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PHYTOCHEMICAL CONTENTS AND BIOLOGICAL PROPERTIES OF *VERNONIA POLYANTHES* LESS

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ABSTRACT: *Vernonia polyanthes* Less. (Asteraceae) is a medicinal plant that has been broadly used in Brazil for the treatment of inflammation and cutaneous damages. The present study evaluated the phytochemical contents and the antioxidant and topical anti-inflammatory activities of the *V. polyanthes* branches. Dried and powdered of *V. polyanthes* branches were exhaustively extracted with ethanol by static maceration followed by partition to obtain the hexane, dichloromethane, ethyl acetate, and butanol fractions. Phytochemical screening and the total phenolic and flavonoid contents were determined using chemical reactions and spectrophotometry, respectively. The antioxidant activity was evaluated by DPPH, reducing power of Fe^{+3} and β-carotene/linoleic acid assays. The topical anti-inflammatory effects of the ethanol extract (EEVPB) and ethyl acetate fraction (EFVPB) were investigated in Croton oil-, arachidonic acid-, and phenol-induced ear edema models. Tannins, flavonoids, coumarins, terpenoids and steroids, saponins and alkaloids were detected in the ethanol extract and/or fractions. In these samples, the total phenolic and flavonoid contents varied from 0.65 ± 0.07 to 12.58 ± 0.67 g/100 g and 0.15 ± 0.02 to 4.75 ± 0.21 g/100 g, respectively. After topical application, EEVPB and EFVPB significantly ($p < 0.01$ or $p < 0.001$) reduced ear edema induced by three phlogistic agents (0.1, 0.5 or 1.0 mg/ear). These results suggest that *V. polyanthes* is an important and promising source of bioactive compounds with relevant biological properties and can be used as a strategy to develop new products for the treatment of skin conditions related to oxidative damage.

INTRODUCTION: Alternative therapies, such as acupuncture, homeopathy, anthroposophy, functional food and herbal medicine have received great attention in recent decades for the treatment of several disorders [1]. Plant extracts, for example, have shown a large therapeutic potential, as well as can be rich in nutritional compounds to cure, among others, inflammatory processes associated with oxidative damage [2, 3]. Under this aspect, natural products represent an important source for the development of new pharmaceuticals and nutraceuticals and bioactive prototypes [4]. These products can be used as systemic and topical agents to produce benefits for the patient's health [5]. *Vernonia polyanthes* Less (Asteraceae family) is a common herbal shrub and small tree found in Brazil and it has been used by bees as a major source in the production of brown propolis commercialized in Brazil for its nutritional and medicinal properties [6]. In folk medicine, *V. polyanthes* has been used as diuretic, hypotensive, antiulcerogenic, antirheumatic, antimicrobial, cicatrizing, cutaneous damage, anti-inflammatory [7], and for the treatment of malaria and fever [8].

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This species has shown antiulcer [9], antinociceptive and anti-inflammatory [7], antihypertensive [10], and antimicrobial [11, 12] activities. In addition, compounds, as 3,7-dimethoxy- 5,3',4'-trihydroxy flavone, piptocarphin A, 3',4'-dimethoxyluteolin, glaucolide A, 3,5-di-O-(E)-caffeoylquinic acid, 4,5-di-O-(E)-caffeoylquinic acid, luteolin, quercetin, protocatechuic acid, quercetin-3-O-β-glucoside, apigenin and isorhamnetin, have been identified in *V. polyanthes* and they have been related to their medicinal properties [8, 12]. Phytochemical screening of the ethanol extract from leaves showed the presence of flavonoids, tannins, coumarins, terpenoids, sterols, saponins and alkaloids [7]. Flavonoids and terpenes, for example, can also act by different mechanisms of inflammation, inhibiting the synthesis or expression of inflammatory mediators [13, 14].

Over the generations, plant-derived natural products have played a significant role in the primary form of health care for a major proportion of the world's population and are an important source of active compounds [15]. A prominent example is the anti-inflammatory activity of *V. polyanthes* that it has been used, especially, for the treatment of rheumatism, cicatrisation, cutaneous damage and inflammation with pharmacological evidences [7, 8].

In this sense, the present investigation was designed to evaluate the antioxidant and topical anti-inflammatory effects of the ethanol extract and/or fractions of *V. polyanthes* branches using *in vitro* and *in vivo* models. In addition, phytochemical screening and total phenolic and flavonoids determination were conducted in order to evidence possible bioactive products.

MATERIALS AND METHODS:

Plant Material: *V. polyanthes* was collected in the Medicinal Garden of the Faculty of Pharmacy, Federal University of Juiz de Fora, Juiz de Fora city, Minas Gerais State, Southeast region of Brazil, in March 2012. A voucher specimen, identified by Dr. Fátima Regina Gonçalves Salimena, was deposited in the Herbarium of the Federal University of Juiz de Fora (CESJ number 10.329). The branches were placed in a drying oven with forced air circulation at 50 ºC for a loss of 90-95% humidity.

Extract Preparation: Dried and powdered branches (580 g) were exhaustively extracted in 95% ethanol (6.0 l) by static maceration for 5 weeks at room temperature with renewal of solvent every 3 days. The ethanol extract was filtered and evaporated under a rotary vacuum evaporator (Rotavapor RII, Büchi, Flawil, Switzerland) at controlled temperature (50–52 ^{o}C). This material was placed into a desiccator with silica to yield 41.60 g. The ethanol extract from *V. polyanthes* branches (EEVPB, 30 g) was suspended in water:ethanol (9:1) followed by liquid/liquid partition with increasing organic solvent polarity: hexane, dichloromethane, ethyl acetate, and butanol. After this procedure, hexane (HFVPB), dichloromethane (DFVPB), ethyl acetate (EFVPB), and butanol (BFVPB) fractions were obtained and they were used to phytochemical screening, total phenolic and flavonoids determination and antioxidant activity [16]. In addition, EEVPB and EFVPB were evaluated for the topical anti-inflammatory activity, since EFVPB produced greater antioxidant effect.

Chemicals: Drugs and reagents used in this study (and their sources) were as follows: Croton oil, arachidonic acid, phenol, dexamethasone, indomethacin, rutin hydrate, trichloroacetic acid, ascorbic acid, gallic acid, Folin-Ciocalteu reagent, Butylated hydroxytoluene (BHT), 2,2-diphenyl-1-picrylhydrazyl (DPPH), linoleic acid and β-carotene (Sigma-Aldrich Co.), pyridine, ethanol, sodium carbonate, aluminum chloride, potassium ferrocyanide, phosphate buffer, tween 20, ferric chloride, dimethyl sulfoxide**,** acetic acid, hexane, dichloromethane, chloroform, ethyl acetate, butanol and acetone (Vetec Química Farm Ltda), and ketamine chloride and xylazine chloride (Syntec).

Phytochemical Screening: Phytochemical screening was performed to detect the presence of tannins, flavonoids, terpenes and phytosterols, saponins, coumarins, anthraquinones, and alkaloids [17]. The tests were based on the visual observation of color change or formation of a precipitate after the addition of specific reagents.

Total Phenolic Determination: In this assay, samples (EEVPB, HFVPB, DFVPB, EFVPB and BFVPB) were oxidized with Folin-Ciocalteu reagent and the reaction was neutralized with

sodium carbonate [18]. This reaction was used to determine the total phenolic content and the gallic acid as reference standard. After 60 min, the absorbance of the resulting blue color was measured at 765 nm. The analysis was performed in triplicate and results were expressed as gram of gallic acid equivalent.

Total Flavonoids Determination: A simple colorimetric method was used for total flavonoid determination using aluminum chloride and rutin as standard [18]. Flavonoid constituents of the samples (EEVPB, HFVPB, DFVPB, EFVPB and BFVPB) reacted with aluminum chloride in the presence of acetic acid, pyridine:ethanol (2:8), and distilled water remaining at room temperature for 30 min. The absorbance of the reaction mixture was measured at 420 nm with a double beam UV/Visible spectrophotometer. The calibration curve was prepared with rutin solutions in ethanol (30 to 2 µg/ml) and result was expressed as gram of rutin equivalent.

DPPH Radical Scavenging Activity: 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was used for determination of free radical-scavenging activity [19]. Different concentrations of EEVPB and fractions (360 to 20 µg/ml) were added, at an equal volume, to methanol solution of DPPH (0.03 mM). After 60 min at room temperature, the absorbance was recorded at 518 nm. The experiment was performed in triplicate and rutin (20 to 0.5 µg/ml) was used as standard control. EC_{50} values denote the concentration (µg/ml) of the samples, which are required to scavenge 50% of DPPH free radicals.

Test of Iron Reducing Power: Reducing power of iron (FRAP) was determined using a serial dilution of EEVPB and fractions (440 to 10 µg/ml) with phosphate buffer pH 6.6, and 1% potassium ferrocyanide that was incubated at 50ºC for 20 min [20]. This mixture received 10% trichloroacetic acid followed by centrifugation at 3000 g for 10 minutes. The supernatant was separated and mixed with distilled water containing 1% ferric chloride. The absorbance of this mixture was measured at 700 nm in triplicate and ascorbic acid (10 to 0.5 µg/ml) was used as reference material. The measurement was considered the possible antioxidant activity.

***β*-Carotene/Linoleic Acid Bleaching Assay:** In this experiment, *β*-carotene diluted in chloroform was mixed with linoleic acid and Tween 20. Using the rotary evaporator (BUCHI, Germany), the chloroform was removed and the mixture was diluted with distilled water to form an emulsion [21]. The emulsion was transferred into different test tubes containing EEVPB and fractions (38.46 to 1.20 µg/ml) and placed in a water bath at 50°C for 2 h. Absorbance of the samples was measured at every 15 min for 105 min at 470 nm using a spectrophotometer (UV-VIS Spectrophotometer, Shimadzu). Butylated hydroxytoluene (BHT) was used as positive control (at the same concentration as samples). All samples were assayed in triplicate and the results were expressed in percentage of inhibition of lipid peroxidation (%) which is the sample concentration providing 50% inhibition of linoleic acid oxidation.

Animals: Male Swiss albino mice (50–70 days, weighing 25–30 g) were used for the experiments. All animals were obtained from Central Biotery of the Federal University of Juiz de Fora and the experimental procedures were performed in the Laboratory of Pharmacology of Natural Products of this institution. The animals were grouped and housed in plastic cages (47 × 34 × 18 cm^3) under a 12 h light/12 h dark cycle at room temperature (22 ±2 °C), fed with the balanced feed (Nuvilab Rodents - Nuvital Nutrients, Colombo, Brazil) and received water. After each procedure, the animals were euthanized with an overdose of ketamine and xylazine. Animal care and the experimental protocol were in accordance to the principles and guidelines recommended by the Brazilian College of Animal Experimentation (COBEA) and the rules of the Council for International Organizations of Medical Sciences. Experiments were approved by the local Ethical Committee (protocol number 037/2010). Due to the results of antioxidant activity related to the phenolic and flavonoid contents, the ethanol extract (EEVPB) and ethyl acetate fraction (EFVPB) were chosen to carry out the anti-inflammatory tests.

Croton oil -Induced Ear Edema: Five groups with eight mice each (n = 8) were separated and 20 µl of 2.5% Croton oil (v/v, diluted in acetone) were administrated topically on the inner surface of the right ear.

To accompany the baseline in the groups, 20 µl of acetone (vehicle) were applied in the left ear [22]. After 15 min, the right ears were treated with EEVPB or EFVPB (0.1, 0.5 and 1.0 mg/ear), and dexamethasone (0.1 mg/ear, positive control), while the negative control (untreated) received 20 µl of acetone. The weight of the ear edema (mg) was evaluated 6 hours of Croton oil application by the difference between the masses of the right and left ears.

Arachidonic Acid-Induced Ear Edema: Edema was induced in mice (n = 8) by topical administration on the inner surface of the right ear using arachidonic acid (AA) (2.0 mg/ear in 20 µl of acetone) [23]. The left ear received 20 µl of acetone as vehicle. After 15 min, the right ear was topically treated with EEVPB or EFVPB (0.1, 0.5 and 1.0 mg/ear in 20 µl of acetone), and indomethacin (2.0 mg/ear in 20 µl of acetone, positive control). The negative control (untreated) received topically 20 µl of acetone on the right ear. As in croton oil test, the weight (mg) of the ear edema was evaluated after 1 hour of AA application by the difference between the masses of the right and left ears.

Phenol-Induced Ear Edema: In this test, the edema was induced in mice (n = 8) by topical administration on the inner surface of the right ear with phenol (10% v/v in 20 µl of acetone), while the left ear received 20 µl of acetone as vehicle [24]. Fifteen min after induction of the edema, the right ear was topically treated with EEVP or EFVPB (0.1, 0.5 and 1.0 mg/ear in 20 µl of acetone), and dexamethasone (0.1 mg/ear in 20 µl of acetone, positive control). Acetone (20 µl/ear, negative control) was topically applied on the left ear. The ear edema was evaluated 2 h after phenol application by means of increase in ear weight (mg).

Statistical Analysis: Data are expressed as mean ± S.E.M. Statistical significance was analyzed by the one-way analysis of variance (ANOVA) followed by the Student Newman-Keuls' or Tukey's test. The T test for correlation was also applied. *P* values below 0.05 were considered significant. The percentage of inhibition was calculated by using: $100 - T \times 100/C$ (%) or $T \times 100/C - 100$(%); where C and T indicate non-treated (vehicle) and drug-treated, respectively. The Graph Pad® Prism 5.0. software was used for statistical analyses.

RESULTS AND DISCUSSION:

Phytochemical Screening: In **Table 1**, positive reactions to tannins, flavonoids, coumarins, terpenoids and steroids, saponins and alkaloids were detected in EEVPB and fractions from *V. polyanthes* branches. Reactions to terpenes and flavonoids were well characterized in specific samples.

TABLE 1: PHYTOCHEMICAL SCREENING OF EEVPB AND FRACTIONS

Chemical classes	Reactions	EEVPB	HFVPB	DFVPB	EFVPB	BFVPB
Tannins	Iron salts	+	-	-	+	+
	Lead salts	+	-	-	+	+
	Copper acetate	+	-	-	+	-
	Alkaloids	-	-	-	-	-
	Gelatine	-	-	-	+	+
Flavonoids	Aluminum chloride	+	-	-	+	+
	Sodium hydroxide	+	-	-	+	+
	Shinoda	+	-	-	+	+
Coumarins	Potassium hydroxide	+	-	-	+	+
Terpenes and steroids	Libermann-Burchard	+	+	-	-	-
	Kedde	+	+	-	-	-
	Baljet	+	+	-	-	-
Saponins	Foam Index	+	-	-	-	+
Alkaloids	Dragendorff	+	-	+	+	-
	Mayer	+	-	+	+	-
	Bouchardat	+	-	+	+	-
	Bertrand	+	-	+	+	-
Anthraquinones	Borntraeger	-	-	-	-	-

EEVPB: Ethanol extract; HFVPB: Hexane fraction; DFVPB: Dichloromethane fraction; EFVPB: Ethyl acetate fraction; BFVPB: Butanol fraction; (+) positive reaction; (-) negative reaction.

Total Phenolic and Flavonoid Contents: As shown in **Table 2**, the total phenolic content varied from 0.65 ± 0.07 to 12.58 ± 0.67 g/100 g and flavonoid ranged from 0.15 ± 0.02 to 4.75 ± 0.21 g/100 g in the *V. polyanthes* branches. The values were statistically different ($p < 0.05$) and the ethyl acetate fraction exhibited the highest total phenolic and flavonoid contents.

TABLE 2: TOTAL PHENOLIC AND FLAVONOID CONTENTS OF EEVPB AND FRACTIONS

Plant extract	Total phenolic (g/100g)	Total flavonoid (g/100 g)
Ethanol extract	6.71 ± 0.32	3.02 ± 0.33
Hexane fraction	0.65 ± 0.07	0.15 ± 0.02
Dichloromethane fraction	1.45 ± 0.05	0.64 ± 0.04
Ethyl acetate fraction	12.58 ± 0.67	4.75 ± 0.21
Butanol fraction	3.65 ± 0.11	1.97 ± 0.05

Each value in the table is represented as mean ± S.E.M. (n = 3). Same letters in the same column indicate that there was no significant difference between the means considering $p < 0.05$ after ANOVA followed of Tukey's test.

DPPH Radical Scavenging and Fe^{+3} Reducing Power Activities: The scavenging effects of the ethanol extract and fractions of *V. polyanthes* on DPPH radical showed that the EC_{50} values were statistically different ($p < 0.05$) and ranged from 58.56 ± 0.34 to 215.47 ± 1.70 µg/ml (**Table 3**). It is also observed that the ethyl acetate and butanol fractions were more active to inhibit the DPPH radical. In addition, the samples produced antioxidant activity on FRAP with EC_{50} values between 123.91 ± 0.44 and 467.94 ± 2.47 µg/ml. Using the DPPH test, EFVPB was more potent in convert Fe (+3) to Fe (+2).

TABLE 3: ANTIOXIDANT ACTIVITY OF EEVPB AND FRACTIONS BY DPPH AND Fe^{+3} REDUCING POWER METHODS.

Plant extract/Chemical	EC_{50} (µg/mL)	
	DPPH	Fe^{+3} Reducing Power
Ethanol extract	126.94 ± 0.42	346.50 ± 0.88
Hexane fraction	215.47 ± 1.70	467.94 ± 2.47
Dichloromethane fraction	175.73 ± 1.25	334.43 ± 2.87
Ethyl acetate fraction	58.56 ± 0.34	123.91 ± 0.44
Butanol fraction	77.35 ± 0.12	146.97 ± 1.21
Rutin	8.73 ± 0.27	-
Ascorbic acid	-	10.94 ± 0.32

Each value in the table is represented as mean ± S.E.M. (n = 3). There was significant difference between the means considering $p < 0.05$ after ANOVA followed of Tukey´s test.

After correlation analysis from fraction values, the line equations and correlation coefficients (r) were: total phenolic and DPPH (y = -11.116x + 182.72 and r = 0.803), total phenolic and FRAP (y = -22.572x + 371.75 and r = 0.758), flavonoids and DPPH (y = -32.431x + 192.67 and r = 0.88) and flavonoids and FRAP (y = -66.745x + 393.63 and r = 0.84).

β-Carotene Bleaching Antioxidant Activity: Based on the results of DPPH and reducing power, the ethanol extract and fractions from *V. polyanthes* branches were also tested on the lipid peroxidation model using the β-carotene/linoleic acid system. The order of oxidation power inhibition was as follows: hexane fraction > dichloromethane fraction > ethyl acetate fraction > butanol fraction > ethanol extract (**Fig. 1** and **Table 4**).

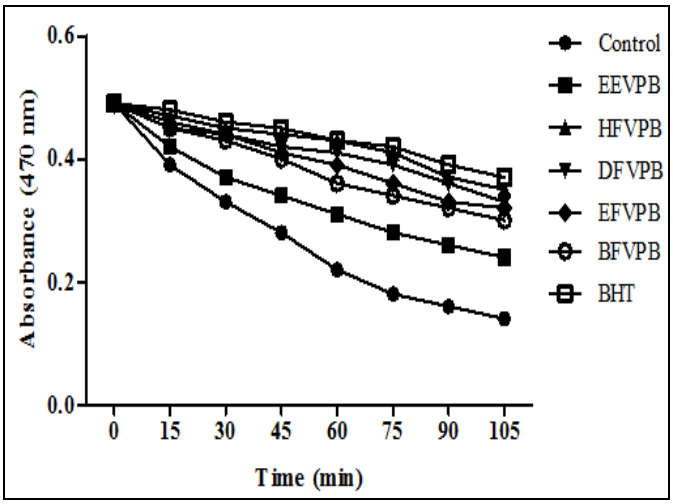

FIG. 1: DECAYMENT OF COLORING OF THE β-CAROTENE/LINOLEIC ACID SYSTEM IN THE PRESENCE OF THE ETHANOL EXTRACT AND FRACTIONS FROM *VERNONIA POLYANTHES* BRANCHES. EEVPB: ETHANOL EXTRACT; HFVPB: HEXANE FRACTION; DFVPB: DICHLOROMETHANE FRACTION; EFVPB: ETHYL ACETATE FRACTION; BFVPB: BUTANOL FRACTION.

TABLE 4: INHIBITION OF LIPID PEROXIDATION OF EEVPB AND FRACTIONS IN BETA-CAROTENE/LINOLEIC ACID SYSTEM.

Plant extract/Chemical	Inhibition of lipid peroxidation (%)
Ethanol extract	29.08 ± 0.75
Hexane fraction	59.13 ± 1.15
Dichloromethane fraction	54.25 ± 0.50
Ethyl acetate fraction	49.95 ± 0.10
Butanol fraction	45.26 ± 0.60
BHT	65.28 ± 0.35

Each value in the table is represented as mean ± S.E.M. (n = 3). There was significant difference between the means considering $p < 0.05$ after ANOVA followed of Tukey's test.

Topical Effects of EEVPB on Croton Oil-, Arachidonic Acid- and Phenol-Induced Mice Ear Edema: The ethanol extract (0.1, 0.5 and 1.0 mg/ear), as reported in **Fig. 2**, when applied topically and compared to negative control group, significantly inhibited the increase in ear weight due to inflammation process caused by Croton oil (45.79, 58.74 and 61.81%, $p < 0.001$, respectively), AA (35.40%, $p < 0.01$; 46.29%, $p < 0.001$; 0.5 and 1.0 mg/ear, respectively) and phenol (39.12, 49.45 and 63.43%, $p < 0.001$, respectively). The anti-inflammatory drugs used as positive controls in those assays, indomethacin (AA) or dexamethasone (croton oil and phenol), were both effective (**Fig. 2**).

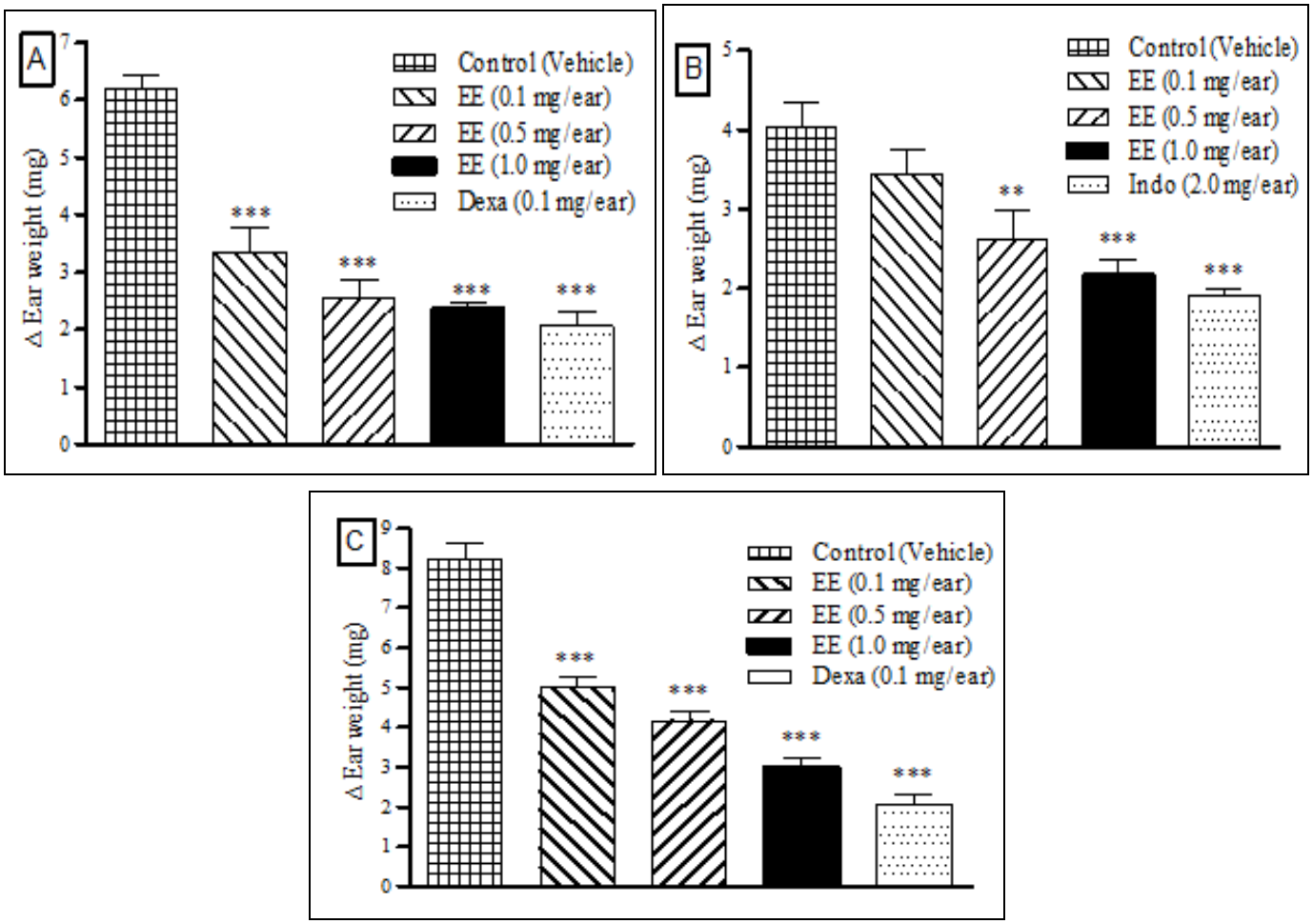

FIG. 2: TOPICAL EFFECT OF EEVPB, DEXAMETHASONE (DEXA) AND INDOMETHACIN (INDO) ON MICE EAR EDEMA INDUCED BY IRRITANT AGENTS. (A) CROTON OIL, (B) ARACHIDONIC ACID AND (C) PHENOL. THE ANIMALS WERE CHALLENGED WITH IRRITANT AGENTS AND THEN TREATED WITH EEVPB (0.1, 0.5 AND 1.0 mg/EAR), DEXA (0.1 MG/EAR, POSITIVE CONTROL GROUP), INDO (2.0 mg/EAR, POSITIVE CONTROL GROUP) AND ACETONE (VEHICLE, NEGATIVE CONTROL GROUP). AFTER APPLICATION OF THE IRRITANTS, THE EAR EDEMA WAS EVALUATED BASED ON THE INCREASE OF EAR WEIGHT (mg), OBTAINED BY THE DIFFERENCE BETWEEN THE RIGHT EAR (INFLAMED) AND THE LEFT EAR (NON-INFLAMED). The bars represent the mean ± S.E.M (n = 8). **$p < 0.01$ and ***$p < 0.001$ represent the significance level when compared with negative control group. ANOVA followed by Student-Newman-Keuls test as post-hoc.

Topical Effects of EFVPB on Croton Oil-Arachidonic Acid- and Phenol-Induced Mice Ear Edema: As showed in **Fig. 3**, EFVPB inhibited the ear edema induced by croton oil, arachidonic acid and phenol. Doses of 0.5 and 1.0 mg/ear of EFVPB reduced the ear edema induced by Croton oil (28.27 and 41.52%, respectively) and phenol (22.81 and 44.17%, respectively). After arachidonic acid application, the animals treated with EFVPB produced a significant response ($p < 0.001$) of 37.62% on the edema reduction when compared to control group using dose of 1.0 mg/ear.

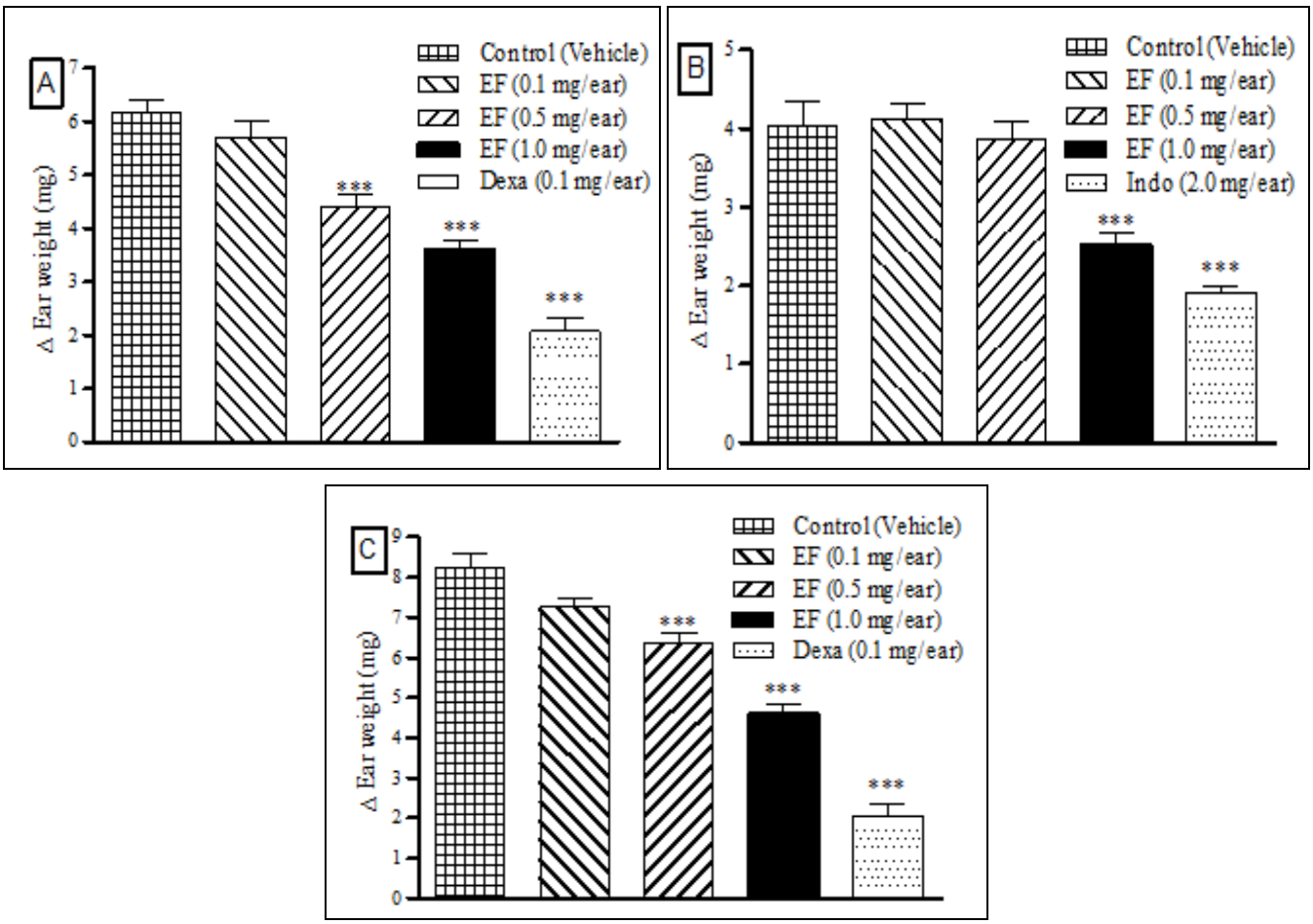

FIG. 3: TOPICAL EFFECT OF EFVPB, DEXAMETHASONE (DEXA) AND INDOMETHACIN (INDO) ON MICE EAR EDEMA INDUCED BY IRRITANT AGENTS. (A) CROTON OIL, (B) ARACHIDONIC ACID AND (C) PHENOL. THE ANIMALS WERE CHALLENGED WITH IRRITANT AGENTS AND THEN TREATED WITH EFVPB (0.1, 0.5 AND 1.0 mg/EAR), DEXA (0.1 mg/EAR, POSITIVE CONTROL GROUP), INDO (2.0 mg/EAR, POSITIVE CONTROL GROUP) AND ACETONE (VEHICLE, NEGATIVE CONTROL GROUP). AFTER APPLICATION OF THE IRRITANTS, THE EAR EDEMA WAS EVALUATED BASED ON THE INCREASE OF EAR WEIGHT (mg), OBTAINED BY THE DIFFERENCE BETWEEN THE RIGHT EAR (INFLAMED) AND THE LEFT EAR (NON-INFLAMED). The bars represent the mean ± S.E.M (n = 8). $^{**}p < 0.01$ and $^{***}p < 0.001$ represent the significance level when compared with negative control group. ANOVA followed by Student-Newman-Keuls test as post-hoc.

The data from phytochemical screening corroborates the chemical description for the *V. polyanthes* [7, 8, 12]. Among the special metabolites detected and quantified, phenols, and especially flavonoids, have been extensively studied for its antioxidant and anti-inflammatory actions [13, 25]. Our results confirmed the positive reactions of phenolic compounds (**Table 1**) and these constituents may be associated with the medicinal properties of *V. polyanthes*. As observed in **Table 2**, the variation of the total phenolic and flavonoid contents in EEVPB, HFVPB, DFVPB, EFVPB and BFVPB was influenced by polarity of solvents, since hexane is able to remove terpenes and steroids and dichloromethane promotes the extraction of lignans, methoxylated flavonoids, sesquiterpenes, lactones, coumarins and triterpenes [16].

In addition, free flavonoids, tannins, xanthones, triterpenic acids, saponins and phenolic compounds are extracted with ethyl acetate, while glycosylated flavonoids, tannins, saponins and carbohydrates are separated by butanol action [16].

Our results suggest that EEVPB and fractions have a promising antioxidant effect since was able to inhibit the stable radical DPPH and chelate iron. Probably, the neutralization of free radicals is associated with the action of phenolic compounds mainly found in the ethyl acetate and butanol fractions. Among others, this effect may be related to the presence of phenolic constituents (phytochemical screening and total phenol and flavonoid contents) in *V. polyanthes* that exhibit mechanism against these radicals [26].

Furthermore, there is a tendency of correlation between the values in **Tables 2** and **3**, especially the fractions.

Considering the data of the lipid peroxidation inhibition (**Fig. 1** and **Table 4**), the results indicate that the potential of *V. polyanthes* to inhibit oxidative processes in emulsified systems should be exploited. The hexane fraction, containing nonpolar compounds, promoted the greatest oxidation inhibition power in the system, suggesting the high capacity of these compounds to scavenge free radicals liberated during linoleic acid oxidation. In this sense, our results showed that ethanol extract and fractions are capable of inhibiting oxidants (superoxide, hydroxyl and peroxyl radicals and hypochlorous acid, singlet oxygen quenching and metal ion chelation), which can cause cellular damages through the peroxidation of unsaturated fatty acids, denaturation of proteins and reaction with carbohydrates and nucleic acids triggering physiological changes related to several diseases [27]. The presence of compounds found in species of *Vernonia*, such as terpenoids, triterpenoid saponins, flavonoids, coumarins, polyacetylenes, and steroids, can confirm these observations [7, 8, 12].

In anti-inflammatory activity studies, the ear edema models have been used as pharmacological tools for the investigation of new drugs useful for the treatment of skin disorders [28]. The medicinal use of *V. polyanthes* for cicatrisation and cutaneous damage was the crucial stimulus for this research. For the first time, EEVPB and EFVPB showed topical anti-inflammatory effect, which can be related to the presence of secondary metabolites, since several compounds (3,7-dimethoxy-5,3’,4’-trihydroxyflavone, piptocarphin A, 3’,4’-dimethoxyluteolin, glaucolide A, 3,5-di-O-(E)-caffeoylquinic acid, 4,5-di-O-(E)-caffeoylquinic acid, luteolin, quercetin, protocatechuic acid, quercetin-3-O-β-glucoside, apigenin and isorhamnetin) have been identified in this plant [8, 12] and this effect may involve different mechanisms in the cutaneous inflammation [29].

The results showed evidences that the *V. polyanthes* branches promote a significant reduction of inflammatory process at croton oil-induced mice ear edema model. The mechanism of this cutaneous inflammation involves the action of 12-*O*-tetracanoilphorbol-13-acetate, an ester of phorbol, that activate the phospholipid-dependent protein kinase C with stimulation of mitogen activated protein kinases (MAPK) and phospholipase A2. In this process, platelet activation factor and arachidonic acid are released and they generate vascular permeability, vasodilation, leukocyte migration, release of histamine and serotonin, and synthesis of prostaglandins and leukotrienes [30]. MAPK also activates nuclear transcription factors (e.g. NF-κB and AP-1) through direct stimulation of pro-inflammatory cytokines, TNF-α, interleukins and metalloproteinases [31].

The pathways of synthesis of these mediators can be inhibited by non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids and leukotriene receptor antagonists [31]. In this sense, the results showed that to reduce the Croton oil-induced ear edema, EEVPB and EFVPB can be inhibiting, in least, one of these inflammatory mediator pathways (**Fig. 2A** and **3A**). However, EEVPB was more active than EFVPB, since the magnitude of the effect was more expressive when compared to dose. Therefore, phenolic and non-phenolic constituents may contribute with this activity.

In the current study, as observed for the indomethacin (positive control), a cyclooxygenase inhibitor, EEVPB and EFVPB were effective in decreasing the arachidonic acid-induced ear edema (**Fig. 2B** and **3B**). Comparing the data of the **Fig. 2B** and **3B**, EEVPB was more potent to inhibit the ear edema than EFVPB, since that dose of 0.50 mg/ear of EEVPB was able to reduce the edema. This is an important result, because the arachidonic acid has been associated with the pathogenesis of skin diseases and its topical application generates inflammatory eicosanoids (prostaglandin E_2 and leukotrienes) and histamine with formation of erythema, edema, blood flow, vascular permeability, and neutrophil accumulation [24].

In the third stage of this study, our results showed that EEVPB and EFVPB significantly reduced the phenol-induced edema of dose-dependent manner and similar to dexamethasone (**Fig. 2C** and **3C**).

Probably, this effect is due to inhibition of the synthesis of inflammatory cytokines, prostaglandins and reactive oxygen species (ROS) generated by phenol. The action of phenol on the skin produces an inflammatory response with characteristic of contact dermatitis with release of cytokines (IL-1α, TNF-α and IL-8), prostaglandins and ROS [30, 32].

In this sense, the antioxidant capacity of phenolic compounds supported by the presence of flavonoids and others secondary metabolites may also justify this finding. Therefore, our results can indicate the potential use of EEVPB and EFVPB for the treatment of contact dermatitis.

CONCLUSION: In conclusion, the current study showed that *Vernonia polyanthes* Less branches have a relevant antioxidant and topical anti-inflammatory effects, which can be associated with the inhibition of inflammatory mediators involving oxidative stress. In addition, these findings corroborate the popular use of this species for the treatment of inflammation and cutaneous damage, and can contribute to the strategies of health policies related to the Traditional and Complementary Medicine.

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