

PHYTOCHEMICAL SCREENING, ANTIOXIDANT, ANTI-CYTOTOXIC AND ANTICANCER EFFECTS OF GALINSOGA PARVIFLORA AND VERNONIA POLYANTHES (ASTERACEAE) EXTRACTS



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ABSTRACT

The study was designed to investigate the chemical composition and the biological effects of *G. parviflora* and *V. polyanthes* ethanolic extracts in vitro. Total content of phenols, flavonoids and tannins was quantified by spectrophotometry; chemical characterization was permed by mass spectrometry (ESI (-) FT-ICR MS and APCI (+) FT-ICR MS analysis). Antioxidant activities were determined by FRAP and Fe²⁺ chelating methods. Extracts cytotoxicity was evaluated in human lymphocytes, sarcoma-180 (S-180) and human gastric adenocarcinoma (AGS) cells, by MTT assay. *V. polyanthes* presented higher total content of tannins and *G. parviflora* presented higher amount of phenols and flavonoids. Chemical characterization showed the presence of flavonoids, phenolic acids and sesquiterpene lactones in *V. polyanthes* extract, and steroids, phenolic acids and fatty acids (Poly Unsaturated Fatty Acids - PUFA) in *G. parviflora* extract. *V. polyanthes* extract stood out in the Fe²⁺ chelation test. *G. parviflora* extract did not present outstanding antioxidant results in the tested protocols. Both species showed a tendency to promote cytotoxicity in human lymphocyte cells. Regarding the antiproliferative effect, both species were able to reduce S-

180 cell viability and *G. parviflora* extract showed high antiproliferative potential in the assay with AGS cells. These findings reinforce the medicinal use of these plants, as well as suggest their potential use for the development of new drugs and for the treatment of cancers.

1. INTRODUCTION

Plants from Asteraceae family produce several secondary metabolites, such as phenolic derivatives, terpenoids, such as sesquiterpene lactones, alkaloids and essential oils [1], [2], [3], [4]. This family is relevant in biochemical, economic and pharmacological aspects [5], [6], [7] and *Galinsoga parviflora* Cav and *Vernonia polyanthes* Less are included in the plant group.

G. parviflora Cav., popularly known as gallant soldier or picão branco, is an herbaceous native from South America, abundant in agricultural areas of temperate and subtropical regions of the world [8], [9]. This plant is considered a non-conventional food plant and its leaves are used in salads, spices and sauteed in countries of North and South America and South Africa [8], [10], [11], [12], [13]. Dried leaves and the juice of *G. parviflora* have been used to treat dermatological disorders and hemorrhages, as well as, are used for analgesia and as an anti-inflammatory. Due to its high vitamin C concentration, this herb is also used to treat colds, flu, cold sores and to prevent scurvy [8],[14], [15], [16]. Phenolic compounds constitute the main class of phytochemicals isolated from this species, however, flavonoids, aromatic esters, diterpenoids, caffeic acid, steroids and phenolic acid derivatives were also isolated in *G. parviflora* [8], [9], [11], [14], [17].

Vernonia polyanthes Less., popularly known as assa-peixe, is a plant native of Brazil, well distributed in several country regions, being found mainly in the Cerrado and Atlantic Forest [4], [18], [19], [20]. *V. polyanthes* is found in open land, pastures, poorly fertile soils and along rivers and roads. It is considered an invasive plant in agricultural activities, being characterized as a honey plant [18], [19], [21], [22]. *V. polyanthes* is used therapeutically as an infusion to treat infections of the respiratory tract, muscle pain, kidney treatments, wounds, sprains, bruises, dislocations, hemorrhoids and uterus infections [18], [20], [23]. In addition, there are in vitro and in vivo tests that demonstrate its diuretic, antihypertensive, anti-hemorrhagic, sedative, abortive, anthelmintic, antiulcerogenic, anti-rheumatic, healing, anti-inflammatory, antinociceptive, antibacterial, antifungal, leishmanicidal and anti-tumor actions [18], [21], [23], [24], [25], [26], [27], [28], [29].

In the search for new compounds with antioxidant action, numerous plants have been evaluated and studied for their capacity to neutralize free radicals [30], which can assist in preventive medicine, delaying the development of chronic diseases [31]. Plants have a variety of molecules capable of capturing free radicals, such as flavonoids, polyphenols, anthocyanins, carotenoids, vitamins and other endogenous metabolites that exhibit antioxidant action [32], [33]. Plant antioxidants can act by different mechanisms, which in general are related to the ability to compete for active sites and cellular receptors, or by modulating the expression of genes encoding proteins involved in intracellular defence mechanisms against oxidative processes in cell structures [34], [35].

Thus, this study aimed to characterize the crude ethanolic extracts of *G. parviflora* and *V. polyanthes* in terms of their chemical composition, by spectrometric analyses, and to evaluate their antioxidant, cytotoxic and antiproliferative effects in vitro, as well as to establish the possible relationships between the chemical composition and biological activities exhibited by the extracts.

2. MATERIALS AND METHODS

Plant extracts

Plant material was collected in Muriaé - MG, identified and a specimen voucher of *G. parviflora* and *V. polyanthes* was deposited at the Herbarium of the Universidade Federal de Viçosa, registration code *vic* 53548 and *vic* 53549, respectively. Both plants were registered with the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen), registration number A8105A. Crude extracts of the total aerial part of the herbs were obtained by exhaustive maceration, with 100% ethanol at room temperature (25 - 30 ° C), protected from light, later filtered and concentrated in a vacuum rotary evaporator.

Chemical Analysis of Extracts

Total Phytochemicals Content

Total content of phenolic compounds was tested according to the Zhang et al. [36], by Folin – Ciocalteu method. The total flavonoid and tannin content were assessed by the protocols of Zhishen et al. [37] and Pansera et al. [38], respectively. The readings were taken on an ELISA microplate spectrophotometer, at the wavelengths recommended for each protocol. All analyses were performed in triplicate and the standards used were the recommended in the protocols.

ESI (-) FT-ICR MS analysis

The ethanolic extracts were analysed by Mass Spectrometry with Fourier Transform Ion Cycle (FT-ICR MS) to determine the chemical profile. The samples were solubilized (1.0 mg.mL^{-1}) in a methanol solution, which was infused at a rate of $2.0 \text{ }\mu\text{L}/\text{min}$ in the negative mode electrospray (ESI). The Solarix model 9.4 T mass spectrometer, Bruker Daltonics, Bremen, Germany, was programmed to operate in a range of m/z 150 – 1500. The conditions of the ESI source used in the analysis were: nebulizer gas pressure of 1.0 bar, capillary voltage 3.8 kV and capillary transfer temperature of $200 \text{ }^\circ\text{C}$. The ion accumulation time was 0.010 s, and each spectrum was acquired by accumulating 32 scans with a 4M time domain (*mega-point*). Mass spectra were processed using Data Analysis software (Bruker Daltonics, Bremen, Germany).

APCI (+) FT-ICR MS analysis

For sample analysis, 1.0 mg of the extracts of *V. polyanthes* and *G. parviflora* were individually solubilized in 1.0 mL of methanol (99.5%, Vetec® Química Fina Ltda, Brazil). The samples were injected directly into the APCI (+) source at a flow rate of $20 \text{ }\mu\text{L.min}^{-1}$. The dynamic range of ion acquisition in the ICR cell was m/z 200 - 1200.

Other APCI source parameters were: voltage in the capillary (cone): 2,100.0 V; end plate offset = - 500 V; drying gas temperature and flow: $180 \text{ }^\circ\text{C}$ and 4 L min^{-1} ; nebulizer gas pressure and temperature: $320 \text{ }^\circ\text{C}$ and 2.0 bar; skimmer = 25 V; collision voltage = - 2 V and corona discharge: 3000 nA. In ion transmission, the ion accumulation time in the hexapole (*ion accumulation time*) and TOF were 0.020 s and a range of 0.850 - 0.900 ms, respectively. Each spectrum was acquired from the accumulation of 32 scans with a time domain of 4M (*mega-point*). Mass spectra were acquired and processed using Data Analysis software (Bruker Daltonics, Bremen, Germany).

Antioxidant Activity

Evaluation of the antioxidant activity of the crude extracts was performed by FRAP and Fe^{2+} chelating activity.

FRAP (*Ferric Reducing Antioxidant Power*) is also known as Iron Reducing Antioxidant Power test. Following the protocol of Rufino et al. [39], with modifications, FRAP reagent was obtained from the combination of 0.3 mM acetate buffer solution, 10 mM TPTZ (2,4,6-Tri (2-pyridyl) 1,3,5-triazine) solution and aqueous chloride solution ferric 20 mM. In 2.0 mL microtubes, it was added 30 μL of the samples, 90 μL of distilled water and 900 μL of the FRAP reagent. Microtubes were vortexed and incubated in an oven at $37 \text{ }^\circ\text{C}$ for 30 minutes. Thus, 250 μL of this solution was added to a 96-well microplate, being performed the same with the blank, reaction control and standards gallic acid, ferrous sulfate and Trolox. The reading was performed on a spectrophotometer for ELISA microplate at 595 nm. The calculation of Antioxidant Activity (AA%) was performed using the equation below, with values expressed in EC_{50} ($\mu\text{g.mL}^{-1}$).

$$AA (\%) = \left(\frac{Abs_{sample} - Abs_{control}}{Abs_{control}} \right) \times 100$$

Fe^{2+} ion chelating activity test was performed as established by Tang et al. [40], with modifications. In a 1.5 mL microtube, it was added 1000 μL of the sample, 50 μL of FeCl_2 and 200 μL of ferrozine. The microtube was vortexed, allowed to react for 10 minutes, the solution was placed in 96-well microplates and read on an Epoch ELISA spectrophotometer at 595 nm. Ascorbic acid, gallic acid and EDTA were used as standards. All tests were performed in triplicate and the calculations for assessing chelating activity (CA%) were based on the equation below, with values expressed in EC_{50} ($\mu\text{g.mL}^{-1}$).

$$CA (\%) = \left(\frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \right) \times 100$$

In vitro cytotoxicity

Cancer cells

Anticancer *in vitro* experiments were performed with sarcoma 180 (S-180) and human gastric adenocarcinoma cells (AGS; ATCC CRL-1739). Sarcoma-180 cells were acquired from the Banco de Células do Rio de Janeiro, Brazil, and the AGS cells were supplied by the Laboratório de Triagem Biológica de Produtos Naturais from UFES. S-180 cells were previously cultured with culture medium RPMI 1640 and AGS cells with DMEM medium, both cell lines supplied with 10% fetal bovine serum. Cells were seeded in 96-well microplate, S-180 at 2.10^5 cells.mL⁻¹ and AGS at 6.10^4 cells.mL⁻¹ in each well, and previously maintained at 37 °C and atmosphere of 5% CO₂.

After 24 hours, cells were treated with seven different concentrations of the extracts for 48h, starting from the initial concentration of 200 µg.mL⁻¹ for *V. polyanthes* and 400 µg.mL⁻¹ for *G. parviflora*. Assay was performed in triplicate and cell viability was assessed using the MTT reduction method. All protocols were in accordance to the Ethics Committee of Humans and Animals Use.

Human lymphocytes

To assess the cytotoxicity in healthy cells, it was used the protocol of Marullo et al. [41], with modifications by Dutra et al. [42]. Peripheral blood was collected from a healthy donor, aged between 18 and 30 years, with free and informed consent. Possible donors with history of recent disease, exposure to radiation or drug use and alcohol ingestion thirty days before blood donating were excluded from donating. All protocols were approved by the Research Ethics Committee of the Universidade Federal do Espírito Santo.

In order to compare the effects of extracts in cancer and healthy cells, human lymphocytes were cultured under the same growth conditions of S-180 and AGS cells. To evaluate the anti-cytotoxicity, human lymphocytes were treated with extract concentrations at 5.00, 25.00 or 50.00 µg.mL⁻¹ and cisplatin at 50.0 µg.mL⁻¹. Following the protocol of pre-treatment, human lymphocytes received extract concentrations and after 24 hours from the incubation received cisplatin; simultaneous treatment, in which the extract concentrations and cisplatin were placed at the same time; and post-treatment, where the lymphocytes were initially treated with cisplatin and, after 24 h, received extract concentrations. Untreated cells were used as a negative control (NC) and cells treated with cisplatin were used as a positive control (PC). To evaluate the perceptual of cytotoxic damage reduction it was used the formula from Serpeloni et al., adapted for Dutra et al. [42], [43]:

$$\% \text{ Reduction} = \frac{(\% \text{ cell viability in A} - \% \text{ cell viability in B}) \times 100}{(\% \text{ cell viability in A} - \% \text{ cell viability in C})}$$

Where “A” is the cell group treated with cisplatin; “B” is the cell group treated with plant extracts more cisplatin; and “C” is the negative control group of cells.

MTT assay

The method is based on the reduction of MTT ((3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazoline bromide) in a violet-colored product (formazan) by the mitochondrial enzyme succinate-dehydrogenase, a reaction that can only occur in viable cells. Thus, after 24h or 48h of the last treatment, human lymphocytes, S-180 and AGS cells were subjected to the cell viability test using the MTT assay.

Microplates were centrifuged at 860 rcf for 10 minutes and the supernatant was discarded. 20 µL of MTT at 5 mg.mL⁻¹ were added to each well and incubated for 3 hours at 37 °C and an atmosphere of 5% CO₂. After the period, the plates were centrifuged at 860 rcf for 5 minutes, the supernatant was discarded and 100 µL of DMSO was added. The reading was performed on an Epoch ELISA spectrophotometer at 595 nm. The experiments were carried out in triplicate and the evaluation of cell cytotoxicity was calculated by the equation below and expressed as a percentage of viable cells (% VC) and IC₅₀ (µg.mL⁻¹):

$$\%VC = \left(\frac{Abs_{sample}}{Abs_{control}} \right) \times 100$$

Statistical analysis

Results were presented as mean \pm standard error of the mean (SE). After verifying the normality of the data, the comparison of means was performed by one-way analysis of variance (ANOVA), followed by Tukey's test ($p < 0.05$). In order to establish relationships between total phenols, flavonoids and tannins content, antioxidant activities, anti-cytotoxicity and anticancer effects of the extracts of *G. parviflora* and *V. polyanthes*, principal component analysis (PCA) and Pearson correlation were performed. For PCA analysis and Pearson correlation, the results of S-180 anticancer effects at the concentration of $50.0 \mu\text{g.mL}^{-1}$ were used, since this concentration corresponds to the best results for anticancer activity.

3. RESULTS AND DISCUSSIONS

Chemical analysis of *V. polyanthes* and *G. parviflora* extracts

In a comparison, the evaluation of the total content indicates that *G. parviflora* presents higher values of phenolic compounds and total flavonoids and that *V. polyanthes* stands out for the total tannin content (Table 1).

Table 1: Total Phenols, flavonoids and tannins in the aerial parts extract of *V. polyanthes* and *G. parviflora*.

Extract	Phenols		Flavonoids		Tannins	
	(mg GAE.g ⁻¹) \pm SE		(mg RE. g ⁻¹) \pm SE		(mg TAE.g ⁻¹) \pm SE	
<i>V. polyanthes</i>	90.11	\pm 0.71	67.04	\pm 4.04	94.9	\pm 0.50
<i>G. parviflora</i>	133.04	\pm 5.45	96.02	\pm 11.45	5.5	\pm 0.10

Values are expressed as mean \pm SE. SE: Standard error; GAE: Gallic Acid Equivalents; RE: Rutin Equivalents; TAE: Tannic Acid Equivalents.

Compounds or classes of metabolites presented in extracts were proposed based on the ions generated, number of unsaturations and rings (DBE) and data from the literature. Fig. 1A and Table 2 show the chemical composition of the extract of the species *V. polyanthes* by the negative ESI ionization source, and Fig. 2 A and Table 3 indicate the results obtained from the APCI (+) FT-ICR MS. In addition, Fig. 1B and Table 4 summarizes the chemical composition of *G. parviflora* by the negative ESI ionization source, and Fig. 2B and Table 5 summarizes the results obtained by the positive APCI ionization source.

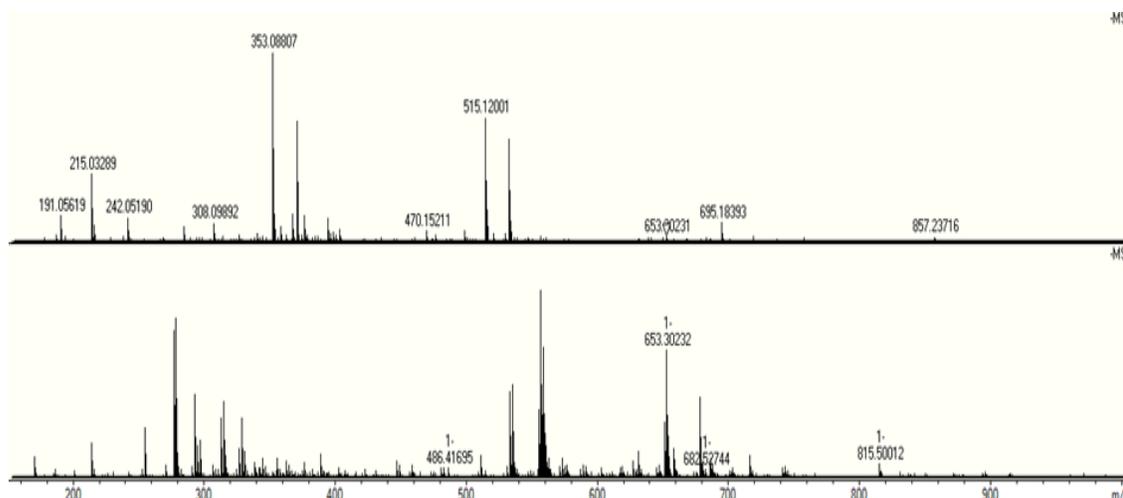


Figure 1: Mass spectra obtained by analyzing the ESI (-) source from top to bottom, of the species *V. polyanthes* and *G. parviflora*.

Mass spectra of *V. polyanthe* is represented by (A); and *G. parviflora* is represented by (B).

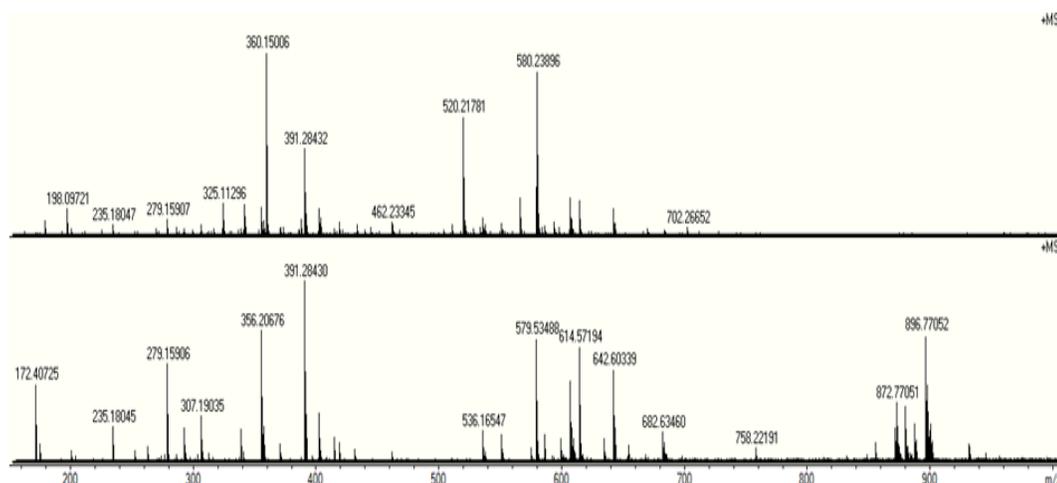


Figure 2: Mass spectra obtained by analyzing the APCI (+) source from top to bottom, of the species *V. polyanthes* and *G. parviflora*.

Mass spectra of *V. polyanthe* is represented by (A); and *G. parviflora* is represented by (B).

Table 2: Compounds identified by ESI (-) FT-ICR MS in the extract of *V. polyanthes*.

[M-H] ⁻ (m/z)	Molecular Formula [M-H] ⁻	Error (ppm)	DBE ^a	Class or proposed substance
179.05618	C ₆ H ₁₁ O ₆	-0.37	1.5	monosaccharide
285.04065	C ₁₅ H ₉ O ₆	-0.19	11.5	luteolin/Kaempferol
353.08807	C ₁₆ H ₁₇ O ₉	-0.74	8.5	monocaffeoylquinic acid
461.07293	C ₂₁ H ₁₇ O ₁₂	-0.83	13.5	luteolin-7-O-glucuronide
477.10437	C ₂₂ H ₂₁ O ₁₂	-1.10	12.5	flavonoid
515.12001	C ₂₅ H ₂₃ O ₁₂	-1.0	14.5	Dicafeoilquinic acid
533.15179	C ₂₂ H ₂₉ O ₁₅	-1.11	8.5	diglycosylated ferulic acid

Table 3: Compounds identified by APCI (+) FT-ICR MS in the extract of *V. polyanthes*

[M-H] ⁻ (m/z)	Molecular Formula [M-H] ⁺ / [M+NH ₄] ⁺	Error (ppm)	DBE ^a	Class or proposed substance
360.15011	C ₁₂ H ₂₆ NO ₁₁	-0.08	0.5	disaccharide (ammonium adduct)
422.18109	C ₂₁ H ₂₈ NO ₈	-0.16	8.5	8β-2 methylacryloyloxy-isohirsutinolide (ammonium adduct)
440.19175	C ₂₁ H ₃₀ NO ₉	-0.20	7.5	Piptocarpine A (ammonium adduct)
482.20235	C ₂₃ H ₃₂ NO ₁₀	0.15	8.5	Glaucolide A (ammonium adduct)
496.21761	C ₂₄ H ₃₄ NO ₁₀	0.22	8.5	10α-Acetóxi-8α-methylacryloyloxy-1 α-13-O-acetate or 1β-methoxyhirsutinolide-13-O-acetate (ammonium adduct)

^aNumber of unsaturations and rings (double bond equivalent)

Table 4: Compounds identified by ESI (-) FT-ICR MS in the extract of *G. parviflora* ethanolic.

[M-H] ⁻ (<i>m/z</i>)	Molecular Formula [M-H]	Error (ppm)	DBE ^a	Class or proposed substance
179.03511	C ₉ H ₇ O ₄	-0.71	6.5	caffeic acid
253.21737	C ₁₆ H ₂₉ O ₂	-0.26	2.5	palmitoleic acid
255.23329	C ₁₆ H ₃₁ O ₂	-1.32	1.5	palmitic acid
277.21768	C ₁₈ H ₂₉ O ₂	-1.36	4.5	linolenic acid
279.23288	C ₁₈ H ₃₁ O ₂	0.26	3.5	linoleic acid
293.21227	C ₁₈ H ₂₉ O ₃	0.06	4.5	fatty acid
297.24391	C ₁₈ H ₃₂ O ₃	-1.32	2.5	fatty acid
327.21768	C ₁₈ H ₃₁ O ₅	0.05	3.5	oxylipine
353.08792	C ₁₆ H ₁₇ O ₉	-0.32	8.5	caffeoylquinic acid
515.11927	C ₂₅ H ₂₃ O ₁₂	0.45	14.5	dicafeoilquinic acid

^aNumber of unsaturations and rings (double bound equivalent).

Table 5: Compounds identified by APCI (+) FT-ICR MS in the extract of *G. parviflora* ethanolic

[M-H] ⁻ (<i>m/z</i>)	Molecular Formula [M-H] ⁺ /[M+NH ₄] ⁺	Error (ppm)	DBE ^a	Class or proposed substance
409.34634	C ₂₉ H ₄₅ O	0.37	7.5	steroid
411.36218	C ₂₉ H ₄₇ O	-0.08	6.5	stigmasterol
413.37805	C ₂₉ H ₄₉ O	-1.59	5.5	β-sitosterol
607.56616	C ₃₉ H ₇₅ O ₄	-0.29	2.5	fatty acid adduct
614.57194	C ₃₇ H ₇₆ NO ₅	-0.23	0.5	fatty acid adduct
896.77052	C ₅₇ H ₁₀₂ NO ₆	-0.39	7.5	triacylglycerol (ammonium adduct)
898.78627	C ₅₇ H ₁₀₄ NO ₆	-0.51	6.5	triacylglycerol (ammonium adduct)

^aNumber of unsaturations and rings (double bound equivalent).

Chanaj-Kaczmarek et al. [9] quantified phenolic compounds and total flavonoids in *G. parviflora* hydromethanolic extract and, compared to our study, obtained low values of phenolic compounds (29.55 mg GAE.g⁻¹) and flavonoids (2.48 mg QE. g⁻¹). It may occur due to the use of different solvents or because the use of ethanol seems to favor the extraction of the phenolic and flavonoid compounds of *G. parviflora*. In addition, differences in climatic and edaphic variables and possible differences in genotypes may have contributed to the variation in the content of secondary metabolites [44], [45], [46].

In previous investigations, chemical analyzes of ethanol extracts from *V. polyanthes* leaves showed lower levels of flavonoids compared to our study [23], [47]. In contrast, in the study by Rodrigues [48] with ethanolic extract of the leaves of *V. polyanthes*, it was described levels of phenolic and flavonoids compounds higher than those presented in our investigation. This finds reinforces the possible influence of different study conditions on the total contents of phenolic and flavonoid compounds.

V. polyanthes is rich in flavonoids, phenolic acids, chlorogenic acids and sesquiterpene lactones [49], [50], [51]. Our results is corroborated by the study of Martucci [21] and Martucci and Gobbo-Neto [52], which indicate the presence of several compounds, such as dicafeoylquinic acid, monocateoylquinic acid and luteolin-7-*O*-glucuronide in *V. polyanthes* extract (Table 2). Other studies also report substances similar to those identified in our study, such as the dicafeoylquinic acid and the flavonoid luteolin [48], [50], and phenolic acids derived from cinnamic acid, such as ferulic acid and caffeine [53].

In the crude extract of *V. polyanthes* different compounds were identified by APCI (+) FT-ICR MS, such as lactone 8β-2 methylacryloyloxy-isohirsutinolide, already described for this species (Table 3) [49], [54]. Similarly, piptocarpine A, glaucolide A, 10α-acetoxy-8α-methylacryloyloxy-1 α-13-*O*-acetate or 1β-methoxyhirsutinolide- 13-*O*-acetate have been identified in previous studies [50], [51], [54].

Other studies indicate that *G. parviflora* exhibits a diversity of flavonoids derived from caffeic acid, steroids, among others [8], [55], [56] which is in accordance to the study of Bazylo et al. [57], with ethanolic extract, that identified phenolic acids, such as caffeic, caffeoylquinic and dicaffeoylquinic acid, using HPTLC; and also in accordance to the results of Dudek et al. [55], with hydrophilic extract of aerial parts, that identified substances derived from caffeic acid, such as 5-*O*-caffeoylquinic acid (chlorogenic acid), 1, 3-*O*-dicaffeoylquinic acid, 3, 5-*O*-dicaffeoylquinic acid, using spectrometric techniques. Meanwhile, the findings presented in our study (Table 5) corroborate those reported by Mostafa et al. [56] and Anwar et al. [58] that indicate the presence of stigmaterol, β -sitosterol and β -sitosterol in *G. parviflora* extracts. In addition, caffeic acid derivatives found in *G. parviflora* has been identified as an important protective factor for dermal fibroblasts against oxidative stress induced by ultraviolet radiation (UVA), by activating the cellular antioxidant system in a study by Parzonko and Kiss [59].

Polyunsaturated fatty acids (linolenic and linoleic acid) and some monounsaturated (palmitoleic, palmitic and ricinoleic acid) were also identified in *G. parviflora* (Table 4), which has not yet been reported for this species. Public health authorities consider nutraceuticals as powerful instruments in maintaining health against nutritional problems and chronic diseases, with improvement in the individual's quality of life [60]. Thus, the presence of fatty acids in *G. parviflora* indicates the promising uses of this herb as a nutraceutical.

On this way, long chain polyunsaturated fatty acids (linolenic and linoleic), PUFAs, compounds not synthesized by humans, must be ingested through the diet (fish, seeds and vegetable oils). PUFA n-3 (linolenic acid) and PUFA n-6 (linoleic acid) acts, respectively, in anti-inflammatory and pro-inflammatory process, therefore, must to be in balance [61], [62], [63].

Despite the controversies, omega-3 fatty acid supplementation has been recommended and studies have reported satisfactory results regarding its regular dietary intake, with favorable effects on triglyceride levels, coagulation and blood pressure, heart rate, cancer prevention, reduction in the incidence of arteriosclerosis and in the prognosis of symptomatic heart failure or myocardial infarction [64], [65], [66].

Antioxidant activity of *V. polyanthes* and *G. parviflora* extracts

Antioxidant activities of *V. polyanthes* and *G. parviflora* extracts, by FRAP and Fe²⁺ chelating activity is shown in Table 6. *G. parviflora* demonstrates lower antioxidant activity, when compared to *V. polyanthes*, in both tests. Following statistical analysis, *V. polyanthes* extract presented antioxidant activity comparable to the standards in the Fe²⁺ chelating test; while the same was not observed for *G. parviflora* extract in tested conditions.

Table 6: Antioxidant activity of *V. polyanthes* and *G. parviflora* ethanolic extracts by FRAP and Fe²⁺ chelating methods.

Sample	EC ₅₀ (µg.mL ⁻¹) ± SE	
	FRAP	Fe ²⁺ chelating
<i>V. polyanthes</i>	183.00 ^d ± 5.30	32.45 ^b ± 0.42
<i>G. parviflora</i>	314.40 ^c ± 2.8	216.20 ^a ± 35.27
Ascorbic acid	-	7.85 ^b ± 1.57
Gallic Acid	101.97 ^e ± 0.55	8.06 ^b ± 0.99
EDTA	-	15.11 ^b ± 0.29
Ferrous sulphate	504.70 ^a ± 2.80	-
Trolox	384.80 ^b ± 2.10	-

Values are expressed as mean ± SE (n = 3); SE: Standard error; values followed by different letters (a, b, c, d or e) differ statistically; ANOVA, post hoc Tukey test (p<0.05).

It was observed in the study of Studzinska-Sroka et al. [16], with hydroalcoholic extract of *G. parviflora*, considerable antioxidant activity in the FRAP assay, with EC₅₀ = 498.20 µg.mL⁻¹. It was also verified in the *G. parviflora* extract, by liquid chromatography (UPLC-PDA), the presence of phenolic acids, such as chlorogenic, caffeic and isovanilic acids, and 4-hydroxybenzoic. In conclusion, the authors stated that the application of *G. parviflora* extract of in cutaneous lesions allowed the healing of wounds and exhibited antioxidant, anti-inflammatory and hyaluronidase inhibitory activities. In addition, studies indicate significant antioxidant activity of *V. polyanthes* extracts, such as in FRAP assay, different to the reported in our study, and correlate antioxidant effects to the levels

of total phenolics and flavonoids, such as rutin and quercetin [23], [47], [58]. *V. polyanthes* extract stood out in the Fe²⁺ chelating test (Table 6), a condition similar to the observed in two fractions of *V. amygdalina* (ethanolic polyphenol and acetone eluate) that exhibited high chelating power [67].

Cytotoxicity of *V. polyanthes* and *G. parviflora* extracts

The cytotoxic activity of *V. polyanthes* and *G. parviflora* in S-180 and AGS cells after 48 hour of treatment was presented as IC₅₀. The results with S-180 cells suggest that *V. polyanthes* extract presented IC₅₀ lower than 5.00 µg.mL⁻¹, while *G. parviflora* presented IC₅₀ = 5.26 ± 1.09 µg.mL⁻¹. Following the results with AGS cells, *V. polyanthes* extract presented IC₅₀ = 8.47 ± 0.55 µg.mL⁻¹ and stands out in relation to *G. parviflora*, with IC₅₀ = 60.97 ± 0.39 µg.mL⁻¹, showing cytotoxic effect against AGS cells. In the experiments with human lymphocytes, *V. polyanthes* extract presented IC₅₀ = 46.42 ± 3.50 µg.mL⁻¹ and *G. parviflora* presented IC₅₀ = 48.48 ± 1.89 µg.mL⁻¹.

In a comparison, *V. polyanthes* and *G. parviflora* extracts showed similar anticancer activity against S-180 cells; however, the extract of *V. polyanthes* was more cytotoxic for AGS cells than the extract of *G. parviflora*. In addition, the cytotoxicity induced by extracts in human lymphocytes did not differ.

Following the protocols of the anti-cytotoxicity, the results showed that in the pre and simultaneous treatment, both species were not able to avoid the cytotoxic damage induced by cisplatin. In the post-treatment, it was observed, for the highest tested concentrations of *V. polyanthes* extract, a tendency to reverse cisplatin induced damage. For *G. parviflora* extract, only the concentration of 25µg.mL⁻¹ showed a promising action against cisplatin damage (Table 7).

Table 7: Percentage of viable human lymphocytes treated with different concentrations of the *V. polyanthes* and *G. parviflora* extracts at different concentrations, following the pre, simultaneous and post-treatment protocols.

Treatment	% Cell viability ± SE					
	Pre-treatment	% Reduction	Simultaneous treatment	% Reduction	Post-treatment	% Reduction
NC	100.00 ± 5.61 ^a	-	100.00 ± 3.32 ^a	-	100.00 ± 7.25 ^a	-
PC	46.81 ± 0.38 ^b	-	77.07 ± 1.59 ^b	-	75.26 ± 0.61 ^b	-
<i>V. polyanthes</i> 5 µg.mL ⁻¹	45.5 ± 1.44 ^b	nd	78.34 ± 2.21 ^b	5.54	70.67 ± 2.47 ^{bc}	nd
<i>V. polyanthes</i> 25µg.mL ⁻¹	48.35 ± 0.96 ^b	2.90	86.62 ± 3.04 ^b	41.65	82.33 ± 4.43 ^{ab}	28.58
<i>V. polyanthes</i> 50µg.mL ⁻¹	56.48 ± 3.81 ^b	18.18	84.39 ± 0.64 ^b	31.92	86.22 ± 1.97 ^{ab}	44.30
<i>G. parviflora</i> 5 µg.mL ⁻¹	47.25 ± 0.22 ^b	0.83	75.80 ± 4.14 ^b	nd	69.61 ± 0.93 ^b	nd
<i>G. parviflora</i> 25 µg.mL ⁻¹	45.05 ± 0.22 ^b	nd	72.93 ± 0.84 ^b	nd	81.98 ± 3.98 ^{ab}	27.16
<i>G. parviflora</i> 50 µg.mL ⁻¹	46.15 ± 0.66 ^b	nd	79.30 ± 3.86 ^b	9.73	77.39 ± 2.45 ^b	8.61

Cisplatin is a highly reactive molecule used in the treatment of cancer due to the ability to bind to proteins and phospholipid membranes. In addition, cisplatin can interact with RNA and DNA, forming adducts that may inhibits the replication, transcription or interrupt the cell cycle and activation of apoptosis, generating genotoxic and cytotoxic effects [68], [69]. Our results suggest that *V. polyanthes* and *G. parviflora* act on human lymphocytes repair mechanism, positively interfering with the maintenance of cellular homeostasis, even after interaction with cisplatin.

To our knowledge, there are no studies involving simultaneously *V. polyanthes* extract, human lymphocytes, S-180 and AGS cells. However, studies with other species of the genus *Vernonia* have demonstrated the promising uses of this group of herbs in trials with several cancer cell lines. An example is the investigation of Amuthan et al.[70], comparing the cytoprotective activity of *V. cinerea* crude aqueous extract and its fractions in normal HEK293 kidney cells and HELA cell lines, against cisplatin-induced cytotoxicity. Also investigating anticancer activity, Siew al. [71], showed that the methanolic extract of *V. amygdalina* leaves showed antiproliferative activity against several cancer cell lines. Traditionally, *V. amygdalina* leaves are already used in popular Indian culture for the treatment of cancer, and based on the excellent results that this species, the authors claim that further studies are needed to understand its potential in the treatment of cancer.

Explorative analyses: correlations between phytochemicals, antioxidant activity and anticancer effects

Results obtained in different assays were correlated by PCA and Pearson correlation coefficient. The first principal component (F1) accounted 76.05% and second principal component (F2) accounted 15.67%, with a total variance of 91.72% (Fig. 3). Total phenol, flavonoid and tannins content, FRAP, Fe²⁺ chelating, anticancer activity against AGS cells and pre-treatment assay were the variables that dominated PC1. S-180 anticancer, simultaneous and post-treatment assays were the variables that dominated PC2. These finds suggest that anticancer activity against AGS cells was correlated with total phenol and flavonoid content and FRAP and Fe²⁺ chelating antioxidant activity, while anti-cytotoxic activity in the pre-treatment protocol was correlated to the total tannins content. Following PCA analysis, it was not possible establish correlations among chemical content of extracts and human lymphocytes cytotoxicity, simultaneous and post-treatment assays. In summary, *V. polyanthes* seems to be correlated with cytotoxicity in AGS cells and anti-cytotoxicity in the post-treatment protocol, while *G. parviflora* seems to be correlated with cytotoxicity in sarcoma 180 cells, and pre and simultaneous treatment.

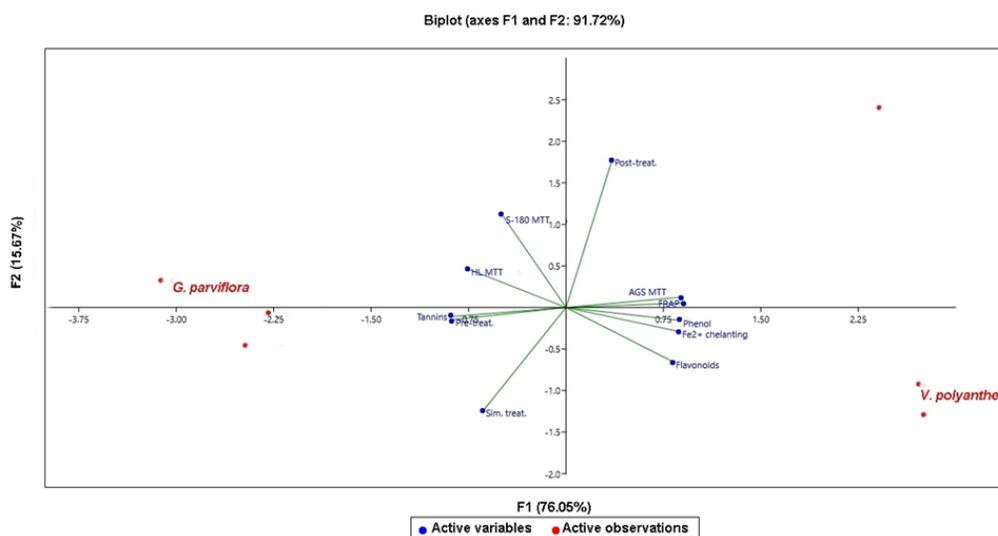


Figure 3: PCA (scores and loading plots, biplot) based on total phenols, flavonoids and tannins content analysed in *V. polyanthes* and *G. parviflora* extracts and their antioxidant activities in FRAP and Fe²⁺ chelating activity and cytotoxic, anti-cytotoxic and anticancer effects by MTT assay. Cell viability was evaluated by MTT assay using human lymphocytes (HL MTT), S-180 (S-180 MTT) and AGS cells (AGS MTT); or after perform the protocols of pre (Pre-treat.), simultaneous (Sim. treat.) and post-treatment (Post-treat.). Values of anti-cytotoxicity effect at the concentration of 50 µg.mL⁻¹ were used to perform Pearson correlation analysis. Values of anti-cytotoxicity effect at the concentration of 50 µg.mL⁻¹ were used to perform PCA analysis.

In Pearson correlation analysis (Table 8), values followed by positive sign suggest a directly proportional relation between the factors. Thus, the positive correlation between phenol and flavonoid total content and FRAP and Fe²⁺ chelating assays suggest the increasing of antioxidant activity, as well as, the positive correlation between phenol and flavonoid and AGS cytotoxicity suggest the increasing of the anticancer effect. In addition, FRAP and Fe²⁺ chelating antioxidants are strongly correlated to the anticancer effects against AGS cells. Anti-cytotoxicity protocols were used to evaluate the ability of extracts inhibit or prevent cytotoxic damage induced by cisplatin. Similarly, considering positive correlations between the total tannin content and pre and simultaneous treatment tests suggest that an increase in tannins availability interferes positively with cell viability of human lymphocytes, as well as, tannins content interfere with human lymphocytes cell viability.

Table 8: Pearson correlation analysis between total phenols, flavonoids and tannins content analysed in *V. polyanthes* and *G. parviflora* extracts and their antioxidant activities in FRAP and Fe²⁺ chelating activity and cytotoxic, anti-cytotoxic and anticancer effects by MTT assay.

	Phenol	Flavonoid s	Tannin s	FRAP	Fe ²⁺ chelating	HL MTT	S-180 MTT	AGS MTT	Pre- treat.	Sim. treat.	Post- treat.
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Phenol	1										
Flavonoids	0.9524	1									
Tannins	- 0.9886	-0.8980	1								
FRAP	0.9917	0.9153	- 0.9982	1							
Fe ²⁺ chelanting	0.9970	0.9683	- 0.9746	0.9788	1						
HL MTT	- 0.8236	-0.8950	0.7811	- 0.8162	-0.8237	1					
S-180 MTT	- 0.5501	-0.5745	0.5133	- 0.5155	-0.5713	0.3568	1				
AGS MTT	0.9871	0.8933	- 0.9999	0.9975	0.9725	- 0.7755	- 0.5116	1			
Pre-treat.	- 0.9414	-0.8744	0.9567	- 0.9685	-0.9188	0.8717	0.4780	-0.9554	1		
Sim. treat.	- 0.6425	-0.3885	0.7505	- 0.7265	-0.5849	0.3524	0.1992	-0.7570	0.7312	1	
Post-treat.	0.3154	0.0712	- 0.4363	0.4158	0.2494	- 0.1239	0.2614	0.4424	- 0.4696	- 0.8318	1

Cell viability was evaluated by MTT assay using: human lymphocytes (HL MTT), S-180 (S-180 MTT) and AGS cells (AGS MTT); or after perform the protocols of pre (Pre-treat.), simultaneous (Sim. treat.) and post-treatment (Post-treat.). Values of anti-cytotoxicity effect at the concentration of 50 µg.mL⁻¹ were used to perform Pearson correlation analysis.

Marzouk and Abd Elhalim [72], using NMR technique, found in the extract of *V. leopoldii* aerial parts compounds previously not reported in the literature, characterized as sesquiterpene lactones, triterpene tetracyclic and apigenin-7-*O*-glucide and luteolin-7-*O*-glucide. According to the authors, chemical and pharmacological investigations have shown that the sesquiterpene lactones found in plants of the genus *Vernonia* present antitumor activities, and some sesquiterpene lactones may exhibit strong cytotoxicity against tumor cell lines.

Sesquiterpene lactones identified in *V. polyanthes* by the APCI (+) FT-ICR MS technique (8β-2 methylacryloyloxy-isohirsutinolide, piptocarpine A and 10α-acetoxy-8α-methylacryloyloxy-1 α-13-*O*-acetate or 1β-methoxyhirsut -*O*-acetate) may have contributed to the antiproliferative effects on S-180 and gastric adenocarcinoma cells. Considering that sesquiterpene lactones are chemical markers of the Asteraceae family and of the genus *Vernonia*, it was expected to find compounds of this chemical class in the extracts of the herbs investigated in our study. In the review of Ghantous et al. [73], sesquiterpene lactones are led clinical trials of cancer, as well as, the authors relate the good performance of these substances in cancer clinical trials to the structure-activity of sesquiterpene lactones (lipophilicity and geometry).

Similar to studies with *V. polyanthes*, to our knowledge, there are not studies in the literature that relate *G. parviflora* extracts to the cytotoxicity in human lymphocyte cells, S-180 and AGS. The study of Bazylko et al. [57] evaluated the potential cytotoxicity of aqueous and ethanolic extracts of *G. parviflora* and its protective effect against damage caused by UV (ultraviolet) irradiation in human skin fibroblast cells. The authors concluded that the ethanolic extract was cytotoxic, presenting intense effect on the generation of reactive species in fibroblasts after UVB irradiation exposure. On the contrary, the aqueous extract exhibited protective activity in fibroblasts, preventing the decrease in proliferative activity and the increase in apoptosis caused by UVA and UVB irradiation, by the inhibition of EROS generation.

Parzonko and Kiss [59] investigated the photo-protective effects of two derivatives of caffeic acid isolated from aerial parts of *G. parviflora* (2,3,5- or 2,4,5-tricafeoilaltrarico acid (TCA) and 2,4- or 3,5-dicafoilglicaric acid (DCG)), in human dermal fibroblasts. In conclusion, the study clearly demonstrated that the derivatives of caffeic acid found in *G. parviflora*, in particular TCA, protect cells against damage caused by UVA radiation.

4. CONCLUSIONS AND RECOMMENDATIONS

Despite belonging to the same family, our analyses suggest that the studied plants present different content of phenols, flavonoids and tannins. It was identified Poly Unsaturated Fatty Acids (PUFA) in *G. parviflora* extract, being the first record of such compound for this plant species. Regarding anticancer effects, both species were able to reduce S-180 cell viability, and *V. polyanthes* extract showed high antiproliferative potential against AGS cells. In the anti-cytotoxic assay, *V. polyanthes* was more efficient in repairing cytotoxic cisplatin-induced damage. Chemical content of *G. parviflora* and *V. polyanthes* seems interfere with antioxidant and cytotoxic effects exhibited for the extracts.

There are several drugs for the treatment of cancer. In this scenario, our results reinforce the use of *G. parviflora* and *V. polyanthes* for medicinal and nutraceutical purposes, as well as suggest their potential use for the development of new drugs for the treatment of cancer. However, further investigations are needed to verify the biological activities of these plants.

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CONFLICT OF INTEREST

The author have declared that no competing interests exist.

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