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# Phytochemical composition and biological screening of two *Lophophytum* species

[Composición fitoquímica y cribado biológico de dos especies de *Lophophytum*]

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**Abstract:** *Lophophytum* species are holoparasites that grow on tree roots. The objectives of the work were to explore the chemical composition of the tubers of two *Lophophytum* species and to analyze the antioxidant, anti-inflammatory and antilithiatic activity of their extracts using *in vitro* methods. The chemical composition was determined by histochemical, phytochemical and TLC tests. In addition, the profile of phenolic compounds was determined by HPLC-MS. The presence of secondary metabolites of recognized activity was demonstrated. The results of the HPLC-MS/MS allowed the tentative identification of catechin, luteolin and glycosides of eriodictyol, naringenin and luteolin in the extract of *Lophophytum leandri* and eriodictyol, naringenin, luteolin and their glycosylated derivatives in *Lophophytum mirabile*. The extracts showed promising antioxidant (DPPH, ABTS and β-carotene-linoleic acid), anti-inflammatory (inhibition of 5-LOX) and anti-urolytic (by bioautographic TLC) activity. It is noteworthy that these are the first results of the phytochemical composition and biological activity of *L. mirabile*. However, *in vivo* studies are required to corroborate these activities.

**Keywords:** Holoparasites; Naringenin; Antioxidant; Anti-inflammatory; Antilithiatic.

**Resumen:** Las especies de *Lophophytum* son holoparásitas que crecen en raíces de árboles. Los objetivos del trabajo fueron explorar la composición química del túber de dos especies de *Lophophytum* y analizar la actividad antioxidante, antiinflamatoria y antilitiásica de sus extractos usando métodos *in vitro*. La composición química se determinó mediante pruebas histoquímicas, fitoquímicas y por TLC. Además, se determinó el perfil de compuestos fenólicos por HPLC-MS/MS. Se demostró presencia de metabolitos secundarios de reconocida actividad. Los resultados del HPLC-MS/MS permitieron identificar tentativamente catequina, luteolina y glucósidos de eriodictiol, naringenina y luteolina en el extracto de *Lophophytum leandri* y eriodictiol, naringenina, luteolina y sus derivados glicosilados en *Lophophytum mirabile*. Los extractos mostraron prometedora actividad antioxidante (DPPH, ABTS y β-caroteno-ácido linoleico), antiinflammatoria (inhibición de la 5-LOX) y antiurolitiásica (por TLC bioautográfica). Es de destacar que estos son los primeros resultados de composición fitoquímica y actividad biológica de *L. mirabile*. Sin embargo, se requieren estudios *in vivo* para corroborar dichas actividades.

**Palabras clave:** Holoparásitas; Naringenina; Antioxidante; Antiinflammatorio; Antilitiásico.

## INTRODUCTION

*Lophophytum* is a genus of holoparasitic plants without chlorophyll, which grows on the roots of certain trees, especially of the Leguminosae family (certain Fabaceae). These species belong to Balanophoraceae family, all of which grow in tropical or subtropical mountainous regions of Asia and South America. The species of the genus *Lophophytum* that occur in South America are mainly distributed in Brazil, Peru, Colombia, Bolivia, Ecuador, Paraguay, and Argentina.

The species that grow in Argentina are *Lophophytum leandri* Eichler (Corrientes and Misiones) and *L. mirabile* Schott & Endl. Subsp. *bolivianum* (Wedd.) B. Hansen (Salta and Jujuy). They are similar species and differ in minor details of the inflorescence. They present a vegetative body or subterranean tuft that lacks differentiation of stem, root, and leaves. They grow almost entirely immersed in the soil at the root of the tree, with only the flower or inflorescence rising like a long pine cone; sharp, erect brownish scales cover the female and male flower (Gonzalez & Mauseth, 2010; Sato & Gonzalez, 2016; Sato & Gonzalez, 2017).

*Lophophytum leandri* resembles a stone as it lies in the earth, hence its name--the stone flower. Pharmacological investigations showed that it contains acids, resin, glucose, albumen, and mucilage, and Dr. Schwabe mentions a tannin of catechu, leucoanthocyanidin substances, and a small amount of bromine and iodine, probably in the form of organic compounds. The natives of South America use the plant as a medicine for a variety of ailments, all of them very vague--as is usual in herbal medicine. Catarrhal jaundice and "insufficient bile" are mentioned by one author, and Dr. Schwabe reported its use in digestive disturbances, liver disease, epilepsy, rickets, and malignant conditions (Raeside *et al.*, 1969). Bracci *et al.* (2012), demonstrated its use as diuretic and hepatic.

Concerning *L. mirabile* subsp. *mirabile* ("flaky potato"), there are records of its use in Mato Grosso, Brazil, for the treatment of gastrointestinal or hepatic disorders (Bieski *et al.*, 2015). The cooked inflorescences were cited with aphrodisiac effects and dry and ground rhizome for the treatment of epilepsy and jaundice (Fraga Falcão, 1975; Coluccio, 2005). Roast inflorescence of *L. mirabile* subsp. *mirabile* is food for the Brazilian natives (Teixeira *et al.*, 2019). In the north-west of Argentina, the properties of its closely related *Ombrophytum subterraneum*, which possesses fleshy and edible infructescence, are well known and used as a home remedy for diseases and disorders of liver and kidney (Gomez Villafañe & Sato, 2019).

In light of this background, the aims of this work were to explore the phytochemical and histochemical compositions of the tuber of *Lophophytum* species of Argentine and analyze the antioxidant, anti-inflammatory and antilithiatic activity of their extracts using different *in vitro* assays to corroborate the popular uses.

## MATERIAL AND METHODS

### *Plant material*

*Lophophytum leandri*, was collected from San Ignacio and Salto Tabay department of Misiones province, Argentina, (voucher N° Sato 114, 421, 422, 423) in 2017 and the tuber of *L. mirabile* Schott & Endl. subs. *bolivianum*, (Wedd.) B. Hansen collected from Calilegua National Park of Jujuy province, Argentina (Sato N° 430, 432, 434, 436). Both species were identified by Sato, H. A. and deposited in the herbarium CTES of the *Instituto de Botànica del Nordeste*, IBONE, Corrientes, Argentina and the herbarium JUA of the *Facultad de Ciencias Agrarias* (UNJu), Jujuy, Argentina.

### *Histochemical screening*

Histological cuts of 25 μm thickness of the fresh tuber were made by a freezing microtome. The cuts were subjected to different histochemical identification tests according to Zarlavsky's (2014) protocols to investigate the presence/absence of various ergastic substances or secondary metabolites. The presence of starch and chitin was determined with Lugol (iodine and potassium iodide) and lipophilic substances with an alcoholic solution saturated with Sudan IV. Tannins were identified by staining with safranine and corroborated by reaction with ferric sulfate. The phloroglucinol test was carried out to determine cellulose/lignin. For the identification of proteins, the Millon reagent was used, cellulose was evidenced with zinc chloride. Saponins were determined using concentrated sulphuric acid, while pectic substances was reveal with 0.1% ruthenium red, and for mucilages was used 1% brilliant cresyl blue.

### *Preparation of extracts*

The tubers were dried at room temperature and pulverized in a composite mortar. The extracts (20 g in 100 mL) were obtained by maceration for 7 days, with frequent agitation, using ethanol 80° as solvent,

and subsequent vacuum filtration. Next, they were allowed to evaporate and dried completely at 40º C. Further stored in a deep freeze and used for analysis.

***Preliminary phytochemical screening***

The presence of alkaloids (Dragendorff's, Bouchardat's and Mayer's reactions), saponins (foam formation), terpenoids (Liebermann–Bouchardat's test), anthraquinones (Borntrager's reaction), tannins (precipitation reactions with gelatin), condensed and/or hydrolyzed tannins /phenolic compounds (with $FeCl_3$), and flavonoids (Shinoda test) were assessed according to qualitative standard methods (Domínguez, 1988). Phytochemical screening using thin-layer chromatography (TLC) was carried out on the ethanol extracts. The extracts were subjected to TLC examination for group determination of the secondary metabolites. In this respect, Dragendroff's reagent for alkaloids, ferric chloride reagent for phenolics, NP/PEG reagent for flavonoids, and vanillin/sulfuric acid reagent for terpenoids were used as detecting agents. Solvent systems for developing ready coated analytical TLC plates (Merck Silica gel 60 F254, 0.25 mm) were selected according to Wagner and Bladt (1996). These systems were: for alkaloids (toluene, ethyl acetate, diethylamine, 3.5:1:5), for phenols (ethyl acetate, formic acid, acetic acid, water, 100:11:11:27), for flavonoids (toluene, ethyl acetate and acetic acid, 36:12:5) and for terpenes (ethyl acetate, methanol and water 81:11:8).

***Determination of total phenols and flavonoids content***

The total phenolic content (TPC) was quantified, according to Singleton *et al.* (1999). Flavonoid content (FC) was determined according to Woisky and Salatino (1998). The results for phenols content and flavonoids content were expressed as gallic acid equivalent (GAE)/g dry extract (DE) and quercetin equivalents (QE)/g dry extract (DE), respectively. The samples were prepared in triplicate for each analysis, and the mean value of absorbance was calculated.

***Identification of the main phenolic compounds***

Identification of compounds was achieved by comparing retention times and their UV–vis spectra from 200 to 400 nm, as well as by the addition of an external standard. The identity of the compounds was tentatively confirmed by HPLC/MS/MS in the ISIDSA (*Instituto Superior de Investigación, Desarrollo y Servicios de Alimentos*-CONICET, UNC, Córdoba, Argentina). A mass spectrometer (quadrupole-TOF system, microTOF-Q11, Series,Bruker, Germany) equipped with a turbo spray source and coupled on line to an HPLC instrument (Agilent Technologies, Germany) fitted with a binary pump, an UV detector, an autosampler and a column oven were used for HPLC-UV-ESI–MS/ MS experiments. The separations were conducted at a temperature of 35° C on a Phenomenex Luna C-18 (Torrance, CA, USA) 5 µm particle size column (250 mm 4.60 mm). The injection volume was 40µL, and the flow rate was 0.4 mL/min. Gradient elution was carried out with a binary system consisting of water/formic acid 0.5% v/v (A) and methanol/formic acid 0.5%, v/v (B). The gradient elution was modified as follows: 0–3 min 20% B, 3–8 min 50% B, 8–20 min 70% B, 21–30 min 80% B, 31-43 min 20% B. The UV detection was performed at 190-600 nm. The conditions of ESI were as follows: drying and nebulizer gas ($N_2$) flow rate and pressure, 8 L/min and 4.0 bar; drying temperature,180° C; capillary voltage, 4500 V. $N_2$ and Ar gases were used as nebulizer and collision gas, respectively. The system was calibrated in the negative ion mode: a capillary voltage of 4500 V for an m/z range from 50 to 1000 m/z. The MS detector was programmed to perform MS/MS scan of the three most abundant ions, using 13.0 eV collision energy. Compass Version 3.1 and Data Analysis Version 4.0 were used for data acquisition and processing, respectively.

The optimization of analysis method was performed using different commercial standards of polyphenols, mainly phenolic acids and flavonoids, such us caffeic acid and ferulic acid (Extrasynthèse, Genay, France), myricetin, kaempferol, p-coumaric acid and naringin (Fluka, United Kingdom), chlorogenic acid, naringenin, apigenin and rutin (Sigma-Aldrich, Steinheim, Germany). The results were compared with previously published spectroscopic data (Fabre *et al.*, 2001; Zhang *et al.*, 2019). The polyphenolic compounds were identified, based on their MS spectra, fragmentation spectra (MS/MS), UV-Vis spectra, exact mass error calculation, as well as bibliographic data and Massbank database.

***Antioxidant activity***

**DPPH$^•$ (2,2-diphenyl-1-picrylhydrazyl) assay**

It was determined following the method described by Lim *et al.* (2007). The reaction mixture contained 1.5 mL of DPPH solution (300 µM in ethanol 70°), plant

extract (10 to 50 µL) and ethanol 80° adjusted at a final volume of 3 mL. Initial absorbance and absorbance after 20min were measured at 514 nm. Ethanol 80° was used as negative control, ascorbic acid, and Trolox as positive control. The results were expressed as a TEAC value in mmol Trolox Equivalent (TE)/g DE and as $IC_{50}$ (half maximal inhibitory concentration in mg/mL).

**$ABTS^{•+}$ [2,2'- azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] decolorization assay**

It was estimated according to Re *et al.* (1999). Briefly, ABTS was dissolved to a 7 mM concentration. ABTS radical cation ($ABTS^{•+}$) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. Afterward, the $ABTS^{•+}$ solution was diluted with ethanol to an absorbance of 0.7 ± 0.02 at 734 nm and equilibrated at 30° C. After the addition of 1.0 mL of diluted $ABTS^{•+}$ solution to 10 µL of samples, the absorbance reading was taken after 6 min. Ethanol 80° was used as negative control, ascorbic acid and Trolox as the positive control. The results were expressed as a TEAC value in mmol TE/g DE and as IC50 (mg/mL). Also, correlation coefficient (R) and coefficient of determination ($R^2$) between TEAC $ABTS^{•+}$ and TPC were calculated using Microsoft Excel.

**β-Carotene-linoleic acid assay**

This experiment was carried out using the method from Emmons *et al.* (1999) with some modifications. β-Carotene (10 mg) was dissolved in 1 mL of chloroform, and 50 µL was added to 530 µL of linoleic acid and 40 µL of Tween 20. Oxygenated deionized water (100 mL) was added and mixed well. Aliquots of 3 mL of the carotene/linoleic acid emulsion were mixed with a sample of the extracts (100 µg /mL) and incubated in a water bath at 50° C. Oxidation of the emulsion was monitored by spectrophotometry UV/vis at 490 nm over a 120 min period. The control sample contained solvent in the place of the extract. Antioxidant activity was expressed as a percentage of inhibition relative to the control from 20 to 120 min incubation period using the following equation:

$$\text{Percent Inhibition (\%)} = 100 \times \left[ 1 - \frac{(as\,t0 - as\,t120)}{(ac\,t0 - ac\,t120)} \right]$$

where *as* and *ac* are the absorbances of sample and control at t = 0 min and t = 120 min, respectively.

The same procedure was done on both BHT (as positive control) and blank. Antioxidant capacities of the extract (100 mg) were compared with those of BHT and blank.

***Anti-inflammatory activity (5-LOX assay)***

The LOX inhibiting activity was assayed spectrophotometrically as described Taraporewala and Kauffman (1990) with minor modifications. Briefly, 100 µL of the enzyme solution (at the final concentration of 200 U/mL) was prepared in borate/chloride buffer pH 9 (500 mL $H_3BO_3$ 0.2M and KCl 0.2M -1:1- y 208 mL NaOH 0.2 N, until 1L with distilled water) and mixed with 10 µL of different concentrations of extracts and then incubated at room temperature for 3 min. The reaction was subsequently initiated by the addition of the 20 µL of substrate solution (linoleic acid, 250 µM), and the reading was recorded for 6 min at 234 nm. Quercetin was used as a positive control. The results were expressed as $IC_{50}$ (µg/mL).

***Detection of antilithiatic potential (TLC bioautographic technique)***

The detection of crystallization inhibition was performed by direct TLC bioautographic method, according to Kale *et al.* (2017). After activation, the TLC plates were loaded in spot type with 20 µL of tri-sodium citrate (positive control), and all plant extract in working range from 20 mg to 100 mg, then they were over layered with 40 mL of 0.8% of bactoagar prepared in $CaCl_2$ (0.2 M). The experimental plates were kept in the moist chamber for 6 h. TLC agar gel plate was then removed from the moist chamber and carefully immersed in a 0.2 M ammonium oxalate solution. Calcium chloride present in agar gel and ammonium oxalate solution will form calcium oxalate (CaOx) crystals by reacting with each other on the gel surface. The region with antilithiatic activity or crystal inhibition (CI) potential shows a clear zone due to CaOx crystallization inhibition. Since silica gel and CaOx crystals are white in color, thus it is difficult to visualize CI zone. This situation has been overcome by using staining

with 0.2% alizarin red that specifically impart a pink color to CaOx crystals. A clear zone of inhibition can be seen by this technique, which confirms the antilithiatic potential of the therapeutic drug as it inhibited CaOx crystal formation. The zones were measured at different concentrations for both positive control and extracts. The areas were calculated by multiplication of the diameters of the halos by 3.14 (π number). The results were recorded in the form of Inhibitory area and calculated in terms of CI (%) using the following formula:

$$CI\ \% = (As-Ac)/Ac \times 100$$

As = Area of the zone of inhibition on TLC bioautographic plate; Ac: Maximum diffusing area for negative control [Ac was calculated as 6 $cm^2$ for these techniques which is constant].

Finally, the results were expressed as $IC_{50}$ (mg/mL).

***Statistical analysis***

For each sample, all values were calculated as the mean of three measurements. Results are presented as mean ± standard deviations (SD). Statistical analysis was performed using a one-way analysis of variance, followed by Tukey's post-hoc test using the SPSS 21.0 statistical package (IBM Corp, Armonk, NY, USA). A P value of less than 0.05 was considered statistically significant.

## RESULTS

The results of the histochemical tests showed similarities in both species, so they are described together, specifying the smallest differences. The starch test was highly positive; cells charged with numerous purplish-blue stained starch grains were observed. The test for lignin was positive, lots of stained purple-red tissue were observed that corresponded mainly to lignified walls of the tuber of both species. The cellulose test was positive, the structures stained intense violet color, and lignified cellular walls of gold yellow were observed. The reaction for protein was negative for both species. The test for fats and oils was highly positive; lipid substances stained red, dispersed inside the cells were detected in appreciable amounts. The test for chitin was negative; there were no structures stained violet in any region of the tuber. Regarding tannins, the test was highly positive, tannosomes stained of blue color -"greenish"- were observed inside the cells of highly variable dimensions, the quantity of them increases from the inner tissues to the outer ones of the tuber. The reaction for saponins was negative. The reaction for mucilages was positive for both species; however, in *L. leandri*, higher intensity of the blue coloration and more stained structures were observed.

The phytochemical analysis in both species showed the existence of several phytoconstituents, including carbohydrates, phenols, tannins, flavonoids, and steroids. As with the histochemical tests, no saponins were found in both extracts. Besides, alkaloid and anthraquinones were found to be absent in both extracts. The results are shown in Table No. 1.

**Table No. 1**
**Phytochemical screening of *Lophophytum* extracts**

| Name of the test | Type of test | *L. leandri* | *L. mirabile* |
|---|---|---|---|
| Alkaloids | Dragendorff | - | - |
| | Mayer | - | - |
| | Burchardat | - | - |
| Carbohydrates | Fehling | + | + |
| Phenols & Tannins | ($FeCl_3$) | + | + |
| Flavonoids | Shinoda test | d | + |
| Saponins | Foam | - | - |
| Steroids/Triterpenoids | Liebermann-Burchard | + | + |
| Anthraquinones | Hydrolised (HCl 2M + $CH_3Cl$ + KOH) | - | - |

**(+) indicates presence, (–) indicates absence of phytochemicals, and (d) shows a doubtful result**

The extract of *L. leandri* presented the highest TPC, with a value of 750.00 ± 11.34 mg GAE/g DE, whereas for *L. mirabile* the value was 107.08 ± 2.45 mg GAE/g DE. Regarding FC, no significant differences were found between the two extracts ($p>0.05$), with 46.29 ± 1.26 mg QE/g DE for *L. leandri* and 51.28 ± 1.65 mg QE/g DE for *L. mirabile*.

Ten polyphenolic compounds were identified in *L. mirabile* extract based on their MS spectra, fragmentation pattern (MS/MS), UV-Vis spectra, as well as bibliographic data and Mass Bank (Table No. 2). The results showed key aglycone fragments of 151, 135 for eriodictyol (peak 8), 151, 177 for naringenin (peak 9), and 241, 175 for luteolin (peak 10). In addition, the loss of 162 Daltons was indicative of hexose (glucose or galactose, the most common sugars found in flavonoids), the loss of 133 Daltons was indicative of pentose (xylose or arabinose, the most common pentoses found in natural products). The remaining seven peaks appeared to be glycosylated derivatives of eriodictyol, naringenin, and luteolin.

**Table No. 2**
**Identification of phenolic compounds in *Lophophytum mirabile***

| Peak | Rt (min) | Experimental [M-H]- (m/z) | Theorical [M-H]- (m/z) | MS/MS Fragmentation (m/z) | UV-vis (λ nm) | Tentative identification | Molecular formula |
|---|---|---|---|---|---|---|---|
| 1 | 13.5 | 611.1634 | 611.1607 | 287 | 284, 327sh | Eriodictyol-O-dihexoside | $C_{27}H_{32}O_{16}$ |
| 2 | 14.2 | 581.1509 | 581.1501 | 287 | 284, 325sh | Eriodictyol-O-pentosil-hexoside | $C_{26}H_{30}O_{15}$ |
| 3 | 15.5 | 449.1078 | 449.1078 | 287 | 284, 325 sh | Eriodictyol-O-hexoside | $C_{21}H_{22}O_{11}$ |
| 4 | 15.8 | 579.1367 | 579.1379 | 285 | 345, 283 | Luteolin-O-pentosil-hexoside | $C_{26}H_{28}O_{15}$ |
| 5 | 16.3 | 565.1567 | 565.1552 | 271 | 283 | Naringenin-O-pentosil-hexoside | $C_{26}H_{30}O_{14}$ |
| 6 | 18.1 | 447.0939 | 447.0922 | 285 | 349, 254, 266 | Luteolin-O-hexoside | $C_{21}H_{20}O_{11}$ |
| 7 | 18.6 | 433.1148 | 433.1129 | 271, 269, 243 | 283, 331 | Naringenin-O-hexoside | $C_{21}H_{22}O_{10}$ |
| 8 | 22.5 | 287.0568 | 287.055 | 225, 201, 177, 151 | 288 | Eriodictyol | $C_{15}H_{12}O_6$ |
| 9 | 24.8 | 271.0617 | 271.0601 | 253, 227, 177, 151 | 288, 332sh | Naringenin | $C_{15}H_{12}O_5$ |
| 10 | 25.1 | 285.041 | 285.0394 | 175, 199, 241 | 348, 289,265 | Luteolin | $C_{15}H_{10}O_6$ |

On the other hand, as regards the phytochemical analysis of the extract from *L. leandri*, the results were not conclusive. The presence of seven polyphenolic compounds is proposed, but only five of them could be identified, possibly due to the low concentration of the other compounds in the sample extract (Table No. 3). Catechin, luteolin, and glucosides of eriodictyol, naringenin, and luteolin were found.

The results of the radical scavenging activity are shown in Table No. 4. *Lophophytum leandri* extract was the most active, with $IC_{50}$ values of 0.06 ± 0.03 mg/mL and 0.03 ± 0.003 mg/mL for DPPH and ABTS, respectively, compared to *L. mirabile* extract with $IC_{50}$ values of 0.09 ± 0.05 mg/mL (DPPH) and 0.06 ± 0.01 mg/mL (ABTS). Both values were higher than those found for ascorbic acid (0.02 mg/mL for DPPH and 0.01 mg/mL for ABTS). The Pearson correlation analysis showed a positive correlation between the TPC and the ABTS

scavenging potential ($R^2$ 0.9713 and 0.9462 for *L. leandri* and *L. mirabile*, respectively).

The results of β-carotene linoleic acid assays are shown in Figure No. 1. The data are expressed as a percent of antioxidant activity (AA%). In this test, the inhibitory effect of ethanolic extracts was lower than BHT at the same concentration (80 ± 4.8%), but both extracts effectively inhibited the linoleic acid oxidation.

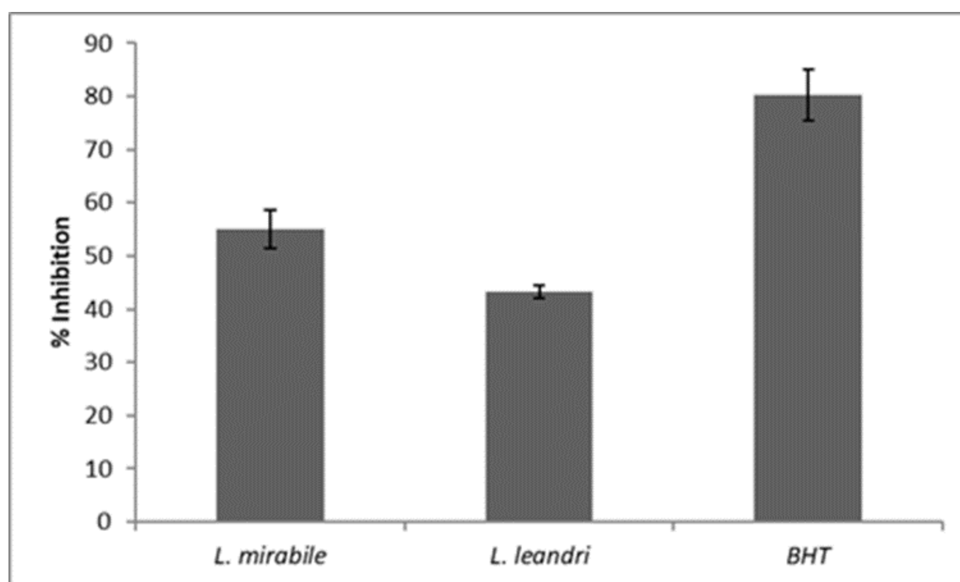


**Figure No. 1**
**Percentage of antioxidant activity (AA %) for the two extracts of Lophophytum**

**Table No. 3**
**Identification of phenolic compounds in *Lophophytum leandri***

| Peak | Rt (min) | Experimental [M-H]- (m/z) | Theorical [M-H]- (m/z) | MS/MS Fragmentation (m/z) | UV-vis (λ nm) | Tentative identification | Molecular formula |
|---|---|---|---|---|---|---|---|
| 1 | 13.9 | 289.0356 | 289.0707 | 245 | 279 | Catechin | $C_{15}H_{14}O_6$ |
| 2 | 15.5 | 449.1118 | 449.1078 | 287 | 284, 325sh | Eriodictyol-O-hexoside | $C_{21}H_{22}O_{11}$ |
| 3 | 18.0 | 447.0978 | 447.0922 | 285 | nd | Luteolin-hexoside | $C_{21}H_{20}O_{11}$ |
| 4 | 18.5 | 433.1199 | 433.1129 | 271 | 280 | Naringenin-O-hexoside | $C_{21}H_{22}O_{10}$ |
| 5 | 25.2 | 285.0406 | 285.0394 | 241, 217, 199, 175 | nd | Luteolin | $C_{15}H_{10}O_6$ |
| 6 | 30.5 | 313.0362 | 313.0707 | 269, 225, 283, 241, 201 | nd | Dihidroxi-dimetoxiflavone? | $C_{17}H_{14}O_6$ |
| 7 | 30.8 | 269.0470 | 269.0444 | 225 | nd | Baicalein? | $C_{15}H_{10}O_5$ |

*In vitro* anti-inflammatory activities showed similar values for *L. leandri* and quercetin control ($IC_{50}$ = 3.24 μg/mL and 2.35 μg/mL, respectively), while *L. mirabile* has shown higher value ($IC_{50}$ = 8,64 μg/mL) compared to control.

Regarding antilithiatic activity, both extracts and tri-sodium citrate (positive control) were analyzed for CaOx crystal inhibition (CI). The results are shown in Table No. 5. These CI values were similar to positive control at 100 mg, indicating biological activity in both extracts, with $IC_{50}$= 59.01 mg/mL ± 1.58 for *L. leandri*, $IC_{50}$ = 60.72 mg/mL ± 4.89 for *L. mirabile* and $IC_{50}$ = 26.48 ± 1.34 mg/mL ± for tri-sodium citrate. The assay showed the dose-dependent inhibition of CaOx inhibition. The results suggest that there are no significant differences between the $IC_{50}$ of the two extracts of *Lophophytum.*

**Table No. 4**
**Total phenols and flavonoids content and scavenger activity of extracts**

| Species | TPC (mg GAE/g dry extract) | FC (mg QE/g dry extract) | IC 50-DPPH test (mg/mL) | TEAC DPPH (µM TE/g DE) | IC 50-ABTS test (mg/mL) | TEAC ABTS (µM TE/g DE) |
|---|---|---|---|---|---|---|
| ***L. leandri*** | **750.00$^{a}$ ± 11.34** | **46.29$^{a}$ ± 1.26** | **0.06$^{a}$ ± 0.03** | **213.64$^{a}$ ± 5.47** | **0.03$^{a}$ ± 0.003** | **106.82$^{a}$ ± 1.23** |
| ***L. mirabile*** | **107.08$^{b}$ ± 2.45** | **51.28$^{b}$ ± 1.65** | **0.09$^{b}$ ± 0.05** | **28.76$^{b}$ ± 0.32** | **0.06$^{b}$ ± 0.01** | **14.38$^{b}$ ± 0.15** |

**Values are expressed as mean ± SD (n=3). Means with superscripts with different letters in the columns are significantly ($p$<0.05) different from each other. TPC, Total Phenolic Content; FC, Flavonoid Content**

**Table No. 5**
**Percent Inhibitory values for Tri-sodium citrate and *Lophophytum* species**

| Concentration (mg/mL) | *L. leandri* | *L. mirabile* | Tri-sodium citrate |
|---|---|---|---|
| 20 | 2.7 ± 0.68 | 1.2 ± 0.51 | 42.2 ± 11.79 |
| 40 | 41.3 ± 5.23 | 38.7 ± 2.62 | 60 ± 3.02 |
| 60 | 59.6 ± 2.62 | 60.5 ± 1.51 | 70.2 ± 1.51 |
| 80 | 69.6 ± 2.22 | 65.7 ± 1.51 | 78.8 ± 3.02 |
| 100 | 81.2± 1.68 | 80.5 ± 2.62 | 83.2 ± 2.62 |
| **$IC_{50}$** | **59.01 ± 1.58** | **60.72 ± 4.89** | **26.48 ± 1.34** |

## DISCUSSION

Phenolic compounds represent one of the most numerous groups of secondary metabolites in plants. They are a heterogeneous family of chemical compounds which can be divided into flavonoids and non-flavonoids. In the non-flavonoid group, the most important are phenolic acid, stilbenes and lignans (Shahidi & Ambigaipalan, 2015). The method of Folin for the quantification of TPC gives an idea about the amount of phenols, but it does not discriminate between the type of phenolic compounds present in the sample (Martin, 2017). No records were found of the quantification of total phenols in these species. However, Nina *et al.* (2020), found that the TPC in a related species (*Ombrophytum subterraneum*) was 242 mg GAE/g CE (crude extract) for the tuber. A greater value than that found in this work for *L. mirabile* but a lot smaller than that found in *L. leandri.*

Previously, the presence of flavonoids has been reported in the rhizomes of *L. leandri* (Weinges *et al.*, 1971). To our knowledge, there are no records of studies of the phytochemical composition of *L. mirabile*. Regarding the profile of phenolic compounds, the results for *L. leandri* are partially consistent with the findings of Bracci *et al.* (2012), who reported the presence of catechin in the ethanolic extract of this species. In contrast, naringenin, rutin, and quercetin-O-glucoside were not found in the present report. Regarding glucosides, Weinges *et al.* (1971) found glucosides of eriodictyol, naringenin, quercetin, and epicatechin, in the alcoholic extract of

dried rhizomes of *L. leandri*. These differences may be due to the method of extraction used in every case. Both extracts possessed intermediate antioxidant activity (50 µg/mL < $IC_{50}$ < 100 µg/mL), with better free radical scavenger activity observed in *L. leandri*. This is according to Phongpaichit *et al.* (2007), who worked with DPPH radical, and argue that extracts that possess $IC_{50}$ values ranging from 50 to 100 µg/mL are considered to exhibit intermediate antioxidant activity. The TPC of these extracts estimated in our study could explain the antioxidant potential of them. The strong positive correlation found in this research between antioxidant activity (TEAC) and TPC supports this hypothesis.

Studies have related plants richer in phenolic compounds show potent radical scavenging activity (Torres *et al.*, 2018). Phenolic compounds, can act as electron donors and can react with DPPH and ABTS free radical and convert them to more stable products (Mathew *et al.*, 2015). Also, the presence of some identified compounds may explain the antioxidant activity of extracts. Naringenin has a well-documented antioxidative activity (Karim *et al.*, 2018). Cavia-Saiz *et al.* (2010), reported a strong capacity to inhibit 50% of ABTS ($IC_{50}$ = 7.9 ± 0.2 µmol/L) for this flavonoid. Eryodictyol and luteolin are also well known for their antioxidant activity (Rajan *et al.*, 2018).

Radical scavenging activities are very important to prevent the harmful effects of free radicals, which can lead to certain health hazards. However, only within the last two decades, has there been an explosive discovery of their roles in the development of diseases, and of the health-protective effects of antioxidants and free radical scavengers (Engwa, 2018; Adwas *et al.*, 2019).

Concerning the antioxidant activity, measured by β-carotene linoleic acid assay, the results showed that there were no significant differences between the AA% of *L. mirabile* extract (52 ± 3.6%) and the AA% of *L. leandri* extract at the same concentration (43 ± 1.2%). This trend of antioxidant activity contradicts to that observed in DPPH and ABTS tests, where *L. leandri* extract exhibited the best values. However, this can be explained by the different mechanisms of action that these extracts could have in the process of inhibition of oxidative reactions.

For this reason, the several methods used here are crucial due to the different array of bioactive components present in the plant species that may be responsible for the antioxidant activity of them. This activity may be based on different mechanisms of action, namely: inhibition of generation and scavenging capacity against ROS or RNS; reducing capacity; metal chelating capacity; singled oxygen quenching activity, hydrogen peroxide decomposition activity. Antioxidants serve as a defensive factor against free radicals' effects in the body (Gulcin, 2020).

Based on our results, the extract of *L. leandri* has better free radical scavenging activity and it is likely that this activity is due to its higher TPC. As previously mentioned, the Folin assay does not discriminate between different types of phenols. Probably the difference in antiradical activity is attributable to non-flavonoid phenolic compounds, such as lignans. Note that in this study, we mainly focused on the search for phenolic acids and flavonoids through HPLC-MS/MS. Lignans are a class of phenolic compounds, widely distributed in the plant kingdom. They exhibit various bioactivities including antioxidant effects that help combat the effects of harmful free radicals (Oroian & Escriche, 2015, Mocan *et al.*, 2016). Whereas there is not much history of the chemical composition of these species, several papers mention the presence of lignans in other species of the Balanophoraceae family, such as, *Balanophora involucrata*, *B. laxiflora*, *Thonningia sanguinea* and *Ombrophytum subterraneum* (She *et al.*, 2013, Quang *et al.*, 2018, Thomford *et al.* 2018, Nina *et al.*, 2020).

These are the first data of antioxidant activity for both species and could explain its popular use, considering the role of natural antioxidants in hepatoprotection. Many plants have been reported to exert antioxidant activity through which they are expected to protect liver injury (Hernandez-Aquino *et al.*, 2018). Naringenin showed hepatoprotective effect via exhibiting antioxidant and free radical scavenging activity against oxidative stress induced by different toxicants such as chemicals, drugs, heavy metals (Karim *et al.*, 2018). Recently, Araya *et al.*, (2019) related the strong free radical scavenging activity of *Cucumis ficifolius* extract to its hepatoprotective effect.

In addition to their ability to quench radicals, polyphenols can affect the activity of enzymes such as LOX that modulate the inflammatory process. Regarding this analysis, the 5-LOX inhibition values indicate a very strong activity according to Baylac and Racine (2003), who create an arbitrary scale of relative *in vitro* activity for medicinal plant extracts ($IC_{50}$ < 10 µg/mL -very strong-, 10 µg/mL < $IC_{50}$ <

30 µg/mL –strong-, 31 µg/mL < $IC_{50}$ < 50 µg/mL -moderate-, 51 µg/mL < $IC_{50}$ < 100 µg/mL –weak- and inactive above 100 µg/mL). 5-LOX is the first enzyme in the metabolic pathway leading to the synthesis of leukotrienes, which are harmful inflammatory substances that scientists believe may have a direct influence on several disease processes. LOXs are sensitive to antioxidants since they are involved in inhibition of lipid hydroperoxide formation due to scavenging of lipidoxy- or lipidperoxy-radicals. This could lead to less availability of lipid hydroperoxide substrate required for LOX catalysis (Paun *et al.*, 2018). Another hypothesis proposed indicated that polyphenols can inhibit lipoxygenase by chelating the iron of the active site of this enzyme and/or by reducing the ferric form of the enzyme to an inactive ferrous form (Ha *et al.*, 2010; Hung *et al.*, 2018). The antioxidant capacity of extracts contributes to reinforce their anti-inflammatory effect. Related to some of the compounds identified in these extracts, Bitto *et al* (2014) demonstrated that a mixed extract containing baicalin and catechin, acts with a significant inhibition of 5-LOX. In addition, results also showed that luteolin was potent inhibitor of 5-LOX (Sroka *et al.*, 2017). Therefore, further research is needed on the concentration of each of these compounds in the analyzed extracts.

Urolithiasis is generally the result of an imbalance between inhibitors and promoters of stone formation in the kidneys. The CI values obtained in this work are better than those found by Kale *et al.* (2017) for other species of popular use in urolithiasis. One possible mode of action of these species may be due to its antioxidant effect. There is *in vivo* evidence that hyperoxaluria induced peroxidative damage to the renal tubular membrane surface provides a favorable environment for individual CaOx crystal attachment and subsequent development of the kidney stones (Jagannath *et al.*, 2012). The antiurolithiatic activity of plant polyphenols is supposed to be due to their diuretic, antioxidant, anti-inflammatory activities, and angiotensin-converting enzyme inhibition (Ahmed *et al.*, 2018). The phytochemical studies have demonstrated that flavonoids and polyphenols are the main active principles in these extracts. In the work of Bracci *et al.* (2012), catechin showed an appreciable diuretic activity; this flavonoid is present in the *L. leandri* extract and could be responsible for this activity.

## CONCLUSIONS

The investigation of these extracts showed promising antioxidant, anti-inflammatory, and anti-urolithiathic properties. It is noteworthy that these are the first results of the phytochemical composition and biological activity of *L. mirabile*. The chemical compounds and biological activities found support the traditional uses of both species. These bioactivities could presumably be attributed to their polyphenolic compositions. However, a quantification of the polyphenols presents in the extracts, further study concerning the presence of lignans and determination of the mechanism of action of the extracts using *in vivo* systems are needed.

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