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Original Article

In vitro antioxidant properties and antimicrobial activity of the ethanolic extract of *Senecio nutans* Sch. Beep. (*Asteraceae*)

[Propiedades antioxidantes *in vitro* y actividad antimicrobiana del extracto etanólico de *Senecio nutans* Sch. Beep. (*Asteraceae*)]

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Abstract

Context: *Senecio nutans* Sch. Beep. is used by the Andean population of Ayacucho-Peru as traditional medicine for various health problems.

Aims: To evaluate the antioxidant and antimicrobial activity of the ethanolic extract of leaves of *S. nutans* against the enteropathogen *Escherichia coli* and the filamentous fungus *Aspergillus niger*.

Methods: The antioxidant capacity of the ethanolic extract was evaluated by ABTS, DPPH, and FRAP assays; the antimicrobial activity was evaluated by the agar diffusion method, and determining the minimum inhibitory concentration (MIC), minimum bactericidal (MBC), and fungicidal concentration (MFC).

Results: Polyphenols, terpenes, and tannins were identified; the total polyphenolic content was 67.91 ± 0.29 mg GAE/g. The ethanolic extract at 20 mg/mL showed moderate antibacterial activity (79.14 ± 0.02% inhibition, MIC and MBC >400 µg/mL on *E. coli*), and antifungal (88.90 ± 0.17% inhibition, MIC and MCF >400 µg/mL on *A. niger*); radical scavenging capacity (ABTS 10.31 ± 0.09 mM TE/g; DPPH 8.28 ± 0.07 IC_{50} µg/mL) and iron reducing power (FRAP 17.72 ± 0.11 mM TE/g). *S. nutans* turned out to be a potential antimicrobial and antioxidant species associated with the presence of its bioactive components, for which further investigation is warranted.

Conclusions: The ethanolic extract of leaves *S. nutans* showed moderate activity against pathogenic bacteria (*E. coli*) and filamentous fungus (*A. niger*), as well as antioxidant activity in three *in vitro* methods.

Keywords: antimicrobial activity; chachacoma; *in vitro* antioxidant activity; phenolic compounds; *Senecio nutans*.

Resumen

Contexto: *Senecio nutans* Sch. Beep. es utilizada por la población andina de Ayacucho-Perú como medicina tradicional en diversos problemas de salud.

Objetivos: Evaluar la actividad antioxidante y antimicrobiana del extracto etanólico de hojas de *S. nutans* frente al enteropatógeno *Escherichia coli* y el hongo filamentoso *Aspergillus niger*.

Métodos: La capacidad antioxidante del extracto etanólico fue evaluada mediante los ensayos de ABTS, DPPH y FRAP; la actividad antimicrobiana se evaluó mediante el método de difusión en agar, y determinando la concentración inhibitoria mínima (MIC), concentración bactericida (MBC) y fungicida mínimo (MCF).

Resultados: Se identificó polifenoles, terpenos y taninos; el contenido polifenólico total fue de 67,91 ± 0,29 mg GAE/g. El extracto etanólico a 20 mg/mL mostró actividad antibacteriana moderada (79,14 ± 0,02% de inhibición, MIC y MBC >400 µg/mL sobre *E. coli*), y antifúngica (88,90 ± 0,17 % de inhibición, MIC y MCF >400 µg/mL sobre *A. niger*); capacidad secuestradora de radicales (ABTS 10,31 ± 0,09 mM TE/g; DPPH 8,28 ± 0,07 IC_{50} µg/mL) y poder reductor del hierro (FRAP 17,72 ± 0,11 mM TE/g). *S. nutans* resultó ser una especie potencial como antimicrobiana y antioxidante, asociado a la presencia de sus componentes bioactivos, por lo que amerita mayor investigación.

Conclusiones: El extracto etanólico de las hojas de *S. nutans* mostró actividad moderada frente a la bacteria patógena (*E. coli*) y hongo filamentoso (*A. niger*), a la vez actividad antioxidante en tres métodos *in vitro*.

Palabras Clave: actividad antimicrobiana; actividad antioxidante *in vitro*; chachacoma; compuestos fenólicos; *Senecio nutans*.

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INTRODUCTION

Plant extracts were the initial source of Mesopotamian pharmacology associating art and magic to mitigate the ailments of the diseases of the time (Chávez et al., 2021); Currently, vegetable plants continue to be an alternative for developing populations (Abbas et al., 2018), It is estimated that two-thirds of the world population use various plant resources as traditional medicine (Gallegos, 2016), and nutraceuticals that support functional foods (Parra et al., 2018). Peru, due to its natural regions of Coast, Andes and Jungle, presents microclimates conducive to the growth of a diversity of plant species with potential bioactive sources and promising drugs with various pharmacological properties (Carbonel et al., 2016; Chávez et al., 2021; Loja et al., 2017).

The genus *Senecio* L. belongs to the *Asteraceae* family, with more than 3000 species distributed throughout the world (Basaid et al., 2020; Paredes et al., 2016), reporting approximately 300 species in South America (Paredes et al., 2016). In the chemical composition of the mentioned genus, the following have been detected: pyrrolizidine alkaloids (with hepatotoxic and carcinogenic activity), sesquiterpenes (bisabolane, cacalol, caryophillane, eremophilane, germacrane, humulane, silphinene), diterpenes, triterpenes, benzofurane, p-hydroxyacetophenones, polyfructans and flavonoids (Paredes et al., 2016). *Senecio nutans* Sch. Beep. is a perennial shrub (30-60 cm high) that grows naturally in the Andean valleys of Peru and Latin America (3500-4800 m.a.s.l) (Juarez et al., 2007); in *S. nutans* several phytochemicals have been identified from the Andes of Peru, including sesquiterpenes (4.6%), cyclic monoterpenes (8.8% Δ^3-carene, 15.5% α-phellandrene, 13.3-26.0% sabinene), alkylbenzene-related monoterpenes (8.8% p- cymene), monoterpene hydrocarbons (12.2-15.1% α-terpinene, 7.5% γ-terpinene) (De Feo et al., 2003). In the species *S. nutans*, from Chile (Toconce II Region of Antofagasta) was found in addition to that described in the species from Peru (0.4% α-phellandrene, 1.1% sabinene, 1.9% p-cymene, 1.8% γ-terpinene) t-cadinol, α-cadinol, caryophyllene, p-cymenol, methyl-cinnamate, linalool, methyl-hydrocinnamate, terpinen-4-ol, α-terpineol, α-terpinene, β-pinene, trans-piperitol, and thymol (Paredes et al., 2016), sesquiterpene lactone (damsine), sesquiterpenes γ- and δ-cadinene and the spirolactone canrenone (Palacios et al., 2022); in popular medicine, these plants are used as a stomach analgesic, anti-inflammatory, antipyretic, antiemetic, vasodilator, wounds, eczema, and in respiratory diseases (asthma, bronchitis, cough, colds) (Basaid et al., 2020; Lopez et al., 2018; Ouchbani et al., 2013).

Antimicrobial resistance to synthetic drugs is a global Public Health problem; therefore, medicinal plants have once again become an alternative since several species naturally synthesize antimicrobial agents (Paredes et al., 2016). Acute diarrheal disease (ADD) in children under five years of age is one of the causes of mortality in developing countries in Africa, Southeast Asia, and Latin America (Gómez-Duarte, 2014). In Peru, diarrhea is one of the three causes of morbidity and mortality in children under one year of age, having decreased in the last three years (CNEPCE, 2022); diarrhea is a response mechanism of the organism against an enteropathogenic agent according to the season (cold months: rotavirus; warm months: enterotoxigenic *Escherichia coli, Vibrio cholerae,* and *Giardia lamblia*) or non-infectious (endocrine, drugs, inflammatory, tumor and due to malabsorption) (Riveros and Ochoa, 2015). Given the need to control this and other diseases, extracts of essential oils from plants of the genus *Senecio* L. have been studied, which have shown antimicrobial activity (Paredes et al. 2016); one of these plant species is *S. nutans*, which is being investigated as an antimicrobial (due to the presence of polyfructans and sesquiterpene lactones) (Joshi et al., 2019; Mishra et al., 2011, Paredes et al., 2016; Parra et al., 2018), and antifungal; and due to the hydroxyl groups of polyphenols, they exhibit antioxidant properties (Galvez et al., 2020; Singh et al., 2016).

After conducting a review in the PubMed-NCBI database on antimicrobial studies and the antioxidant activity of *S. nutans*. In Peru, it has been shown that the phytochemical and experimental pharmacology studies of this plant species are limited. It is part of the traditional medicine of the Andean inhabitants of the Pallca annex, Chipao district, Lucanas province, Ayacucho region, so that deserves to study it; and based on its potential antimicrobial and antioxidant activity, propose the separation of secondary metabolites, elucidate its chemical structure, and establish the chemical structure/activity relationship, so that it is a pharmacotherapeutic and economically viable alternative, like other natural products of plant origin (Basaid et al., 2020), without promoting pseudoscience in our country. Therefore, the objective was to evaluate the antioxidant and antimicrobial activity of the ethanolic extract of *Senecio nutans* against the enteropathogen *Escherichia coli* and the filamentous fungus *Aspergillus niger*.

MATERIAL AND METHODS

Chemical and reagents

All the solvents (dichloromethane, methanol, and ethanol), of analytical grade (99.5%), were purchased from Beaker brand (USA); ammonium hydroxide, potassium persulfate, sodium carbonate, dimethyl sulfoxide, sodium acetate, ferric trichloride, acetic acid, hydrochloric acid, anhydrous sodium sulfate, and Folin Ciocalteu reagent of Merck brand (Germany); gallic acid, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), DPPH (2,2-diphenyl-1-picrylhydrazyl), TPTZ [2,4,6-Tris(2- pyridyl)-s-triazine], ABTS (2,2'-azino-bis-(3-ethyl benzothiazoline-6-ammonium sulfonate) brand Sigma-Aldrich (USA). The bacterial and fungal strains were *Escherichia coli* (ATCC 25922), *Salmonella enterica* (ATCC 29934), *Aspergillus niger* (ATCC 9029), and *Candida albicans* (ATCC 60193), provided by the CERTILAB SAC laboratory, Lima, Peru. Dimethylsulfoxide (DMSO), Mueller Hinton Broth, Mueller Hinton Agar (Merck).

Plant material

The leaves of *S. nutans* were collected during April 2019 from plants that grew naturally in the Pallcca annex, Chipao district (14°21′58″S, 73°52′35″W, at 3452 m.a.s.l.), Lucanas province, Ayacucho region, Peru. The plant was authenticated by a Taxonomic Biologist from the Department of Biology, Faculty of Sciences, San Luis Gonzaga National University of Ica. A supporting specimen was deposited in the Herbarium of the Natural History Museum of the National University of San Marcos, Lima, Peru, record No. 148-USM-2019.

Plant extract preparation

The leaves were cleaned and dried in the shade for 15 days. The dry leaves of the plant species were pulverized, then 500 g of plant powder was weighed and left to macerate for 15 days with periodic agitation to optimize the extraction of secondary metabolites, using 96° ethanol as extraction solvent in sufficient quantity to cover vegetable powdery. The liquid extract obtained was filtered and dried with a rotary evaporator (Heidolph model LABOROTA 4000, German) at 40°C, obtaining a dry extract. This extract was kept at 4°C until use (Alvarado et al., 2022; Casian et al., 2015; Chávez et al., 2021).

Phytochemical study, total polyphenol content and antioxidant activity

Phytochemical study

Secondary metabolites were identified by coloration and/or precipitation reactions in solvents of different polarities. In fraction A, tannins (non-nitrogenated phenolics) were identified; a 1% HCl solution was added to fraction A1, it was stirred, filtered, and an insoluble part and an acid solution were obtained; the insoluble part was washed with distilled water until neutral pH, subsequently dissolved with 5 mL of dichloromethane, anhydrous sodium sulfate was added and filtered, obtaining fraction B (with it, steroids and quinones were identified); the acidic solution was filtered, 25% ammonium hydroxide was added, and dichloromethane was added for extraction, forming two phases (dichloromethane and aqueous). The dichloromethane phase was washed with distilled water, anhydrous sodium sulfate was added, it was filtered, and fraction C was obtained (terpenes were identified); the aqueous phase was saturated with 5 g of anhydrous sodium sulfate, and a mixture of dichloromethane: ethanol (3:2) for extraction, forming an organic and an aqueous phase. The organic phase was washed with anhydrous sodium sulfate solution and saturated with 1 g of anhydrous sodium sulfate, filtered to obtain fraction D (alkaloids were identified); to the aqueous phase (a result of dichloromethane extraction: ethanol), the aqueous residue was added, generating fraction E and with it condensed tannins (flavonoids) were identified (Chávez et al., 2021).

Total polyphenol content using the Folin-Ciocalteu method

A gallic acid (GA) calibration curve was prepared with a concentration range of 1-7.5 µg/mL. The vegetable ethanolic extract was evaluated at 0.9 mg/mL. 0.3 mL of sample was reacted with 0.45 mL of Folin-Ciocalteu 0.2 N reagent for 5 min. After that time, 0.45 mL of sodium carbonate (Na_2CO_3) 20% and ultrapure water (HPLC grade) q.s.f. 3 mL were added and stirred to homogenize. It was left to react for 30 min in the dark and at 25°C. The experiment was performed in triplicate, and the absorbance's were measured at a wavelength of 750 nm (Spectrophotometer Peak Instrumental, model C-7100, USA). The total polyphenolic content was expressed in mg of gallic acid equivalent (GAE)/g of ethanolic extract (Maungchanburi et al., 2022).

Antioxidant activity

ABTS radical scavenging assay: To 10 µL of plant extract sample, 990 µL of ABTS reagent (2,2'-azino-bis-(3-ethyl benzothiazoline-6-ammonium sulfonate) with potassium persulfate was added. The mixture was left to react for 4 min and at 37 °C. The experiment was carried out in triplicate. The mixture of the sample and the $ABTS^{\bullet+}$ cationic radical solution was measured at a wavelength of 734 nm (Spectrophotometer Peak Instrumental, model C-7100, USA); the initial absorbance of ABTS was 0.690. Trolox was used as a reference compound in a concentration range of 0.0312-0.5 mM. Results were expressed as mmol Trolox equivalents/g (TEAC: Trolox equivalent antioxidant capacity) (Ramos-Escudero et al., 2012; Sarmiento et al., 2022).

DPPH radical scavenging assay: A volume of 2.9 mL of 100 mM DPPH solution was added to 100 µL of plant extract diluted in dimethylsulfoxide (DMSO), obtaining a final volume of 3 mL. It was then homogenized in a vortex for 30 s. The mixture was reacted for 30 min protected from light. The reaction sample (extract + DPPH), and the blank (methanol) were measured in triplicate at a wavelength of 517 nm (Spectrophotometer Peak Instrumental, model C-7100, USA); the initial DPPH absorbance was 1.035. Trolox was used as a reference compound at a concentration of 0.0312-0.5 mM. The antioxidant activity was expressed as the value of the 50% inhibitory concentration (IC_{50}). The DPPH radical solution was prepared at a concentration of 100 mM in 80%($^{v}/_{v}$) methanol (Ramos-Escudero et al., 2012; Sarmiento et al., 2022).

Ferric Reducing Antioxidant Power (FRAP): A volume of 1.5 mL of FRAP solution is added to 50 µL of ethanolic extract sample, homogenized in a vortex for 30s, then incubated at room temperature for 30 min. The study sample (extract + FRAP solution) and the FRAP solution were measured in triplicate at a wavelength of 593 nm (Spectrophotometer Peak Instrumental, model C-7100 USA). Trolox was used as a reference compound at a concentration of 0.0312-1.0 mM. The final absorbance was obtained by subtracting the absorbance value of the initial FRAP solution. Results are expressed as mM Trolox/g dry weight of plant extract. The FRAP solution was prepared by mixing 25 mL of acetate buffer (300 mM, pH 3.6), 2.5 mL of 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution diluted with 40 mM HCl and trichloride ferric solution ($FeCl_3.6H_2O$) 20 mM (Ramos-Escudero et al., 2012; Sarmiento et al., 2022). All the antioxidant tests were performed in triplicate.

***In vitro* antimicrobial inhibition assay**

Antibacterial activity by agar diffusion method

Bacterial strains were activated in Mueller Hinton broth (MHB) at 37 °C in a water bath with shaking for 24 h. Afterward, each strain was adjusted to a concentration of 10^8 CFU/mL by comparison with the standard 0.5 of the McFarland scale using a spectrophotometer at 580 nm, which was prepared by mixing 9.95 mL of 1% H_2SO_4 and 0.05 mL of 1% $BaCl_2$. Subsequently, 1000 µL of each bacterial culture were seeded in Petri plates that previously contained 20 mL of melted Mueller-Hinton agar (MHA), distributed evenly with a Drigalsky spatula. Plates were allowed to dry for 5 min, then six 6 mm diameter wells were made using a sterile punch. In each plate, 50 µL of negative control alcohol 50%($^{v}/_{v}$) was added, and four sample concentrations of the ethanolic extract of *S. nutans* leaves, the concentrations were defined by previous tests according to the bacterial species (*E. coli* 2.5, 5.0, 10 and 20 mg/mL, *S. tiphy* 33.75, 45, 60 and 80 mg/mL), and positive control ciprofloxacin (5 µg/mL). Plates were refrigerated for 30 min, then incubated at 37 °C for 24 h. Antimicrobial activity was detected by measuring the zone of inhibition (including well diameter) in mm using a calibrated Vernier. The tests were performed in triplicate for each strain. (Benites et al., 2011; CLSI, 2018; De Zoysa et al., 2019).

Identification of the minimum inhibitory concentration (MIC) of S. nutans

Volumes of 80 µL of Müeller-Hilton broth (CMH) plus 20 µL of extract sample at concentrations of 25, 50, 100, 200, and 400 µg/mL were added to each well of the microplate, then 100 µL of bacterial inoculum was added to a concentration of 10^8 CFU/mL according to the McFarland scale. In the well of column 1, 200 µL of negative control for sterility (190 µL CMH+ 10 µL DMSO) was added; and in the last column of the positive control (100 µL of CMH + 100 µl of inoculum) was added. Ciprofloxacin was dissolved with CMH and tested at the following concentrations: 64, 49, 24.50, 12.25, 6.12, 3.06, 1.53, 0.76, and 0.38 µg/mL. 200 µL of each antimicrobial concentration was added to each well. Negative control of sterility (200 µL of MHC) and positive control (100 µL of MHC and 100 µL of bacterial inoculum) were considered. The final volume of all wells was 200 µL. All plates were incubated at 37°C for 24 h. To assess bacterial growth (blue-purple to pink color variation was observed). The MIC is measured visually as the lowest extract concentration at which the initial blue-purple coloration was maintained (no visible growth). All tests were in triplicate, and the average of three values was reported as MIC and expressed in µg/mL (CLSI, 2018;

Hossain et al., 2022; Kowalska-Krochmal and Dudek-Wicher, 2021).

Identification of the minimum bactericidal concentration (MBC) of S. nutans

With a micropipette, 100 µL of the sample was taken from the wells in which no visible growth of the bacteria was observed (blue-purple coloration), and they were seeded in Petri plates with MHA. A volume of 100 µL of bacterial inoculum and ciprofloxacin was used as a positive control, and MHA without inoculum and without antimicrobial as a negative control. The plates were incubated at 37°C for 24 h. The reading of the results was carried out in those plates where the extracted sample was able to completely eliminate the bacterial development, comparing it with the positive control. The average of three values was reported as MBC (Centurión-Hidalgo et al., 2013; CLSI, 2018; Tenorio-Abreu et al., 2015).

Antifungal activity by agar diffusion method

For antifungal activity, the following concentrations of the ethanolic extract of leaves of *S. nutans*: 20 and 40 mg/mL. The cultures of two types of filamentous fungi were resuspended in a physiological solution; then, the suspensions were adjusted to a concentration of 10^6 conidia/mL. 20 mL of Sabouraud dextrose agar melted at 45 °C was used, which were aseptically mixed with 1 mL of the fungal suspension (1 × 10^4 CFU/mL) in 100 mm × 15 mm Petri dishes. Subsequently, 11 mm diameter wells were made with a sterile punch. In each well, 100 µL of plant extract was inoculated at a concentration of 20 mg/mL for *Aspergillus niger*, 40 mg/mL for *Candida albicans*. It was left to stand for 30 min at room temperature, and incubated at 28°C for 7 to 14 days, depending on the fungus under evaluation, then the reading was made, recording the diameter of the inhibition halos. The tests were performed in triplicate, a positive control group (fluconazole for *A. niger*; ketoconazole for *C. albicans*), and negative control (ethanol, which is the extracting solvent) (Badaracco et al., 2020).

Identification of the minimum inhibitory concentration (MIC) of S. nutans

Microplates containing 100 µL of samples and controls (fluconazole 0.125-64 µg/mL) and ketoconazole (0.125-32 µg/mL); subsequently, 100 µL of inoculum suspension of each of the fungal species was added. They were incubated at 37°C for 24 h for *Candida albicans* strains and 72 h for *Aspergillus niger*. The results were read visually, and a color change from purple to pink or colorless was considered positive. The lowest concentration that did not undergo color change was considered as the MIC value, and for the final MIC report, the average of three experimental values was considered (CLSI, 2018; Liu et al., 2007).

Identification of the minimum fungicidal concentration (MFC) of S. nutans

With a micropipette, 10 µL of the sample was taken from the wells of the microplates in which no fungal growth was observed, and they were seeded in Petri with Sb-Agar; subsequently, they were incubated at 28°C for 2-5 days, growth was observed, and the minimum MFC fungicide concentration was determined (Badaracco et al., 2020; CLSI, 2018).

Statistical analysis

The studies were performed in quadruplicate. The data were expressed as mean ± standard deviation (SD) and its 95% confidence interval (95%CI). The relationship between the total content of phenols and antimicrobial inhibition and the relationship between the antioxidant activity assays and the total content of bioactive compounds was established by applying the Pearson correlation coefficient. The Statistical Software GraphPad Prism 9. Version 9.1.2 was used.

RESULTS AND DISCUSSION

In this study, it has been identified through coloration reactions and precipitation of secondary metabolites from the ethanolic extract of the leaves of *Senecio nutans*, as non-nitrogenous phenolic tannins (fraction A), condensed tannins (E) and terpenes (fractions B and C). The most representative are phenols and terpenes, which are involved in biological activity. The known general chemical structure of phenols, terpenes, alkaloids, and condensed tannins is incorporated (Table 1). Regarding the identification of the terpenes of the ethanolic extract of the present study, this is based on what was reported in two previous studies carried out with the same species, which grow in different Andean areas of Peru: De Feo et al. (2003) identified 25 chemical compounds as cyclic monoterpenes (Δ^3-carene, α-phellandrene, sabinene) in the oil from the aerial parts of *S. nutans*, from the Andean zone of the Colca District, Arequipa, Peru (3500 m.a.s.l.) monoterpene hydrocarbons (α-terpinene), alkylbenzene related to monoterpenes (p-cymene); In the plant sample collected from the high provinces of the Department of Arequipa, Peru (4800 m.a.s.l.), 46 chemical compounds were identified: α-phellandrene, sabinene, p-cymene, α-terpinene, and γ-terpinene; oxygenated monoterpenes (terpinen-4-ol) and 15 sesquiterpenes. Juarez et al. (2007) studied the oil from the leaves of *S. nutans* from the Andean zone of Huaral-Rauma, Lima, Peru (4200 m.a.s.l.). They identified 75 chemical compounds, the most representa-

tive being p-cymene, α-phellandrene, β-pinene, α-pinene, sesquiterpenes, and phenolic terpenes (methyl cinnamate).

Based on the result of the identification of hydrolyzable tannins (phenolic acids) such as gallic acid, the total polyphenol content (TPC) was estimated using the Folin-Ciocalteu method, whose value is 67.91 ± 0.29 mg GAE/g (Table 2). All vegetable plants containing a high concentration of polyphenols are a likely source of antioxidants (Zhang et al., 2019) with an ability to scavenge free radicals (Jaberian et al., 2013). This species had a higher content of TPC compared to the ethanolic extract of the aerial parts of *S. nutans*(20.58 ± 0.59 mg GAE/g) (Parra et al., 2018). The antioxidant capacity of the phenolic and condensed tannins of the plant extract was also evaluated using three *in vitro* methods (Table 2): by the cationic radical $ABTS^{\bullet+}$ chromophore acceptor of an electron (10.31 ± 0.09 mM TE/g); by the FRAP assay that is based on the transfer of an electron from the study sample to the ferric ion (Fe^{3+}) of the TPTZ-Fe^{3+} to reduce it to ferrous ion Fe^{2+} (17.72 ± 0.11 mM TE/g); by means of the $DPPH^{\bullet}$ free radical assay that accepts an electron from the antioxidants, in this case from the polyphenolic compounds of the ethanolic extract of the leaves of *S. nutans* (8.28 ± 0.07 µg/mL IC_{50}), a low IC_{50} value indicates high antioxidant power. In a previous study carried out by Parra et al. (2018) demonstrated the antioxidant capacity of the aerial parts of the ethanolic extract of *S. nutans* (ABT 0.01301 mM TE/g and FRAP 0.02765 mM TE/g). This indicates that its polyphenolic components are reducing agents (electron donors) or metal chelators (Al-Mansoub et al., 2021), with the ability to eliminate hydroxyl radicals, superoxide anion, and others (Zhang et al., 2019). There is a significant relationship between antioxidant activity and total polyphenol content, specifically between TPC/DPPH and TPC/ABTS, as there is a 96.57% and 79.53% linear relationship, respectively. The probability shows that the results are statistically significant ($p<0.05$) (Fig. 1).

Table 1. Presence of secondary metabolites in the ethanolic extract of *S. nutans*.

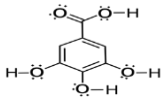
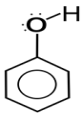
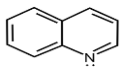
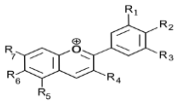
Fractions	Secondary metabolites	General chemical structure	Assay	Results	Observation
A	Non-nitrogenated phenols		Shinoda	+	Reddish yellow coloration
	Phenols and tannins		Ferric chloride	+	Bluish-green coloration
B	Terpenes		Lieberman Burchard	+	Pink coloration
C	Terpenes		Lieberman Burchard	+	Pink coloration
D	Alkaloids		Dragendorff	+	Slight orange precipitate.
			Mayer	+	White or pale-yellow precipitate.
			Hager	+	Yellow precipitate.
E	Condensed tannins		Shinoda	+	Reddish yellow coloration

Table 2. Total polyphenol content, effect of the ethanolic extract of *Senecio nutans* Sch. Beep. on radical scavenging capacity of ABTS, DPPH and FRAP.

Assay	Results			Reference compounds
	Mean ± SD	CV%	95%CI	
TPC (mg GAE/g)	67.91 ± 0.29	0.42	0.2811	Gallic acid
ABTS (mM TE/g)	10.31 ± 0.09	0.91	0.0919	Trolox
DPPH (IC_{50} µg/mL)	8.28 ± 0.07	0.84	0.0684	Trolox
FRAP (mM TE/g)	17.72 ± 0.11	0.60	0.1038	Trolox

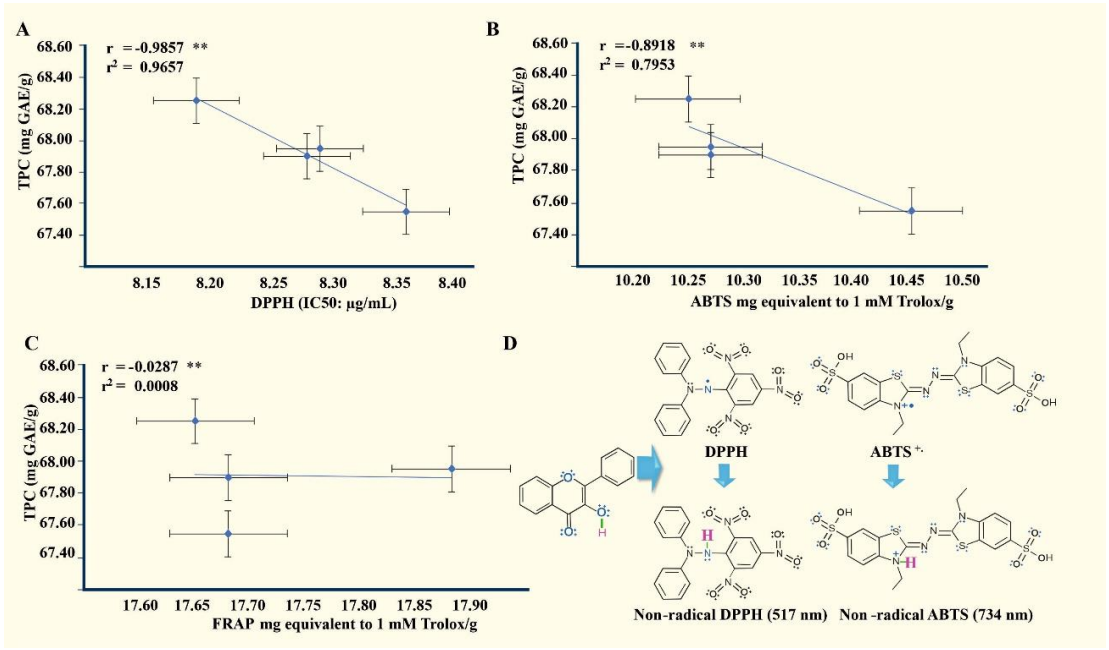


Figure 1. Pearson's correlation coefficient of the total polyphenol content/antioxidant activity of DPPH **(A)**, ABTS **(B)**, and FRAP **(C)**.

**The analysis of variance (ANOVA) for all trials shows that the difference in means is statistically significant ($p<0.05$). Figure 1D proposes the transfer of a polyphenol hydrogen ion to the DPPH and ABTS radicals.

Table 3. Percentage and diameter of inhibition, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of ethanolic extract of *S. nutans.*

Microoganism	Type	Extract concentration	Extract inhibition		MIC	MBC or MFC	Standard antibiotic	
		Concentration (mg/mL)	(%) (Mean ± SD)	Diameter (Mean ± SD mm)	(µg/mL)	(µg/mL)	%	Diameter (mm)
Escherichia coli	Gram-	20	79.14 ± 0.02	19.67 ± 1.10	>400	>400	100	24.93
Salmonella tiphy	Gram-	80	29.39 ± 0.14	16.97 ± 0.50	>400	>400	100	57.83
Aspergillus niger	Fungus	20	88.90 ± 0.17	25.07 ± 0.74	>400	>400	100	28.83
Candida albicans	Fungus	40	37.79 ± 0.51	16.40 ± 0.75	>400	>400	100	42.80

Table 3 describes the results of the potential antimicrobial activity of the ethanolic extract of *S. nutans* leaves. Moderate antibacterial activity was detected against *E. coli* (gram-negative pathogen) 79.14% inhibition, MIC and MBC > 400 µg/mL; and antifungal activity against *Aspergillus niger* (88.90% inhibition and 25.07 mm diameter of inhibition) with MIC and MCF > 400 µg/mL. Fig. 2 shows the antibacterial and antifungal study at four concentrations of plant extract: 2.5, 5.0, 10, and 20 mg/mL. It was studied against *E. coli* (Fig. 2A) and *A. niger*, and the positive control was fluconazole (Fig. 2B); 33.75, 45, 60 and 80 mg/mL against *S. typhi* (Fig. 2C); At 16.87, 22.50, 30and 40 mg/mL, the plant extract was evaluated on *C. albicans* (Fig. 2D), and ketoconazole was used as a positive control. The positive antibacterial control was ciprofloxacin (5 µg/mL). Fig. 3 shows the antimicrobial activity of the ethanolic extract of *S. nutans* leaves evaluated *in vitro* by the agar diffusion method.

In various studies, it has been described that *S. nutans* have antibacterial activity (Parra et al., 2018), which is due to the presence of oxygenated terpenes (Juarez et al., 2007). Santander et al. (2015) demonstrated the bactericidal activity of the metabolite 4-hydroxy-3-(3-methyl-2-butenyl) acetophenone (4HMBA) isolated from *S. nutans* against *S. aureus* at clinically relevant levels. However, the ethanolic extract of *S. nutans* did not show antibacterial activity against Gram-negative bacteria (*Salmonella, Escherichia*), but it did show antibacterial activity against Gram-positive bacteria (*Listeria, Bacillus* and *Streptococcus* species). The same year, Soberón et al. (2015) reported that the 4HMBA metabolite extracted from *S. nutans* inhibits filamentation and reduces the thickness of the cell wall of *C. albicans* at a concentration of 5 mg/L com-

pared to fluconazole 0.5 mg/L. Holetz et al. (2002) proposed that vegetable plant extracts should be evaluated considering three levels of antimicrobial activity based on MIC: good activity if the MIC is <100 µg/mL, moderate activity from 100 to 500 µg/mL, weak activity from 500-1000 µg/mL, and when it is greater than 1000 µg/mL the extract is considered inactive. In our study, it has been shown that the ethanolic extract of the leaves of *S. nutans* presents moderate activity (>400 µg/mL) according to the levels proposed by Holetz et al. (2002). Our findings are similar to those reported by Paredes et al. (2016)

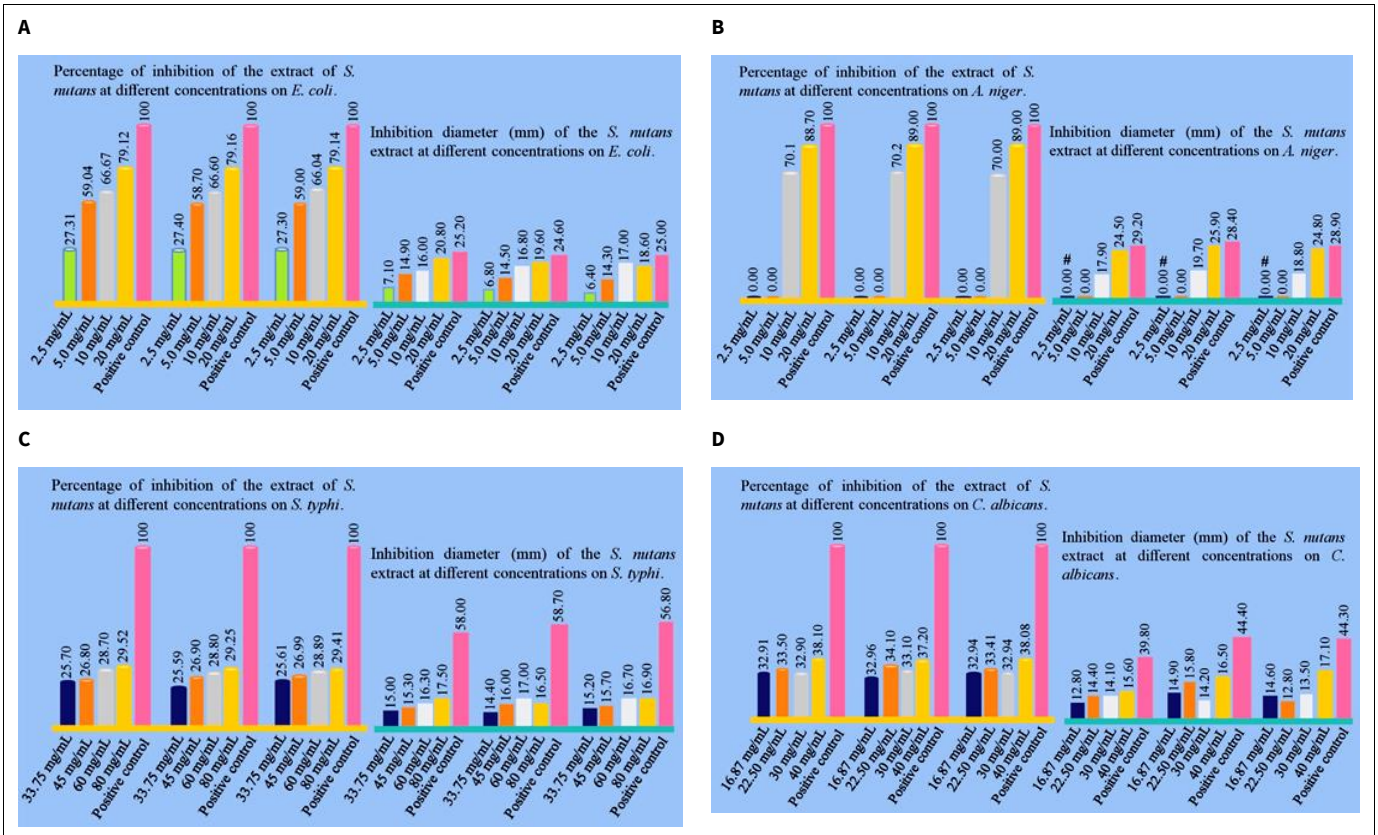


Figure 2. Percentage and antimicrobial inhibition at four concentrations of the extract of leaves of *Senecio nutans* Sch. Beep.

Concentrations of 2.5, 5.0, 10 and 20 mg/mL were studied against *E. coli* **(A)** and *A. niger*, and the positive control was fluconazole **(B)**; concentrations of 33.75, 45, 60 and 80 mg/mL against *S. typhi* **(C)**; At 16.87, 22.50, 30 and 40 mg/mL, the plant extract was evaluated on *C. albicans* **(D)**, and ketoconazole was used as a positive control. The positive antibacterial control was ciprofloxacin (5 µg/mL).

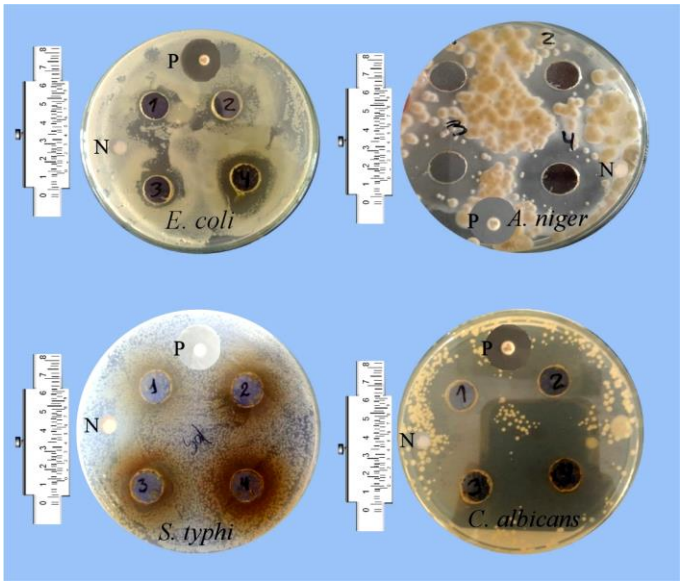


Figure 3. Photograph of the culture and inhibition of bacteria and fungi in Petri dishes and their inhibitory activity.

The 4 concentrations of plant extract evaluated are listed as 1, 2, 3 and 4. Negative control (N), positive control (P) as ciprofloxacin antibacterial, fluconazole as antifungal against *A. niger*, and ketoconazole against *C. albicans*.

who have shown that the MIC value of the essential oil of *S. nutans* is 400 µg/mL, specifying that said activity is due to methyl cinnamate and p-cymenol. Previously, Juarez et al. (2007) reported that *S. nutans* oil has low activity on *E. coli* (MIC = 100 µg/mL), probably due to the low content of oxygenated terpenes in the oil. Subsequently, Benites et al. (2011) indicated that the MIC of the essential oil of leaves and stems of *S. atacamensis* Phil., on *E. coli* was not possible to detect at a concentration of 200 µg/mL. Recently Galvez et al. (2020) showed that the essential oil of the aerial parts of *S. nutans* is inactive in *Aspergillus*, and moderately active against the fungus *Fusarium*; This activity would be due to its chemical components (sabinene, α-phellandrene, o-cymene, β-pinene).

A review carried out by Valdivieso-Ugarte et al. (2019) it was shown that the essential oils of vegetable plants, including *S. nutans*, mainly have monoterpenes, sesquiterpenes, eugenol, cinnamaldehyde, and safrole; being the monoterpenes responsible for interfering with the multiplication and development of microorganisms. The active metabolites of this plant species have different sites of action on pathogens, which hinders the development of microbial resistance (Galvez et al., 2020; Singh et al., 2016).

The limitations of the present investigation are in the type of ethanolic plant extract and the use of only the leaves. Other biases that can lead to confusion include not extracting the secondary metabolites and evaluating their antimicrobial activity. Notwithstanding the foregoing, the results obtained show antioxidant and antimicrobial activity on two pathogens, which is why it is worth continuing to study this plant species by extracting secondary metabolites, elucidating its chemical structure, synthesizing the chemical structure, and carrying out preclinical studies that demonstrate bioactivity, to propose a clinical study in humans as a possible drug, and/or as an antifungal additive in the food industry, and without negative environmental impact.

CONCLUSION

The ethanolic extract of the leaves of *S. nutans* Sch. Beep. showed antimicrobial activity against pathogenic bacteria (*E. coli*) and filamentous fungus (*A. niger*), as well as antioxidant activity, demonstrated in three *in vitro* methods. Both activities are associated with the presence of bioactive components.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR CONTRIBUTION:

Contribution	Surco F	García JA	Bendezú MR	Laos D	Panay JF	Valle M	Palomino JJ	Yarasca PE	Loja B	Alvarado AT
Concepts or ideas	x	x	x	x	x	x	x	x	x	x
Design	x	x	x	x	x	x	x	x	x	x
Definition of intellectual content	x	x	x							x
Literature search				x	x	x	x	x	x	
Experimental studies	x	x	x				x		x	x
Data acquisition	x	x		x						
Data analysis	x					x	x	x		x
Statistical analysis		x					x		x	
Manuscript preparation	x	x	x							x
Manuscript editing	x	x	x	x						x
Manuscript review	x	x	x	x	x	x	x	x	x	x

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