Pharmacognostic study of *Vernonia ferruginea*, *Vernonia polyanthes* and *Vernonia westiniana species*

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Ely Eduardo Saranz Camargo[1]

ABSTRACT

Introduction: Medicinal plants have always been a reason for discussion, the knowledge of their use dates back to past times in the history of civilization. *Vernonias* are species known as "assa-peixe", and *Vernonia polianthes*, popularly, is widely used for the treatment of various pathologies. This study aims to carry out pharmacognostic methods to identify species of *Vernonia ferrugínea*, *Vernonia polyanthes* and *Vernonia westiniana* The identification of a species becomes difficult when the plants are acquired in the form of powders, dry extract or in liquid form such as tinctures and extracts. In this way, pharmacognostic tests were carried out, facilitating the identification of the species. Methodology: Based on physical and chemical methods, described in the Brazilian Pharmacopoeia VI edition and on the website of the Brazilian Society of Pharmacognosy, some tests were carried out to identify secondary metabolites present in the species: *Vernonia ferrugínea*, *Vernonia polyanthes* and *Vernonia westiniana*. Results and Discussion: In the results obtained, chemical reactions and physical methods were found, which provided safe identification of the species studied. In the determination of the ash content, it is possible to differentiate them, being more evident through chemical methods, than through reactions, the presence of flavonoid glycosides, alkaloids, terpenes and saponnic compounds was determined. Another practical method that provided an identification of the compound 3,7dimethyl-quercetin, isolated by high performance liquid chromatography (HPLC), was the comparative thin layer chromatography (CCCD), which verified the presence in *Vernonia polyanthes* and was not found in *Vernonia westiniana*. From the same isolated substance it was possible to quantify it in methanol extracts and in the infusion, and higher concentrations were found in extracts of *Vernonia ferruginea* and lower concentrations in infusion of *Vernonia polyanthes*. Conclusion: From this pharmacognostic study, it was possible to establish a method of identification of Vernonias species, however, for better safety it is necessary to use more technologically modern methods.

Keywords: Medicinal Plant, Pharmacognostic Control, Identification, Herbal Medicines.

[1] Professor and researcher at the Faculty of Medicine of Ji-Paraná - FAMEJIPA and at the Estácio University Center of Ji-Paraná - RO

INTRODUCTION

The use of medicinal plants is as old as the history of civilization itself. Entire peoples often associated the healing power of medicinal plants with magic and religious rituals. Second, Cordeiro et al., 19961, reported in their manuscript that, even today, Western folk medicine performs rituals where they mix knowledge of the healing properties of medicinal plants with rituals and prayers to expel diseases1.

Brazil has the largest medicinal flora in the world, however, there was a period in which medicinal plants were discredited. This occurred due to the advancement of the pharmaceutical industry in the world, corroborating a new concept of medicine, often palliative2.

Currently, the use of medicinal plants is increasingly widespread, not only in Brazil, but also in other countries, especially in Europe. A few years ago, only the population of the interior, who cultivated both edible and medicinal plants, used them to cure the most diverse diseases through knowledge acquired from grandparents or great-grandparents3.

Although the Brazilian flora is recognized as one of the most important, in numerical, economic, ornamental, ecological and medicinal terms in the world, there are few works that deal with this subject4.

Some species are easily found, distributed throughout the national territory, despite this, there is a major problem in the lack of quality of plant material, which in most cases there is no reliable identification5. In magistral pharmacies, inputs, products derived from medicinal plants, are purchased in the form of tinctures or powders, which are hardly tested to assess quality and/or botanical identification6.

A species widely used in folk medicine is *Vernonia polyanthes*, mainly for the treatment of bronchitis, respiratory system disorders and, also against kidney problems7,8. It is also indicated for rebellious coughs, flu, skin conditions, muscle pain, rheumatism9. Because they have morphological similarities with two other species, *Vernonia ferruginea and Vernonia westiniana*, it is of paramount importance to carry out pharmacognostic studies to determine quality assurance, such as: comparative chromatography in thin layer, total ash, acid insoluble ash, for the confirmation of the species *Vernonia polyanthes*, often misclassified by laymen9.

METHODOLOGY

The species, *Vernonia ferruginea* , *Vernonia polyanthes* and *Vernonia westiniana,* were collected in the vicinity of São José do Rio Preto – SP, identified at the Institute of Biosciences – UNESP, in partnership with the botany department of the Educational Institution. After collection and identification, weeding was carried out and after separating the leaves and flowering sums, they were subjected to drying in an oven of circulating air at 37º C for 48 hours.

After drying, the species were sprayed and stored in plastic bags and left in stock for pharmacognostic tests. For this work, a bibliographic survey was also carried out, using articles available in the following databases: lilacs, web of science, pubmed and scielo, with the objective of knowing the secondary metabolites present in the respective species, facilitating their pharmacognostic identification. The execution of the tests was based on the methodologies described in the Brazilian Pharmacopoeia VI edition10, and on the website of the Brazilian Society of Pharmacognosy (SBF), in the link ensino11.

PHYSICAL METHODS

The determination of moisture in the powders of the species: *Vernonia ferruginea*, *Vernonia polyanthes* and *Vernonia westiniana* was based on the method described in the Brazilian Pharmacopoeia10. The characterization of the method is based on the loss due to desiccation in an oven and aims to determine the amount of volatile substances of any nature eliminated under the conditions specified in the monograph10. The quantitative determination of the total ashes was also performed according to the method described in the Brazilian Pharmacopoeia10, with a quantity of powder from each of the species being evaluated, placed in a porcelain crucible, previously desiccated and the mass recorded. The respective samples were incinerated at a temperature of 450°C, then, after cooling in a desiccant, weighing was carried out and the percentage of ash of the respective powders of the species used was calculated.

In the test to determine the moisture index, a 50mL becker was used for each of the species, in the form of powder, being left in an oven at 105º C for 6 hours, after which they were placed in a desiccator for cooling. Subsequently, the beckers were taken, with the help of tongs, the masses were individually evaluated, 1 g of each species was placed in the beckers, separately and taken to the greenhouse at 105º C for 1 hour, determining the respective masses to verify the moisture content10.

After determining the total ashes, the crucibles containing the ashes of the respective species were added to each one, certain amounts of 7% hydrochloric acid were added to each one and placed in heating for 5 minutes. After this time, they were removed from heating, left on the countertop, covered with clock glass, until cooled, and after that, the respective residues of each crucible were transferred to filter paper and sent for drying on a hot plate, After drying, they were incinerated, in crucibles, in the muffle until a constant weight in determining the concentration of the ashes insoluble in acid10.

The determination of the total flavonoid levels was carried out according to the method described in the article by Silva (2016)[12], in which aliquots of 0.5ml, in triplicate of each sample of the hydroalcoholic extracts of *Vernonia ferruginea* , *Vernonia polyanthes* and *Vernonia westiniana*, that an equal volume of methanol solution of 5% aluminum chloride (AlCl3) was added. It was

allowed to rest for 15 minutes and was read in a UV spectrophotometer (Bel Photomic) at a wavelength of 420nm. The total flavonoid content was determined using a calibration curve with quercetin, purchased on the market, at concentrations of 0, 25, 50, 75, 100 mg/ml, considering a variation of +/- 5%[12]. From the equation of the line obtained in the curve of the pattern graph, the total flavonoid content was calculated, and the results were expressed in mg of quercetin per 100g of each of the species of *Vernonia ferrugínea*, *Vernonia polyanthes* and *Vernonia westiniana*.

Comparative thin layer chromatography (CCCD) was used to identify the presence of 3,7 dimethoxy-quercetin, isolated in methanol extract of *Vernonia polyanthes* and also found in *Vernonia ferruginea*, in infusion of *Vernonia polyanthes* and *Vernonia westiniana*. The method consists of obtaining infusions of the mentioned species, applying them on a silica gel plate, separately, with the isolated substance (3,7 dimethoxy-quercetin), used as a marker.

After application of the extracts and quercetin on the plate, it was placed in a glass vat, containing BAW eluent (butanol, acetic acid and water) in the respective concentrations of 65:25:15, impregnating the tub for elution of the applied extracts. After it reached the upper line, the plate was removed from the eluent, allowed to dry for a few minutes and developed with saturated solution of ceric sulfate and resublimated iodine saturated in a glass vat.

In the quantification of the substance, 3.7 dimethyl quercetin, isolated in methanol extract of *Vernonia polyanthes*, in another work, a Waters Millipore chromatograph was used, equipped with a binary pump system model 501 Waters, UV detector (Waters model 486), Phenomenex C-18 column in reverse phase (250 x 4.60 mm, 5 µ) and mobile phase composed with methanol and water. The samples used were obtained from the extracts of *Vernonia ferrugínea* and *Vernonia polyanthes*, and in the latter, an infusion for quercetin quantification was also repaired, by construction of a calibration curve, using five different concentrations of the standard.

The quantitative analysis of 3,7 dimethyl quercetin, present in the methanol extracts of *the polyanthes and ferruginea species*, was used using the external standard method. Obtained in standard solution of the substance isolated in the methanol extract of *Vernonia polyanthes*, in the following concentrations: 2.0, 0.26, 0.035, 0.0047, 0.00063mg/mL. The sample was prepared from the MeOH extract by collecting 100mg of the extract and diluting it in 1mL of methanol. The infusion was prepared at 10% (w/v) of the dried plant in boiling distilled water until and left until cooled.

CHEMICAL METHODS

The chemical methods used in the identification of the secondary metabolites present in the species studied were based on the Brazilian Pharmacopoeia and on the website of the Brazilian Society of Pharmacognosy.

In the determination of cardiotonic glycosides, the following reactions were performed: Liebermann-Burchard reaction, pentagonal lactonic ring identification reaction: Kedde reaction and 2-deoxysugar identification reaction: Keller-Kiliani reaction. In the flavonoid compounds, the Shinoda or Cyanidine reactions, reaction with aluminum chloride, reaction with ferric chloride, reaction with sodium hydroxide, as well as in the anthraquinones, possible Borntraeger reactions were analyzed, being a microsublimation process.

In the saponin glycosides, a physical process was used that consists of agitation of the solution to detect foam formation. In the chemical process, general reactions, the Rossol reaction, the Micthell reaction, the Rosenthalen reaction, the reaction with Sulfo-vanille reagent were performed. In the specific reactions, the Liebermann-Burchard reaction was performed, reactions performed from chloroform solution. A general reaction was also performed, with trichloroacetic acid and specific Salkowski reaction.

Analysis of alkaloids, metabolite responsible for different antimicrobial activities, was performed from the sprayed plant and after extraction with dilute sulfuric acid and chloroform, it was detected with neutral lead acetate. For the determination of tannins, general reactions are observed: reaction with 2% ferric chloride, reaction with neutral lead acetate. Specific reactions with lead acetate and glacial acetic acid and reaction with ferric chloride.

RESULTS AND DISCUSSION

Pharmacognostic tests were proposed in order to identify species of *Vernonia ferrugínea*, *Vernonia polyanthes* and *Vernonia westiniana*., because they are morphologically similar and that, in magistral pharmacies, are acquired in the form of dry powder and microprocessed, or even in dry extract. Thus, it becomes difficult to identify the species, so pharmacognostic tests are of great value for this determination.

The species *Vernonia ferruginea*, despite having a large amount of flavonoids, is very similar to *Vernonia polyanthes*, therefore, to perform most of the pharmacognostic tests, the species *Vernonia polyanthes* and *Vernonia westiniana* were used, because they have similar morphological characteristics.

In the performance of the tests to determine water losses, as described in materials and methods, using the leaves and stalks of *Vernonia polyantes* and in the same way for *Vernonia westiniana,* values of desiccation losses of 8.8% and 10% were obtained, respectively. The moisture index can directly influence ash index and other tests.

In the quality control of medicinal plants, a simple, easily reproducible test that offers good security in the identification of species, and the index of total ash and acid insoluble ash. According to the analyses described in materials and methods, the result obtained clearly showed a difference in

the ash content of the two species (Table 1), while the ash content of *Vernonia polianthes* reached levels between 0.32g of total ash and 0.10g of acid-insoluble ash, the indices obtained with *Vernonia westiniana* reached values of 0.39g and 0.09g, respectively and in *Vernonia polyanthes* , values of 0.17g and 0.11g were obtained, respectively.

Thus, the ash index presents itself as a simple and fast way to differentiate between the species: *Vernonia ferruginea*, *Vernonia polyanthes* and *Vernonia westiniana.* With this result, it can be concluded that the total ash index test and acid-insoluble ash can clearly differentiate the species of Vernonias studied.

Table 1 - Total and acid-insoluble ash of *Vernonia ferriginea, Vernonia polyanthes and Vernonia westiniana.*

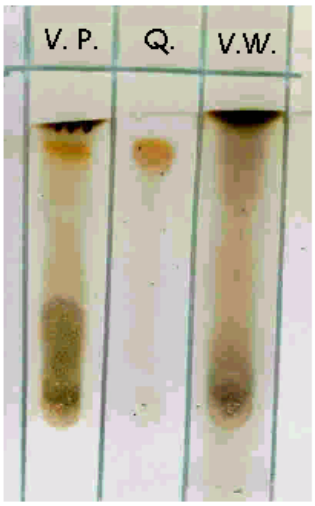
Species	**Total ash**	**Acid insoluble ash**
Vernonia polyanthes	0.32g (10.7%)	0.10g (3.33%)
It's a bit of a bit of a fun way to do it.	0.39g (13.0%)	0.09g (3.0%)
Vernonia ferruginea	0.17g (7.12%)	0.11g (3.52%)

The corresponding hydrogen potential (pH) obtained in both the infusion and the methanol extract of *Vernonia polyanthes and Vernonia westiniana* was 5.78 and 7.27, respectively. As there were no significant differences in the infusion with the methanolic extract, it can be stated that *Vernonia polyanthes* has a higher acidity index than *Vernonia westiniana.*

The use of medicinal plants is directly related to the manipulation of magisterial formulas in pharmacies, and in the vast majority of cases they are acquired in the form of powders (dried and ground), making it difficult to identify the plant macroscopically. A quick and practical method for pharmacies to identify vetal drugs is the CCCD method of methanol extract and infusion of *Vernonia polyanthes*, using the commercial standard of 3,7 dimethyl quercetin, which can be easily found in the market.

The methodology proved to be effective for the proposed test, because with the use of a specific developer for flavonoids, the presence of the substance in the extract and infusion of *Vernonia polyanthes* is clear (Figure 1), the method can still be guaranteed, since 3,7 dimethyl quercetin is not present in another similar species of the genus, studied so far.

Figure 1 – CCCD of the methanol extract of *Vernonia polyanthes and Vernonia westiniana* with a standard of 3,7 dimethyl quercetin.



V.P. – Extrato metanólico de *Vernonia polyanthes.*

Q. – Standard 3.7 dimethyl quercetin in methanol.

V.W. – Extrato methanolica of *Vernonia westiniana.*

After the isolation of the metabolite 3,7 dimethyl quercetin, in methanol extract of *Vernonia polyanthes*, taken as an external standard, it was used to quantify the presence in methanol extracts of *Vernonia polyanthes and Vernonia ferruginea species* and infusion of *Vernonia polyanthes.*

The calibration curve allows the linearity of the detector (UV-vis) to be verified within the ranges of concentrations evaluated, where a correlation index value of 0.99959 was obtained. This means that the method used in the analysis of the substance studied obeys a linear correlation in the ranges of concentrations considered.

According to data obtained in the calibration curve, from linear regression it was determined the quantification of the substance 3,7 dimethyl quercetin, present in the extracts MeOH of *Vernonia polyanthes and Vernonia ferruginea*, obtaining, respectively, the concentration of 0.13mg/mL and 0.54mg/mL in each extract and in the infusion of *Vernonia polyanthes* the concentration of 0.063mg/mL.

In determining the amount of flavonoids present in the extracts, it is a good indicator to evidence pharmacological effects, since they are considered antioxidants and anti-inflammatories and are found in most plant species. Thus, it is important to quantify this metabolite for safe and effective use of the species studied, to evidence the therapeutic activity.

The results obtained in the quantification of flavonoids, for the species: *Vernonia ferruginea*, *Vernonia polyanthes* and *Vernonia westiniana,* based on spectrophotometric methods, in the quantification of quercetin, provided percentage (w/w) of the flavonoid in each medicinal species studied. Thus, according to the results obtained in the tests, it was determined that, in *Vernonia ferriginea,* the percentage of 1.21% was assessed, for the species *Vernonia polyanthes*, the percentage value was 0.75% and in the species *Vernonia westiniana* the percentage of the presence of the flavonoid, determined from quercetin, was 0.05%, which characterizes that this species does not present significant amounts of flavonoids, shown in Table 2.

Table 2 - Percentages of total flavonoid concentrations, based on the values obtained with quercetin as a marker.

Medical plant	Flavonoid content obtained
Vernonia ferruginea	1.21% m/m
Vernonia polyanthes	0.75% m/m
Vernonia westiniana	0.05% m/m

In the determination of cardiotonic glycosides, according to the Liebermann-Burchard reaction, it showed positive results for the species *Vernonia ferruginea*, *Vernonia polyanthes* and *Vernonia westiniana,* observing a slightly reddish border ring, indicating the presence of cardenolides and bufadienolids. This reaction is characteristic of steroid and triterpenoid compounds, due to the fact that the reactant promotes dehydration and dehydrogenation of the fundamental nucleus, which results in derivatives with conjugated double bonds and, therefore, stained. For this reason, this characteristic is common in the compounds cardenolides and bufadienolides.

The determination of flavonoid glycosides, based on the Cyanidine test, is characteristic of the largest number of substances of this class, with positive results for the species: *Vernonia ferruginea* and *Vernonia polyanthes,* because according to the quantification of total flavonoids, the species *Vernonia westiniana*, did not present significant quantities in its constitution, corroborated the finding, according to the test result, of the occurrence of red coloration in the sample. The study with ferric chloride and sodium hydroxide showed negative results, and inconclusive results in the case of the reaction with aluminum chloride, because no change in the color of the reaction was observed.

In tests to determine the presence of anthraquinone derivatives, they are usually orange in color. In the tests carried out, based on the Borntraeger reaction, which should present a reddish-pink color indicating the presence of these anthraquinone derivatives, no change in color was observed. Therefore, the result for the presence of anthraquinones was negative in all species studied. In the microsublimation process, after heating in a plate under a metal ring, crystals should be presented for positive results, so the result obtained was considered negative, confirming the result of the previous reaction, with no identification of crystals in any of the species.

Another test that offers a safe identification is the determination of saponin glycosides, using the physicochemical method, which, after uninterrupted agitation of the test tubes, containing the diluted samples, for 5 seconds, the formation of foam was observed in all tubes, corroborating the indication of the presence of saponin compounds. Even after leaving the extracts to rest for 30 minutes, the foam formation remained stable in two species, *Vernonia ferruginea*, *Vernonia polyanthes,* considered positive for the reaction of Rossol, Mitchell, Rosenthalen which showed a slight reddish-brown reaction. In the species *Vernonia westiniana*, the test was negative, because the foam formed did not remain stable after 30 minutes.

In the reactions of Sulfo-vanillic and trichloroacetic acid, the results were negative for the species, as they did not present specific coloration, while in the Salkowski tests the species *Vernonia ferruginea* and *Vernonia polyanthes,* presented triterpenoidal nucleus and Liebermann-Burchard steroidal nucleus due to the staining that the samples presented, which confirms the presence of saponin compounds in the extracts.

The presence of alkaloids in the samples also evidences many of the therapeutic actions of the species studied, and the result obtained in the reaction with neutral lead acetate was positive, as it showed the formation of white precipitate in the samples of: *Vernonia ferruginea*, *Vernonia polyanthes* and *Vernonia westiniana*, which indicated the presence of alkaloids in the formation of insoluble complexes.

The tannins, present in the Vernonias species, were detected from the reaction with ferric chloride, which presented characteristic coloration, green for condensed tannins or catechic for the samples of *Vernonia ferruginea*, *Vernonia polyanthes* and *Vernonia westiniana,* indicating the presence of hydrolyzable tannins. In the test with neutral lead acetate, it showed a whitish precipitate confirming the presence of hydrolyzable tannins in the three species, as well as in the reaction with lead acetate and glacial acetic acid confirming the presence of hydrolyzed tannins.

CONCLUSION

Thus, it is concluded that the tests performed to identify the species of *Vernonia ferruginea*, *Vernonia polyanthes* and *Vernonia westiniana,* are favorable for the metabolite compounds found in the species, which corroborate the quality assurance, contributing greater safety to the magistral pharmacies that use powders for the preparation of formulations.

However, it is known that these tests are not completely conclusive, and there should be more accurate control, with isolation and identification of the compounds, by technologically modern methods and with accurate and safe results. Thus, for greater reliability of quality control, a phytochemical study of the species should be carried out.

REFERENCES

1. Cordeiro, R., Nunes, V. Do Amaral, & Almeida, O. R. (1996). *Plantas que curam* (Vol. 1). Editora Três.

2. Lorenzi, H., & Matos, F. J. A. (2021). *Plantas medicinais no Brasil: Nativas e exóticas* (3ª ed.). Instituto Plantarum.

3. Budke, M. R., Budo, M. L. D., Silva, F. M., & Ressel, L. B. (2011). Plantas medicinais: O saber sustentado na prática do cotidiano popular. *Escola Anna Nery, 15*(1), 1-8.

4. Fonseca, M. C. M. (2012). *Epamig pesquisa: Produção de plantas medicinais para aplicação no SUS*. Espaço para o produtor.

5. Albuquerque, U. P., Silva, J. S., Campos, J. L. A., Souza, R. S., Silva, T. C., & Alves, R. R. N. (2013). The current status of ethnobiological research in Latin America: Gaps and perspectives. *Journal of Ethnobiology and Ethnomedicine, 9*(72), 1-9.

6. Camargo, D. G. K., Caetano, J. N., & Camargo, E. E. S. (2022). Identification and pharmacognostic control of the species: Mentha x villosa, Lippia alba, Cymbopogon citratus and Melissa officinalis. *Brazilian Journal of Development, 8*(10), 65329-65345. https://doi.org/10.34117/bjdv8n10-026

7. Feleti, S. M. V., Aleluia, R. L., Gervásio, S. V., Dutra, J. C. V., Oliveira, J. R. P., Gonçalves, R. C. R., Jamal, C. M., Kuster, R. M., Brasileiro, B. G., & Battituci, M. C. R. (2020). Phytochemical screening, antioxidant, anti-cytotoxic and anticancer effects of *Galinsoga parviflora* and *Vernonia polyanthes* (Asteraceae) extracts. *International Journal of Research - Granthaalayah, 8*, 84–98.

8. Gallon, M. E., Jaiyesimi, O. A., & Gobbo-Neto, L. (2018). LC-UV-HRMS dereplication of secondary metabolites from Brazilian Vernonieae (Asteraceae) species supported through in-house database. *Biochemical Systematics and Ecology, 78*, 5–16.

9. Leite, K. R., & Camargo, E. E. S. (2015). Pharmacognostic study of the species *Achillea millefolium L.*, *Cotyledon orbiculata L.*, and *Ocimium selloi*. *Rojasiana, 14*(1), 1-8.

10. Brasil, Agência Nacional de Vigilância Sanitária. (2019). Resolução de Diretoria Colegiada - RDC nº 298, de 12 de agosto de 2019: Dispõe sobre a aprovação da Farmacopeia Brasileira, 6ª edição. *Farmacopeia Brasileira, V1, V2* e Atualizações. Disponível em: https://www.gov.br/anvisa/pt-br/assuntos/farmacopeia/farmacopeia-brasileira. Acesso em: 16 de julho de 2024.

11. Brasil, Sociedade Brasileira de Farmacognosia. (2024). Ensino. *Sociedade Brasileira de Farmacognosia*. Disponível em: http://www.sbfgnosia.org.br/index.html. Acesso em: 16 de julho de 2024.

12. Silva, J. B., Costa, K. M. F. M., Coelho, W. A. C., Paiva, K. A. R., Costa, G. A. V., Salatino, A., Freitas, C. I. A., & Batista, J. S. (2016). Quantificação de fenóis, flavonoides totais e atividades farmacológicas de geoprópolis de *Plebeia aff. flavocincta* do Rio Grande do Norte. *Pesquisa Veterinária Brasileira, 36*(9), 874-880. https://doi.org/10.1590/S0100-736X2016000900014