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Determination of *in vitro* antimicrobial activity of Peruvian medicinal plants

MSc. thesis

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DECLARATION

This is to certify that this thesis entitled “Determination of in vitro antimicrobial activity of Peruvian medicinal plants”, submitted in partial fulfilment for the award of the MSc. Degree in Tropical Crop Management and Ecology under Department of Crop Science and Agroforestry, Czech University of Life Sciences Prague written by LIZETH JOHANA CHINCHAY CHUMBE, is my own work unless otherwise referenced or acknowledged.

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LIZETH JOHANA CHINCHAY CHUMBE

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ABSTRACT

Peruvian medicinal plants are commonly used because of their positive effects on some human ailments and in some cases, in domestic animals. In some regions, they are used to reduce inflammation of different parts of the body, as analgesics, for digestive disorders (against indigestion and diarrhoea, and as anthelmintic and laxatives), as diuretics, for cardio-vascular problems, for respiratory infections and in some native communities for their wound-healing properties. Nevertheless despite this rich tradition of plants use in the folk medicine, antimicrobial activity and active compounds of medicinal plants from various parts of Peruvian Amazon such as Purus district have not yet been studied for the treatment of microorganisms associated diseases. The aim of this study was to determine *in vitro* antimicrobial activity of ethanol extracts of five Peruvian medicinal plant species (*Anomospermum grandifolium, Caamembeca spectabilis, Casearia pitumba, Piper heterophyllum* and *Solanum sessile*), selected based on ethnobotanical information on their traditional use to treat infectious diseases. The broth microdilution method was used and activities against pathogenic microorganism such as *Candida albicans, Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa,* and *Staphylococcus aureus* were assessed. Among all plants tested, *Casearia pitumba, Piper heterophyllum* and *Solanum sessile* showed antimicrobial properties, inhibiting most Gram-positive bacteria with minimum inhibitory concentrations ranging from 128 to 512 µg/ml. The results of this work suggest *Casearia pitumba, Piper heterophyllum* and *Solanum sessile* as prospective materials for further work on the isolation and identification of active compounds which are responsible for the antimicrobial activity of the most effective medicinal plants. These findings indicate that further studies on chemical and biological properties of their active components should be performed.

Keywords: Antimicrobial activity, broth microdilution method, Peruvian medicinal plants.

RESUMEN

Las plantas medicinales peruanas, son comúnmente usadas por el efecto positivo hacia algunas enfermedades en humanos y en algunos casos en animales domésticos. En algunas regiones son usados para reducir la inflamación de diferentes partes del cuerpo, como analgésicos, para desordenes digestivos (antiparasitario, anti diarreico y laxante) como diurético, para el sistema cardiovascular, para infecciones respiratorias y en algunas comunidades nativas, algunos árboles tienen propiedades para cicatrizar heridas. Sin embargo a pesar de la rica tradición de las plantas usadas en la medicina, en varias partes de la Amazonia peruana, el tratamiento de microorganismos asociados con enfermedades, como en el distrito de Purus, donde la actividad antimicrobiana y los compuestos activos de las plantas medicinales aun no han sido estudiados. El objetivo de este estudio fue determinar la actividad antimicrobiana *in vitro* de cinco especies de plantas medicinales peruanas (*Anomospermum grandifolium*, *Caamembeca spectabilis*, *Casearia pitumba*, *Piper heterophyllum* y *Solanum sessile*), seleccionadas en base a la información etnobotánica, en el uso tradicional para tratar enfermedades infecciosas; del cual fue determinado por el método de microdilución en caldo contra microorganismos patógenicos tales como *Candida albicans, Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa*, y *Staphylococcus aureus*. Entre todas las plantas ensayadas *Casearia pitumba*, *Piper heterophyllum* and *Solanum sessile* mostraron propiedades antimicrobianas, inhibiendo en su mayoría a las bacterias de Gram-positivo, con un rango de concentración mínima inhibitoria de 128 a 512 µg/ml. Los resultados de este trabajo sugieren que *Casearia pitumba, Piper heterophyllum* y *Solanum sessile* como materiales prospectivos para el trabajo posterior en el aislamiento e identificación de compuestos activos que son responsables de la actividad antimicrobiana de las plantas medicinales más eficaces. Estos hallazgos indican que deben realizarse estudios adicionales sobre las propiedades químicas y biológicas de sus componentes activos.

Palabras clave: Actividad antimicrobiana, método de micro dilución en caldo, plantas medicinales peruanas.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ATCC	American Type Culture Collection
CSIS	Centre for Strategic & International Studies
CLS	Clinical and Laboratory Standards Institute
CTE	Comité de Tuberculosis Extremadamente
DGE	Dirección General de Epidemiologia
DMSO	Dimethyl sulfoxide
EOL	Encyclopedia Of Life
IDSA	Infectious Diseases Society of America
MIC	Minimum inhibitory concentrations
NIAID	National Institute of Allergy and Infectious Diseases
WHO	World Health Organization
WNV	West Nile virus

1. INTRODUCTION

1.1. Infectious diseases

Infectious diseases are disorders caused by pathogenic microorganisms, such as bacteria, viruses, fungi or parasites. Many of these organisms live in and on our bodies and in certain conditions, may cause diseases which are spread from one person to another directly or indirectly (WHO, 2016). These are the leading causes of mortality in all regions of the world and are responsible for 15 million of deaths each year (Gaspar, 2016). Most of these deaths are in low and in middle income countries and result from preventable and treatable diseases such as diarrhoea, lower respiratory tract infections (such as tuberculosis), HIV/AIDS and malaria (CSIS, 2016). Some of these diseases affect the entire body while other infections affect only one organ or system of the body such as the eyes, ears, digestive tract, lungs, reproductive or urinary tracts (IDSA, 2016). The rapid increase in the incidence of infectious diseases in the population is due to recent emergence of pathogenic microorganisms, such as *Staphylococcus aureus*, West Nile virus (WNV) and H1N1 influenza virus which are methicillin-resistant and the re-emergence of pathogenic microorganisms that causes diseases such as dengue, malaria and polio (NIAID, 2009).

In Peru infectious diseases such as tuberculosis have high incidence being the first country in America with the highest incidence of multidrug-resistant and extensively drug-resistant tuberculosis (MDR TB and XDR TB) between 80-90% (Resistente CTE, 2008). Among other infectious diseases in Peru is malaria which is contracted in endemic regions within the country, incidence being 47% in Amazonia, 18% in the central jungle and 12% in the northern coast. About 15% of the cases were found in travellers returning from Africa. *Plasmodium vivax* was the most commonly identified and predominated (71%) as the etiology of severe malaria in cases acquired in Peru (Grande et al., 2007 and Llanos-Chea et al., 2015). Another infectious disease is dengue which is considered to be a major public health problem in Peru, particularly affecting urban and suburban communities in the

Amazon region with between 20 to 30% of incidence (Paredes-Esquivel et al., 2016 and DGE, 2015).

1.1.1. Classification

Clinicians tend to classify infectious diseases according to their most common important clinical manifestation and by the organ systems that are primarily affected such as diarrheal diseases, respiratory diseases, central nervous system infection, cardiovascular infection and sepsis while microbiologists, tend to classify infectious diseases according to the characteristics of the causative organism such as bacterial, viral and fungal infectious diseases. Epidemiologists normally classify infectious diseases according to two important epidemiologic characteristics namely, their modes of transmission and the reservoir of the organism (Nelson et al., 2007)

1.1.2. Pathogenic microorganisms

A pathogenic microorganism is considered to be any microorganism capable of injuring its host. These microorganisms are viruses, bacteria, mycobacteria, fungi, protozoa, and some helminths (Medical Dictionary, 2009) of which the main pathogenic microorganisms causing infectious diseases are viruses, bacteria and fungi (Nelson et al., 2007). This underscores the important need for their control, for prevention of the diseases and to understand the nature of these pathogens. For this study, bacteria and fungi were used and will be emphasized.

Bacteria as prokaryotes with a size of 0.2-10µm present with no internal organelles such as mitochondria (Lowy, 2016; Mishra and Agrawal, 2013). Most bacteria have cell walls, but some such as *Mycoplasma spp*. are devoid of cell walls. Bacteria are divided into two major groups: cocci and bacilli. Cocci (singular coccus) are spherical and may occur as single coccus, as a pair (diplococcus) as in the case of *Neisseria gonorrhoeae*, as a cluster as in the case of *Staphylococcus aureus* or as a chain of several cocci as in *Streptococcus spp*. Bacilli (singular bacillus) are rod-shaped bacteria presenting variation in their size and shape. Based on their reactions to Gram staining (colour), cocci and bacilli are divided into

two groups: Gram-positive (purple stain) and Gram-negative (red stain). The Gram-positive bacterial cell wall is made of a thick layer of peptidoglycan with some embedded teichoic acid while the outer layer of the Gram-negative bacterial cell wall is made of a thick layer of lipopolysaccharide, some phospholipids and with a small amount of peptidoglycan (Mishra and Agrawal, 2013). Commonly, bacteria can live extracellularly, but some bacteria (for example *Salmonella typhi, Neisseria gonorrhoeae, Legionella, Mycobacterium, Rickettsia, Chlamydia and Chlamydophila spp*) reside and replicate intracellularly (Schlecht and Bruno, 2015). In addition, bacteria have aerobic and anaerobic members. Several bacteria can grow under both conditions and are called facultative anaerobes (Mishra and Agrawal, 2013). With these characteristics bacteria can inhabit a variety of environments, including extremely cold and hot areas. However, many bacteria prefer the temperature of the healthy human body (NIAID, 2009), thereby causing most of the human diseases.

Bacterial infections are caused when the bacteria release chemical substances called toxins, which can damage tissues reproducing rapidly in the body and can cause diseases such as strep throat, tuberculosis and urinary tract infections, bacteria that cause infections include: *Streptococcus sp.*, *Staphylococcus sp.* and *Escherichia coli* and these infections can be treated with antibiotics. However, inappropriate use of antibiotics has helped to create strains of bacterial diseases that are resistant to treatment with different types of antibiotic medications (Steckelberg, 2014) as in the case of multidrug-resistant tuberculosis for which there were 480 000 recorded cases in 2013 (WHO, 2015).

Fungi are eukaryotic organisms that include unicellular microorganisms such as yeasts and (as moulds) multicellular fungi that produce familiar fruiting forms known as mushrooms. Fungi are nonmotile, with filamentous presentations that lack plastids and photosynthetic pigments. Most of fungi are saprophytes and some parasitize other organisms; they absorb their food in solution, through their cell walls; using their hyphaes that secrete digestive enzymes that break down the substrate, enabling the fungus to absorb the nutrients contained within the substrate (EOL, 2008). Fungi are sources for antibiotics (such as penicillin) used in medicine and for various enzymes such as cellulases, pectinases, and

proteases important for industrial use or as active ingredients of detergents. Many fungi produce bioactive compounds called mycotoxins, such as alkaloids and polyketides that are toxic causing diseases to animals and humans (Patterson, 2016). Fungi can be found in the air, soil, water and on plants. Some live in the human body, usually without causing illness (NIAID, 2009). Fungal infectious diseases (also called mycoses) are classified as opportunistic or primary; these infections develop in immune compromised hosts, and can be systemic or local (Revankar and Sobel, 2014), affecting skin, nail, body hair, internal organs such lungs (for example, *Aspergillus fumigatus* fungi that can cause aspergillosis) and body systems such as the nervous system (NIAID, 2009).

1.1.3. Treatments

Currently to treat infectious diseases, there are different therapies that are used including drugs or vaccines (Waheed et al., 2016). For the treatment of viral infections such as HIV, patient care and moral support as well as antiretroviral therapy are involved (OMICS, 2016). Somehow, for other viral infections, some antiviral drugs such as amantadine, oseltamivir, rimantidine and zanamivir are used, for example, for the treatment of influenza virus (Gale Encyclopedia of Medicine, 2008). In the case of bacterial infections treatment is by administering antibiotics to the patients (OMICS, 2016), the agents that are used for bacterial infections are clinically organized into six groups namely, penicillins, cephalosporins, tetracyclines, aminoglycosides, macrolides and fluoroquinolones (Harvey et al., 2007). In fungal infections, the use of antifungal therapy is limited to the use of amphotericin B, flucytosine, and a handful of clinically available azole agents (Limper et al., 2011). Currently antifungal agents such as voriconazole have become the primary treatment for most forms of invasive aspergillosis. Posaconazole offers a broad antifungal spectrum, and echinocandins are fungicidal against most *Candida* species (Spanakis et al., 2006).

1.1.4. Plants-derived products for treatment of microbial diseases

Throughout history people have used plants for healing and prevention of diseases. Existing records about medicinal plants, and archaeological reports suggest even earlier use of medicinal plants (Sharma, 2013). Currently, there is a wide use of drugs that contain plant extracts. According the World Health Organisation (WHO), of 252 drugs considered as basic and essential, 11% are exclusively of plant origin and a significant number are synthetic drugs obtained from natural precursors (Rates, 2001). The drugs of plant origin belong to a wide diversity of classes of secondary metabolites (terpenoids, alkaloids and phenolic compounds) (Kuete, 2010). Some examples are vincristrine and vinblastine from *Catharanthus roseus*, atropine from *Atropa belladonna*, morphine and codeine from *Papaver somniferum* (Rates, 2001). Artemisin and quinine are isolated from *Artemisia annua* and *Cinchona*, respectively which are used in the prevention and treatment infectious diseases such as malaria (Dvorkin-Camiel and Whelan, 2008). Shikimic acid is isolated from *Illicium verum* and is used as a base material for production of Tamiflu; this is an antiviral medicine for treating the H5N1 virus as well as influenza A and B infections (Lee and Balick, 2006; Bertelli, 2006).

1.2. South American Traditional Medicine

South America, has a long medicinal tradition that has been more preserved in Andean countries (Ecuador, Peru and Bolivia). As a great reference, Kallawaya Indians of Bolivia have medicinal practice that is rooted in the Tiahuanaco (400–1145), Mollo (1145–1435), Inca (1438–1532), Spanish (1532–1825) and Bolivian Republic (1825–1997) periods of the Andes region (Lunny, 1997).

Presently, traditional medicine is still practiced. For example, about 71% and 40% of the population use traditional medicine in Chile and Colombia, respectively (Bussmann and Sharon, 2006). The traditional practice incorporates diverse remedies and practices such as the use of herbs, amulets, guinea pigs (cuys) and incantations to eliminate disease or cleanse the body. Ecuadorian healers examine the urine in a clear container illuminated

by candlelight, viewing a fresh egg that has been passed over a patient's body, and looking at a guinea pig's innards after the animal has been held over the sick person's body for signs of the ailment. Peruvian and Bolivian indigenous communities have some diagnostic techniques in common. For example, when an organ of the examined animal appears to be infected, it is believed that the same organ is infected in the human patient (Chelala, 2009).

1.3. Peruvian Traditional Medicine

The use of medicinal plants has been practiced since the Inca period, and has been characterized to be magical and mystical. Incas had a wide knowledge of natural medicine. They knew the benefits of many products of natural origin, some examples being huairuro seeds (*Ormosia coccinea*), vilca seeds (*Anadenanthera colubrina*), molle resin (*Schinus*), used against depression, coca (*Erythroxylum coca*) which has anaesthetic effects, for pain relief and the use of some hallucinogenic plants such as San Pedro (*Echinopsis pachanoi*), Ayahuasca (*Banisteriosis caapi*) and Peyote (*Lophophora williamsii*). Some cultures as Mochica (I–VI AC) illustrated these diseases in their ceramics (Luna, 2016). Places as Callejón de Huaylas and other near-by valleys were influenced by pre-Incaic cultures, such as Chavin de Huantas (900-200 BC) in the use of traditional natural medicine (Gonzales, 2014).

Peruvian traditional medicine is widely practiced in local communities, as an own health resource, even for people living in urban setting (Gonzales et al., 2014) whereby is a large repository of traditional knowledge, adopted from extinct or endangered indigenous cultures (Phillips and Gentry, 1993). In Andean regions, Northern Peru is believed to be the centre of the Central Andean Health Axis (Camino, 1992; Bussmann et al., 2011). In Amazonian regions, traditional medicine and healing is also practiced (Phillips and Gentry, 1993; Jovel et al., 1996). The traditional plants are sourced from the mountain forests, especially the Andean highlands or the Amazon, which are often known by their Quechua names (Bussmann and Sharon, 2006). Mestizos Amazon have a long tradition of curanderismo, a healing practice that gives care and provides treatment of specific

psychopathological conflicts created by their culture and also related to the practice of sorcery; both are used for the diagnosis of illnesses and the administration of traditional medicine (Phillips and Gentry, 1993). These healing practices are associated with the spirits of the forest or 'supay'; it is believed that the use of 'magical' plants allows the curanderos or healers to communicate directly with powerful beings (Luna and Amaringo, 1991). In indigenous communities, everything is animated, personified and conceived to be capable of interacting with humans: plants, animals, rivers, lakes, rocks, the earth, the sun and the moon are all animated by the holy forces of nature this is expressed in the quechua prayer: "tayta inti (sun) is our father and mama kiua (moon) our mother, pacha mama is mother earth; amaru is the river, the water of life, and wammani, ilia, and mallhu kuntur are the messenger spirits, the visible signs of man's communion with the infinite cosmos" (Jovel et al., 1996). Currently there is no official registry of traditional medicine practitioners, also there is no law prohibiting traditional medicine (Gupta et al., 2010).

1.3.1. Medicinal Plants

In Peru the use of medicinal plants is common because of their positive effects on some ailments in humans and, in some cases, domestic animals. Some Provinces of the Andes of Peru use medicinal plants. For example, in Canta the population use over 87 species of the plants for traditional medicine; thirty-four of these species (including *Ageratina stembergian, Baccharis genistelloides, Baccharis salicifolia, Cestrum auriculatum, Erodium cicutarium, Jaltomata bicolor, Plantago major, Plantago myosuros, Polylepis racemosa, Rumex conglomeratus, Solanum americanum, Solanum pentlandii*)are used to reduce inflammation of different parts of the body, 16 (including *Ambrosia arborescens, Baccharis latifolia, Oreomyrrhis andicola*) are described as analgesics, 23 (including *Aristeguietia discolour, Baccharis genistelloides, Chenopodium ambrosioides, Foeniculum vulgare, Krameria lappacea, Ruellia floribunda, Senecio collinus*) are used for digestive disorders (to cure indigestion, as laxatives, as antidiarrhoeal and as anthelmintic), five namely, *Perezia coerulescens, Perezia multiflora, Perezia pinnatifidia Spilanthes leiocarpa* as diuretic and five (including *Salvia cruikshanksii*) for the cardio-vascular diseases(De-La-Cruz et al., 2007). Within Andean regions the use of medicinal plants in

terms as mode of preparation, mode of remedies administration, and sickness treated, has demonstrated a great homogeneity in Andean pharmacopeia (Poblette, 1992). The northeast of the Peruvian Andes also has an old tradition of herbal healing, some of which are: *Adiantum capillus-veneris, Culcitium canescens var. canescens, Schinus molle, Senecio culcitioides* and *Senecio tephrosioides,* used for respiratory tract infections, *Jungia panicultada* and *Sambucus peruviana* used as anti-inflammatory, *Krameria canescens* used as antidiarrheic and anti-inflammatory and *Mentzelia cordifolia* which is used as an analgesic and cicatrizant (Hammond et al., 1998).

In the Peruvian jungle some trees have properties for wound-healing, well known in folk medicine. For example, the red viscous latex from the Sangre de grado tree *(Croton lechleri)* contains the alkaloid taspine, responsible for the cicatrizant activity as well as other species such as *Anredera diffusa, Jatropha curcas and Peperomia galioides,* which are very used by the indigenous people as cicatrizants (Villegas et al., 1997). There are other plants as *Uncaria tomentosa* used as anti-inflammatory and antioxidants (Gonçalves et al., 2005). Some plants such as *Ardisia guayanensis*, *Aristolochia leuconeura*, *Brugmansia suaveolens, Cornutia microcalycina*, *Croton draconoides, Jatropha curcas*, *Zygia longifolia*are are used for depurative practices in Chazuta valley (Sanz-Biset and Cañiqueral, 2013). In Yanesha community (a Peruvian Amazonian ethnic group) malaria and leishmaniasis are endemic, and the symptoms are well known by the community. They use plants to treat these diseases. Approximately 94 species are used for preparation of remedies for the treatment of malaria or cutaneous leishmaniasis, some of which are *Begonia parviflora, Bidens pilosa, Carica papaya, Cestrum racemosum, Euphorbia heterophylla, Iribachia alata, Jacaranda copaia, Munnozia hastifolia, Pityrogramma calomelanos, Urera lacinata, Verbena littoralis,* and *Vismia sp.* (Valadeau et al., 2009).

1.3.2. Medicinal plants used for Cashinahua ethnical group (Huni kuin)

The Cashinahua (*kasha* = "bat", and *nawa* = foreign; but they call themselves *huni* = "man", *kuin* = "real") are an Indian tribe of the South American tropical rain forest living

near the headwaters of the Yurua-Purus river systems in the state of Acre, western Brazil (Figure 1). This ethnic group represents the culturally more conservative part of the tribe whose ancestors migrated into Peru more than 60 years ago in an effort to avoid the Brazilian rubber traders (Johnston et al., 1969; Santos-Granero and Barclay, 1994). Its population amounts to approximately 3,500 inhabitants, distributed in 13 Peruvian communities and in 11 indigenous communities or areas in Brazil (Santos-Granero and Barclay, 1994).

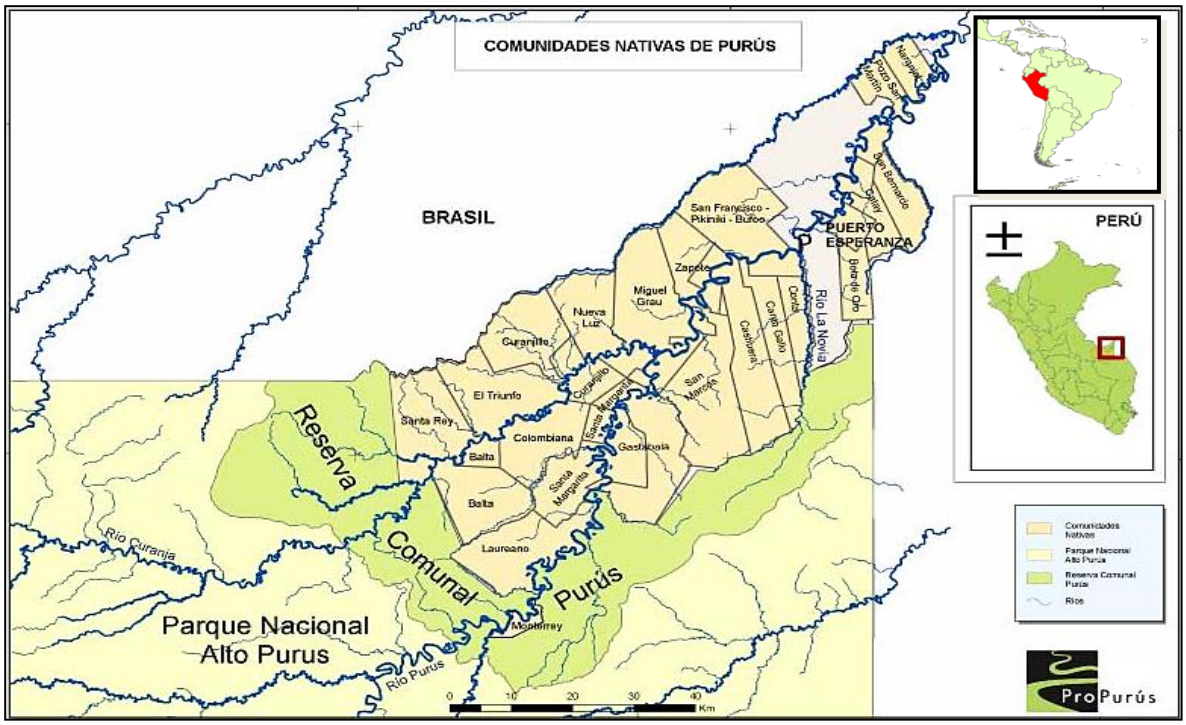

Figure 1. Map of native communities in Purus (Groth, 2014).

Plants play an eminent role in the social and ritual life in Cashinahua. Herbalists are very well appreciated because they can treat diseases with herbs which most of the time are efficient. When the patients do not respond to the treatment of herbs, people normally suspect that the spirits are responsible for the disease; in these cases they go to the shaman to discover the cause of the disease (Santos-Granero and Barclay, 1994).

Healers and shamans with their manifold methods and attributed powers, are classified by the Cashinahua of the Purus River into two groups: *dauya*, "the one with medicine" who

kills and heals through the use of medicinal plants; and the *mukaya*, "the one with bitterness" who heals and eventually kills with the help of spirits (*yuxin*) through a bitter substance (*muka*) that is a materialization of *yuxin* power. While the first specialist is initiated in his art by men, the second depends on the *yuxin* themselves to confirm his power (Whitehead and Wright, 2004). Nevertheless plants are considered to be imbued and as well be vehicles of spirit matter and energy (*yuxin*) (Kensinger, 1995).

Three plants play significant roles in shamanism and ritual practice for treatment to heal disease. These include tobacco (*Nicotiana* spp., *rustica* is most common in rituas), ayahuasca (*Banisteriopsis caapi* and various additives of *Psychotria viridis* or "chacruna"), and Datura (*Brugmansia* spp.). Tobacco is the ritual plant par excellence of Amazonia (Wilbert, 1987; Harner, 1973). Datura is widely dispersed throughout Amazonia but plays a notable role in the shamanic complexes of Andean cultural groups as well (Schultes and Raffuf, 1992; Schultes et al., 2001). Ayahuasca, containing *Psychotria viridis* leaves and other additives, is in large part unique to Amazonia (Schultes and Raffuf, 1992).

1.3.3. Ethnobotanical and Ethnopharmacological Studies

A number of ethnobotanical and ethnopharmacological studies have been carried out in some regions of Peru including Ancash (Hammond et al., 1998), Lima (Rehecho et al., 2011,De-la-Cruz et al., 2007), Loreto (Jovel et al.,1996; Estevez et al., 2007; Ruiz et al., 2011; Odonne, 2013), Madrede Dios (Lawrence et al., 2005;Ayme, 2014), Ucayali (Polesna et al., 2011), and Piura (De Feo, 2003). Nevertheless there is no national pharmacopeia, in the registration system for herbal medicines. There is no information as to the number of herbal medicines registered (Gupta et al., 2010).

1.3.4. Antimicrobial Activity of Peruvian Plants

A number of reports have been published about antimicrobial activities of different extracts from selected Peruvian plants against different microorganisms. The methods mostly used in studies were agar-diffusion and broth microdilution, the most used diluent being ethanol. Antimicrobial activity in previous studies was determined against *Bacillus*

subtillis, Bacillus cereus, Bacteroides fragilis, Candida albicans, Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus epidermidis, Streptococcus pyogenes and *Staphylococcus aureus, Trichophyton mentagrophytes, Microsporum gypseum, Sporothrix schenckii, Corynebacterium diphteriae* and *Clostridium histolyticum* and many others (Rojas et al., 2003; Kloucek et al., 2005; Neto, 2002; Langfield et al., 2004, Kloucek et al., 2007; Bussmann et al., 2008; Andersson et al., 1997; Neto et al., 2002; Valadeau et al., 2009; Bussmann et al., 2010).

Antimicrobial activities of some Peruvian plants as *Abuta grandifolia, Banisteriopsis caapi, Cestrum auriculatum, Croton lechleri Hura creoitans,Iryanthera lancifolia, Lepechinia meyenii, Ophryosporus peruvianus, Pterocarpus rohrii, Miconia salicifolia, Naucleopsis glabra* and a few more others were confirmed (Rojas et al., 2003; Bussmann et al., 2010, Kloucek et al., 2007).

1.4. Characteristics of Studied Plant Species

1.4.1. *Anomospermum grandifolium* Eichler. (Menispermaceae)

Synonyms: *Abuta macrocarpa* Moldenke, *Anomospermum ulei* Diels, *Elissarrhena grandifolia* (Eichler) Diels and *Elissarrhena longipes* Miers.

Vernacular name: Isun betun iti, cipo lingua.

Botanical description: A vine with large dimensions when mature, often reaching the tops of the highest trees; fruiting pedicels slender, 8-9 mm. long, glabrate; fruit oblong-ellipsoid, asymmetric at base, about 2.5 cm. long, 1.3-1.5 cm. wide, the exocarp coriaceous, lucidous, glabrous, fuscous or nigrescent in drying, the mesocarp pulpy, the endocarp osseous, areolate-rugose, longitudinally sulcate in a median circumferential line and each half thus produced again sulcate with a channel of similar depth and more or less paralleling the median one, 4-5 mm, removed from it at all points except the base, with a basal longitudinally sulcate like the endocarp, glabrous, obtusely rounded at both ends (Krukoff and Moldenke, 1938).

Natural habitat and geographic distribution: The specie has a wide range throughout Amazonian Brazil and Peru. In Brazil it occurs on terra firma and varzea land (Krukoff and Moldenke, 1938)

Traditional use: as poison in Guiana (Fanshawe, 1949).

Antimicrobial activity: Saponins were isolated from the methanol extract of the stem of *Anomospermum grandifolium* (Du et al., 2003) where antimicrobial activity screening of the compound of saponins, revealed antifungal properties against *Candida albicans* ATCC 3153 (Plaza A. et al., 2003).

Chemical composition: the stems of *Anomospermum grandifolium* reported curarizing activity of the tertiary and quaternary alkaloids (King, 1948) but no alkaloids have yet been identified (Pelletier, 1999) and from stems of *Anomospermum grandifolium* were isolated two new dammarane saponins and new lupane saponine (Du et al., 2003).

1.4.2. *Caamembeca spectabilis* (DC.) J.F.B. Pastore (Polygalaceae).

Synonyms: *Polygala spectabilis.*

Vernacular name: Camembeca

Botanical description: Erect herbs and sub shrubs reaching up to 1 m. Petiole (4,4–)6–12,5(–14) × (1–)2,8–4 cm in variable shape as lanceolate, narrow-lanceolate, elliptic, oblanceolate, acuminate leaves. Raceme 5-10 cm. Flowers 1–1,2 × 0,9–1,1 cm. Sepals all free. 5 petals, two rudimentary, two lateral and one modified. Capsules lightly winged. Seeds 5-6 x 2-4 mm (width at base), triangular, pubescent (Barea Pastore and da Silveira, 2016).

Natural habitat and geographic distribution: Is endemic of the Amazon region (Bolivia, Colombia, Guyana, Peru, Venezuela and in Brazil (Pastore and Silveira, 2016). Distributed in Colombia (Caquetá, Vaupés), Peru, Guyana, Surinam, French Guiana, Bolivia (Cochabamba, La Paz), Venezuela (Amazonas, Bolivar), N-Brazil (Amapa, Para, Amazonas, Acre), NE-Brazil (Paraiba, Pernambuco, Bahia), C-Brazil (Mato Grosso do

Sul), SE-Brazil (Rio de Janeiro) (Pastore, 2012).

Traditional use: Leaves are prepared in tea, juice, juice with honey, infusion; used in treatment of diarrhea, diabetes, child dysentery, vaginal discharge. Whole plant is prepared in tea and bath in treatment for haemorrhoids (Coelho M., 2009) and amoebal infection (Andrade et al., 1977; Peres and Nagem, 1997).

1.4.3. *Casearia pitumba* Sleumer (Flacourtiaceae)

Synonyms: *Casearia macrophylla* Vahl, *Casearia macrophylla* var. *barbatula* J. F. Macbr., *Casearia microphylla* Dennst., *Casearia timbuchi* J. F. Macbr. Ex Ll. Williams, *Pitumba edulis* A. Rich. ex Eichler, *Pitumba guianensis* Aubl. and *Pitumba guayannensis* Aubl., *Samyda pitumba* Poir.

Botanical description: Shrub or tree to 15 m tall, trunk beige to brown. Inflorescences: sessile, fasciculate, few to many-flowered, in foliate or defoliate axils of young twigs. Anther conective apiculate, very long-hispid, the hairs erect, wiry, about as long as the anther itself and projecting conspicuously beyond the anther. Leaves: 9.5-13.5 cm x 3.5-5 cm, 2-3 times as long as broad, chartaceous, elliptic to obovate, symmetric, dried colour various, secondary veins, branching from midvein at narrower angle (30-60°). Fruit: 2,5-3 cm diameter globose and glabrous capsule, yelowish-green to orange at maturity, drying dark brown. Seed: 12-13 mm long, compressed ovoid-trigonal (Zmarzty S., 2007).

Natural habitat and geographic distribution: Amazonian South America (Zmarzty S., 2007).

Chemical composition: The seeds of *Casearia pitumba* contain pitumbin (terpenoid) (Guittet et al., 1988; Xia et al., 2015).

1.4.4. *Piper heterophylum* Ruiz & Pav. (Piperaceae)

Synonyms: *Piper buchtienii* C. DC., *Piper buchtienii* var. *charopampanum* (C. DC.) Yunck., *Piper charopampanum* C. DC., *Piper dispansum* Trel., *Piper ellitptico-oblongifolium* Trel., *Piper obovatum* var. bolivianum C. DC., *Piper punctatum* Ruiz &

Pav., *Piper rurrenbaqueanum* Trel., *Piper suspectum* Trel. and *Schilleria heterophylla* (Ruiz & Pav.) Kunth.

Vernacular name: Paychané, chuikúnogi, carpunya

Botanical description: Shrub 4 m hig, thin, glabrous. Stem erect, articulate, knotted, different articulations. Alternate ramification (Ruiz & Pavon, 1802). Leaves are moderately petiolate, elliptic-oblong, apex shortly acuminate and the base is equilateral (Pax & de Candolle, 1991; Ruiz & Pavon, 1802). Petiole 10 mm, densely hairy in the base, 3 m long peduncle. Flower composed of four stamens. Berries attached to the lower base (Pax & de Candolle, 1911).

Natural habitat and geographic distribution: West of Amazonia and Pacific slopes of the Andes in Colombia (Chocó); in river beaches, gallery forests, and terra firme, frequently in sandy soils (Daly et al., 2006).

Traditional use: Leaves are used in the Tacana community to treat fever, kidney pain (Valdivia et al., 2009; Bourdy et al., 2000), anti-inflammatory, as disinfectant to wounds and for skin infections (Quisbert, 2007).

Antimicrobial activity: Never investigated scientifically for the antimicrobial activity from the leaves (Valdivia et al., 2009).

Chemical composition: Little information is available on *Piper heterophyllum* at least on a chemical basis (Lopez et al., 2008). From the ethanol extract of the inflorescences of *Piper heterophyllum*, two new prenylated protocatechuic acid derivatives were isolated and characterized; they were named arieianoic acid and arieianol (Green et al., 1999). Also essential oils from the leaves of *Piper heterophyllum* contain mainly monoterpenes: 1,8-cineole (eucalyptol), α-pinene (pinene) and asaricine (Lopez et al., 2008).

14.5. *Solanum sessile* Ruiz & Pav. (Solanaceae)

Synonyms: *Pheliandra herthae* Werderm, *Solanum dibrachiatum* Van Heurck & Mull. Arg., *Solanum grandifolium* C.V. Morton, *Solanum marmellosanum* Bitter, *Solanum*

pulchrum Dunal and *Solanum pulchrum* var. *peruvianum* Dunal.

Vernacular name: Perú, Amazonas "maikuanim", San Martin: "sacha congompe", "sanango", Loreto "asna panga".

Botanical description: Shrubs to small trees, 2-8 m tall, young stems and leaves glabrous, older stems glabrate, bark of older stems reddish-brown, later greyish. Leaves large, obovate, widest distal to the middle, usually in the distal third, glabrous above, occasionally with minute uniseriate trichomes along the veins beneath, this condition extremely variable, major leaves 15-40 cm long, 9-20 cm wide, with 9-11 pairs of primary veins, these raised above, the apex acute, the base variable, minor leaves differing from the major ones in shape and size, orbicular to obovate, 2,5-13,0 cm long, 1,5-9,0 cm wide, the apex acute, the base rounded to acute. Inflorescences terminal and overtopping the leaves at the shoot tips, later and opposite the leaves, complex and many times furcate, 6-25 cm long, 20-100 flowered, sparsely to densely puberulent with erect uniserate trichomes. Berries globose, green, 1,0-1,5 cm in diameter, in dry specimens woody and hard, fruiting pedicels erect and woody, calyx lobes slightly accrescent and woody in fruit. Seeds are ovoid-reniform, 2.5-4.0 mm long, 2-3 mm wide (Knapp, 1991).

Natural habitat and geographic distribution: On the eastern of the Andes from Colombia to southern Perú, extending into Amazon Brazil. Altitudinal range from 100 m to 1800 m in the Amazon basin of the central Andes-Peru (Knapp, 1991).

Despite the rich tradition of plants use in folk medicine in various parts of the Peruvian Amazon such as Purus district for the treatment of microorganisms associated diseases, , the antimicrobial activity and active compounds of these medicinal plants have not yet been studied. Based on ethnobotanical and pharmacological data summarized above, it is plausible to expect that some species used in the district for the treatment of infectious diseases would produce significant antimicrobial effect.

2. OBJECTIVES

The aim of this work is to determine *in vitro* antimicrobial activity of ethanol extracts obtained from five medicinal Peruvian plants traditionally used for treatment of diseases likely to be associated with microorganisms in Purus district, against pathogenic microorganisms by the broth microdilution method.

The specific aims are as follows:

- Determination of the minimum inhibitory concentration (MICs) of ethanol extracts from *Anomospermum grandifolium, Caamembeca spectabilis, Casearia pitumba, Piper heterophyllum, and Solanum sessile*, using the broth microdilution method.
- Identification of the most prospective plants, as potential source of bioactive constituents for further study.

3. MATERIAL AND METHODS

3.1. Plant Material

The selection of plants was based on local reports of plants used in folk medicine by the indigenous people in Purus (Cashinahua) to cure diseases likely to be associated with pathogenic microorganisms.

The leaves of *Anomospermum grandifolium, Caamembeca spectabilis, Casearia pitumba, Piper heterophyllum* and *Solanum sessile* were collected from Purus (southeast Peru), by Jana Horácková between May – June 2015. Voucher specimens authenticated by Maria Elena Chuspe Zans MSc. (Biol.) at National Intercultural University of the Amazon (UNIA) in Ucayali-Peru, where voucher specimens were deposited. The ethnomedicinal indications obtained from direct interviews with the Cashinahua indigenous people (Huni Kuin) in Alto Purus together with botanical names, families and local names of tested plants are summarized in Table 1.

Table 1 Ethnobotanical data of tested Peruvian medicinal plants

Species (Family)	Voucher number	Local name	Part used	Ethnomedicinal uses*
Anomospermum grandifolium Eichler (Menispermaceae)	HOR 286	Isun betun iti	Leaves	Urinary infection, kidney disorders o inflammation, epilepsy, stomach ache
Caamembeca spectabilis (DC.) J.F.B. Pastore (Polygalaceae)	HOR 202	Bata pei dentupa	Leaves	Conjunctivitis, aphthae, vaginal infection, eyesight problems, snakebite (jergon)

Species (Family)	Voucher number	Local name	Part used	Ethnomedicinal uses*
Casearia pitumba Sleumer (Flacourtiaceae)	HOR 249	Shipin tun akai bata	Leaves	snakebite (jergon)
Piper heterophyllum Ruiz & Pav. (Piperaceae)	HOR 222	Bixta kuma katsis	Leaves	faintness, syncope, vomiting
Solanum sessile Ruiz & Pav. (Solanaceae)	HOR 244	Xau bata	Leaves	alligator and crocodile bite – infection, snakebite (shushupe), snakebite (shaku dunu)

* Ethnomedicinal indication obtained from direct interviews with indigenous group (Cashinahua) in Purus.

3.2. Preparation of Extract

About 15 g of dried plant materials of each species was finely ground using Grindomix (GM100 Retsch Germany) and extracted at room temperature in 80% ethanol (450 ml) using a laboratory shaker for 24 hours.

Each extract was subsequently filtered using the filtering apparatus and concentrated to dry state using a vacuum rotary evaporator, Rotavapor R-200 (Buchi, Switzerland) at 40°C. Dried residues were dissolved in 100% dimethyl sulfoxide (DMSO) to obtain a concentration of 51.2 mg/ml stock solution of each extract and stored at – 20 °C until tested. The yield (%) of dried residues of each extract obtained and used as a starting material is shown in Table 2.

Table 2 Yield of Peruvian plants extracts (in 450 ml of 80% ethanol) after evaporation

Species	Yield (g)	Yield (%)
Anomospermum grandifolium	2.4671	16.4
Caamembeca spectabilis	6.0771	40.5
Casearia pitumba	2.0483	13.7
Piper heterophyllum	2.1462	14.3
Solanum sessile	1.4812	9.9

3.3. Microorganisms

Antimicrobial activity was evaluated against 4 bacterial strains and one yeast that were obtained from the American Type Culture Collection (ATCC). Bacterial strains were selected as representative of both classes of Gram-positive and Gram-negative bacteria, the microbial strains used were *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 29213. The yeast strain used in this study was *Candida albicans* ATCC 10231.

Escherichia coli, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* were grown in Mueller-Hinton broth (Oxoid), which was enriched with glucose for *Enterococcus faecalis* cultivation. Microorganism cultures were stored in Mueller-Hinton broth at 4 °C until use.

3.4. Inoculum preparation

The microorganisms were re-cultured suspended in 10 ml of Mueller-Hinton broth at 37°C for 24 h in *Candida albicans* for 48 h. Using Densi-La-Meter II (Lachema, Czech Republic), the turbidity of bacterial suspension was adjusted to 0.5 McFarland standard (which represents 1.5×10^8 bacteria/ml).

3.5. Susceptibility test

In order to assess the susceptibility of microorganisms to Tetracycline as antibiotic control, the range of concentrations of the antibiotic used was dependent on the strain sensitivity of the microorganisms. The concentrations of Tetracycline used for the bacterial strain of *Staphylococcus aureus* and *Escherichia coli* used ranged from 2 to 0.0625 µg/ml and 64 to 2 µg/mlfor *Enterococcus faecalis* and *Pseudomonas aeruginosa*. In the case of the yeast *Candida albicans* Tioconazole of concentrations of between 4 to 0.125 µg/ml was used as antibiotic

3.6. Antimicrobial assay

In vitro antimicrobial activity was measured by the broth microdilution method (Jorgensen et al., 1999; CLSI, 2009) using microtiter plates (96 wells) modified according to the recent recommendations for more effective assessment (Cos et al., 2006). Subsequently two-fold dilution of each sample on microtiter plate was prepared at extracts concentrations ranging from 512 to 16 µg/ml. Each well was inoculated with 5 µl of bacterial suspension at a density of 10^7CFU/ml. The microtiterplates were incubated at 37°C for 24 hours and in the case of the yeast *Candida albicans*, for 48 hours and then checked for the minimum inhibitory concentrations (MICs).

The growth of microorganisms was determined with Multiscan Ascent Microplate Reader (Thermo Fisher Scientific, Waltham, USA) at 405nm. The Minimum inhibitory concentrations (MICs) were then calculated based on the density of the growth control and were expressed as the lowest extract concentrations that resulted in $\geq$80 % reduction in bacterial growth compared to the extract – free growth control. Results reported in this study were expressed as the median/mode of MICs obtained from three independent experiments that were assayed in triplicate.

4. RESULTS

Three of five ethanol extracts of plants selected according to their traditional use in Purus district for treatment of infection-associated diseases, showed antimicrobial activity at least against three of the five microorganisms at concentrations ranging from 128 to 512 µg/ml (Table 3).

Extracts of *C. pitumba*, *P. heterophyllum* and *S. sessile*, had the broadest spectrum of antimicrobial and antifungal action. While *P. heterophyllum* produced the higher antibacterial effect among all tested plants showing a range of MICs from 128 µg/ml to 512 µg/ml against two Gram-positive bacteria (*E. faecalis* and *S. aureus*) and *C. albicans. C. pitumba* inhibited the growth of *S. aureus* by concentrations ranging from 256 µg/ml to >512 µg/ml and *S. sessile* inhibited growth of the *C. albicans* with MICs of 512 µg/ml. No antimicrobial activity was observed for extracts of *A. grandifolium* and *C. spectabilis*, even at the highest concentration tested (512 µg/ml).

In correspondence with previous reports, Gram-negative bacteria have been found to be more resistant than Gram-positive bacteria, probably, because of differences in cell wall morphology of these two groups (Silhavy et al., 2010). The two Gram-negative bacteria *E. coli* and *P. aeruginosa* were found to be resistant to all extracts tested in this study. The Gram-positive bacterium *S. aureus* was inhibited only by two extracts (*C. pitumba* and *P. heterophyllum*) at MIC 256 µg/ml. *E. faecalis* was inhibited only by one extract (*P. heterophyllum*) at MIC 128 µg/ml. *C. albicans* was inhibited by two extracts (*P. heterophyllum* and *S. sessile*) at the highest MIC 512 µg/ml.

Significant antimicrobial activity of the tested plant species *C. pitumba, P. heterophyllum* and *S. sessile*, supports wide traditional use reports from Purus district.

According our best knowledge no report on antimicrobial properties and chemical constituents of the leaves of this plant could be obtained from the literature. Whereby, this new observation calls for investigation on *C. pitumba, P. heterophyllum* and *S. sessile* to isolate and characterize its active antimicrobial constituents.

Table 3. Minimum inhibitory concentrations (µg/ml) of ethanol extracts from medicinal plants of Purus district, Peru.

Species[a]	**SA**	**EF**	**EC**	**PA**	**CA**
A. grandifolium	-[b]	-	-	-	-
C. spectabilis	-	-	-	-	-
C. pitumba	256	-	-	-	-
P. heterophyllum	256	128	-	-	512
S. sessile	-	-	-	-	512
Positive antibiotic control	0.5[c]	8[c]	0.5[c]	8[c]	2[d]

[a] SA, *Staphylococcus aureus,* EF, *Enterococcus faecalis,* EC, *Escherichia coli*, CA, *Candida albicans,* PA, *Pseudomonas aeruginosa*;[b]- not active (> 512 µg/ml); [c] tetracycline; [d] tioconazole.

5. DISCUSSION

The most interesting plant tested in our study seems to be *P. heterophyllum*, because its extract showed the strongest antimicrobial effect against two bacterial species (*S. aureus* and *E. faecalis*) and one yeast (*C. albicans*) with MICs ranging from 128 to 512 µg/ml. The leaves of the genus *Piper* contain various classes of phytochemicals such as alkaloids, carbohydrate, protein, flavonoids, tannins, sterols, phenols, and polyphenols; as it can be illustrated in the case of *P. betle* (Chakraborty and Shah, 2011; Datta et al., 2011). And also *P. betle* oil was found to have significant antibacterial and antifungal activity against *S. aureus* and *C. albicans* with MIC values of: 125 µg/ml and 250 µg/ml respectively (Caburian and Osi, 2010). In another study, methanol extract of the leaves of *P. betle* showed antimicrobial activity against *S. aureus* and *E. coli* by agar disk diffusion method (Nair and Chanda, 2008).

With regard to chemical components, in other species of the same genus as *P. officinarum*, some components were identified in the essential oil of the leaf. These components were beta-caryophyllene (11.2%), alpha-pinene (9.3%), sabinene (7.6%), beta-selinene (5.3%) and limonene (4.6%). The antimicrobial activity of the leaf oil showed weak activity against *P. aeruginosa* and *E. coli* with MIC values of 250 µg/ml (Salleh et al., 2012). Bicyclogermacrene (21.88 %), β-caryophyllene (20.69 %), myrcene (52.60 %) and linalool (15.89 %) were identified as major constituents in essential oil from leaves of *P. cernuum* and *P. regnellii* (Costantin et al., 2001). Both essential oils inhibited *in vitro* growth of *S. aureus* and *C. albicans* but not *P. aeruginosa* when tested using agar diffusion method (Constantin et al., 2001).

In the case of black peppers in this genus, the major constituent is piperine. It is a bioactive compound and has been reported to be the major contributors to the antimicrobial activity of spices (Chaudhry and Tariq, 2006). As in the case of *P. longum* (black pepper), three compounds (piperlonguminine, piperine and pellitorine) of this specie were isolated of which piperine showed active antibacterial activity against *Staphylococcus aureus* (Srinivasa Reddy et al., 2001). In another black pepper as *P. nigrum* L. the predominant

compound obtained from ethanolic extract were piperine and piperic acid, of which piperic acid was the most effective with the minimum inhibitory concentration (<325 µg/ml) against antibacterial strains (Zarai et al., 2013)

Based on the chemotaxomomical relationship, it is possible to suppose that some of the above mentioned active constituents may be responsible for antimicrobial effects of the extract from leaves of *P. heterophyllum* observed in this study.

C. pitumba possessed antimicrobial activity only against *S. aureus* with MIC of 256 µg/ml. *Casearia* genus contain various phytochemicals in the leaves as alkaloids, flavonoids, carbohydrates, glycosides, protein, as in the case of *C. tomentosa* (Tyagi et al., 2017). Also previous phytochemical screenings demonstrated that the *Casearia* plants mainly contain clerodane diterpenoids presented in leaves and twigs that were identified in the *C. sylvestris* extracts (Ferreira et al., 2011), sesquiterpenoids, phenylpropanoids and other constituents from different chemical classes (Xia et al., 2015). Some other species of the genus (*C. costulata, C. grewiifolia, C. multinervosa* and *C. grayi*) were observed to inhibit *C. albicans, E. coli, P. aeruginosa* and *S. aureus* growth using broth dilution method at concentration of 20 µg/ml (Mosaddik et al., 2004).

Therefore, we can suppose that these compounds can be responsible for antimicrobial effect of *C. pitumba.*

S. sessile showed certain growth-inhibitory effect only against *C. albicans* (MIC 512 µg/ml). In previous report of Herrera et al. (2007), the saponin compound of *S. chrysotrichum* leaves was found to be effective against *C. albicans.* This compound may also be responsible for anticandidal effect of *S. sessile*. According to previous studies, leaves of other species and of the same genus (e.g. *S. nigrum* and *S. trilobatum*)contain some compounds such as alkaloids, flavonoids, saponins, glycosides, terpenoids, tannins and phytosterols (Doss et al., 2009; Gogoi and Islam 2012; Musto et al., 2015; Swapna and Kannabiran, 2006). These species presented antimicrobial activity by disc diffusion method and agar dilution assay against *S. aureus* and *E. coli* at very broad range of MICs from 60

to 6 000 µg/ml (Swapna and Kannabiran, 2006; Doss et al., 2009; Aliero and Afolayan, 2006) and different *C. albicans* strains with MIC from 200 to 800 µg/ml (Herrera et al., 2013). This can explain the no antibacterial effect of *S. sessile* at the level 512 µg/ml observed and broth microdilution method that was assayed in this study.

A. grandifolium and *C. spectabilis* didn't produce any antimicrobial activity against any pathogenic microorganism. Nevertheless according to previous studies, the stem of *A. grandifolium* showed antimicrobial activity against *C. albicans* ATCC 3153 (Plaza A. et al., 2003) due to some compounds such as alkaloids (King, 1948; Pelletier, 1999) and saponins (Du et al., 2003) in the stem. But it could be possible that these secondary metabolites are not present in the leaves. There are no reports on antimicrobial study of the leaves of these plants that could be obtained from literature.

6. CONCLUSIONS

In our study, the antimicrobial activities of extracts from five Peruvian medicinal plant species were assayed. The antimicrobial screening was carried out with the aim of verifying traditional uses of these species found in the Purus District (South east, Ucayali Region). The extracts from *P. heterophyllum*, *C. pitumba*, and *S. sessile* showed certain degree of antimicrobial activity.

The ethanol extract of *P. heterophyllum* appears to be the most promising candidate for further work on isolation and identification of active compounds, because it appears to be active against a broader range of microbial species (*S. aureus, E. faecalis* and *C. albicans*) whereas MICs of this plant extract ranged from 128 to 512 µg/ml. However, the ethanol extracts of the other two plant species *C. pitumba* and *S. sessile* should also be given adequate attention since even the inhibition of a single or few microbial species might reveal unexpected properties when the active compound(s) are isolated and purified.

These findings indicate that further studies on chemical and biological properties of their active components should be performed.

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ANNEX 1: Detailed results of antimicrobial susceptibility testing of Peruvian medicinal plants

Pathogen	Microorganisms/Minimum inhibitory concentration (µg/ml)															Antibiotic		
	Anomospermum grandifolium			*Caamembeca spectabilis*			*Casearia pitumba*			*Piper heterophyllum*			*Solanum sessile*					
	1st exp.	2nd exp.	3rd exp.	1st exp.	2nd exp.	3rd exp.	1st exp.	2nd exp.	3rd exp.	1st exp.	2nd exp.	3rd exp.	1st exp.	2nd exp.	3rd exp.	1st exp.	2nd exp.	3rd exp.
Staphylococcus aerues	>512	>512	512	>512	>512	>512	256	256	256	512	256	256	>512	512	512	0.5	1	0.5
	>512	>512	>512	>512	>512	>512	256	256	256	512	256	256	>512	256	>512	0.5	0.5	0.5
	>512	>512	>512	>512	>512	>512	256	256	256	512	256	256	>512	512	>512	0.5	0.5	1
Mode/Median	>512	>512	>512	>512	>512	>512	256	256	256	512	256	256	>512	512	>512	0.5	0.5	0.5
	>512			>512			256			256			>512			0.5		
Enterococcus faecalis	>512	>512	>512	>512	>512	>512	256	>512	>512	64	64	128	>512	>512	>512	4	4	16
	>512	>512	>512	>512	>512	512	512	>512	>512	128	64	64	512	>512	512	8	4	8
	>512	>512	>512	>512	>512	>512	256	>512	>512	256	64	256	>512	>512	>512	8	8	16
Mode/Median	>512	>512	>512	>512	>512	>512	256	>512	>512	128	64	128	>512	>512	>512	8	4	16
	>512			>512			>512			128			>512			8		
Escherichia coli	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	0.5	0.5	1
	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	0.5	0.5	1
	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	0.5	0.5	2
Mode/Median	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	0.5	0.5	1
	>512			>512			>512			>512			>512			0.5		
Pseudomonas aeruginosa	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	8	16	8
	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	8	8	8
	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	8	16	8
Mode/Median	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	8	16	8
	>512			>512			>512			>512			>512			8		

Pathogen	Microorganisms/Minimum inhibitory concentration (μg/ml)															Antibiotic		
	Anomospermum grandifolium			*Caamembeca spectabilis*			*Casearia pitumba*			*Piper heterophyllum*			*Solanum sessile*					
	1st exp.	2nd exp.	3rd exp.	1st exp.	2nd exp.	3rd exp.	1st exp.	2nd exp.	3rd exp.	1st exp.	2nd exp.	3rd exp.	1st exp.	2nd exp.	3rd exp.	1st exp.	2nd exp.	3rd exp.
Candida albicans	>512	>512	512	>512	>512	512	>512	>512	512	512	512	512	256	>512	512	2	4	2
	>512	>512	512	>512	>512	512	512	>512	>512	512	512	512	512	512	512	2	4	2
	>512	>512	512	>512	>512	>512	512	>512	>512	512	>512	>512	512	512	512	2	4	2
Mode/Median	>512	>512	512	>512	>512	512	512	>512	>512	512	512	512	512	512	512	2	4	2
	>512			>512			>512			512			512			2		

ANNEX 2: Photographic illustrations of plant samples

Original photos by Jana Horackova, 2012