

Flavonoids, Terpenes and the Anti-Oxidant Activity of *Diplostephium phyllicoides* (H.B.K.) Wedd.

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Abstract

Objective: To purify and identify secondary metabolites of *Diplostephium phyllicoides* and determine the antioxidant capacity through the DPPH^{*} and ABTS⁺⁺ techniques. **Methods:** Extracts were obtained by soxhlet of the different organs with solvents of increasing polarity and by means of column chromatography, triterpenes and flavonoids were purified, which were identified by spectroscopic techniques. **Findings:** It is purified and identified in leaves: Urs-12-ene-3, 28-diol (Uvaol), 5, 4'-dihydroxy-7-methoxy-flavone (Genkwanin) and 5, 6,4'-trihydroxy-7-methoxy-flavone (Sorbifolin). In the stems were found Bauer-7-en-3b-yl acetate (Bauerenol acetate) and in the flowers, friedelan-3-one (Friedelin) and 3, 3', 4', 5, 7-pentahydroxyflavone (Quercetin). The anti-oxidant activity was determined by the DPPH^{*} and ABTS⁺⁺ free radical assay, with the polar extracts showing a greater activity. The ethanol extract showed an IC₅₀ of 13.80 mg/L MeOH, with the DPPH^{*} assay and 10.14 mg/L MeOH, with the ABTS⁺⁺ assay. The extract of ethyl acetate showed an IC₅₀ of 28.32 mg/L MeOH, with the DPPH^{*} assay and 10.86 mg/L MeOH, with the ABTS⁺⁺ assay method. **Application:** The species *Diplostephium phyllicoides* possesses a high antioxidant activity in the polar fractions, which present the flavonoids, positioning the species with a high medicinal potential.

Keywords: ABTS⁺⁺, DPPH^{*}, *Diplostephium phyllicoides* (H.B.K.) Wedd., Flavonoids, Terpenes

1. Introduction

The *Diplostephium* genus belongs to the Asteraceae family, which is made-up of shrubs or small trees¹, with a pubescent or woolly indumentums. Leaves that are alternate, whole and linear to oblong, with the edges more or less revolute and a coriaceous texture. The inflorescence is paniculate, or somewhat corymbose, with small heterogamous, radiated or discoid flower heads that have few or many flowers, which are involucre, bell-shaped or hemispheric, with bracts in a 4-7 series that are imbricate. A flat or slightly convex receptacle, bare or sometimes with tiny paleas; female flowers in the radius that are uni- or multi-serial a long or short ligulate corolla, 2-3 toothed, violaceous, with a narrow

papillose tube. Branches with linear styles with a papillose edge; an ovulated and fertile ovary; perfect discoid flowers which are meager to numerous; a tubular corolla, five-toothed, which may be yellow, greenish or violet; anthers which are saggitate at the base; a papillose style with short, incrassate branches or long, linear or hispid, subulate ones. The ovary is usually sterile. They have narrow, discoid achenes with 3-5 costas, the ones on the radius more compressed an abundant pappus, the external one with short or scaly aristas, the internal one with longer hairs²⁻³. The *Diplostephium* genus has been phylogenetically broken down into twelve series: *Denticulata*, *Crassifolia*, *Coriacea*, *Huertasina*, *Floribunda*, *Shultziana*, *Rosmarinifolia*, *Phyllicoidea*, *Rupestria*, *Saxatilia*, *Lavandulifolia* and *Anactinota*⁴.

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Diplostephium phylicoides (H.B.K.) Wedd is a species which is nearly exclusive to the páramos (high Andean moors) of the departments of Cundinamarca and Boyacá, at altitudes between 2,800 and 3,500 meters above sea level, and at times reaching 4,000. It is a low bush, with showy capitula with shiny purple, violet or bluish ligules. It has dense woolly-tomentose branches and dense leaves, with revolute, reflective or spatulate margins, which are whitish-yellow on the back⁵.

It is known that *Diplostephium phylicoides*⁶, has an anti-bacterial activity and there are mentions of chemical studies for the *D. Meyenii*⁷, *D. Ericoides* and *D. Floribundum*⁸, species which report the presence of clerodanes, ent-clerodanes, diterpenes and diterpene lactones. For *D. cinereum*, there are reports of two new benzodihydrofurans isolated from the resinous exudate, 13 (2-methylpropane dioxy)-taxol and 13-[(R) - 3 -hydroxyl -3-phenylpropionicoxy]-taxol⁹. In the different species of the *Diplostephium* genus, studies have detected the presence of tannins, lignanes, coumarins, quinones, xanthenes, phenolic acids, flavones and flavonoids, which may act as donors of hydrogen which prevent or control the development of cerebral-vascular strokes or neurodegenerative diseases¹⁰⁻¹⁴.

2. Materials and Methods

Vegetal material, flowering plants were collected along the road between Bogotá and Choachi in a place known as the páramo de Cruz Verde (Cundinamarca, Colombia) at an altitude of between 3, 300 and 3,500 masl. To determine its taxonomical classification, a sample was sent to the Institute of Natural Sciences of the Universidad Nacional de Colombia, where it was identified as *Diplostephium phylicoides* (H.B.K.) Wedd. and registered with the number Col-324495.

2.1 Extraction

The extraction process was done material, which was previously dried, and ground. From 534 grams of dried and ground leaves and 348 grams of the flowers of *Diplostephium phylicoides* (H.B.K.) Wedd., an extraction was made with a Soxhlet extractor, using petroleum ether, dichloromethane, ethyl acetate and ethanol, successively, and the different extracts were concentrated under reduced pressure.

2.2 Fractioning of the Extracts

A solid-liquid extraction was made from the extracts obtained with petroleum ether, dichloromethane, ethyl acetate and ethanol, and the extract was mixed with Silica gel 60 (Kieselgel 60. 0,063 0.2 mm/70 -230 mest ASTM) and extracted in a Soxhlet extractor, using solvents of different polarities (petroleum ether, dichloromethane, ethyl acetate and ethanol). The fractions in each solvent were concentrated with a rotary evaporator. The compounds were isolated with a chromatograph; identified by physical-chemical methods and by UV, IR, ¹HRMN, ¹³CRMN, MS spectroscopy; and confirmed by comparing the data with those reported in the literature.

2.3 Anti-oxidant Activity

The anti-oxidant activity of the extracts and fractions of the leaves of *Diplostephium phylicoides* (H.B.K.) Wedd. which were obtained was determined by the ABTS⁺⁺ (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) radical cation decolorization assay and the DPPH* radicals (2,2-Diphenyl-1-picrylhydrazyl) decolorization assay, comparing their coefficients of 50% inhibition (IC₅₀) and their relative anti-oxidant activity (AAR%), with patterns like ascorbic acid, rutin and trolox¹⁵. The concentrations of the extracts, which used, were 12.5, 25, 62.5, 125 and 250 mg/LMeOH).

3. Resultes

The ether extract of the leaves was flocculated with ethanol and dried the extract was subjected to 7g chromatography in a column packed with 60 G. silica gel eluted with petroleum and then with petroleum mixtures with an increasing polarity (AcOEt (20:1, 9:1, 8:2, 6:4) and finally AcOEt). In the 52-54 petroleum fractions, a white solid was obtaining from the AcOEt 8:2, which the photo chromatography revealed to be a fine layer in two stains, which were then separated by preparative chromatography. The less polar substance showed the following electroscopic data:

IR ν_{\max} (KBr), cm⁻¹: 3400 (OH); 2900 y 2850 (CH aliphatic); 1660 (R₂C=CHR),

¹HRMN (300 MHz, CDCl₃, TMS): δ ppm(H): 0.82(6H, s); 0.98(6H,s); 1.04(6H,s); 1.12(3H,s); 3.40(2H,dd, AB, HO-CH₂-); 3.71(1H,d,HO-CH-); 5,18(1H,t,-C=C-),

¹³CRMN (75 MHz, CDCl₃, TMS): δ ppm(C): 138.41(13); 125.074(12); 79.005(3); 68.845(28); 55.266(5); 54.100(18); 47.732(9); 42.097(14); 40.091(8); 39.414(4); 38.872(19); 38.872(20); 38.791(1); 38.032(17); 36.948(10); 35.186(22); 32.883(7); 30.659(21); 28.139(23); 27.291(15); 26.052(2); 23.350(16); 23.350(29); 21.256(27); 18.356(6); 23.350(30); 16.784(26); 15.595(24); 15.595(25); 23.500(11).

EIMS (70 eV m/z (rel. int.). 442[M]⁺: 55(12.1); 69(15.3); 81(12.8); 95(13.3); 105(10.2); 107(10.1); 119(12.0); 133(26.2); 203(100.0); 204(17.2); 207(14.1); 234(18.0). All of these electroscopic data coincide with those reported for Uvaol; (urs-12-en-28-diol) [C₃₀H₅₀O₂], reported¹⁶⁻¹⁹ (Figure 1).

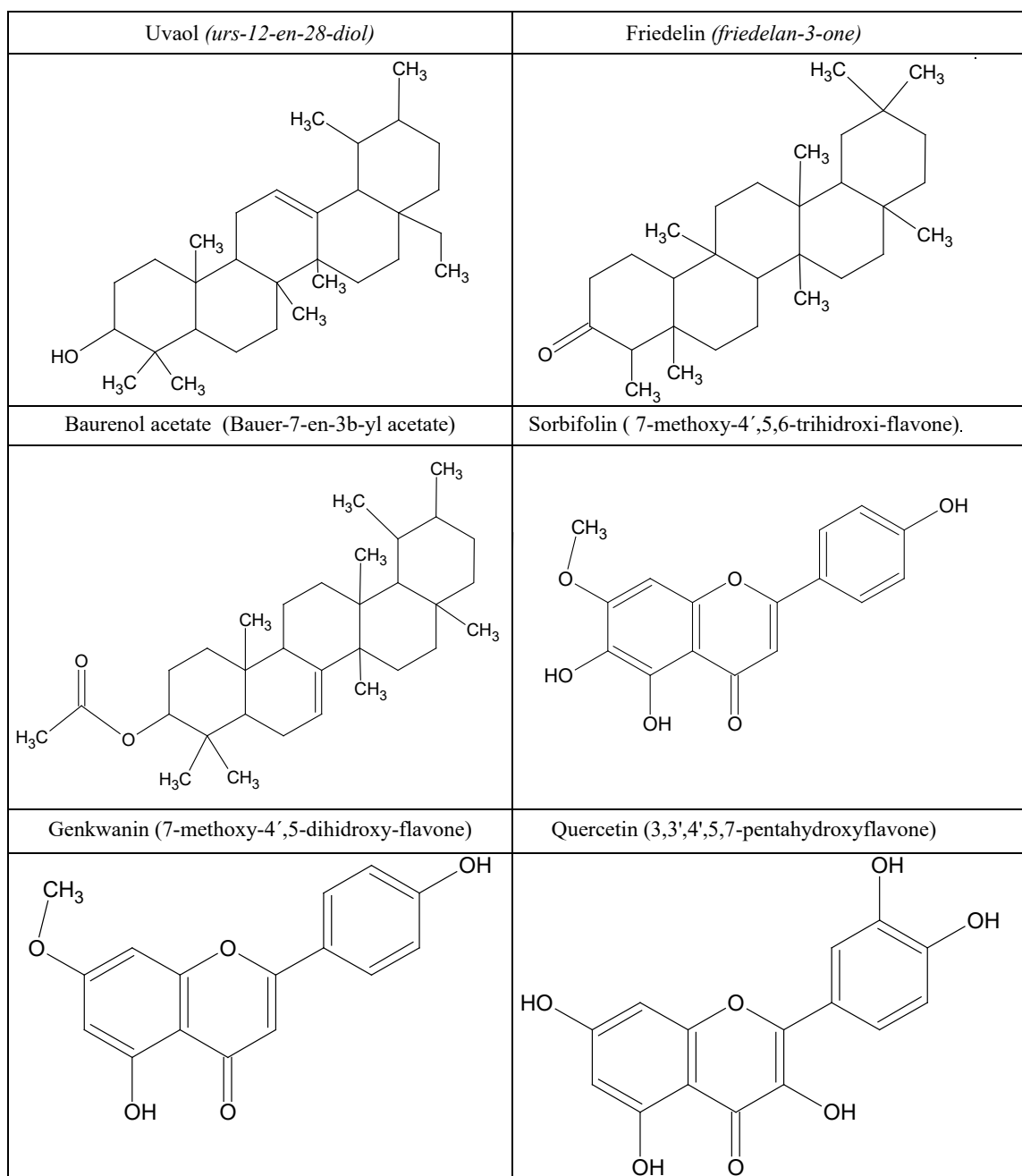


Figure 1. Purified compounds of *Diplostegium phyllicoides* (H.B.K.) Wedd.

With the ether extract of the stems the same procedure was used, which obtained a precipitate whose electroscopic data were, (Figure 1):

IR ν_{\max} (KBr), cm^{-1} : 3400(OH); 2950, 2850 (CH aliphatic); 1730(-C=O),

$^1\text{HRMN}$ (300 MHz, CDCl_3 , TMS): δ ppm(H): 0.79(3H, s); 0.85(3H,s); 0.85(3H,s); 0.91(3H,s); 0.99(3H,s); 1.06(3H,s); 1.49(3H,s); 1.51(3H,s); 2.04(3H,O-CO-R); 4.52(1H,m,-O-CH); 5.42(1H,m,RC=CH),

$^{13}\text{CRMN}$ (75 MHz, CDCl_3 , TMS): δ ppm(C): 145.53; 116.357; 81.200; 55.058; 50.659; 48.274; 41.311; 38.005; 37.815; 36.623; 35.430; 35.159; 32.504; 32.124; 32.124; 31.582; 29.711; 29.250; 28.952; 27.543; 25.673; 24.237; 24.020; 23.668; 22.692; 22.529; 21.256; 16.893; 15.812; 13.075.

EIMS (70 eV m/z (rel. int.) 468[M]⁺: (24.0); 453(15.5); 393(13.5); 301(15.1); 290(20.8); 289(100.0); 255(10.2); 241(13.2); 230(13.8); 229(69.1); 205(22.7); 187(10.0); 159(12.0); 149(17.5); 147(13.5); 145(11.7); 137(12.1); 135(25.6); 133(21.3); 123(35.7); 121(25.0); 119(26.5); 109(39.2); 107(29.7); 105(20.6); 95(48.7); 93(20.9); 91(13.9); 83(16.4); 81(35.3); 79(13.6); 69(53.5); 67(18.9); 57(10.2); 55(29.0); 43(31.6); 41(10.0). From an analysis of their electroscopic data, we concluded that it was Bauerenol acetate (3-acetate-bauer-7-eno) [$\text{C}_{32}\text{H}_{52}\text{O}_2$]²⁰.

In the 113-123 chromatographic fractions of the petroleum extract of the flowers (AcOEt 8:2) some transparent crystals were obtained whose spectroscopic data were:

IR ν_{\max} (KBr), cm^{-1} : 2900(OH); 2900, 2850 (CH aliphatic); 1710 (C=O); 1660, 1450 y 1390 (CH),

$^1\text{HRMN}$ (300 MHz, CDCl_3 , TMS): δ ppm(H): 0.72(3H,s,Me); 0.87((3H,s,Me); 0.89(3H,s,Me); 0.96(3H,s,Me); 0.98(3H;s,Me); 1.00(3H,s,Me); 1.06(3H,s,Me); 1.19(3H;s,Me),

$^{13}\text{CRMN}$ (75 MHz, CDCl_3 , TMS): δ ppm(C): 212.902(3); 59.663(10); 58.358(4); 53.226(8); 42.995(18); 42.175(5); 41.568(2); 41.447(6); 39.807(14); 39.352(22); 38.441(13); 37.591(9); 36.133(16); 35.738(19); 35.465(11); 35.040(30); 32.945(21); 32.550(12); 32.186(28); 31.852(29); 30.577(15); 30.091(17); 28.239(20); 22.317(1); 20.282(27); 18.673(26); 18.339(7); 17.975(25); 14.699(24); 6.805(23). When we analyzed their electroscopic data, we concluded that it was Friedelin (Friedelan-3-one) [$\text{C}_{30}\text{H}_{50}\text{O}$]²¹.

With the fracture in crystallization process, a yellow solid was obtain from the EtOH extract of the leaves: its spectroscopic data were:

UV in MeOH, λ_{\max} (nm): 273.0 – 335.5; (AlCl_3); 280.0 – 302.0 – 346.0 – 340.0; (AlCl_3 – HCl) 280.0 – 301.0 – 356.0 (NaOMe) 267.0 – 328.5 – 396.7; (NaOAc) 274.5 – 381.0; (NaOAc – H_3BO_3) 274.0 – 338.0; (H_3BO_3) 273.0 – 335.5,

$^1\text{HRMN}$ (300 MHz, CDCl_3 , TMS): δ ppm (H, C): 2.5 (d6); 3.5 (H_2O); 3.75 (3H, s, OMe, C-7); 6.59 (1H,s,C-8); 6.75 (1H.S,C-3); 6.9 (2H,d,C2', C-6'); 7.92 (2H,d,C-3', C-5'); from the AA'-XX' system; 13.0(1H,m,C-5) When its electroscopic data was analyzed, we concluded that it was sorbifolin (5,6,4'-trihydroxy-7-methoxyflavone)²².

Another yellow solid was obtained, with the following data:

UV in MeOH, λ_{\max} (nm): 268.8 – 334.5; (AlCl_3)276.0 – 300.0 – 346.0 – 380.0; (AlCl_3 – HCl) 277.0 – 301.0 – 346.0 – 380.0; (NaOMe) 269.0 – 302.0 – 382.0; (NaOAc) 268.8 – 349.5; (NaOAc – H_3BO_3) 268.8 – 336.5; (H_3BO_3) 268.8 – 334.2.

$^1\text{HRMN}$ (300 MHz, CDCl_3 , TMS): δ ppm (H, C): 2.54(d6); 3.52 (H_2O); 3.86 (1H,s,OMe, C-7); 6.40 (1H,d,H-6); 6.78 (1H.d,H-8); 6.85 (1H,s,OH); 6.96 (2H,d,H-2', H-6'); 8.00 (2H,d,H-3',H-5'), of the AA'-XX' system; 13.0(1H,m,H-5). When the spectroscopic data were analyzed, we concluded that it was Genkwanin, (5,4'-dihydroxy-7-methoxy-flavone)²³.

The EtOH extract of the ethyl acetate fraction of the flowers spontaneously precipitated into a yellow solid whose spectroscopic data were:

UV en MeOH, λ_{\max} (nm): 254.8 – 270.4 – 294.7 – 399.4; (AlCl_3) 271.8 – 303.2 – 335.1 – 44.9; (AlCl_3 – HCl) 266.7 – 301.8 – 358.3 – 424.1; (NaOMe) 276.0 – 332.5 – 397.3; (NaOAc) 276.0 – 324.0 – 388.9; (NaOAc – H_3BO_3) 262.1 – 301.2 – 383.5; (H_3BO_3) 256.5 – 297.4 – 368.5. When its spectroscopic data were analyzed, we concluded that it was Quercetin (3, 3', 4', 5, 7-pentahydroxyflavone)²⁴.

Anti-oxidant Activity

Figure 2 shows the percentages of catchment from the extracts of the leaves of *D. phyllicoides* with the DPPH* technique, which confirms that the catchment percentage

of the ethyl acetate extract is 50% higher from 62.5 mg/LMeOH onwards and that of the ethanol extract, from 25 mg/LMeOH onwards. On the basis of a comparison between the concentration curves and the catchment percentage obtained from the leaf extracts, the IC_{50} 's of each extract were obtained, which showed a catchment percentage greater than 50% for the DPPH[•] technique. In addition, confirmed a value of 13.80 mg/LMeOH for the ethanol extract and 28.32 mg/LMeOH for the ethyl acetate extract, with an anti-oxidant capacity of 0.07 for the first and 0.04 for the second.

When we compared the results obtained from the IC_{50} of ascorbic acid 1.13 mg / LMeOH with those from the Rutin 5.88 mg / LMeOH, we found an antioxidant capacity of 0.88 and 0.17, Respectively, confirming that the leaf ethanol extract *Diplostephium phyllicoides* (H.B.K.) Wedd., presents a better inhibitory concentration (IC_{50})

with this method. The relative antioxidant activity (AAR), for the DPPH[•] technique, was determined by comparing the antioxidant capacities of the analyzed extracts with the antioxidant capacities of the standards. The ethanol extract has an AAR of 0.08 compared to the ascorbic acid and 0.43 compared to the rutin, for the extract of ethyl acetate, 0.04 of AAR compared to the ascorbic acid and 0.21 in comparison with the Rutin. This relative antioxidant activity, the higher the value, the greater the antioxidant capacity in mg/LMeOH.

With the ABTS^{•+} technique, we confirmed that the dichloromethane, ethyl acetate and ethanol extracts, at 25mg/LMeOH onwards, had catchment percentages greater than 50% (Figure 3): The IC_{50} 's of each extract which showed a catchment percentage greater than 50% with the ABTS^{•+} technique were: 10.14 mg/LMeOH for the ethanol extract and 10.86 mg/LMeOH for the ethyl

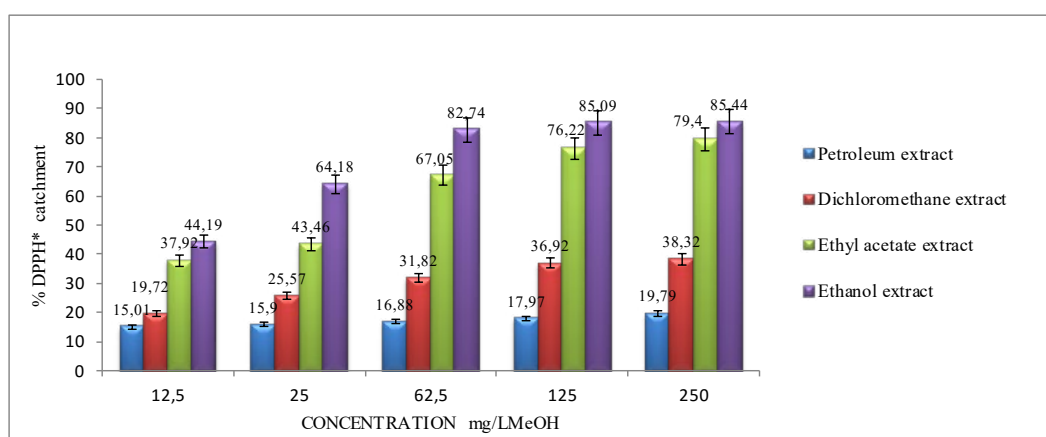


Figure 2. Comparison of the catchment percentage of all the extracts from the leaves of *Diplostephium phyllicoides* (H.B.K.) Wedd., using the DPPH[•] radical decoloration method.

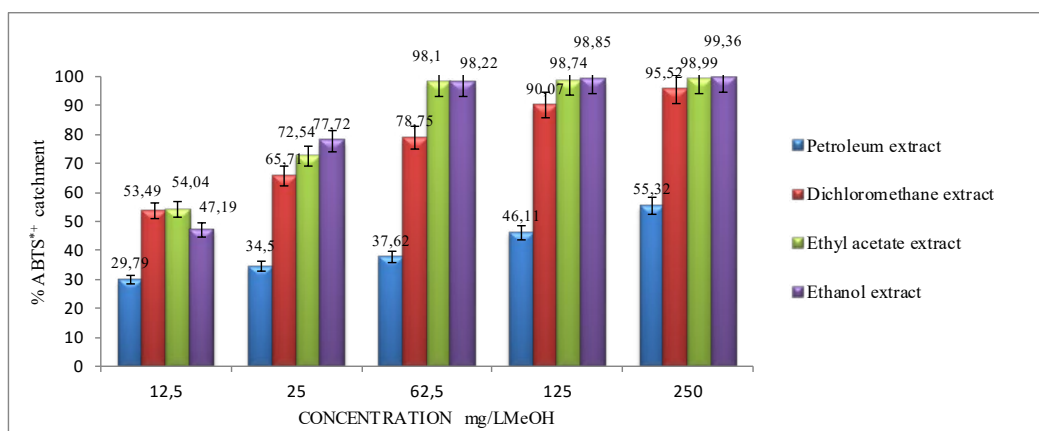


Figure 3. Comparison of the extracts from the leaves of *Diplostephium phyllicoides* (H.B.K.) Wedd., using the ABTS^{•+} radical decoloration method.

acetate extract, with an anti-oxidant capacity of 0.10 for the former and 0.09 for the latter. The above confirms that the ethyl acetate extract of the leaves of *Diplostephium phyllicoides* (H.B.K.) Wedd. shows a better inhibitory concentration (IC_{50}) with this method.

The IC_{50} of the ascorbic acid was 1.41 mg/LMeOH, the trolox 2.32 mg/LMeOH and the rutin, 2.48 mg / LMeOH. The relative antioxidant activity (AAR) of the ethanol extract was 0.14 compared to that of ascorbic acid, 0.23 compared to that of trolox and 0.24 compared to that of the Rutin, while that of the acetate extract of ethyl was 0.13 compared to ascorbic acid, 0.21 compared to trolox and 0.23 compared to routine. The higher the value of this antioxidant activity, the stronger the antioxidant capacity.

4. Analysis and Conclusions

From the petroleum extract of *D. phyllicoides*, the *Uvaol* was purified; (*urs-12-en-28-diol*): [$C_{30}H_{50}O_2$], which has been reported in the following species: *Diospyros montana*²⁵, *D. lotus*¹⁷, *Diospyros maingayi* and *D. sSingaporensis*, *Nepeta eriostachia*²⁶ and *N. aragonensis*¹⁸ and *Lavandula officinalis*²⁷. Another terpin purified from the ether extract of the stems was *Bauerenol*; (*3-acetate-baur-7-eno*): [$C_{32}H_{52}O_2$]; a compound reported for species like *Maytenus ilicifolia* and *Maytenus aquifolium*²⁸. With the ether extract of the flowers, *Friedelin* (*friedelan-3-one*): [$C_{30}H_{50}O$] was purified, which has been reported for species like: *Maytenus diversifolia*^{21,29}. The flavonoids were obtained from the polar extracts. In the fraction of ethyl acetate from the leaves, *Genkwanin* was purified (*apigenin-7-methyl*): it has been reported for *Larrea tridentata* and *L. divaricata*; the galactoside of *Genkwanin* has been isolated in *Larrea tridentata* and *L. divaricata*²³ and *Duabanga sonneratioides*³⁰. *Sorbifolin* (*7-methoxy-4'*, *5*, *6*-, *trihydroxi-flavone*) has been reported in *Pleocarpus revolutus*²². *Quercetin* (*3*, *5*, *7*, *3'*, *4'*- *Pentahydroxyflavone*) was found in the ethyl acetate fraction of the ethanol extract of the flowers: it has been reported for many *Asteraceae*. The most polar extracts of the leaves of *Diplostephium phyllicoides* (H.B.K.) Wedd. showed an anti-oxidant activity, which is related to the presence of flavonoids in those extracts. There have been few scientific studies of the *Diplostephium phyllicoides* species and there are no reports in them of the following triterpenes: *Uvaol*, *Friedelin* and *Bauerenol* acetate nor of the following flavonoids: *Genkwanin*, *Sorbifolin* and *Quercetin*.

5. Acknowledgments

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6. Conflict of Interests

The authors declare that this article does not present any conflict of interests.

7. Bibliography

1. Del Vitto LA, Petenatti EM. Asteraceas de importancia economica y ambiental. primera parte - Sinopsis morfológica y taxonómica, importancia ecológica y plantas de interés industrial. *Multequina*. 2009 July/ December; 18(2):87-115.
2. Von Humboldt FHA, Bonpland AJA, Kunth CS. *Nova Genera et Species Plantarum*. Paris: Lutetiae Parisiorum. 1820; 4:1-312.
3. Bentham GH. *Genera Plantarum and Exemplaria Imprimis in Herbaris Kewpsibus servata definita*. Londini. 1880; 3(pt 1):1-459.
4. Cuatrecasas J. *Prima Flora Colombiana 3. COMPOSITAE-ASTERACEAE*. *Journal of Plant Taxonomy and Geography*. 1969; 24(1):1-335.
5. Weddell HA. *Chloris Andina*. Paris: P. Bertrand. 1857; 1:1-206.
6. Ávila L, Baquero E, Murillo E, Vi-a A. Actividad Antibacteriana de *Diplostephium tolimense* Cuatrec. (*Asteraceae*) frente a *Staphylococcus aureus*. *Revista de la Universidad de Química Farmacéutica*. 2006; 13 (1):55-60.
7. Bittner M, Schuster A, Jakupovic S. Diterpenes from *Diplostephium meyenii*. *Phytochemistry*. 1991; 30 (4):1329-30. crossref
8. Zdero C, Bohlmann F, King R. Clerodane derivatives from *diplostephium*. *Phytochemistry*. 1992 January; 31(1):213-16. crossref
9. Urzua A, Torres R, Andrade L, Delle Monache, Delle Monache F, Brioso JL, Sanchez-Ferrando F, Parella T. Benzodihydrofurans in the resinous exudate from *Diplostephium cinereum*. *Boletín de la Sociedad Chilena de Química*. 2001 March; 46(1):77-80.
10. Halliwell B. Free radicals and anti-oxidants: a personal view. *Nutrition Reviews*, New York. 1994 August; 52(8 pt 1):253-65.
11. Halliwell B, Aeschbach R, Loinger J, Aruoma OI. The characterization on anti-oxidants. *Food and Chemical Toxicology*. 1995 July; 33(7):601-17. crossref

12. Betteridge DJ. What is oxidative stress? *Metabolism*. 2000 February; 49(2-Suppl 1):3-8.
13. Youngson R. *Antioxidantes y Radicales Libres*. Editorial EDAF, S.A. Madrid, España. 2003; p. 1-175.
14. Marwah RG, Fatope MO, Mahrooqi RA, Varma GB, Abadi HA, Burtamani-Al SKS. Anti-oxidant capacity of some edible and wound healing plants in Oman. *Food chemistry*. 2007; 101(2):465-70. [crossref](#)
15. Rodríguez R, Oscar A, Torrenegra RD. The anti-oxidant activity of extracts and fractions of *Chromolaena bullata* (klatt) king & robinson. *Pharmacologyonline*. 2017 May; 1:98-105.
16. Komae H, Hayashi N. Terpenes from *Actinodaphne*, *Machilus* and *Neolitsea* species. *Phytochemistry*. 1972 March; 11(3):1181-82. [crossref](#)
17. Bin Zakaria M, Jeffrey JAD, Waterman PG, Zhong SM. Naphthoquinones and triterpenes from some Asian *Diospyros* species. *Phytochemistry*. 1984; 23(7):1481-84. [crossref](#)
18. Carstenn-Lichterfelde CV, Rodríguez B, Valverde S. Triterpenes and fatty acids from *Nepeta aragonensis*. *Phytochemistry*. 1973; 12(12):3002-03. [crossref](#)
19. Passannanti S, Paternostro M, Piozzi F, Barbagallo C. Diterpenes from the Genus *Amaracus*. *Journal of Natural Products*. 1984 September; 47(5):885-89. [crossref](#)
20. Meksuriyen D, Dhammika NNP, Phoebe CH Jr, Corde GA. Two triterpenes from *Davidsonia pruriens*. *Phytochemistry*. 1986; 25(7):1685-89. [crossref](#)
21. Gunatilaka AAL, Nanayakkara NPD, Wazeer Mohammed IM. ¹³C NMR Spectra of some D: A-Friedo-olananes. *Phytochemistry*. 1983; 22(4):991-92. [crossref](#)
22. Silva M, Wiesenfeld A, Sammes PG, Tyler TW. New sesquiterpenes from *Pleocarphus revolutus*. *Phytochemistry*. 1977; 16(3):379-85. [crossref](#)
23. Sakakibara M, DiFeo D Jr, Nakatani N, Timmermann B, Mabry TJ. Flavonoid methyl ethers on the external leaf surface of *Larrea tridentata* and *L. divaricata*. *Phytochemistry*. 1976; 15(5):727-31.
24. Harborne JB, Mabry TJ. *The Flavonoids*. *Advances in Research*, London: New York: Chapman and Hall. 1982; p. 1-718. [crossref](#)
25. Dutta PK, Dutta NL, Chakvavarti RN. Sterols and Triterpenes of *Diospyros montana*. *Phytochemistry*. 1972; 11:1180-81. [crossref](#)
26. Bhandari SPS, Garg HS, Agrawal PK, Bhakuni DS. Ursane Triterpenoids from *Nepeta eriostachia*. *Phytochemistry*. 1990; 29(12):3956-58. [crossref](#)
27. Hatem NA, Najah ZM. Isolation and elucidation of some chemical constituents of *Lavandula officinalis*. *Journal of Chemical and Pharmaceutical Research*. 2016; 8(3): 394-401.
28. Cordeiro P, Vilegas J, Lanças F. HPLC-MS Analysis of Terpenoids from *Maytenus ilicifolia* and *Maytenus aquifolium* ("Espinheira Santa"). *Journal of the Brazilian Chemical Society*. 1999; 10(6):523-26. [crossref](#)
29. Nozaki H, Suzuki H, Hirayama T, Kasainryjiwu RY, Lee KH. Antitumour triterpenes of *Maytenus diversifolia*. *phytochemistry*. 1986 January; 25(2): 479-85.
30. Sharma SC, Shukla YN, Tandon JS, Dhar MM. Genkwanin 4'-galactoside and other constituents from *Duabanga sonneratioides*. *phytochemistry*. 1974; 13:527-28.