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Unveiling the Bioactive Potential: Antiproliferative, Antimicrobial, and Antioxidant Properties of Polar Extracts From Six Ecuadorian Medicinal Flora

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ABSTRACT

Ecuador is a biodiverse country where ancestral traditions are still maintained. One such tradition is practiced by the Saraguros population in southern Ecuador, who continue to use plants for therapeutic purposes, recognizing nature as a source of therapeutic compounds. In the present work, we studied six plant species used in traditional medicine, some of which lack scientific evidence supporting their use. The selected species were *Alternanthera porrigens*, *Lepidium chichicara*, *Iresine herbstii*, *Indigofera suffruticosa*, *Schinus molle*, and *Vernonanthura patens*. We evaluated their antiproliferative, antioxidant, and antimicrobial activities. From each species, we obtained three microextracts (hydroalcoholic, methanolic, and dichloromethane–methanolic) through consecutive maceration. Antiproliferative activity was evaluated using the SRB assay in five cell lines (A549, HBL-100, HeLa, SW1573, and T-47D). The minimum inhibitory concentration and antimicrobial potential were determined using the broth microdilution technique against a set of 10 strains, and antioxidant capacity was determined using the ABTS and DPPH assays. The results show that microextracts of the *Schinus molle* species exhibited the greatest antiproliferative activity. Regarding antimicrobial activity, the aqueous and methanolic extracts of *Lepidium chichicara* were the most active. Finally, concerning antioxidant activity, the aqueous extracts of *Schinus molle* and *Vernonanthura patens* demonstrated the highest activity. These findings provide scientific evidence for the medicinal use of plants by the Saraguro people, highlighting *Schinus molle*, *Lepidium chichicara*, and *Vernonanthura patens* as promising sources of natural antiproliferative, antimicrobial, and antioxidant agents.

1 | Introduction

Ecuador is recognized as a global biodiversity hotspot, boasting a remarkable variety of plant species within its borders (El-Saadony et al. 2023). For centuries, traditional medicine has relied on plant-derived compounds. The Saraguro ethnic group, part of Ecuador's Kichwa community and located in the southern Andean region of Loja province, exemplifies this tradition. The Saraguros maintain their cultural heritage, including the use of traditional medicinal plants for treating various ailments, contributing to their well-being and potentially increased longevity (Armijos et al. 2014). This ancestral knowledge has significantly contributed to scientific advancements in healthcare. Research has demonstrated that various plant structures, such as roots, leaves, stems, and fruits, contain potent secondary metabolites like tannins, flavonoids, alkaloids, and glycosides, which exhibit

Abbreviations: ATCC, American Type Culture Collection; MIC, minimum inhibitory concentration; NCI, National Cancer Institute; TEAC, Trolox equivalent antioxidant capacity.

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antimicrobial, antioxidant, and cytotoxic activities (El-Saadony et al. 2023; Armijos et al. 2014; Hossain et al. 2022).

In this study, we investigated six plant species belonging to five different families to ensure botanical diversity, all recognized for their medicinal properties. These species are *Alternanthera porrigens*, *Lepidium chichicara*, *Iresine herbstii*, *Indigofera suffruticosa*, *Schinus molle*, and *Vernonanthura patens* (Figure 1).

Interestingly, a review of the literature on pharmacological studies of these selected plant species reveals a gap in the scientific validation of their traditional medicinal uses. While research on related genera has identified promising bioactive compounds, such as the flavonoids (luteolin, rhamnosylvitexin, kaempferol, and quercetin) reported in several *Alternanthera* species (Singla et al. 2022; Arellano Quintanilla et al. 2017) and the diverse array of compounds (phenols, flavonoids, terpenoids, alkaloids, saponins, and glucosides) found in the *Lepidium* genus, including glucosinolates known for their nutritional and therapeutic (antimicrobial, antioxidant, anticancer, anti-inflammatory) properties (Mazumder et al. 2016; Gonzales et al. 2014), specific scientific investigation into our chosen species remains limited. Similarly, *Iresine herbstii* is reported to contain various compounds (flavonoids, glycosides, alkaloids, carbohydrates, saponins, tannins, phenols, phytosterols, proteins, resins, and thiols) in its leaves and stems (Dipankar et al. 2011; Schmidt et al. 2009). The *Indigofera* genus, traditionally used for gastrointestinal disorders, inflammation, pain, skin conditions, and respiratory and infectious diseases, has yielded around 200 identified compounds, predominantly terpenoids, flavonoids, and nitrogen-containing compounds, with studies demonstrating antimicrobial, insecticidal, phytotoxic, antiulcer, hepatotoxic, teratogenic, and cytotoxic activities (Rahman et al. 2018; Gerometta et al. 2020). *Schinus molle* has been shown to contain flavonoids, terpenes, anthraquinones, alkaloids, tannins, phenols, and saponins (Bekele and Yimam 2023; Ados Santos et al. 2009). Finally, chemical analysis of the aerial parts of *Vernonanthura patens* has identified compounds such as hexadecanoic acid, tetracosanoic acid, tetradecanoic acid, aristolone, α-amyrin acetate, lupeol acetate, lupeol, lupeol palmitate, and stigmasta-5,22-dien-3-ol (Manzano Santana et al. 2014; Santana et al. 2012).

The following table (Table 1) summarizes key information regarding the selected plant species, including their traditional medicinal applications. Recognizing the limited existing data on the biological activities of some of these species, the present



FIGURE 1 | Ecuadorian plants recognized for their medicinal properties investigated in this study.

TABLE 1 | General characteristics and ethnobotanical use of the selected plant species.

Scientific name	Family	Vernacular name (Ecuador)	Ethnobotanical use	Citation
Alternanthera porrigens	Amaranthaceae	Moradilla	This fresh plant is used to treat kidney and lung ailments, improve blood circulation, and address warts, hay fever, and allergies	Singla et al. (2022), Arellano Quintanilla et al. (2017)
Lepidium chichicara	Brassicaceae	Chichira negra, Alpa chichira	The sap is taken to cure bad air and prevent relapses and colic in women in labor. The leaves are cooked to treat disorders of the urogenital system	Mazumder et al. (2016), Gonzales et al. (2014)
Iresine herbstii	Amaranthaceae	Hojas de sangre	This plant serves as a pH indicator, insecticide, and fabric dye. Historically, it was used for divinatory practices. Furthermore, it exhibits anti-inflammatory, anti-flu, analgesic, diuretic, sedative, and tonic properties	Dipankar et al. (2011), Schmidt et al. (2009)
Indigofera suffruticosa	Fabaceae	Añil, indigo	This plant is used as a febrifuge, antispasmodic, diuretic, abortifacient, and analgesic, and to treat stomach and urinary problems, jaundice, and ulcers. It also has purgative, sedative, and insecticidal properties	Rahman et al. (2018), Gerometta et al. (2020)
Schinus molle	Anacardiaceae	Pirul, falso pimiento, molle	The essential oil from the leaves is used in home remedies for cataracts and corneal spots, as well as in the treatment of venereal and genitourinary diseases. An infusion of the bark is used to reduce inflammation and help heal ulcers; it also has insect repellent properties	Bekele and Yimam (2023), Ados Santos et al. (2009)
Vernonanthura patens	Asteraceae	Chilca	Cooked leaves are used to combat malaria and in postpartum treatment. They are also used for headaches, wound cleaning and healing, leishmaniasis treatment, and in the preparation of anti-venoms. As a leaf poultice, they are used against athlete's foot. Indigenous healers have also referred to its usefulness in treating certain types of cancer	Manzano Santana et al. (2014), Santana et al. (2012)

study aimed to establish their potential antiproliferative, antibacterial, and antioxidant properties.

2 | Materials and Methods

2.1 | Plant Material

The selected species were *Alternanthera porrigens*, *Lepidium chichicara*, *Iresine herbstii*, *Indigofera suffruticosa*, *Schinus molle*, and *Vernonanthura patens*. The aerial material of the species was collected in the province of Loja, canton of Loja, and the parish of Santiago, between the months of July 2021 and January 2022, under the research permit issued by the Ministry of Environment, Water, Ecological Transition of Ecuador with code MAAE-ARSFC-2022-200. All specimens were identified by Dr. Fanny Tinitana and deposited in the herbarium of the Universidad Técnica Particular de Loja (HUTPL). Table 2 shows the location data in Global Positioning System (GPS) coordinates and voucher specimen data.

TABLE 2 | Collection data of plants investigated in this work.

Plant species	Place of collection	UTM	Voucher number
Alternanthera porrigens	Loja–Loja	3° 59′ 12″ South–79° 12′ 12″ West	HUTPL 14441
Indigofera suffructicosa	Loja–Loja	3° 59′ 12″ South–79° 12′ 12″ West	HUTPL 14443
Iresine herbstii	Loja–Santiago	3° 47′ 22″ South–79° 17′ 20″ West	HUTPL 14440
Lepidium chichicara	Loja–Loja	3° 59′ 12″ South–79° 12′ 12″ West	HUTPL 14446
Schinus molle	Loja–Loja	3° 59′ 26″ South–79° 12′ 0″ West	HUTPL 14445
Vernonanthura patens	Loja–Loja	3° 59′ 12″ South–79° 12′ 12″ West	HUTPL 14442

Abbreviations: HUTPL = Herbarium of Universidad Técnica Particular de Loja, UTM = Universal Transverse Mercator.

2.2 | Extraction of Plant Material

The preparation of the extracts of the six plant species followed our in-house methodological approach (Lacret et al. 2022). The ground plant material of each plant species (10 g) was subjected to maceration in 300 mL of methanol–water (1:1, v/v, MW) for 24 h. This mixture was then filtered, and the remaining plant material was subjected to a second maceration in 300 mL of methanol (M), followed by filtration and a final maceration in 300 mL of dichloromethane–methanol (1:1, v/v, DM). The maceration was carried out under dark conditions and at room temperature. Each resulting solution was concentrated under reduced pressure to evaporate the least volatile solvent. The methanol and dichloromethane extracts were resuspended in water, and all the extracts were lyophilized, obtaining the MW, M, and DM extracts, respectively. Each extract was then weighed and stored at 4 °C until further processing. Extraction yields are reported in Table 3.

2.3 | Cells, Culture, and Seeding

The human solid tumor cell lines used in this study: A549 (lung), HBL-100 (breast), HeLa (cervix), SW1573 (nonsmall cell lung), and T-47D (breast) were provided by Prof. Godefridus J. Peters (VU Medical Center, Amsterdam, The Netherlands). The cell lines were cultured in 35 mm dishes using Roswell Park Memorial Institute (RPMI) 1640 cell culture medium supplemented with 5% fetal bovine serum (FBS) and 2 mM L-glutamine and incubated at 37 °C, with 5% CO_2 and 95% humidified air. To perform the antiproliferative test, the exponential growth phase of the cells must be considered, and they were trypsinized and resuspended in a medium containing antibiotic (100 units of penicillin G and 0.1 mg of streptomycin per mL). The cell suspensions were counted with Moxi Z. After counting, dilutions were made to obtain cell densities suitable for inoculation in the appropriate material plates or microplates.

2.4 | Antiproliferative Tests

To perform the antiproliferative test, the National Cancer Institute (NCI) guidelines (Skehan et al. 1990) were considered, and the sulforhodamine B (SRB) assay (Skehan et al. 1990; Orellana and Kasinski 2016) was used. For the cell lines A549, HBL-100, HeLa, and SW1573, 2500 cells/well were cultured in a 96-well plate, while 5000 cells/well were used for T-47D. For our assays, we started from an initial concentration of 100 mg/mL of each of the three extracts (MW, M, and DM) obtained for each of the plant species. Subsequently, final test concentrations of 250, 25, and 2.5 μg/mL were prepared from the initial concentration through serial dilutions. The incubation time was 48 h. The optical density of each well was measured at 530 nm (primary) and 620 nm (secondary) using a microplate reader (Power Wave XS, BioTek, Winooski, VT, USA). Antiproliferative activity, expressed as 50% growth inhibition (GI_{50}) values, was calculated according to NCI formulas (Skehan et al. 1990).

TABLE 3 | Dry residue (%) obtained after the sequential maceration of plant material.

Plant	Extract			Total
	MW	M	DM	
Alternanthera porrigens	12.1	4.9	1.9	18.9
Lepidium chichicara	16.8	1.7	0.6	19.1
Iresine herbstii	24.9	3.6	0.6	29.2
Indigofera suffruticosa	17.9	2.4	3.7	23.9
Schinus molle	16.6	6.2	0.2	23.1
Vernonanthura patens	14.0	3.1	7.5	24.7

Abbreviations: DM = dichloromethane:methanol mixture (1:1), M = methanol, WM = methanol:water mixture (1:1).

The cytotoxicity of the different extracts was categorized based on the following criteria: $GI_{50} \leq 20$ μg/mL = highly cytotoxic, GI_{50} ranged from 21 to 200 μg/mL = moderately cytotoxic, and $GI_{50} >$ 201 μg/mL = weakly cytotoxic (Niksic et al. 2021).

2.5 | Antimicrobial Assay

The minimum inhibitory concentration (MIC) and antimicrobial potential were determined using the broth microdilution technique (Cartuche et al. 2024). A set of 10 strains from the American Type Culture Collection (ATCC) was used for the assay. These strains are characterized as opportunistic human microorganisms responsible for common human infectious diseases and are widely used worldwide for antimicrobial testing. Three coccobacterial bacteria (*Enterococcus feacalis* ATCC 19433, *Enterococcus faecium* ATCC 27270, and *Staphylococcus aureus* ATCC 25923), four rod-shaped bacteria (*Listeria monocytogenes* ATCC 19115, *Escherichia coli* (O157:H7) ATCC 43888, *Pseudomonas aeruginosa* ATCC 10145, *Salmonella enterica subs enterica serovar Thypimurium* ATCC14 028), one microaerophile rod-shaped bacteria (*Campylobacter jejuni* ATCC 33560), and two fungi (*Candida albicans* ATCC 10231, *Aspergillus niger* ATCC 6275), were selected as microbial test models. Concentrations ranging from 2000 to 15.62 μg/mL were achieved using the serial double dilution method, with a final inoculum concentration, expressed as colony-forming units (cfu) per mL, of 5×10^5 cfu/mL for bacteria, 2.5×10^5 cfu/mL for yeasts, and 5×10^4 spores/mL for sporulated fungi. The culture broth was Mueller Hinton II (MH II) for bacteria and Sabouraud both for fungi, respectively. The *Campylobacter jejuni* culture was activated by adding 5% horse serum in thioglycollate medium; incubation conditions were microaerophilic (Campygen sachet, Thermo Scientific) at 37° C for 48 h. The broth microdilution test was performed in MH II supplemented with 5% horse serum (Thermo) under the same conditions as described above. Commercial antimicrobial agents served as positive controls: ampicillin solution (1 mg/mL) for *E. faecalis*, *E. faecium*, *and S. aureus*; ciprofloxacin solution (1 mg/mL) for *P. aeruginosa*, *S. enterica*, and *E. coli;* erythromycin solution (1 mg/mL) for *C. jejuni*; and amphotericin B solution (250 μg/mL) for *C. albicans* and *A. niger*.

2.6 | Antioxidant Capacity

2.6.1 | The 2,2-Diphenyl-1-Picrylhydrazyl Radical Scavenging Assay

The procedure was adapted from the methodology reported by Thaipong et al. (2006), with minor modifications (Valarezo

et al. 2022). We started with the preparation of a stable solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in MeOH at a concentration of 625 μM. The working solution was prepared by adjusting an aliquot of the stable solution in methanol until an absorbance of 1.1 ± 0.02 was reached. The reading was performed using a microplate reader (EPOCH, BioTek, Winooski, VT, USA) at an absorbance of 515 nm and 96-well multiwell plates were used for the test. 270 μL of the working solution was allowed to react with 30 μL of each of the extracts (methanol/water mixture (WM, 1:1), methanol (M), and dichloromethane/methanol mixture (DM, 1:1)) for 1 h at room temperature in the dark. The same procedure was followed for the blanks and the positive control (Trolox). A solution representing 100% of the DPPH radical was prepared with 270 μL of working solution and 30 μL of methanol. Next, 10 mg of each extract was dissolved in 1 mL of methanol, and a twofold serial dilution method was employed to obtain eight sample concentrations. The half-scavenging capacity (SC_{50}), defined as the sample concentration required to scavenge 50% of the radical, was calculated from the corresponding adjusted curve using GraphPad Prism 8.0.1 (nonlinear regression model).

2.6.2 | The 2,2-Azino-Bis (3-Ethylbenzothiazoline-6-Sulfonic Acid) Radical Scavenging Assay

The assay was adapted from the methodology described by Thaipong et al. (2006) and Arnao et al. (2001), with minor modifications (Valarezo et al. 2022). We started by preparing a stable solution of the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical by mixing equal volumes of a 7.4 μM aqueous solution of ABTS and 2.6 μM potassium peroxydisulfate and reacting in the dark for 24 h. The working solution was prepared by adjusting an aliquot of the stable solution in methanol until an absorbance of 1.1 ± 0.02 was reached. A microplate reader (EPOCH, BioTek, Winooski, VT, USA) was used to monitor the progress of the reaction at 734 nm for 60 min. 96-well multiwell plates were used for the test. The same procedure as described for DPPH was followed for the ABTS assay. The SC_{50} was calculated also, as described for DPPH assay.

3 | Results and Discussion

The continuous search for plant substances that can lead to the development of new therapeutic drugs for treating cancer, diabetes, or infectious diseases is a major concern and research focus for various groups worldwide (Bravo Velásquez 2014). Recognizing the remarkable plant diversity present in South America, we focused on Ecuador, a country that harbors approximately 10% of the world's biodiversity (Pohle and Reinhardt 2004), making it a significant source for this type of study. It is also important to mention that in countries like Ecuador, the ancestral knowledge of indigenous peoples provides a relevant and complementary resource for biodiscovery programs. These cultures transmit knowledge of the medicinal use of plants from generation to generation (Atanasov et al. 2015), and several drugs have already been derived from this knowledge. We used pharmacological criteria to select four species (*Alternanthera porrigens*, *Iresine herbstii*, *Schinus molle*, and *Indigofera suffruticosa*), and an empirical approach, employing ethnobotanical criteria, allowed us to select two additional species (*Lepidium chichicara* and *Vernonanthura patens*) (Saddiqe et al. 2020).

3.1 | Preparation of Plant Microextracts

The extraction yield is an important pharmacognostic parameter to consider when evaluating a plant as a potential source of bioactive compounds, and furthermore, the proper choice of solvents is critical for the outcome of the process. In this context, we have established a sequential extraction protocol that prioritizes the isolation of hydrophilic compounds (Lacret et al. 2022). Our reasoning for this approach stems from the traditional ingestion of medicinal plants as teas.

Sequential maceration at room temperature was selected as the extraction technique to ensure the preservation of thermolabile bioactive compounds that might otherwise degrade under the high temperatures of Soxhlet extraction. This gentle, cold-extraction approach allows for a more accurate representation of the plants' biological potential, mimicking the traditional preparation methods used by the Saraguro community. Furthermore, the use of a sequential solvent system of increasing polarity facilitated the preliminary fractionation of metabolites while minimizing the formation of chemical artifacts often associated with high-energy extraction methods.

We selected water, methanol, and dichloromethane as solvents, considering the wide range of specialized metabolites that could be obtained from the plants under investigation. Therefore, approximately 10 g of each dry plant material was sequentially macerated under the same conditions using solvents of increasing polarity. First, a WM mixture (1:1) was used, followed by M and finally a DM mixture (1:1). After the extraction process, three microextracts were obtained from each plant (WM, M, and DM), resulting in a total of 18 microextracts. For each microextract, the dry mass was weighed, and the extraction yield was calculated and expressed as a percentage. The results are shown in Table 3. Our results showed that the MW and M microextracts from the aerial parts of the six selected plant species yielded the highest amounts. Conversely, the DM microextracts provided the lowest yields. From this, we can infer that these plant species generally possess abundant compounds with medium- to high-polarity characteristics. Specifically, *Iresine herbstii* exhibited the highest yield overall. Subsequently, the 18 microextracts were subjected to biological evaluation.

3.2 | Antiproliferative Activity

For the antiproliferative evaluation of the 18 microextracts (WM, M, and DM), NCI guidelines were followed, testing the extracts on a panel of five human tumor cell lines: A549, HBL-100, HeLa, SW1573, and T-47D. The maximum test concentration was set at 250 μg/mL (Monks et al. 1991; Geran 1972). Table 4 presents the results as GI_{50} values. A subsequent analysis using radar chart (Figure 2) visually illustrates these GI_{50} data, suggesting two key points: first, that the active compounds within each extract may belong to different chemical families and second, that the observed activity exhibits cell line specificity. The radar charts reveal distinct patterns of cell-line sensitivity. For instance, the M-extract of *S. molle* showed a broader spectrum of activity

TABLE 4 | Antiproliferative activity (GI_{50}, μg/mL) of the microextracts against human solid tumor cells.

Plant	Extract	Cell line				
		A549	HBL-100	HeLa	SW1573	T-47D
Alternanthera porrigens	MW	250	250	250	250	250
	M	153	106	184	172	**2.5**
	DM	250	**16**	236	250	250
Lepidium chichicara	MW	152	250	182	250	250
	M	70	62	67	123	91
	DM	56	**36**	**38**	121	52
Iresine herbstii	MW	250	250	250	250	250
	M	250	250	250	250	250
	DM	82	73	57	78	250
Indigofera suffruticosa	MW	250	250	250	250	250
	M	74	**29**	181	**37**	250
	DM	**46**	**16**	121	250	250
Schinus molle	MW	71	**42**	**30**	95	83
	M	**19**	**8.6**	**13**	**14**	**25**
	DM	50	**11**	250	62	70
Vernonanthura patens	MW	250	250	250	250	250
	M	250	250	221	250	250
	DM	158	125	66	140	125

Note: Values under 50 μg/mL are represented in bold.
Abbreviations: DM = dichloromethane:methanol mixture (1:1), M = methanol, WM = methanol:water mixture (1:1).

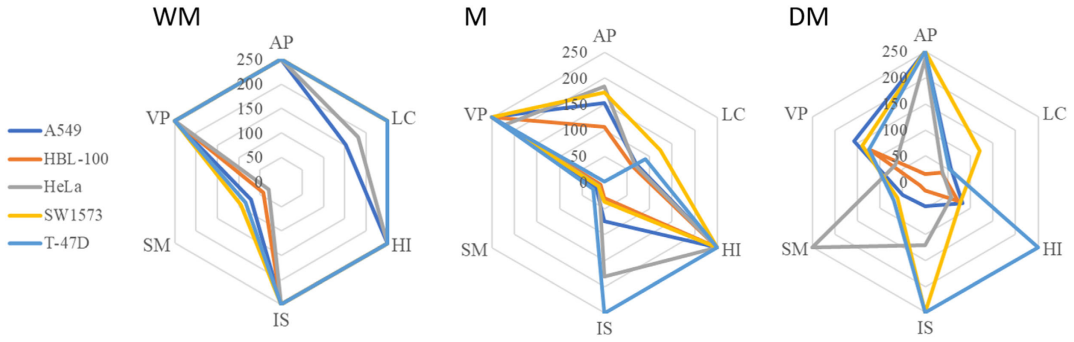


FIGURE 2 | Radar chart graph of the antiproliferative data given in Table 4. DM = dichloromethane:methanol mixture (1:1); M = methanol; WM = methanol:water mixture (1:1).

across all five lines, whereas *A. porrigens* exhibited more localized effects. This selectivity suggests that the extracts do not act through simple nonspecific toxicity but rather through interactions between specific polar metabolites and cellular pathways. The efficacy of *S. molle* against both breast (HBL-100) and cervix (HeLa) lines supports its ethnobotanical use in treating complex genitourinary diseases.

The two species with the greatest antiproliferative activity were *Lepidium chichicara*, followed by *Schinus molle*.

Our results are particularly interesting because *Lepidium chichicara* has not been previously reported to exhibit the antiproliferative effects we observed, with a GI_{50} ranging from 36 to 250 μg/mL. Instead, another member of the *Brassicaceae* family, *Lepidium meyenii* (commonly known as Peruvian maca), has been studied more extensively. Research on *L. meyenii* has demonstrated significant inhibition of cell viability in the G361 melanoma cell line, and its aqueous extract has been shown to be particularly rich in secondary metabolites (Kasprzak et al. 2024). The literature reports that glucosinolates, the main group of active compounds in *Lepidium meyenii*, have been evaluated across various tumor cell lines, including breast (MCF-7 and MDA-MB-231), prostate (PC-3), lung (A-549), cervix (HeLa), and colon (HCT116). These studies have shown a 50% reduction in cell viability (GI_{50}) at concentrations ranging from approximately 33 to 80 μg/mL (Kasprzak et al. 2024). Additionally, in bladder cancer (UM-UC-3) and glioblastoma (LN229) cell lines, glucosinolates have demonstrated a GI_{50} between 110 and 190 μg/mL, indicating their potential cytotoxic effects in these models (Bassan et al. 2018). The radar charts (Figure 2) reveal distinct patterns of cell-line sensitivity. For instance, the M-extract of *S. molle* showed a broader spectrum

of activity across all five lines, whereas *A. porrigens* exhibited more localized effects. This selectivity suggests that the extracts do not act through simple nonspecific toxicity but rather through interactions between specific polar metabolites and cellular pathways. The efficacy of *S. molle* against both breast (HBL-100) and cervix (HeLa) lines supports its ethnobotanical use in treating complex genitourinary diseases.

Similar effects to those observed for *S. molle* in our study have been reported in the literature. For instance, essential oils extracted from the leaves and fruits of *S. molle* have been found to contain α- and β-phellandrenes as their main components. Furthermore, studies have demonstrated the antiproliferative activity of essential oil, ethanol, and aqueous extracts from the fruits and leaves against the Caco-2, HCT116, MCF-7, and T-47D cell lines. Among these, the essential oil and ethanol extract exhibited a dose-dependent inhibition, with half maximal inhibitory concentration (IC_{50}) values ranging from 21 to 65 μg/mL (Aboalhaija et al. 2019). Moreover, another study on the essential oil from *S. molle* leaves reported pronounced in vitro cytotoxic activity against the K562 ($IC_{50} = 0.89$ μg/mL) and NCI-ADR/RES ($IC_{50} = 3.40$ μg/mL) human tumor cell lines (Simionatto et al. 2011).

3.3 | Antimicrobial Activity

To evaluate the antimicrobial activity of the microextracts, we used the broth microdilution method against a panel of 10 human-opportunistic microorganisms comprising three coccobacterial bacteria (*E. feacalis*, *E. faecium*, and *S. aureus*), four rod-shaped bacteria (*L. monocytogenes*, *E. coli*, *P. aeruginosa*, and *S. enterica*), one microaerophile rod-shaped bacteria (*C. jejuni*), and two fungi (*C. albicans*, *A. niger*). Table 5 lists only the microextracts that demonstrated activity. This activity was observed in only 5 of the 10 microorganisms tested. The hydroalcoholic (MW) and methanolic (M) extracts of *Alternanthera porrigens* showed modest inhibitory potential against *Listeria monocytogenes* and *Enterococcus faecium*, respectively. Similarly, the hydroalcoholic and methanolic extracts of *Lepidium chichicara*, the aqueous extract of *Schinus molle*, and the hydroalcoholic and methanolic extracts of *Vernonanthura patens* displayed weak activity against certain microorganisms. Table 5 also includes the MIC values for standard antibiotics for comparison of potency. Based on the obtained results, the plants under study do not appear to be a promising source of novel natural antibiotics.

There is currently no evidence in the reviewed literature concerning the antimicrobial activity of the specific *Alternanthera* species under study. However, activity has been reported for the related species, *Alternanthera brasiliana*. The crude aqueous extract of *A. brasiliana* leaves (in 70% ethanol) and its dichloromethane, ethyl acetate, and butanolic fractions all exhibited moderate activities against the pathogens *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Saccharomyces cerevisiae*, and *Prototheca zopffi* (Pereira et al. 2007).

In the current study, the hydroalcoholic and methanolic extracts of *Lepidium chichicara* were active against *Campylobacter jejuni*. While the MIC values did not reach the level of reference antimicrobials, a moderate antibacterial effect was noted at 500 and 1000 μg/mL for the hydroalcoholic extract. Additionally, the methanolic extract showed activity against *Aspergillus niger* (2000 μg/mL). To date, few other reports of antimicrobial activity for *Lepidium chichicara* have been found in the literature. However, other species within the same genus, such as *Lepidium sativum*, have been extensively evaluated. *L. sativum* extracts reported an MIC of 47.5 μg/mL against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Candida albicans*. Only *Salmonella enterica* required a higher concentration, showing an MIC of 90 μg/mL (Alqahtani et al. 2019). Another study evaluated the ethanol extract of *Lepidium spinosum* against several multidrug-resistant pathogens (*S. aureus* MRSA, *E. faecalis*, *E. coli*, *P. aeruginosa*, *A. baumannii*, *C. albicans*, *C. glabrata*, and *C. krusei*). The researchers found an inhibitory effect on microbial growth at concentrations between 25 and 200 μg/mL, suggesting that *L. spinosum* is a potential natural antimicrobial agent (Mohammed et al. 2022).

The methanolic extract of *Schinus molle* exhibited activity against *Enterococcus faecalis* and *Enterococcus faecium* (2000 μg/mL). This plant has been widely studied, with several research groups reporting its antimicrobial potential, particularly against Gram-negative bacteria like *Escherichia coli* (İlgün et al. 2023; Abrha and Unnithan 2014). Specifically, one study determined that a methanolic extract of *S. molle* had antimicrobial activity at concentrations of 90, 180, and 900 μg/mL against *S. aureus*, *E. faecalis*, *E. coli*, and *C. albicans*, respectively. They demonstrated that this extract could inhibit the growth of bacteria and fungi, showing greater activity against Gram-positive and Gram-negative bacteria (Bekele and Yimam 2023).

Regarding the results for *Vernonanthura patens*, the hydroalcoholic extract was active against *Campylobacter jejuni*, and the methanolic extract showed slight antifungal activity against *Aspergillus niger*. The biological study of this species is limited, with few reported results. Existing literature primarily references its antimalarial activity against *Plasmodium falciparum* (strain Itg2) (Blair and Madrigal 2005) and antileishmania activity from its leaves (Céline et al. 2009). In a separate study, the chemical compounds of the hexane fraction were analyzed for their antifungal activity. This led to the identification of lupeol and epilupeol, which were highly active (80%–100%) against two important fungal pathogens, with epilupeol successfully inhibiting the growth of *Fusarium oxysporum* completely (Santana et al. 2012).

Taken as a whole, while the MIC values were higher than those of pure reference antibiotics like ciprofloxacin, it is essential to consider that these are crude extracts where active molecules are present in a complex matrix. The moderate activity of *V. patens* against *C. jejuni* (1000 μg/mL) is a promising "hit" for an unrefined extract. These results serve as a bioguided compass, indicating that future fractionation of the polar phase is necessary to isolate the potent antimicrobial leads hidden within the total polyphenol pool.

3.4 | Scavenging Radical Capacity

The extracts of the selected plant species exhibited moderate-to-weak antioxidant potential. Of all the extracts tested, the

TABLE 5 | Minimum inhibition concentration (MIC, μg/mL) values of total extracts against human-opportunistic microorganisms.

		Cocci bacteria		Rod-shaped bacteria	Microaerophile rod-shaped bacteria	Sporulated fungi
Plant	**Extract**	***Enterococcus faecalis***	***Enterococcus faecium***	***Listeria monocytogenes***	***Campylobacter jejuni***	***Aspergillus niger***
Alternanthera porrigens	MW			1000		
	M		500			
Lepidium chichicara	MW				500	
	M				1000	2000
Schinus molle	MW	2000	2000			
Vernonanthura patens	MW				1000	
	M					2000
Reference antibiotic						
Ampicillin		0.78	<0.39			
Ciprofloxacin				1.56		
Erythromycin					15.6	
Amphotericin B						<0.098

Note: Standard antibiotic MIC values (for potency comparison) are also shown.
Abbreviations: M = methanol, MIC = minimum inhibition concentration, WM = methanol:water mixture (1:1).

hydroalcoholic extract of *Schinus molle* was the most active, yielding SC_{50} values of 48.20 ± 0.68 μg/mL in the ABTS assay and 51.15 ± 0.15 μg/mL in the DPPH assay. For comprehensive analysis, the Trolox equivalent antioxidant capacity (TEAC) was also determined from the ABTS data and is expressed as μM Trolox/g (Table 6). Trolox served as the positive reference control for all assays.

Our results are comparable to those of a previous study on the root extract of *Schinus molle*, which determined its antioxidant activity using the DPPH method and reported an IC_{50} (i.e., SC_{50}) value of 54.41 μg/mL (Me et al. 2024). This potent activity is likely attributable to the high content of total phenols, flavonoids, and tannins in the root, specifically compounds such as rutin, hyperoside, kaempferol, quercetin, quinic acid, and gallic acid identified in that extract.

To further explore the relationships between pharmacognostic and biological parameters, a Pearson correlation analysis was performed between extraction yield, antiproliferative activity (mean GI_{50}), and antioxidant capacity (mean SC_{50}). A weak positive correlation was observed between yield and GI^{50} values ($r = 0.47$, $p < 0.05$), suggesting that while polar solvents provide higher extraction yields, the potency of the antiproliferative effect is not directly proportional to the amount of extracted mass. Notably, no significant correlation was found between antioxidant capacity and antiproliferative activity ($r = -0.02$, $p > 0.05$). This lack of statistical correlation underlines the independence of the biological mechanisms involved and justifies the use of radar charts (Figure 2) to visually integrate these distinct pharmacological profiles. The multidimensional nature of the data confirms that the radical-scavenging properties and growth-inhibitory effects likely arise from different phytochemical constituents, necessitating independent bioguided fractionation to isolate the specific leads for each biological activity.

Taking all the results, it is evident that the Ecuadorian flora used by the Saraguro community represents a significant reservoir of polar bioactive compounds. The high extraction yields obtained using methanol and hydroalcoholic mixtures, combined with the potent biological responses observed, suggest that the therapeutic efficacy of these plants is primarily driven by hydrophilic secondary metabolites. This chemical profile is particularly relevant as it justifies the traditional preparation methods—such as infusions and decoctions—practiced by ancestral healers. While these crude extracts represent complex mixtures, the correlation between their high polarity and their multifaceted roles as antiproliferative, antioxidant, and antimicrobial agents provides a robust scientific foundation for their traditional use. In particular, the significant antioxidant capacity of *S. molle* and *V. patens* in these polar fractions suggests that the traditional "cooking" of leaves effectively extracts hydrophilic secondary metabolites—likely polyphenols and flavonoids—responsible for their therapeutic reputation.

TABLE 6 | Half scavenging capacity (SC_{50}) of extracts.

Plant	Extract	SC_{50}, μg/mL	
		ABTS	DPPH
Alternanthera porrigens	MW	>1000	>1000
	M	528.99 ± 0.53	538.14 ± 0.67
	DM	105.36 ± 1.23	607.09 ± 1.26
Lepidium chichicara	MW	153.64 ± 0.27	315.18 ± 1.08
	M	117.63 ± 1.28	310.09 ± 0.84
	DM	(–)	(–)
Iresine herbstii	MW	437.27 ± 0.58	963.59 ± 1.30
	M	>1000	>1000
	DM	(–)	(–)
Indigofera suffruticosa	MW	143.68 ± 0.59	615.85 ± 0.93
	M	>1000	>1000
	DM	47.79 ± 1.35	>1000
Schinus molle	MW	48.20 ± 0.68	51.15 ± 0.15
	M	463.59 ± 1.04	134.63 ± 1.95
	DM	(–)	(–)
Vernonanthura patens	MW	58.64 ± 0.97	142.05 ± 0.85
	M	314.58 ± 1.40	585.24 ± 1.68
	DM	499.87 ± 0.67	>1000
Reference compound		SC_{50}, μM	
Trolox		23.27 ± 1.05	29.99 ± 1.04

Note:(–) Activity was higher than 1000 μg/mL or insolubility issues occurred.
Abbreviations: ABTS = 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid, DM = dichloromethane:methanol mixture (1:1), DPPH = 2,2-diphenyl-1-picrylhydrazyl, M = methanol, WM = methanol:water mixture (1:1).

TABLE 7 | Correlation between ethnobotanical uses and experimental bioactivity.

Plant species	Traditional ethnobotanical use	Key biological result	Correlation and potential
Schinus molle	Cataracts, genitourinary diseases, inflammation, and ulcers	Top antioxidant and broad-spectrum antiproliferative	High antioxidant capacity in polar extracts supports its traditional use as an anti-inflammatory and healing agent.
Lepidium chichicara	Urogenital disorders, colic, and "bad air" prevention	Best antimicrobial (active against *C. jejuni*) and significant antiproliferative	Validation of its use in urogenital infections; its cytotoxic profile suggests a high content of bioactive glucosinolates
Vernonanthura patens	Cancer treatment (healer reports), malaria, and wound healing	High antioxidant activity and antifungal potential	Scientific backing for its use in chronic diseases and wound care due to its radical scavenging properties
Alternanthera porrigens	Kidney/lung ailments and blood circulation.	Moderate antimicrobial and selective antiproliferative effects.	The polar nature of the active extracts aligns with traditional intake as a tea for systemic conditions
Indigofera suffruticosa	Ulcers, inflammation, and urinary problems	Moderate antiproliferative activity	Supports its reputation as a cytotoxic agent, potentially useful in managing abnormal cell growth in traditional medicine

A standout result of this study is the antiproliferative and antimicrobial activity of *L. chichicara*, a species with scarce prior scientific documentation. While specific phytochemical quantification was not performed, the biological profile observed (activity against *C. jejuni* and solid tumor lines) is consistent with the presence of glucosinolates, the chemical markers of the *Lepidium* genus. These nitrogen–sulfur polar compounds are known for their cytotoxic and antibiotic properties, providing a plausible chemical explanation for the efficacy of Saraguro's use of this plant for urogenital and digestive disorders. As summarized in Table 7, there is a clear synergy between the traditional knowledge of the Saraguro people and the experimental bioactivity observed in the polar fractions.

While this study focused on the biological fingerprint of these species, the correlation between high polar yields and potent bioactivity provides indirect but strong evidence of a rich polyphenolic and glycosylated composition. This pharmacological baseline is a prerequisite for the more targeted chemical characterization now justified by these findings.

4 | Conclusions

In summary, this study investigated the antiproliferative, antimicrobial, and antioxidant properties of polar microextracts from six Ecuadorian medicinal plants traditionally used by the Saraguros population. The extraction process demonstrated that most compounds are of medium-to-high polarity, as the hydroalcoholic (WM) and methanolic (M) microextracts generally provided the highest yields across all species, with *Iresine herbstii* exhibiting the overall highest yield.

The microextracts of *Lepidium chichicara* and *Schinus molle* showed the greatest antiproliferative activity against the five tested human tumor cell lines. The activity observed for *L. chichicara* is particularly notable, as its antiproliferative effects have not been widely reported previously. Overall, the plants tested were not identified as a strong source of novel natural antibiotics, as most microextracts displayed only moderate-to-weak activity. The hydroalcoholic and methanolic extracts of *Lepidium chichicara* showed activity against *Campylobacter jejuni*, while the methanolic extract of *Schinus molle* was active against *Enterococcus faecalis* and *Enterococcus faecium*. The hydroalcoholic extract of *Schinus molle* was the most potent antioxidant in both the ABTS and DPPH assays. This activity is supported by literature suggesting the presence of bioactive phenols, flavonoids, and tannins in *S. molle* extracts.

This study provides scientific validation for the traditional use of the Ecuadorian medicinal plants by the Saraguros community. The significant antiproliferative activity observed for *Lepidium chichicara* warrants further investigation. Future work should focus on bioguided fractionation and isolation to identify the specific compounds responsible for the most promising activities. This will allow for a better understanding of the modes of action and facilitate the development of new plant-derived therapeutic agents.

Author Contributions

María I. Ramírez: conceptualization, methodology, formal analysis, investigation, data curation, writing – original draft preparation, writing - review and editing. **Aday González-Bakker:** methodology, formal analysis, investigation, writing – original draft, writing – review, and editing. **Adam N. Khan:** methodology, formal analysis, investigation, writing – original draft, writing – review, and editing. **José M. Padrón:** conceptualization, methodology, formal analysis, investigation, resources, data curation, writing – original draft, writing – review and editing, supervision, project administration, funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Ethics Statement

The authors declare that no experiments were conducted on humans or animals for this research.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data is available upon request to the corresponding author.

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