In vitro tissue culture, preliminar phytochemical analysis, and antibacterial activity of *Psittacanthus linearis* (Killip) J.K. Macbride (Loranthaceae)

Cultivo de tejidos *in vitro*, análisis fitoquímico preliminar y actividad antibacteriana de *Psittacanthus linearis* (Killip) J.K. Macbride (Loranthaceae)

Juan Pablo Balladares Ballona*, Guillermo Eduardo Delgado Paredes**, Marcelo Luis Wagner, Consuelo Rojas Idrogo*****

DOI: 10.15446/rev.colomb.biote.v21n2.83410

ABSTRACT

Hemiparasitic plants commonly known as mistletoe (muérdago in Spanish) in the families Santalaceae and Loranthaceae are common in various kinds of plants or trees, and many hemiparasitic plants are used for medicinal purposes in various parts of the world. The objective of the present work, carried out in *Psittacanthus linearis* (suelda con suelda), a representative species in the seasonally dry forest (SDF) from the north of Perú, was to study aspects of *in vitro* tissue culture, carry out preliminary phytochemical analysis, and assess antibacterial activity. Seeds of individuals of *P. linearis*, which used *Prosopis pallida* (algarrobo) as host plant, were collected and used to induce *in vitro* seed germination, clonal propagation, callus induction and organogenesis. Stems, leaves and fruits of individuals of *P. linearis* were dried, powdered, and subjected to ethanol extraction. Posteriorly the extract was first recovered with ethanol and the remnant with chloroform, which formed the ethanolic and chloroformic fraction. A preliminary phytochemical screening was performed and preliminary antibacterial studies with *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* were carried out and their results are discussed. This is the first report about *in vitro* tissue culture, phytochemical analysis and antibacterial activity of *P. linearis*. The results may have important implications for understanding physiological and biochemical interactions between host and hemiparasitic species as well as *P. linearis* with *P. pallida* and other SDF species.

Key words. Catechin and cyanidin, hemiparasitic plant, *Prosopis pallida*, 'suelda con suelda', *Staphylococcus aureus*.

RESUMEN

Las plantas hemiparásitas o 'mistletoe' o 'muérdago' son comunes en varios grupos vegetales o árboles, perteneciendo a las familias Santalaceae and Loranthaceae y muchas plantas hemiparásitas son usadas como medicina en varios lugares del mundo. El objetivo del presente trabajo realizado en *Psittacanthus linearis* or 'suelda con suelda', especie representativa en el bosque estacional-

* Lic., Facultad de Ciencias Biológicas, Universidad Nacional Pedro Ruiz Gallo. juanpi3n_74@hotmail.com.

** PhD., Profesor Principal, Facultad de Ciencias Biológicas, Universidad Nacional Pedro Ruiz Gallo, Ciudad Universitaria, Juan XXIII No. 391, Lambayeque, Perú. guidelg2015@yahoo.es. ORCID: 0000-0001-5769-8209.

*** PhD., Profesor Titular, Cátedra de Farmacobotánica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina, Junín 956 Piso 4 (1113) Ciudad Autónoma de Buenos Aires, República Argentina. mwagner@ffyb.uba.ar. ORCID: 0000-0003-3225-9765.

**** MSc., Profesora Principal, Facultad de Ciencias Biológicas, Universidad Nacional Pedro Ruiz, Ciudad Universitaria, Juan XXIII No. 391, Lambayeque, Perú. crojasi2002@yahoo.es. ORCID: 0000-0003-3525-6711.

mente seco (BES) del norte del Perú, fue estudiar algunos aspectos en el cultivo de tejidos *in vitro*, el análisis fitoquímico preliminar y su actividad antibacterial. Semillas de *P. linearis* teniendo a *Prosopis pallida* 'algarrobo' como hospedero, fueron colectadas y utilizadas en la germinación *in vitro*, propagación clonal, inducción de callos y procesos organogénicos. Tallos, hojas y frutos de plantas silvestres fueron secados, pulverizados y sometidos a extracción con etanol y el extracto fue recuperado primero con etanol y el remanente con cloroformo formando las fraciones etanólica y clorofórmica. Se realizó un estudio fitoquímico y antibacteriano preliminar utilizando *Staphylococcus aureus*, *Escherichia coli* y *Pseudomonas aeruginosa* y los resultados son discutidos. Este trabajo es el primer estudio sobre cultivo de tejidos, análisis fitoquímico y actividad antibacteriana de *P. linearis*. Los resultados obtenidos tienen importantes implicancias para el conocimiento de las interacciones fisiológicas y bioquímicas entre las especies hospederas y las plantas hemiparásitas, como *P. linearis* con *P. pallida* y otras especies del BES.

Palabras clave. Catequina y cianidina, planta hemiparásita, *Prosopis pallida*, 'suelda con suelda', *Staphylococcus aureus*.

Recibido: agosto 6 de 2018 **Aprobado:** octubre 10 de 2019

INTRODUCTION

The order Santalales consists of 10 families and ca. 2000 species. The largest family is Loranthaceae (900), followed by the Santalaceae (400), Viscaceae (300), and Olacaceae (250). None of the six remaining families has more than a hundred species (Cronquist,1988). According to the system proposed by the Angiosperm Phylogeny Group, the family Loranthaceae is classified with Olacaceae, Balanophoraceae, and Santalaceae, and others families in the order Santalales, Core Superasterids (APG IV, 2016).

Loranthaceae is pantropical, with ca. 65 genera and 900 species of epiphytic, photosynthetic and hemiparasites plants that adhere to branchs of trees by means of haustoria to absorb water and nutrients. Hemiparasitic plants are commonly known as mistletoe (Lorenzi, 2000; Pennington *et al*., 2004) or 'suelda con suelda' (Peruvian vernacular name). There are 11 genera and ca. 60 species in Peru. The only arborescent genus is *Gaiadendron* G., which is a root parasite (Pennington *et al*., 2004). *Psittacanthus linearis* (Killip) J.F. Macbr. attached to following forest species of the seasonally dry forest: *Prosopis pallida* (Willd.) Kunth (Fabaceae) (algarrobo), *Acacia macracantha* Willd. (Fabaceae) (faique), *Salix humboltiana* Willd. (Salicaceae) (sauce) and others species.

A parasitic plant is an angiosperm (flowering plant) that directly attaches to another plant via haustorium, which is a specialized structure that forms a morphological and physiological link between the parasite and host (Nickrent & Musselman, 2004; Yoshida *et al*., 2016). Hemiparasitic plants have an ambiguous relationship with their hosts, which on the one hand, are the sources of inorganic nutrients but, on the other hand, can compete with the hemiparasites for light. Consequently, hemiparasitic plants have a unique way of acquiring resource that combines parasitism of other species with their own photosynthetic activity, so that despite their active photoassimilation and green habit, they acquire substantial amount of carbon from their hosts (Těšitel *et al*., 2009). It was investigated a model of the spatial distribution of true mistletoe, (*Cladocolea loniceroides* (Tiegh.) Kujit, using classical statistics, spatial statistics and geostatistics in the green areas of Tlalpan Delegation - Mexico City to analyze the correlation among mistletoe-hosts (Espinoza-Zúñiga *et al*., 2019).

A study of the hemiparasitic angiosperm *Thesium humile* Vahl (Santalaceae) assessed physiological changes in the root before and after the attachment to the host plant (*Triticum vulgare*). This obliged angiosperm root hemiparasite can live in an autotrophic state for several weeks before joining the host. *T. humile* is able to take up water and nutrients ions from the soil, but has very high levels of Na and low levels of P (Fer *et al*., 1994). The functional relationships between aerial and root parasitic plants and their woody hosts and consequences for ecosystems were recently discussed, and gross comparisons of nutrient content between infected and uninfected hosts, or parts of hosts, have been widely used to infer basic differences or similarities between host and parasites (Bell & Adams, 2011). A study of the hemiparasite *Santalum album* L. (Santalaceae) and its hosts in southern China compared two non-N_2-fixing hosts [*Bischofia polycarpa* (H. Lév.) Airy Shaw (Phyllanthaceae) and *Dracontomelon duperreanum* Pierre (Anacardiaceae)] and two N_2-fixing hosts (*Acacia confusa* Merr. and *Dalbergia odorifera* T.C. Chen, both species within Fabaceae) with respect to the growth characteristics and nitrogen nutrition of *S. album* (Lu et *al*., 2014).

Studies about *in vitro* tissue culture, phytochemical analysis and antibacterial activity are scarce in the species of Loranthaceae, and in the case of *P. linearis* are non-existent.

Deeks *et al*. (1999) reviewed *in vitro* tissue culture of parasitic flowering plants in genera of the Loranthaceae family such as *Amyema* Tiegh., *Amylotheca* Tiegh., *Dendrophthoe* Mart., *Nuytsia* R. Br. ex G. Don, *Scurrula* L., *Tapinanthus* (Blume) Rchb, and *Taxillus* Tiegh., and discussed the applications of tissue culture techniques in studies of the biology and host-pathogen interactions.

Callus induction, seedlings (with haustorial discs, holdfasts and plumular leaves), embryogenic callus, shoots, and somatic embryos, were the main results obtained (cf. Deeks *et al.*, 1999). Relevant results have been reached for *Dendrophthoe falcata* (L. f.) Ettingsh, one of the most studied species (Ram *et al.*, 1993), and for *Amyema miquelii* (Lehm. ex Miq.) Tiegh., *A. quandang* (Lindl.) Tiegh., and *A. pendula* (Sieber ex Spreng.) Thieg., where callus was induced, and the seedlings formation with several structures was obtained (Hall *et al*., 1987).

Several species of Loranthaceae are of ethnobotanical importance and are used as medicinal plants in various regions of the world, especially in Africa and India. African mistletoes of the Loranthaceae (*Globimetula* Tiegh., *Phragmanthera* Tiegh., *Agelanthus* Tiegh. and *Tapinanthus*, and Viscaceae family as *Viscum* L.) are hemiparasitic plants and their preparations in the form of injectable extracts, infusions, tinctures, fluid extracts or tea bags are widely used in various cultures and in almost every continent to treat or manage health problems including hypertension, *diabetes mellitus*, inflammatory conditions, irregular menstruations, menopause, epilepsy, arthritis, and cancer (Adesina *et al*., 2013). A preliminary phytochemical screening of the methanolic extract of *Helicanthes elastica* (Desr.) Danser (Loranthaceae) which grows on the host plants *Nerium indicum* Mill. (= *N. oleander* L.) (Apocynaceae) and *Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg. (Euphorbiaceae) revealed the occurrence of various constituents such as glycosides, saponins, tannins and phenols in both hosts (Kumar & Mathew, 2014). On the other hand, a *in vitro* study about the anti-diabetic properties from hemiparasitic species of *D. falcata* revealed that its plant's leaves extracts had inhibitory activity on the key enzyme alpha-amylase, which enzyme breaks the large strach molecules that produces free glucose and simultaneously increases the blood sugar level, and consequently hyperglycemia (Naskar *et al*., 2019). Likewise, the cloroform fraction and crude extract from *Loranthus acaciae* Zucc grown in Saudi Arabia showed anti-diabetic, anti-inflammatory and antioxidant activities (Noman *et al*., 2019). Leaves from *Scurrula parasitica* L., quercetin, quercitrin, kaempferol 3-*O*-α-L-rhamnoside, (+)-catechin compounds, together with ethyl acetate and methanol extracts exhibited effective antioxidant activities against DPPH(2,2-diphenyl-1-picrylhydrazyl), ABTS[2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] and FRAP (Ferric reducing antioxidant potential), while *n*-hexane and other compounds were inactive (Muhammad *et al*., 2019).

Other preliminary phytochemical, physicochemical, and antimicrobial studies of *Loranthus elasticus* Desv. [= *Helicanthes elasticus* (Desv.) Danser] associated to the neem tree (*Azadirachta indica* A. Juss.) (Meliaceae) and collected from the local fields of Salem district of Tamil Nadu, India, were performed (Krishnaveni *et al*., 2016). An ethnobotany study of seven hemiparasitic Loranthaceae species: *Tapinanthus bangwensis* (Engl. & K. Krause) Danser, *T. belvisii* (DC.) Danser, *T. sessilifolius* var. *glaber* Balle (= *Tapinanthus praetexta* Polhill & Wiens), *Phragmanthera capitata* (Spreng.) Balle, *P. capitata* var. *alba*, *Globimetula braunii* (Engl.) Danser and *G. dinklagei* subsp. *assiana* Balle [= *Globimetula assiana* (Balle) Wiens & Polhill], all of which are used in traditional medicine in the Sud-Comoé region (Côte d'Ivoire) (Amon *et al*., 2017). Likewise, an ethnobotanical analysis of parasitic plants (Parijibi) in the Nepal Himalaya, a hotspot of botanical diversity, housing 15 families and 29 genera of plants with parasitic or mycoheterotrophic habit (O'Neill & Rana, 2016).

On the other hand, in miscellaneous studies, African mistletoe [*Tapinanthus dodoneifolius* (DC.) Danser] [= *Agelanthus dodoneifolius* (DC.) Polhill & Wiens] commonly called 'Kauchi' in Hausland (Northern Nigeria) is currently used ethnomedicinally in the inhibition of the growth of *Agrobacterium tumefaciens*, *Escherichia coli*, *Salmonella* sp., *Proteus* sp., and *Pseudomonas* sp., species of bacteria associated with gastrointestinal tract and wound infections (Deeni & Sadiq, 2002). The thin layer chromatography (TLC) phytochemical screening in some Nigerian Loranthaceae as *Phragmanthera*, *Globimetula* and *Tapinanthus* genus, collected from the field was carried out with a view to ascertaining chemical constituents present and determining their importance in the taxonomic delimitation of the taxa (Wahab *et al*., 2010). In another study in *Phragmanthera capitata* 11 endophytic fungi species from four genera: *Aspergillus* (six species), *Penicillium* (three species), *Trichoderma* (one species) and *Fusarium* (one species). In the same study the phytochemical analysis revealed the presence of flavonoids, anthroquinones, tannins, phenols, steroids, coumarins and terpenoids, and absence of alkaloids and saponins in all the extracts (Ladoh-Yemeda *et al*., 2015). Micromorphological studies of the Loranthaceae *P. capitata* with scanning electron, light, and energy dispersive X-ray (EDX) microscopies revealed a paracytic type of stomata, oval-shaped lenticels, densely packed stellate trichomes, tracheary elements, which are tightly packed with granules believed to be proteins, and deposits chiefly composed of Si, Al, K, and Fe (Ohikhena *et al*., 2017). Likewise, qualitative and quantitative phytochemistry, micro and macro-elements and microscopy of *Tapinanthus dodoneifolius* (African mistletoes) collected from 'guava' (*Psidium guajava* L.) (Myrtaceae), 'rubber' (*Hevea brasiliensis*), and 'orange' [*Citrus sinensis* (L.) Osbeck] (Rutaceae) trees was carried out with the purpose of comparing their pharmacological and biological contents. The phytochemical analysis of African mistletoes showed the presence of oxalate, phytate, saponin, alkaloid, glycoside, and tannin, which were present in proportions that varied with their hosts (Idu *et al*., 2016).

The aim of the present study was to evaluate some aspects of *in vitro* tissue culture, preliminar phytochemical analysis and antibacterial activity of *Psittacanthus linearis* collected from the host *Prosopis pallida*, a representative species of the Seasonally Dry Forest of Northern Peru, due to its high tolerance to drought and salinity and produce fruits with high nutritional value.

MATERIALS AND METHODS

Plant materials and seed desinfection. Seeds and whole plants with red flowers of [*Psittacanthus linearis* (Killip) J.K. Macbride] 'suelda con suelda'(Loranthaceae), in *Prosopis pallida* 'algarrobo' (Figure 1a) were collected from a natural population in Pítipo district of Ferreñafe province, Lambayeque-Peru. The plant specimens were identified by Dr. Santos Llatas Quiroz and botanist José Ayasta Varona from the PRG Herbarium of the Faculty of Biological Sciences of the Universidad Nacional Pedro Ruiz Gallo (Lambayeque, Peru). Specimens were deposited in the Herbarium PRG with the voucher number 18013. The seeds were manually dehusked and surface sterilized by immersion for 1 min in ethanol 70% (v/v) and 5 min in sodium hypochlorite solution (Clorox®) 5.25% (v/v) containing a few drops of polyoxyethylene sorbitan monolaurate (Tween 20®), followed by five rinses of 1 min each with sterile distilled water.

Culture media and culture conditions. All media consisted of full-strenght MS (Murashige & Skoog, 1962) salt formulation containing the following ingredients: 1.0 mg L^{-1} thiamine.HCl, 100 mg L^{-1} myo-inositol, 3% sucrose, and 0.6% agar-agar. The disinfected seeds were germinated aseptically in the MS formulation supplemented with 3-indol acetic acid (IAA) and 6-benzilaminopurine (BAP). The seeds were scored daily for germination and the breakthrough of the radicle from the seed coat were used as the criterion for germination (Côme, 1968). In the leaves and apical buds elongation, BAP and IAA-GA_3 (Gibberellic acid) combinations were applied in several concentrations. In the callus induction, three types of auxins [2,4-D (2,4-dichlorophenoxyacetic acid), NAA (naphthalene acetic acid), and IAA] were applied in several concentrations, and for organogenesis, IAA-BAP were also applied in several concentrations. The pH of all the culture media was adjusted to 5.7±0.1 with KOH or HCl before autoclaving. For all experiments, 15 mL of the medium was transferred to 150x25 mm test tubes, covered with polypropylene tops, and autoclaved for 20 min at 121 °C and 1.05 kg cm^{-2}. One explant was cultured per tube. Cultures were incubated at 26±2 °C under a 16-h photoperiod with the light intensity of 70 µmol m^{-2} s^{-1} photosynthetic active radiation provided by cool white fluorescent tubes; only the callus cultures were incubated in a dark room.

Phytochemical analysis. Stems, leaves, and fruits of *P. linearis* were oven-dried separately at 40 °C and powdered (5 to 10 mm of particle diameter). The dried powder was subjected to extraction with 150 mL of 96° ethanol (three extractions of 50 mL) during 24 h. All extracts were evaporated to dryness under reduced pressure. The extract was recovered with ethanol and the remnant with chloroform to form the ethanolic and chloroformic fractions. Subsequent analysis included colorimetric quantification of total polyphenols, determination of proteins and Total Radical-Trapping Antioxidant Parameter (TRAP) (Lissi *et al*., 1992, Wagner *et al*., 1998), and characterization of flavonoids by means of two-dimensional chromatography.

Calorimetric quantification of total polyphenols. Samples (stems, leaves and fruis) of 1 g air-dried material were extracted with MeOH (50:50) for 48 h and dark conditions. For this process 3 mL ferric chloride and 3 mL potassium ferrocyanide were used, and the readings were made in photocolorimeter with an absorbance of 720 nm. The standard curves were established with tannic acid.

Detection of proteins by Sodium Dodecyl sulfate Polyacrylamide gel Electrophoresis (SDS-PAGE). SDS-PAGE was performed according to the method of Laemmli (1970) using a discontinuous system of two layers: 10% polyacrylamide resolving gel and 4% polyacrylamide stackin gel. Samples (leaves) of 1 g air-dried material were extracted with 10 mL of 20 mM Tris-HCl buffer pH 8.0, 1 mM phenylmethylsulfonyl fluoride (PMSF), 5 mM β mercaptoethanol (βME) and 1% polyvinylpyrrolidone

(PVP). Electrophoresis was carried out at 150 mA/gel for 45 min using a Mini Protean II Electrophoresis System (Bio Rad, USA), and gels were stained with silver. Molecular weight standars SDS-VII (Sigma Chem. Co., St Louis MO) were used (Wagner *et al*., 1998).

Determination of Total Reactive Antioxidant Potential (TRAP). Total Reactive Antioxidant Potential (TRAP) was measured by luminol-enhanced chemilumininescence (Lissi *et al*., 1992). The reaction medium consisted of phosphate buffer 100 mM (pH 7.4), 20 mM 2,2'-azo-*bis*(2 -amidinopropane) (ABAP), 10 µM luminol in 0.1 M NaOH. Incubation of the mixture at room temperature generates a nearly constant light intensity that was measured directly in a Packard *tri*-Carb scintillation counter with the circuite coincidence out of mode. The system was calibrated with catechin, quercetin and ascorbic acid.

Caracterization of flavonoids. For this analysis, 5.0 mg samples (stems and leaves) of methanolic extracts from *P. linearis* were disolved in 10 mL MeOH (80:20). They were purified or fractionated by column chromatography and analyzed by TLC and UV spectroscopy (Mabry *et al*., 1970; Waterman & Mole, 1994).

We also compared protein profiles of leaves of *P. linearis* (PH) and *Ligaria cuneifolia* from different hosts 20, 24, 30 and 3. L. cuneifolia has been studied with respect to its anatomical, phytochemical and immunological properties (Wagner *et al*., 1998), and in Argentina leaf and stems infusions have been used as a substitute of *Viscum album* based on their putative depressive effect on high blood pressure.

Antibacterial activity. Three strains of Gram positive (*Staphylococcus aureus*) and Gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria, were isolated from clinical samples from the Microbiology Laboratory (Faculty of Biological Sciences of the Universidad Nacional Pedro Ruiz Gallo, Lambayeque, Peru) and identified by microscopic observation and biochemical tests. In the dilutions and inoculum preparations, the ethanolic and chlorophormic fractions of wild plants were weighed and disolved in sterile distilled water to prepare the required dilutions of 0.0 (control), 50, 100, 200 and 300 µg mL^{-1} per disc. Inocula of bacterial species were prepared in nutrient medium Müller Hinton and kept under incubation at 37 °C for 8 h for sterilization control. The cultures were then refrigerated at 2-8 °C. For the Kirby-Bauer Disc Diffusion Test, 1 mL of inoculum suspension was spread uniformily over the agar medium using a sterile glass rod to get uniform distribution of bacteria. Sterile discs of 5 mm diameter prepared with Whatman paper filter N° 41 were impregned with different concentrations of 0.0, 50, 100, 200 and 300 µg mL^{-1} per disc of plant extract. The discs were placed on the medium and incubated at 37 °C for 24 h. Antibacterial activity was determined by measuring the width of the inhibition zone (mm). Equivalent concentrations to the tube N° 5 McFarland nephelometer which corresponding to a density of $1.5x10^{8}$ ufc mL^{-1}, were used.

Statistical analysis. The data (means ± SE, *n* = 10) were subjected to one-way analysis of variance (ANOVA) and the Tukey HSD multiple range test $P \leq 0.05$ level, in order to compare treatment means. All analyses were performed with SPSS version 11.5 (SPSS Inc., Chicago, IL, USA).

RESULTS

In vitro tissue culture

***In vitro* seed germination**. Data of the *P. linearis* seed evaluation are presented in the table 1. The contamination rate in some treatments was of 10%, and *Aspergillus* and *Cladospora* were observed; the browning was observed between 10 to 20%, in all the treatments tested. Cotyledons emerged in 7 days, and complete expansión of cotyledons occured between 50 and 60 days. After 60 days, 100% of seeds treated with 2.0 mg L^{-1} BAP germinated and reached the maximum leaf elongation (5.2 mm) and the largest diameter of the holdfast (6.7 mm) after 90 days, the elongation of the leaves was 12.3 mm (Figure 1b). In other experiment, 100% of seeds adhered to host branches *Acacia macracantha* (faique) germinated after 7 days (Figure 1c).

Leaves elongation. The highest leaves elongation (8.8 mm) was observed in MS culture medium supplemented with 0.02 mg L^{-1} IAA and 0.02 mg L^{-1} GA_3, with an average (40%) of seedlings with 5-7 leaves per explant (germinated seed more holdfast). We also observed a significant formation of green and compact callus (70%), with organogenic appearance (Table 2), after 60 days of culture (Figure 1d).

Callus induction and organogenesis. Callus induction (90%) in physiological condition (+++) was observed in MS culture medium supplemented with 2.0 mg L^{-1} BAP, evaluated over 150 days of culture; likewise, 10-25% of organogenic callus (indirect organogenesis) showed

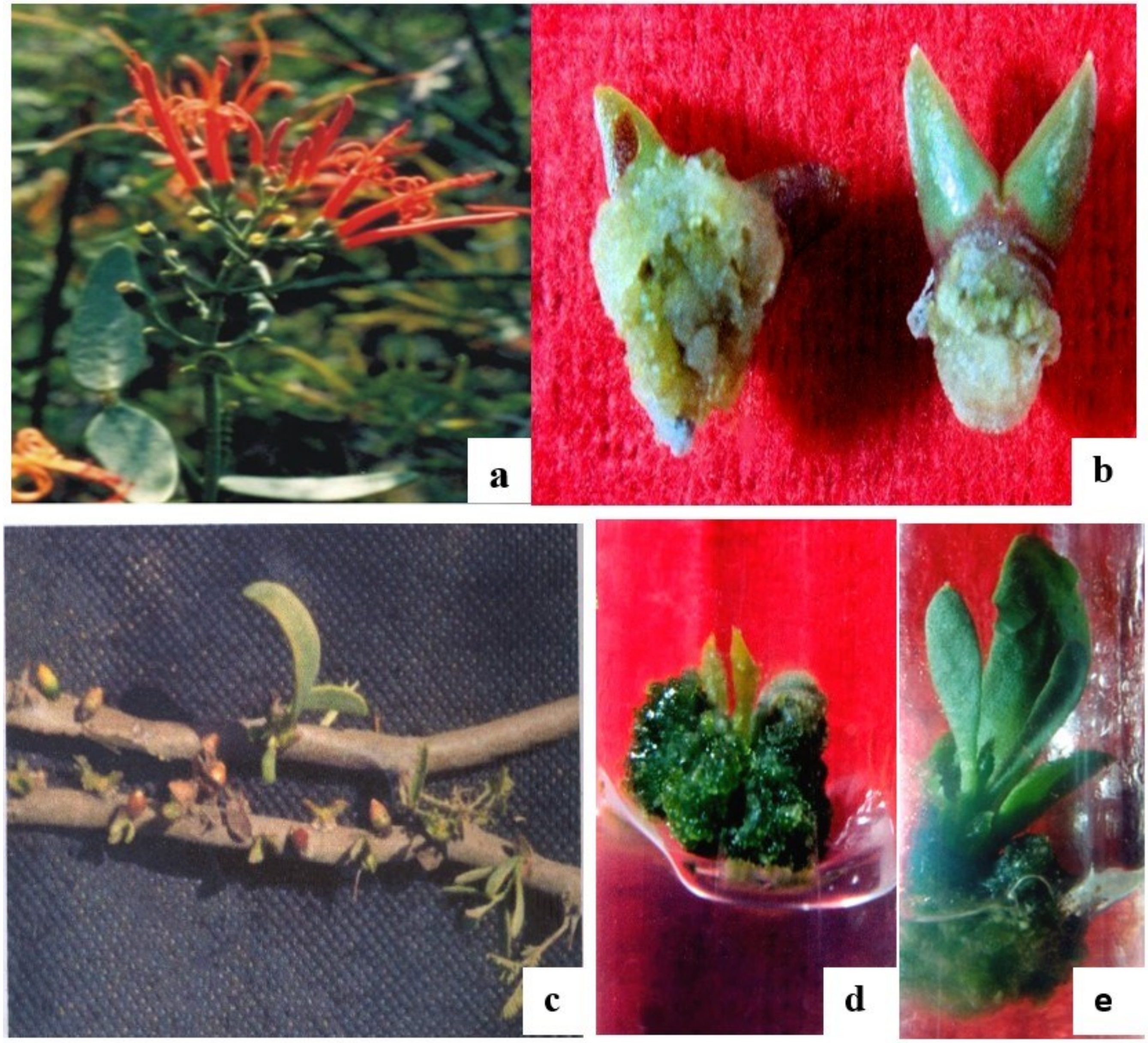

Figure 1. Plants and various process in *in vitro* tissue culture of *Psittacanthus linearis*. **a.** Adult plant of *P. linearis* ('suelda con suelda') on host *Prosopis pallida* ('algarrobo'). **b.** Germinated seeds with holdfast formation and green and compact callus, in culture medium supplemented with 2.0 mg L^{-1} BAP, **c.** Seeds of *P. linearis* adhered to branches of the host, observing some growing seedlings, **d.** Green callus formed in the holdfast, in culture medium supplemented with 2.0 mg L^{-1} BAP, showing organogenic primordia, **e.** Seedling obtained from seed, propagated in culture medium supplemented with 2.0 mg L^{-1} BAP, after 120 days of culture.

structures similar to apical buds (Table 3, figure 1e). Other formulations of culture medium – IAA (0.5, 1.0 and 2.0 mg L^{-1}), NAA (0.5, 1.0 and 2.0 mg L^{-1}) and 2,4-D (0.125, 0.25 and 0.5 mg L^{-1}) – showed between 20-40% green and compact callus formation.

Phytochemical analysis

In the determination of total polyphenols, employing tannic acid as a standard, we observed a higher concentration in red flowers (171.51 mg/dry mass) and a lower concentration in leaves (36.27 mg/dry mass); in stems and fruits the concentración of tanic acid was 42.62 and

Table 1. *In vitro* seeds germination of *Psittacanthus linearis* after 60 days of culturea.

Plant growth regulators (mg L^{-1})		Cont.[b] (%)	Brown.[c] (%)	Seed germ.[d] (%)	Morphogenic responses			
IAA	BAP				Size of leaves (mm)	Diameter of the holdfast (mm)	Seed with leaves[e] (%)	Compact green callus (%)
-	-	10	20	100	3.0±0.2[c]	4.6±0.3[cd]	40 (1-2)	20[c]
-	0,2	10	20	100	1.9±0.2[d]	4.7±0.4[cd]	30 (1-2)	50[b]
-	2	0	10	100	5.2±0.4[a]	6.7±0.2[a]	60 (1-2)	90[a]
0,2	0,2	0	20	100	3.7±0.5[b]	5.0±0.3[c]	50 (3-4)	60[b]
0,2	2	0	10	100	2.4±0.3[d]	6.1±0.3[b]	30 (5 to >)	60[b]

[a]Means followed by the same letters do not significantly differ according to a Tukey test at a 5% probability.
[b]Cont., Fungal contamination; [c]Brown., browning; [d]Seed germ., seed germination
[e]1-2 leaves; 3-4 leaves; 5 to > leaves

146.70 mg/dry mass, respectively. In the characterization of flavonoids, two glycosidated (kaempherol glicoside and quercetin glicoside) and two non-glycosidated (kaempherol and quercetin) were found. The color reactions indicated the presence of free and glycosidated quercetin and free and glycosidated kaemferol (Figure 2a). In the amylic fraction of the proanthocyanidins analysis where was used the acid treatment of the methanolic extract of stems and leaves, was detected cyanidin, where the procyanidin (monomer catechin) by acid treatment is transformed into cyanidin (Figure 2b). Likewise, in a comparative study on the detection of proteins in leaves of *P. linearis* and leaves of *Ligaria cuneifolia* (R. et P.) Tiegh., a species already studied in our laboratory, from different hosts of *L. cuneifolia* (20, 24, 30 and 31) was observed that the band 22.6 kD presents in *P. linearis* is absent in specimens of *L. cuneifolia* (Figure 2c).

Antibacterial activity

Among the three bacterial species tested, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, each with three strains, respectively, only strains of *S. aureus* showed sensitivity to the extracts of *P. linearis*. Additionally, the ethanolic extracts were found to be more active than the chloroformic extracts. Fruit extracts were more active than stem and leaves extracts. Concentrations of 300 μg mL^{-1} were more active than concentrations of 200 and 100 μg mL^{-1}, and strain S1 of *S. aureus* was more sensitive than strains S2 and S3 (Table 5). The highest inhibition halo (16.7 mm) was observed in the ethanolic extract of fruits at concentrations of 100, 200 and 300 μg mL^{-1} when tested against strain S1 (Table 5, figure 2d).

DISCUSSION

In the most 'mistletoes' of the Loranthaceae, a radicle emerges during seed germination which attaches and penetrates the host. The plumule (embryogenic shoot) emerges from between the two cotyledons (Bajaj, 1970). This dependency of the parasite on host stimulus for seed germination and the chemical factors initiating haustorium formation were studied in tissue culture (Bhatnagar, 1987).

Between the decades 70s to 90s, several classic studies using tissue culture were performed in various species of the Loranthaceae such as *Amyema* and *Amylotheca*, two genera of Australian mistletoes. Hall *et al*. (1987) studied the effects of hormone and nutrients on development and morphology of leaves, callus and seedlings grown on modified MS medium containing IAA or NAA in *Amyema* and found that seedlings formed plumular leaves and haustorial discs *in vitro*. Likewise, mature embryos from *Amylotheca dictyophleba* (F. Muell.) Tiegh. on White's medium, with casein hydrolysate and IAA, developed seedlings with holdfasts, haustorial disc, and plumular leaves (Bajaj, 1970). *D. falcata* in White's medium developed undifferentiated and embriogenic callus embryoids, buds (shoot, floral), and seedlings with holdfasts and haustorial discs (Ram & Singh, 1991). *Nuytsia floribunda* R. Br. in White's medium, with casein hydrolysate, IBA, and KIN, produced

Table 2. Size and number of leaves in the holdfast of *Psittacanthus linearis* after 60 days of culture.

Plant growth regulators (mg L^{-1})			Morphogenic responses				Compact green callus (%)
IAA	BAP	GA_3	Size of leaves (mm)[a]	Number of leaves (%)			
				2-abr	5-jul	8 to >	
-	2	-	6.2±0.3[b]	60[a]	30[a]	10[b]	80[a]
0,02	0	0,02	8.8±0.5[a]	30[b]	40[a]	30[a]	70[a]

[a]Means followed by the same letters do not significantly differ according to a Tukey test at a 5% probability.

Table 3. Callus induction and indirect organogenesis in the holdfast of *Psittacanthus linearis* after 150 days of culture.

Culture days	Plant growth regulators (mg L^{-1})		Callus induction[a] (%)				Indirect organogene sis (%)
	IAA	BAP	-	+	++	+++	
90	0,2	2	10[b]	10[b]	80.0[a]	0.0[c]	0.0[d]
90	1	2	20[a]	0.0[c]	60.0[b]	20.0[b]	0.0[d]
90	2	2	20[a]	40.0[a]	40.0[c]	0.0[c]	0.0[d]
150	-	2	10[b]	0.0[c]	0.0[d]	90[a]	10[c]
180	-	2	10[b]	0.0[c]	0.0[d]	90[a]	25[b]
210	-	2	10[b]	0.0[c]	0.0[d]	90[a]	35[a]

[a]-, without callus formation; +, callus < 5 mm Ø; ++, callus between 6-10 mm Ø; +++, callus > 10 mm Ø.

[a]Means followed by the same letters do not significantly differ according to a Tukey test at a 5% probability.

shoots, roots, embryogenic callus, and somatic embryos (Nag & Johri, 1969). *Scurrula pulverulenta* (Wall.) G. Don mature endosperm on White's medium, with casein hydrolizate and IAA, produced callus, shoots, seedlings with haustoria, and somatic embryos (Bhojwani & Johri, 1970). *Tapinanthus bangwensis* (Engl. & K. Krause) Danser, on White's medium produced callus, shoots, and seedlings with embryo germination and holdfast formation occurring without a host (Onofeghara, 1972). In both, *Taxillus vestitus* (Wall.) Danser and *T. cuneatus* (Heyne) Danser several factors that affecting organogenesis were studied and it was also founded that the development of shoot buds and haustoria was influenced by growth regulator combinations (Johri & Nag, 1970; Nag & Johri, 1976). In all these studies, morphogenic processes were initiated with seed germination and holfast induction and continued with callus formation and elongation of the cotyledons, as was observed in the present study with *P. linearis*, where only callus formation and elongation of cotyledonal leaves were the most relevant results. In another parasitic species, western hemlock dwarf mistletoe [*Arceuthobium tsugense* (Rosend.) G.N. Jones], the most evolutionarily specialized genus of Santalaceae (ex Viscaceae), seedlings developed from split radicles and split holdfasts after 5 months in culture (Deeks *et al*., 1997). In the present study a similar or longer period of time was required to induce holdfast with green callus for the seedlings of *P. ligularis*.

Phytochemical constituents of the leaves of African mistletoe (*Tapinanthus dodoneifolius*), an ethnomedicinal plant of Northern Nigeria, obtained from 14 different hosts, showed the common occurrence of anthraquinones, saponins, tannins, a rare presence of alkaloids and the absence of phlobatannins in the hemi-parasite (Deeni & Sadiq, 2002). Likewise, preliminary phytochemical screening of the methanolic extracts of *H. elastica* in

Table 4. Qualitative phytochemical analysis of several plant samples of *Psittacanthus linearis* in *Prosopis pallida*.

A. Total Polyphenols							
Plant samples				Tannic acid (mg of dry mass)			
Stem				42,62			
Leaf				36,27			
Red Flower				171,51			
Fruit				146,7			
B. Rf values and maximum absorption in methanol to kaempferol and quercetin							
	Rf (x 100) in			Maximum absorption			
Flavonol (Flavonoids)	BAA	CAA	Forestal	In MeOH			
Kaempferol	83	40	55	266	367		
Quercetin	65	15	43	255	370		
C. Rf values and absorption maxima for cyanidin							
	Rf (x 100) in				Maximum absorption		
Secondary metabolite	BAA	Formic	Forestal	HOAC 15%	MeOH/ HCl	E440/ Máx (%)	MeOH/ HCl $+AlCl_3$
Cyanidin	65	28	47	60	536	19	552
D. Antioxidant activity of the hydroalcoholic leaf extract							
Plant sample	mMol ascorbic acid/g	mMol catechin/g	mMol quercetin/g				
Psittacanthus	2,05	0,32	0,42				
Ligaria	1,56	0,74	1,19				

BAA: n butane: acetic acid: water (4:1:5, upper phase)
CAA: chloroform: acetic acid (2:1, saturated with water)
Forestal: hydrochloric acid (CC): acetic acid: water (3:30:10)
Rf: Distance covered by the standard/versus the solvent
Formic: formic acid: acetic acid: water (9:2:3)

hosts, *N. indicum* and *H. brasiliensis*, revealed the occurrence of various constituents as alkaloids, glycosides, flavonoids, phenols, tannins, sterols, triterpenoids, diterpenes and carbohydrates, but not proteins or amino acids (Kumar & Mathew, 2014). In another study, phytochemical analysis of endophytic fungi from stems of *Phragmanthera capitata*, harvested in *Theobroma cacao* L., revealed the presence of flavonoids, anthroquinones, tannins, phenols, steroids, coumarins and terpenoids, but absence of alkaloids and saponins, in all acetate ethyl extracts (Ladoh-Yemeda *et al.*, 2015). Phytochemical constituents from *L. elasticus* presented in the Neem Tree (*A. indica*), namely alkaloids, amino acids, anthocyanin, carbohydrates, cardiac glycosides, coumarins, diterpenes, emodins, fatty acids, phlobatannin, phenols, saponin and terpenoids, were presented in methanolyc extract, whereas the flavonoids, glycosides, leucoanthocyanin, phytosterol, proteins, steroids and tannin were absent in methanol extract. Small amounts of saponins were noticed in alcohol and water extracts, and in water extracts of the leaves shows moderate amounts of gums and mucilage (Krishnaveni *et al.*, 2016). The phytochemical analysis of *L acaciae* led to the isolation and characterization of quercetin 3-*O*-*β*-D-glucopyranoside, quercetin 3-*O*-*β*-(6-*O*-galloyl)-glucopyranoside, (-) catechin, and catechin 7-*O* -gallate. Among these compounds quercetin 3-*O*-*β*-D-glucopyranoside, quercetin 3-*O*-*β*-(6-*O*-galloyl)-glucopyranoside and catechin 7-*O*-gallate, were isolated for the first time from this species (Noman *et al.*, 2019). In *Plicosepalus curviflorus* (Oliv.) Tiegh., a semiparasitic plant grown in Saudi Arabia, traditionally used as a cure for diabetes and cancer in human, a flavonoid quercetin, (-)-catechin, and a flavane gallate 2S,3R-3,3´,4´,5,7-pentahydroxyflavane-5-O-gallate, were isolated from the aerial parts (Orfali *et al.*, 2019). Cold extractions of *S. parasitica* leaves were carried out using n-hexane, ethyl acetate and metanol to obtain the crude extracts, and purification of the extracts led to isolation of quercetin, quercitrin, kaempferol 3-*O*-α-L-rhamnoside, (+)-catechin, lupeol 5, lupeol palmitate, β-sitosterol, squalene, octacosane, octadecane and eicosane (Muhammad *et al.*, 2019). Only polyphenols and four flavonoids, two glycosidated (kaempherol glicoside and quercetin glicoside) and two non-glycosidated (kaempherol and quercetin), were detected in the study carried out on *P. linearis*, without deepening in the isolation and identification of other secondary metabolites.

In the study on the relationship between mistletoes-hosts, thirty field collections of specimens of the genus *Phragmanthera*, *Tapinanthus* and *Globimetula* (Loranthaceae), obtained from various localities of Nigeria, with several hosts plants being parasitized by the mistletoes, were phytochemical screened. Most of the samples were slightly positive for alkaloids, anthraquinone-related compounds, terpenoids and terpenoid-related compounds; however, ketonic compounds were of rare ocurrence in all the samples (Wahab *et al.*, 2010). Analysis of *Tapinanthus dodoneifolius* (DC.) Danser, collected from *P. guajava*, *H. brasiliensis* and *Citrus sinensis*

Table 5. Antimicrobial activity of both chlorophormic and ethanolic extracts of various plant samples of *Psittacanthus linearis* against *Staphyloccus aureus* strains[a].

Posit.	Chlorophormic Extract			Ethanolic Extract		
	Bact. strain	Conct. (µg mL^{-1} per disc)/ Plant sample	Inhibition halos (mm)/Signif.	Bact. strain	Conct. (µg mL^{-1} per disc)/ Plant sample	Inhibition halos (mm)/Signif.
1	S1	300/Stem	13.0^{a}	S1	100/Fruit	16.7^{a}
2	S2	300/Stem	13.0^{a}	S1	200/Fruit	16.7^{a}
3	S3	300/Stem	12.7^{ab}	S1	300/Fruit	16.7^{a}
4	S1	200/Stem	12.3^{abc}	S3	300/Leaf	15.0^{b}
5	S1	300/Fruit	12.0^{abc}	S2	300/Fruit	14.7^{b}
6	S2	200/Stem	12.0^{abc}	S3	300/Fruit	14.7^{b}
7	S1	100/Stem	11.7^{abcd}	S1	50/Fruit	14.3^{c}
8	S2	300/Fruit	11.3^{abcde}	S2	200/Fruit	14.3^{c}
9	S1	200/Fruit	11.3^{abcde}	S3	200/Leaf	14.3^{c}
10	S2	100/Stem	11.3^{abcde}	S1	300/Stem	14.3^{c}
11	S3	200/Stem	11.3^{abcde}	S2	100/Fruit	14.0^{cd}
12	S2	200/Fruit	11.0^{abcdef}	S3	100/Leaf	13.7^{d}
13	S3	100/Stem	$10.7^{abcdefg}$	S1	200/Stem	12.6^{e}
14	S2	50/Stem	10.3^{bcdefg}	S3	200/Fruit	12.0^{f}
15	S1	50/Stem	10.0^{cdefgh}	S3	100/Fruit	11.3^{g}
16	S3	300/Fruit	10.0^{cdefgh}	S1	300/Leaf	11.3^{g}
17	S3	50/Stem	9.3^{defghi}	S2	300/Stem	11.3^{g}
18	S3	200/Fruit	9.3^{defghi}	S1	200/Leaf	11.0^{gh}
19	S1	100/Fruit	9.0^{efghi}	S2	300/Leaf	10.7^{hi}
20	S2	100/Fruit	8.7^{fghi}	S2	200/Stem	10.6^{i}
21	S1	50/Fruit	8.3^{ghi}	S1	100/Stem	10.0^{i}
22	S1	300/Leaf	8.3^{ghi}	S3	300/Stem	10.0^{i}
23	S2	50/Fruit	8.3^{ghi}	S3	50/Fruit	9.7^{i}
24	S2	300/Leaf	7.7^{hi}	S2	200/Leaf	9.7^{i}
25	S3	300/Leaf	7.7^{hi}	S1	50/Stem	9.3^{k}
26	S1	200/Leaf	7.3^{i}	S3	200/Stem	9.0^{k}
27	S2	200/Leaf	7.0^{i}	S1	100/Leaf	8.3^{l}
28	S3	200/Leaf	7.0^{i}	S2	100/Stem	7.0^{m}
29	S1	50/Leaf	0.0^{j}	S2	100/Leaf	7.0^{m}
30	S1	100/Leaf	0.0^{j}	S3	100/Stem	7.0^{m}
31	S2	50/Leaf	0.0^{j}	S3	50/Leaf	6.7^{m}
32	S2	100/Leaf	0.0^{j}	S1	50/Leaf	6.0^{n}
33	S3	50/Leaf	0.0^{j}	S2	50/Stem	6.0^{n}
34	S3	50/Fruit	0.0^{j}	S2	50/Leaf	6.0^{n}
35	S3	100/Leaf	0.0^{j}	S3	50/Stem	6.0^{n}
36	S3	100/Fruit	0.0^{j}	S2	50/Fruit	1.1^{o}

[a]Posit., Position; Bact. Str., Bacterial Strein; Conct. (µg/D), Concentration (µg/Disc); Inhib./Signif., Inhibition/Significance.

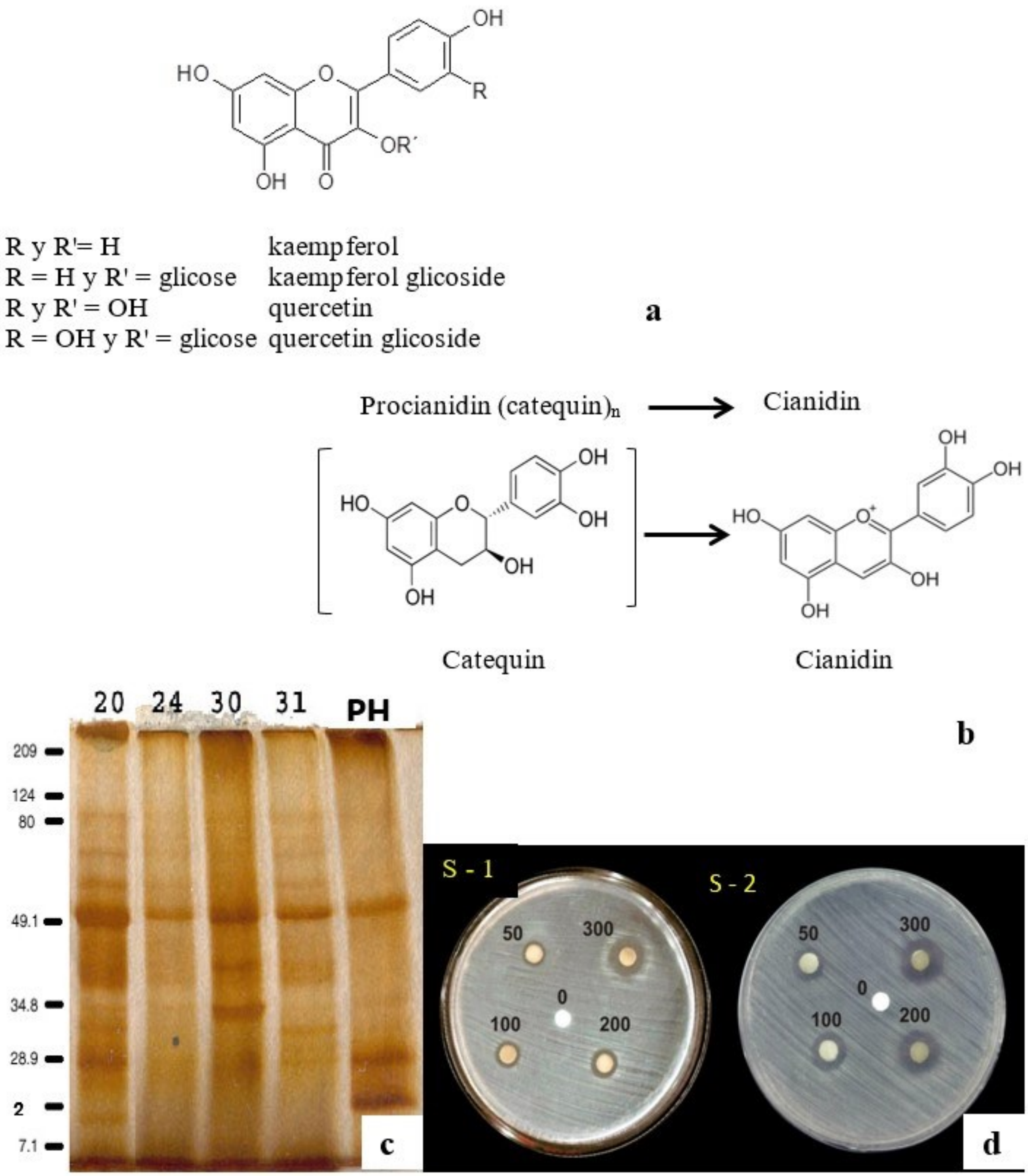

Figure 2. Structure of some chemical compounds detected in *Psittacanthus linearis* and antibacterial activity of the ethanol extract on strains S1 and S2 of *S. aureus* **a.** Chemical structuren of flavonoids quercetin and kampferol, **b.** Chemical structure of the catechin that by acid treatment originates cyanidin, **c.** Comparative protein profile between leaves of *P. linearis* (PH) and leaves of *Ligaria cuneifolia* from different hosts 20, 24, 30 y 31 and **d.** Antibacterial activity of the ethanolic extract of stems on strains S1 and S2 of *S. aureus*.

(L.) Osbeck showed the presence of oxalate, phytate, saponin, alkaloid, glycoside and tannin (Idu *et al.*, 2016). Certainly, not only is very necessary the study of the relation *P. linearis* with *P. pallida* (algarrobo) but also with other species of the seasonally dry forest such as *A. macracantha* (faique), *Loxopterygium huasango* Spruce ex Engl. (hualtaco), and others.

In all of these studies, the presence of phenols was common; flavonoids, proteins, and amino acids were only detected in some species, which indicates that the species of the Loranthaceae family present a broad spectrum of secondary metabolites. Likewise, the occurrence of different protein bands between the species of *P. linearis* and *L. cuneifolia* can be partially used in the differentiation of genera, as indicated in the study by Wahab *et al*. (2010) who also concluded that chemical characters of thirty field collections from various Loranthaceae species may only be used as supporting evidence in the identification and delimitation of the taxa. However, studies of proteins in Loranthaceae species are scarce. Proteins from *L. elasticus* were reported, but without specific identification (Krishnaveni et al., 2016). In the trials on total antioxidant activity (TRAP), in leaf extract of *P. linearis* exhibited a decrease in chemiluminescence when was compared with *L. cuneifolia* (Wagner *et al*., 1998). This phenomenon was for a period proportional to the amount of oxidants present in the sample, a process that occurred until the regeneration of the luminol radicals, expressing the results in mMol of catechin. In other species of Loranthaceae in Peru, these comparative methods can also be used for the differentiation of genera and species.

There are few studies about the antimicrobial activity exerted by extracts of Loranthacaeae species. The antimicrobial activity of *L. elastica* extracts showed that all the organisms are inactive in the organic solvents up to a level of 200 µg mL^{-1}, and both Gram negative (*Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) and Gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) organisms show activity in the methanol and ethanol extracts at a concentration of 25 µg mL^{-1}; the ethanol and methanol extracts show positive for all organisms tested (Krishnaveni *et al*., 2016). In another study, screening of the *Tapinanthus dodoneifolius*, obtained from 14 different hots, revealed a wide spectrum of antimicrobial activities against drug-resistant bacteria such as *Agrobacterium tumefaciens*, *Bacillus* sp., *Escherichia coli*, *Salmonella* sp., *Proteus* sp. and *Pseudomonas* sp., all of which are known to be associated with either crown gall or gastrointestinal tract and wound infections (Deeni & Sadiq, 2002). Likewise, all extracts and isolated compounds of *S. parasitica* leaves showed weak activity on antimicrobial activity against two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and a fungi (*Aspergillus niger*) with the exception of quercetin which exhibited moderate activity against *P. aeruginosa* with MIC and MBC value of 250 µg/mL (Muhammad *et al*., 2019). In the study with *P. linearis*, although the results were only significant with strains of *Staphylococcus aureus*, its is necessary to carry out other tests with different strains of bacterial species of wide prevalence in hospital infections.

CONCLUSION

In this work, carried out in *Psittacanthus linearis* (suelda con suelda), a representative species of the BES of Lambayeque (Peru), we found that *in vitro* tissue culture allows germination of seeds, clonal propagation, callus induction and organogenesis. Likewise, some phytochemical aspects were studied and their biological activity on certain bacterial pathogens was demonstrated.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. Deborah Woodcock from Marsh Institute of Clark University, and to MSc Alain Monsalve Mera for English improvements.

LITERATURE CITED

Adesina, S. K., Illoh, H. C., Johnny, I. I. & Jacobs, I. E. (2013). African mistletoes (Loranthaceae); ethnopharmacology, chemistry and medicinal values: an update. *African Journal of Traditional Complementary and Alternative Medicine*, 10: 161-170.

APG (The Angiosperm Phylogeny Group). (2016). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Botanical Journal of the Linnean Society*, 181: 1-20.

Bajaj, Y. P. S. (1970). Growth responses of excised embryos of some mistletoes. *Z. Phlanzenphysiologie*, 63: 408-415.

Bell, T. L. & Adams, M. A. (2011). Attack on all fronts: functional relationships between aerial and root parasitic plants and their woody hosts and consequences for ecosystems. *Tree Physiology*, 31: 3-15.

Bhatnagar, S. P. (1987). *In vitro* morphogenic responses of mistletoes. In: Weber, H.C & Forstrenter, W (eds.). Procedings of the 4th International Symposium on Parasitic Flowering Plants. Pp. 105-108. Marburg, Germany: Philips University.

Bhojwani, S. S. & Johri, B. M. (1970). Cytokinin-induced shoot bud differentiation in mature endosperm of *Scurrula pulverulenta*. *Z. Pflanzenphysiology*, 63: 269-275

Côme, D. (1968). Problèmes de terminologie posés par la germination et ses obstacles. *Bulletin de la Societé Française de Physiologie Végétale*, 14: 3-9.

Cronquist, A. (1988). The Evolution and Classification of Flowering Plants. The New York Botanical Garden. 555 p.

Deeks, S. J., Shamoun, S. F. & Punja, Z. K. (1997). *In vitro* culture of western hemlock dwarf mistletoe. In: Sturrock, R (ed.). Proceedings of the 45th Annual Western International Forest Disease Work Conference. Prince George, B.C., 74.

Deeks, S. J., Shamoun, S. F. & Punja, Z. K. (1999). Tissue culture of parasitic flowering plants. Methods and Applications in Agricultura and Forestry. *In Vitro Cellular & Development Biology – Plant*, 35: 369-381.

Deeni, Y. Y. & Sadiq, N. M. (2002). Antimicrobial properties and phytochemical constituents of the leaves of African mistletoe (*Tapinanthus dodoneifolius* (DC) Danser) (Loranthaceae): an ethnomedicinal plant of Hausaland, Northern Nigeria. *Journal of Ethnopharmacology*, 83: 235-240.

Espinoza-Zúñiga, P., Ramírez-Dávila, J. F., Cibrián-Tovar, D., Villanueva-Morales, A., Cibrián-Llanderal, V. D., Figueroa-Figueroa, D. K., & Rivera-Martínez, R. (2019). Modelación de la distribución espacial del muérdago (Santalales: Loranthaceae) en las áreas verdes de la delegación Tlalpan, México. *Bosque*, 40:17-28.

Fer, A., Russo, N., Simier, P., Marie-Claire, A. & Thalouarn, P. (1994). Physiological changes in a root hemiparasitic angiosperm, *Thesium humile* (Santalaceae), before and after attachment to the host plant (*Triticum vulgare*). *Journal of Plant Physiology*, 143: 704-710.

Hall, P. J., Letham, D. S. & Barlow, B. A. (1987). The influence of hormones on development of *Amyema* seedlings cultured *in vitro*. In: Weber, H.C. & Forstrenter, W. (eds.). Procedings of the 4th International Symposium on Parasitic Flowering Plants. Pp. 285-291. Marburg, Germany: Philips University.

Idu, M., Ovuakporie-Uvo, O. & Nwaokolo, M. J. (2016). Phytochemistry and microscopy of *Tapinanthus dodoneifolius* (DC) (Danser) (Santalales: Loranthaceae) (African mistletoes) from guava, rubber and orange host trees. *Brazilian Journal of Biological Sciences*, 3: 27-35.

Johri, B. M. & Nag, K. K. (1970). Endosperm of *Taxillus vestitus* Wall: A system to study the effect of cytokinins in vitro in shoot bud formation. *Current Science*, 39: 177-179.

Krishnaveni, T., Valliappan, R., Selvaraju, R. & Prasad, P. N. (2016). Preliminary phytochemical, physicochemical and antimicrobial studies of *Loranths elasticus* of *Loranthaceae* family. *Journal of Phramacognosy and Phytochemistry*, 5: 7-11.

Kumar, A. & Mathew, L. (2014). Comparative account of the preliminary phytochemical aspects of *Helicanthes elastica* (Desr) Danser growing on two diferentes hosts. *Journal of Pharmacognosy and Phytochemistry*, 3: 218-221.

Ladoh-Yemeda, C. F., Nyegue, M. A., Ngene, J. P., Benelesse, G. E., Lenta, B., Wansi, J. D., Mpondo, M. E. & Dibong, S. D. (2015). Identification and phytochemical screening of endophytic fungi from stems of *Phragmanthera capitata* (Sprengel) S. Balle (Loranthaceae). *Journal of Applied Biosciences*, 90: 8355-8360.

Laemmli, U. K. (1970). Clevage of structural proteins during assembly of the head of the Bacteriophage T4. *Nature*, 227: 680-685.

Lissi, E., Pascual, C. & Del Castillo, M. D. (1992). Luminol luminescence induced by 2,2'-azo-*bis* (2-amidinopropane) thermolysis. *Free Radical Research Communications*, 17: 299-311.

Lorenzi, H. (2000). Weeds of Brazil: Terrestrial, Aquatic, Parasitic and Toxic Herbs. 3rd Edn. Plantarum Institute, Nova Odessa, Brazil.

Lu, J. K., Xu, D. P., Kang, L. H. & He, X. H. (2014). Host-species-dependent physiological characteristics of hemiparasitic *Santalum album* in association with N_2-fixing and non-N_2-fixing hosts native to southern China. *Tree Physiology*, 34: 1006-1017.

Mabry, T. J., Markham, K. R. & Thomas, M. B. (1970). The Systematic Identification of the Flavonoids. Springer: Berlin.

Muhammad, K. J., Jamil, S. & Basar, N. (2019). Phytochemical study and biological activities of *Scurrula parasitica* L (Loranthaceae) leaves. *Journal of Research in Pharmacy*, 23:522-531.

Murashige, T. & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologie Plantarum*, 15: 473-497.

Nag, K. K. & Johri, B. M. (1969). Organogenesis and chromosomal constitution in embryos callus of Nuystia floribunda. *Phytomorphology*, 19: 405-408.

Nag, K. K. & Johri, B. M. (1976). Experimental morphogenesis of the embryo of *Dendrophthoe*, *Taxillus*, and *Nuystia*. *Botanical Gazette*, 37: 378-390.

Naskar, A. K., Ray, S., Parui, S. M. & Mondal, A. K. (2019). Studies on an *in-vitro* investigation of anti diabetic property of a hemiparasitic taxa *Dendrophthoe falcata* (L.f.) Ettingsh (Loranthaceae). *Pharmacognosy Journal*, 11:699-704.

Nickrent, D. L. & Musselman, L. J. (2004). Introduction to Parasitic Flowering Plants. The Plant Health Instructor. DOI: 10.1094/PHI-I-2004-0330-01. *Update 2016*.

Noman, O. M., Mothana, R. A., Al-Rehaily A. J., Al qahtani, A. S., Nasr, F. A., Khaled, J. M., Alajmi, M. F. & Al-Said, M. S. (2019). Phytochemical analysis and anti-diabetic, anti-inflammatory and antioxidant activities of *Loranthus acaciae* Zucc. grown in Saudi Arabia. *Saudi Pharmaceutical Journal*, 27:724-730.

Ohikhena, F. U., Wintola, O. A. & Afolayan, A. J. (2017). Micromorphological studies of the Loranthaceae, *Phragmanthera capitata* (Sprengel) Balle. *Journal of Botany*, Article ID 5603140, 9 pages.

O'Neill, A. R. & Rana, S. K. (2016). An ethnobotanical analysis of parasitic plants (*Parijibi*) in the Nepal Himalaya. *Journal of Etnobiology and Ethnomedicine*, 12: 14-29.

Onofeghara, F. A. (1972). The effects of growth substances on the growth of *Tapinanthus banguensis* (Loranthaceae) *in vitro*. *Annals of Botany*, 36: 563-570.

Orfali, R., Perveen, S., Siddiqui, N. A., Alam, P., Alhowiriny, T. A., Al-Taweel, A. M., Al-Yahya, S., Ameen, F., Majrashi, N., Alluhayb, K., Alghanem, B., Shaibah, H. & Khan, S. I. (2019). Pharmacological evaluation of secondary metabolites and their simultaneous determination in the Arabian medicinal plant *Plicosepalus curviflorus* using HPTLC validated method. *Journal of Analytical Methods in Chemistry*, Volume 2019, Article ID 7435909, 8 pages.

Pennington, T. D., Reynel, C. & Daza, A. (2004). Illustrated Guide to the Trees of Peru. Ed. dh. 817 p.

Ram, R. L. & Singh, M. P. N. (1991). *In vitro* haustoria regeneration form embryo and *in vitro*-formed leaf callus cultured in *Dendrophthoe falcata* (L.f.) Ettings. *Advances of Plant Sciences*, 4: 48-53.

Ram, R. L., Sood, S. K. & Singh, M. P. N. (1993). *In vitro* ontogeny, requeriments, control and physiology of shoot bud regeneration in *Dendrophthoe falcata* (L.f.) Ettings. *Advances in Plant Sciences*, 6: 115-127.

Těšitel, J., Plavcová, L. & Cameron, D. D. (2010). Interactions between hemiparasitic plants and their hosts. The importance of organic carbon transfer. *Plant Signaling & Behavior*, 5: 1072-1076.

Wahab, O. M., Ayodele, A. E. & Moody, J. O. (2010). TLC phytochemical screening in some Nigerian Loranthaceae. *Journal of Pharmacognosy and Phytotherapy*, 2: 64-70.

Wagner, M., Fernández, T., Varela, B., Álvarez, E., Ricco, R., Hajos, S. & Gumi, A. (1998). Anatomical, phytochemical and immunological studies on *Ligaria cuneifolia* (R. & P.) Tiegh (Loranthaceae). *Pharmaceutical Biology*, 36: 1-9.

Waterman, P. G. & Molle, S. (1994). Analysis of Phenolic Plant Metabolites. Backwell Scientific Publications: Oxford.

Yoshida, S., Cui, S., ichihashi, Y. & Shirasu, K. (2016). The haustorium, a specialized invasive organ in parasitic plants. *Annual Review of Plant Biology*, 67: 643-667.