong antimicrobial effects of the extract of *N. glabra.*

Pterocarpus rohrii has been found to possess activity predominantly against Gram-positive bacteria. Not much information about *P. rohrii* is known, but species from the same family, *P. indicus* was tested previously and showed a wide spectrum of activity against bacteria and protozoa used [127]. In former research *P. indicus* was active against *C. albicans* [128]. According to our results, the extract was barely active against this yeast. In the same study the leaf extract showed a low activity against *P. aeruginosa* and *E. coli,* and as it has been reported in this work, *P. rohrii* posessed low antimicrobial action against these bacteria too (MIC 4 mg/ml against *E.coli* and 8 mg/ml against *P. aeruginosa*). Finally, in the previous report *P. indicus* was found inactive against *S. aureus* and *B. subtilis* and according to our results we found the extract slightly active against *S. aureus* but with an important antimicrobial activity against *B. subtilis,* showing a MIC of 0.25 mg/ml.

Since the Brazil nut (*Bertholletia excelsa*) has long been a common food, rather than an herbal remedy, it hasn't been the subject of any clinical research outside of that concerning

its selenium content [41]. The seeds also contain higher amount of ascorbic acid, chlorine, iodine and tin, known for antimicrobial activity [54, 168]. The extract of *B. excelsa* showed a high selective inhibition only of Gram-positive bacteria ranging from 0.25 to 1 mg/ml. This may be due to presence of phytosterols stigmasterol and sitosterols, which previously displayed antimicrobial activity inhibiting strongly *Staphylococcus mutans*, *S. aureus* and *S. epidermidis* [169].

Crude extract of *Phyllanthus amarus* demonstrated significant *in vitro* antibacterial activity against *Staphylococcus aureus, Micrococcus luteus* [107] and *Escherichia coli* [110]. Methanol extract of leaves inhibited *Staphylococcus aureus* at concentration of 1 mg/ml [108], *Pseudomonas aeruginosa* at 2 mg/ml [109]. This data are in line with results presented in this study , althoug the MICs for mentioned bacteria were slightly higher in our case, what could be caused by using a different method (the antimicrobial tests mentioned above were performed by disc diffusion method at agar plates). Among compounds isolated from *Phyllanthus amarus*, tannins corilagin, geraniin and gallic acid [170] showed *in vitro* activity against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* [171, 172] and also quercetin, quercitrin, rutin, limonene [54] and phyllanthin [110] are described as responsible for antimicrobial effect.

Ethanol extract of *Piper aduncum* previously showed inhibitory activity in agar-well diffusion tests against the AIDS-related pathogens *Cryptococcus neoformans*, *Mycobacterium intracellularae*, *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa* [117] and mediate activity against oral pathogens has been reported [118]. Ethanol solution of essential oil from stems of *Piper angustifolium* (synonym) exhibited moderate bacteriostatic and fungistatic activities against *Trichophyton mentagrophytes*, *Pseudomonas aeruginosa, Candida albicans* and *Escherichia coli* [119]. In contrast, in this study moderate activity against Gram-positive and no inhibition of Gram-negative bacteria and yeast has been noticed. Dihydrochalcones asebogenin, piperaduncins A, B and C, flavonone sakuranetin, anodendroic acid methyl ester, carotenoid lutein and benzoic acid derivates posessed antimicrobial properties against a variety of microorganisms including *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Cryptococcus neoformans*, *Mycobacteria intracellulare*, *Micrococcus luteus* and *Pseudomonas aeruginosa* [117, 121, 122].

High antifungal activity but no antibacterial and anticandidal activity of methanol and methylene chloride extracts from *Terminalia catappa* aerial part [146] has been reported. On the other hand different authors [145] investigated roots of *T. catappa* and detected good antimicrobial activity of chloroform and methanol extracts against *Escherichia coli* and *Staphylococcus aureus*, which correlates to our results. The phytochemical studies on *T. catappa* bark and leaves demonstrate the presence of tannins and flavonoid glycosides [149]. Among them, gallic acid, corilagin, ellagic and gallic acid and rutin showed *in vitro* antibacterial activity [172, 173].

The results of the screen indicate a broad spectrum of activity demonstrated by *Cordia alliodora.* Its ethanol extract inhibits on the same level Gram-positive as well as more resistant Gram-negative bacteria and the yeast. The antifungal effect is consistent with results from earlier studies, where dichloromethane extract from the root bark of *C. alliodora* exhibited activities against the phytopathogenic fungus *Cladosporium cucumerinum* the yeast *Candida albicans* [66]. Phenylpropanoid and prenylated hydroquinone from the root bark were found to be responsible for antifungal properties [64].

Cipollini [134] described antifungal properties of *Solanum* sp. fruit glycoalkaloids, what could explaine a significant inhibition of *Candida albicans* (MIC 1 mg/ml). Some other species of *Solanum* genus were bioactive against *Bacillus subtilis* (MIC 0.31 mg/ml) in agar-well diffusion assay [135], what conformes to strong inhibition of Gram-positive bacteria performed by *Solanum mammosum* in our tests.

Moderate inhibitory effect predominantly against Gram-positive bacteria has been performed by *Dipteryx odorata*, what correlates with its popular use in treating skin infections. Data on antimicrobial properties are lacking. Only anticandidal activity of coumarin and retusin, that can be isolated from bark, has been described in literature [174]. From substances with known antimicrobial properties, salicylic acid and triterpen lupeol [175, 176] are present in *D. odorata.*

Uncaria tomentosa has a broad spectrum of bioactivity, ranging from anti-inflammatory to antiviral [25]. Antibacterial activity has been reported previously on *Photobacterium phosphoreum*, *Salmonella typhimurium* and *Bacillus subtilis* with weak response and no

inhibition against *Candida albicans* and *Sacharomyces cerevisiae* [153]. Isolated oxindol alkaloid isopteropodine was active only against the Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*, with a MIC value of 150 μg/ml and 250 μg/ml, respectively [154]. This is consistent with high inhibitory effect selective to Gram-positive inhibitory obtained for *U. tomentosa* ethanol extract tested.

The extracts of *Brunfelsia grandiflora*, *Caesalpinia spinosa*, *Dracontium loretense* and *Equisetum giganteum* showed only weak antimicrobial activity, mainly against Gram-positive bacterial strains. The effect of *B. grandiflora* could be employed by scopoletin, a psychoactive furocoumarin which is claimed to be anti-inflammatory and antimicrobial [50]. Scopoletin isolated from *Foeniculum vulgare* was active against several bacteria [177, 178] From other compounds isolated from *Brunfelsia* sp. farnesol and geraniol performed antimicbrobial activity [179, 180]. Pods of *C. spinosa* contain up to 25 % of gallic acid [59] and previously this tannin isolated from *Acalypha wilkesiana*, *Acalypha hispida* and *Rubus ulmifolius* showed *in vitro* antimicrobial activity when tested against *Bacillus cereus*, *Staphylococcus aureus,* and *Escherichia coli* [172, 181]. Antimycotic activity of aqueous, dichloromethane and methanol extracts of *Equisetum giganteum* at concentrations of 50 and 100 mg/ml was evaluated by the paper disk-agar diffusion method, but did no show any activity. Antimicrobial activity of aqueous extract was tested at concentration 62.5 μg/ml using the agar-well diffusion method against *Staphylococcus aureus* and *Escherichia coli* with no significant results [83, 84]. In case of *D. loretense*, no comparable studies were performed. The detailed study on phytochemical composition has not previously been performed.

6.2. Common antimicrobial plants

In intension to demonstrate the potential of Peruvian medicinal plants, five plant commonly described in literature as source of antimicrobial agents [1, 3, 54, 161, 182] were chosen and tested by the same method. Numerous studies refering about biological activity of curcumin from *Curcuma longa* [183-186], hypericin and hyperforin from *Hypericum perforatum* [187, 188], fabatin from *Vicia Faba* [189, 190], resveratrol from *Vitis vinifera* [191-193] and allicin from bulbs of *Allium sativum* [194-198] were published.

In previous study, aqueous solutions of *H. perforatum* were found to be active against Gram-positive bacteria, with good activity towards *Staphylococcus aureus*, with a MIC range of 1.3 to 2.5 mg/ml [199]. According to our results the ethanol extract of *H. perforatum* was also inhibiting predominantly Gram-positive bacteria (MICs ranging from 0.25 to 64 mg/ml), whereas *Staphylococcus aureus* was inhibited by concentration of 2 mg/ml. It has been suggested that hyperforin appears to be the active antimicrobial constituent of *H. perforatum* exhibiting methicillin-resistant strains of *Staphylococcus aureus* with a MIC value of 1.0 μg/ml [199].

In study of Papadopoulou et al. [200] wine extracts tested using agar well diffusion method was effective against *Staphylococcus aureus* and less effective against *Escherichia coli* and *Candida albicans*. Also, *Candida albicans* was resistant to more wine extracts. Our work confirms that ethanol extract demostrate no inhibition activity against *Candida albicans* and also against Gram-negative bacteria. Moreower, *in vitro* studies on *V. vinifera* showed that growth of the bacteria strains *Staphylococcus aureus*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa* was inhibited at 171 - 342 μg/ml of resveratrol in the solvent DMSO [201]. According to our results ethanol extract of *V. vinifera* inhibited predominantly *Staphylococcus aureus* with a MIC of 32 mg/ml and *Enterococcus faecalis* with a MIC of 16 mg/ml.

Fabatins have shown to be active against both Gram-negative and Gram-positive bacteria, but inactive against the yeasts *Sacharomyces cerevisiae* and *Candida albicans* [190]. Corresponding with this roport, our results demostrate that *Vicia faba* ethanol extract is mainly effective against Gram-positive bacteria e.g. *Enterococcus faecalis* (MIC 8 mg/ml), *Staphylococcus aureus* (MIC 32 mg/ml) and not effective against yeast. No inhibitory effect against Gram-negative bacteria has been observed in conformity with results from literature.

Previous studies showed that *Curcuma longa* oil and ethanol extract (10–200 mg/ml) have inhibitory effects on the growth of *Staphylococcus albus* and *Staphylococcus aureus*, while curcumin (2.5–50 mg/ml) was inhibiting only *S. aureus* [202]. Considering *C. longa* antibacterial effects it shows inhibition activity only against Gram-positive bacteria with no positive effects against Gram-negative bacteria and the yeast. Although a certain

inhibitory activity against *S. aureus* with MIC of 2 mg/ml was achieved, *Bacillus cereus* was the most susceptible bacteria in this study (MIC 0.25 mg/ml).

Regarding *Allium sativum*, there is a great number of studies on its antimicrobial activity. The inhibitory effect of crude extracts against Gram-positive strains (MIC range 35 to 142 mg/ml) and allicin (mean MIC 27.5 μg/ml) was tested [203]. Hovewer, our results showed week inhibitory activity of ethanol extract against only two bacteria *Bacillus cereus* and *Bacillus subtilis* with MIC 8 mg/ml and yeast with 16 mg/ml. Ajoene, a garlic-derived sulfur-containing compound, demonstrated antimicrobial activity against Gram-positive bacteria, such as *B. cereus, B. subtilis* and against Gram-negative bacteria, such as *Escherichia coli*. Ajoene also inhibited yeast growth at concentrations below 20 μg/ml [204, 205]. In comparison to other studies, no positive results with garlic extracts against Gram-negative bacteria were achieved in this study.

6.3. Comparision of the most effective plants

Comparing the data of two most active extracts of both groups of plants (*Maytenus macrocarpa* and *Naucleopsis glabra* in contrast to *Hypericum perforatum* and *Curcuma longa*), the Peruvian medicinal plants were significantly more effective against organisms tested. While *M. macrocarpa* and *N. glabra* inhibited all strains at MICs ranging from 0.0625 to 4 mg/ml), *H. perforatum* and *C. longa* extracts tested in concentration range from 0.0625 to 64 mg/ml were active only against 67 % and 55 % respectively microorganisms tested resulting in MICs of 0.25 to 64 mg/ml. According to these results obtained in antimicrobial screening performed, the crude extracts of Peruvian plants proved to be up to 1000 times more active against microbial strains used in the tests.

Following the stated theory that experiments with quantities higher than 1 mg/ml for extracts or 0.1 mg/ml for isolated compounds should be avoided, whereas the presence of activity is very interesting in the case of concentrations below 100 μg/ml for extracts and 10 μg/ml for isolated compounds [1], only *Hypericum perforatum* and *Curcuma longa* are able to fulfil these conditions for inhibiting *Bacillus cereus* at MIC 0.5 mg/ml. On the other hand, our results for Peruvian plants showed thirteen extracts (81 %) to have MICs of 1 mg/ml or lower. Very promising is the activity of *Naucleopsis glabra* against *Streptococcus pyogenes,* with MIC value 0.0625 mg/ml, and could be interesting in case of *N. glabra* against *Bacillus cereus, Bacillus subtilis, Enterococcus faecalis, Staphylococcus aureus* and *Staphylococcus epidermidis* and *M. macrocarpa* against *Bacillus cereus, Bacillus subtilis, Staphylococcus epidermidis, Streptococcus pyogenes* and *Escherichia coli,* due that both achieved MICs values of 0.125 mg/ml.

7. Conclusions

Antimicrobial study of sixteen Peruvian plants, all of them selected based on their relevant ethnomedical use, has provided various extracts with strong activity against several pathogenic microorganisms. The results of this study support the folkloric use of many of these plants.

According to our best knowledge, this is the first report on antimicrobial activity of extracts from *Bertholletia excelsa, Brunfelsia grandiflora, Cordia alliodora, Dipteryx micrantha, Maytenus macrocarpa, Naucleopsis glabra, Pterocarpus rohrii* and *Solanum mammosum.*

The ethanol extracts of *Abuta grandifolia, Maytenus macrocarpa, Naucleopsis glabra* and *Pterocarpus rohrii* exhibited the most promissing results suggesting their potential use in food or pharmaceutical industry for development of new antimicrobialy effective herbal-based nutraceuticals, functional foods, food additives and pharmaceutical or veterinary preparations.

Additionally, a comparative study of five common higher plants described in literature as source of strong antimicrobial agents, namelly *Curcuma longa*, *Hypericum perforatum, Vicia faba*, *Vitis vinifera* and *Allium sativum*, was carried out in this study. According to the resuls obtained, the Peruvian medicinal plants crude extracts were significantly (up to 1000 times) more active, what makes them a promising source of new antimicrobial agents. Howewer, it is still unknown, which compounds are responsible for their significant biological activity. Thus further bioassay-guided isolation and identification of the active principles of these plants are required.

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9. Appendices

Appendix 1. *Abuta grandifolia*

Appendix 2. *Abuta grandifolia* - inflorescence

Appendix 3. *Bertholletia excelsa*

Appendix 4. *Bertholletia excelsa* – fruits

Appendix 5. *Brunfelsia grandiflora*

Appendix 6. *Caesalpinia spinosa*

Appendix 7. *Cordia alliodora* –typical white-gray trunk

Appendix 8. *Cordia alliodora* – inflorescence

Appendix 9. *Dipteryx micrantha* – buttressed trunk

Appendix 10. *Dipteryx micrantha*

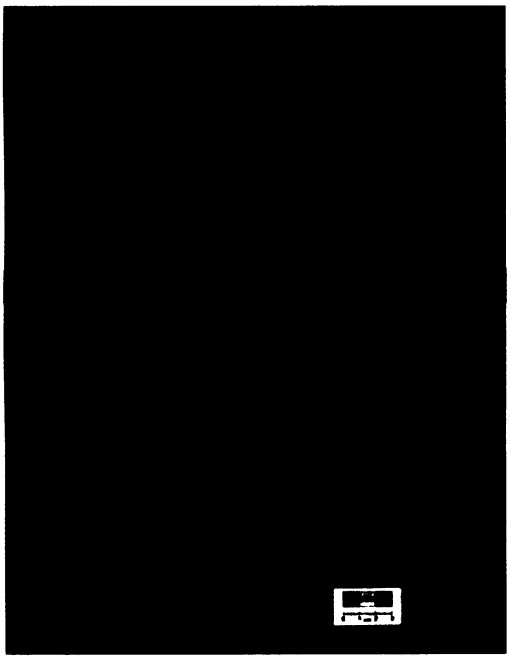
Appendix 11. *Dracontium loretense* – seeds

Appendix 12. *Dracontium loretense* – rhizome

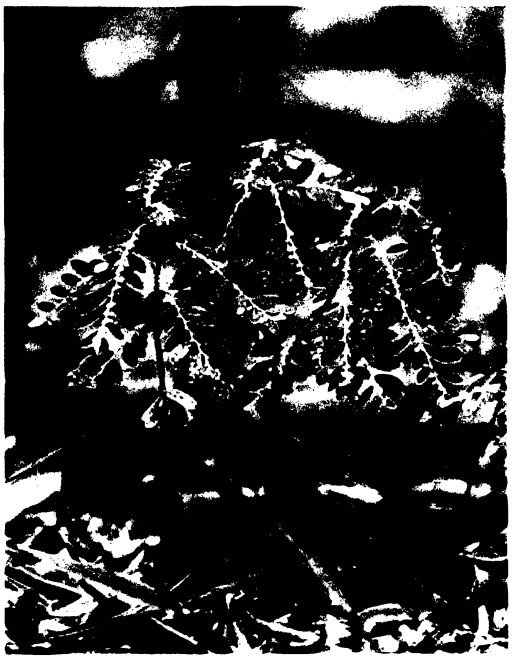
Appendix 13. *Equisetum giganteum*

Appendix 14. *Maytenus macrocarpa*

Appendix 15. *Naucleopsis glabra* – branch



Appendix 16. *Phyllanthus amarus*



Appendix 17. *Piper aduncum*

Appendix 18. *Pterocarpus rohrii* – inflorescence

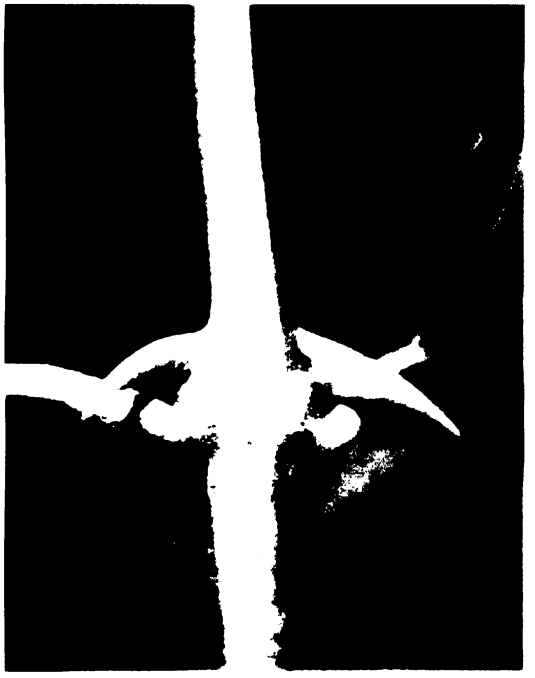
Appendix 19. *Solanum mammosum*

Appendix 20. *Solanum mammosum* – fruit

Appendix 21. *Terminalia catappa*

Appendix 22. *Terminalia catappa* – fruits

Appendix 23. *Uncaria tomentosa* – typical thorns



Appendix 24. *Uncaria tomentosa*