International Journal of Health Sciences
Available online at www.sciencescholar.us
Vol. 8 No. 1, April 2024, pages: 83-91
e-ISSN: 2550-696X, p-ISSN: 2550-6978
https://doi.org/10.53730/ijhs.v8n1.14812

Phytochemical Study of Two Plants: Calendula Officinalis and Berberis Vulgaris and Evaluation of Their Antioxidant Activities with DPPH Test

Fatna Gougue [a], **Saad Boutaiba** [b]

Manuscript submitted: 07 December 2023, Manuscript revised: 10 February 2024, Accepted for publication: 14 March 2024

Corresponding Author [a]

Keywords

antioxidant activity;
Berberis vulgaris;
Calendula officinalis;
DPPH test;
phytochemical;

Abstract

Plants have been the basis of traditional medicine throughout the world and continue to provide new remedies for humanity, so a great effort has been made to use available experimental techniques to identify natural antioxidants from plants. Several authors have examined the beneficial uses of these plant species. In order to contribute to the valorization of the Algerian flora, we are interested in this study by the phytochemical study of two plants Calendula officinalis and Berberis vulgaris which are much used in the traditional medicine in the region of Djelfa, and evaluate their antioxidant activity with the DPPH test. The qualitative phytochemical examination of *Berberis* vulgaris root showed the presence of alkaloids, tannins, sterols, triterpenes, and reducing compounds in large quantities, and it also revealed lower amounts of coumarins, terpenoids, saponins, and mucilage. Phytochemical studies of the dried petals of calendula officinalis revealed the presence of tannins, alkaloids, Sterols and Triterpenes, and saponosides. The tests for flavonoids and coumarins were negative on our extract. The antioxidant activity of Berberis vulgaris root was measured by the DPPH free radical scavenging technique. The extract of total alkaloids which show higher percentages of inhibition at low concentrations with EC50 is 0.26 g/l. EC50 of polyphenol extract of Calendula officinalis is 3.17 g/l.

[a] Department of Biology, Faculty of Natural and Life Sciences, Djelfa University BP3117, Djelfa, Algeria
[b] Department of Biology, Faculty of Natural and Life Sciences, Djelfa University BP3117, Djelfa, Algeria & Ecology and Development of Steppe Ecosystems Laboratory, Faculty of Natural and Life Sciences, Djelfa University BP3117, Djelfa, Algeria

Contents

1 Introduction

Today, plants have been the basis of traditional medicine throughout the world for thousands of years and continue to provide new remedies; Algeria is known for its wide plant diversity with 3183 species the majority of them are endemic plants due to its climatic and topographic diversity. When one lists active plant ingredients, which might include alkaloids, flavonoids or glycosides, essential oils (Ekwenye, 2006), tannins (Dahanukar et al., 2000), and some other unusual substances.

The antioxidant activity of plant polyphenols has had positive effects on neurological disorders on the basis of in vitro observations (Moosmann & Behl, 1999; Parr & Bolwell, 2000). Plant polyphenols might also display distinctive anticarcinogenic, antimutagenic, and cardioprotective effects due to their free radical scavenging properties (Santos-Buelga & Scalbert, 2000; Alghazeer et al., 2008). The alkaloidal content of the root of Berberis vulgaris, especially berberine, is generally claimed to be responsible for its beneficial effects. These justify the interest in discovering new natural antioxidant molecules to replace synthetic antioxidants in foods or medicinal materials. In this focus, our work is the phytochemical study to determine the major secondary metabolites and evaluate the antioxidant activity of different extracts of Berberis vulgaris and Calendula officinalis plants in the Djelfa region (country of Algeria).

2 Materials and Methods

Material vegetal

Two plants Berberis vulgaris (Berberidaceae) and Calendula officinalis were obtained in Djelfa (steppe region in Algeria in 2019). The part used in this study is the root of Berberis vulgaris stored in a dark place at room temperature but the part used is the petals of Calendula officinalis which were dried under light and at room temperature followed by manual crushing and then packaged in kraft paper bags.

Extraction of total alkaloids from the root of Berberis vulgaris

The extraction was done according to the protocol of Mezouar et al (Mezouar et al., 2014). Forty grams of the ground plant material is extracted by 400 ml of 10% acetic acid in methanol. The extract is macerated for 24 hours with agitation and protected from light. After filtration, the extract is concentrated with a rotavapour to a quarter of its initial volume, then precipitated by the addition of concentrated ammonium hydroxide. The two phases obtained are extracted with chloroform until complete exhaustion, and the chloroform phase is evaporated to dryness at 40 °C. The residue obtained is stored at 4 °C (Mezouar et al., 2014).

Extraction of total polyphenols from Calendula officinalis flowers

Dried calendula flowers (5 g) were extracted with 70% methanol (250 ml) at room temperature for 24 h. After filtration the extract was concentrated under reduced pressure and the resulting product was fractionated using ethyl acetate (Ćetković et al., 2003). A ratio of 70% of methanol is required to inactivate polyphenol

oxidases, enzymes involved in the oxidation of polyphenols, which leads to the browning phenomenon (Chirinos et al., 2007).

Phytochemical tests

The main chemical constituents were characterized by colour reactions and observations under ultraviolet light, using analytical techniques described in the literature (Bruneton J, 1999).

DPPH radical scavenging activity assay

Radical scavenging activity of plant extracts against stable DPPH_ (2,2-diphenyl-2-picrylhydrazyl hydrate) was determined spectrophotometrically by the slightly modified method of Brand-Williams et al. (1995), The evaluation of the antioxidant capacity is carried out as follows: The plant extract (0.1ml) is added to 2.9 ml of DPPH (2,2-diphenyl-1-picrylhydrazyl) at 0.04 g/1 in methanol solution. The reading is taken at 517 nm, after 30 minutes of incubation in the dark. The free radical solution is freshly prepared. All measurements are repeated at least three times to minimize errors; The antioxidant activity of the extract was expressed as an EC50 value defined as the concentration (in l M) of the extract that inhibited the formation of DPPH radicals by 50%. Triplicate measurements were carried out and their activity was calculated by the percentage of DPPH scavenged. The DPPH radical scavenging activity obtained was compared with that of ascorbic acid (Adams et al., 2007; El-Naili et al., 2008; Jabbari & Gharib, 2012).

Thin layer chromatography of alkaloids of Berberis vulgaris roots

15 mg of total alkaloids are solubilized in 0.5 ml of chloroform and deposited on a silica plate. The system used was chloroform/methanol (9/1) (v/v). The chromatogram was observed using a UV lamp at 366 nm. The retention factor was calculated using the following formula:
Frontal RF ratio = Distance travelled by the component/Distance travelled by the eluent

Thin layer chromatography of polyphenols from Calendula officinalis flowers

Dried calendula flowers (5 g) were extracted with 70% methanol (250 ml) at room temperature for 24 h. Aliquots of the extract obtained (20% v/v) were evaporated to the dry state (methanol extract). Thin layer analysis is performed on a silica gel glass plate (4×10 cm). A volume of 1 µL of methanol extract is spotted on the TLC plate. The solvent system used for the polyphenols is ethyl acetate: formic acid: acetic acid: and distilled water 100 : 11 : 11 : 26 The chromatogram is observed under visible light and UV light at 366 nm. The retention factor was calculated using the following formula:
Frontal RF ratio = Distance travelled by the component/Distance travelled by the eluent.

3 Results and Discussions

Extraction of total alkaloids from the root of Berberis vulgaris

The extraction of total alkaloids from the root of Berberis vulgaris gives a yield of 31.7%, which shows the richness of the plant in alkaloids, if we compare our results with other works (Mezouar et al., 2014). we can see that our plant is very rich in alkaloids

Extraction of total polyphenols from Calendula officinalis flowers

The extraction of polyphenols from Calendula officinalis petals gives a yield of 26.5 %. The extract is in the form of orange crystals.

Phytochemical tests
Phytochemical tests of Berberis vulgaris

Phytochemical tests of Berberis vulgaris root revealed the presence of tannins, alkaloids, sterols, and Triterpenes, coumarins, and saponosides. The tests for flavonoids, and anthocyanins, were negative in our extracts (table 1).

Table 1
Results of phytochemical tests on the root of Berberis vulgaris

Chemical family		Result
Phenolic compounds	Flavonoïdes	-
	Anthocyanines	-
	Tanins	+++
Alcaloïdes	/	+++
Coumarines	/	+
Anthraquinones	/	-
Sterols et triterpenes	/	+++
Terpenoïdes	/	+
Saponosides	/	++

These results are similar to those obtained by Meliani et al. (2011), who detected the presence of tannins, alkaloids, saponosides, and sterols in the root of Berberis vulgaris

Phytochemical tests of Calendula officinalis

Phytochemical studies of the dried petals of Calendula officinalis revealed the presence of tannins, alkaloids, Sterols and Triterpenes, and saponosides. (table 2)

Table 2
Results of phytochemical tests of Calendula officinalis

Chemical family		Result
Phenolic compounds	Flavonoïdes	-
	Anthocyanines	-
	Tanins	+
Alcaloïdes	/	+
Coumarines	/	-
Sterols et triterpenes	/	+

The tests for flavonoids and coumarins were negative in our extract, According to the results of Kaur et al. (2015), the phytochemical study of calendula officinalis extract showed the presence of flavonoids, saponins, carbohydrates, amino acids, and phenols. The absence of flavonoids in our extracts although the orange colour of the petals is due to flavonoids can be explained according to Jabbari & Gharib (2012), by the fact that flavonoids are practically insoluble in water, but they are often soluble in organic solvents. This is a frequent problem today, as new molecules in drug discovery are less water-soluble and more lipophilic. The mixed-solvent procedures using mainly mixtures of organic solvents in water provide a good alternative for poorly or non-soluble compounds (Jebril, 2008; Shekarabi et al., 2022; Fatehi et al., 2005; Ivanovska & Philipov, 1996).

Free radical (DPPH) scavenging activity of alcaloides extract of Berberis vulgaris

Table 3
Free radical (DPPH) scavenging activity of alcaloides extract compared to the antioxidant controls

Extract	EC_{50} (g/l)
alkaloids extract	0.26
Vitamin C	0.058

Figure 1 shows the variation of the concentration of alcaloides versus inhibition. By the same protocol as Mezouar et al. (2014), we obtained an EC50 of alkaloids equal to 0.26 g/l and they obtained an EC50 equal to 2.8 mg/ml of our extracts having an important antioxidant activity compared to the results obtained by Mezouar et al. (2014).

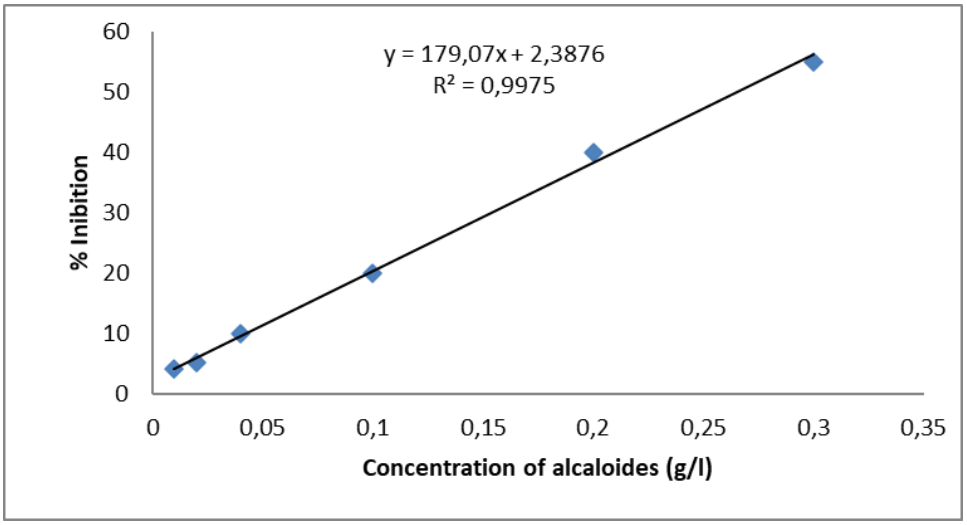

Figure 1. Variation of the concentration of alcaloides versus inhibition

Free radical (DPPH) scavenging activity of alcaloides extract of Calendula officinalis

Table 4
Free radical (DPPH) scavenging activity of polyphenols extract of Calendula officinalis compared to the antioxidant controls

Extract	EC_{50} (g/l)
Polyphenols extract	3.17
Vitamin C	0.058

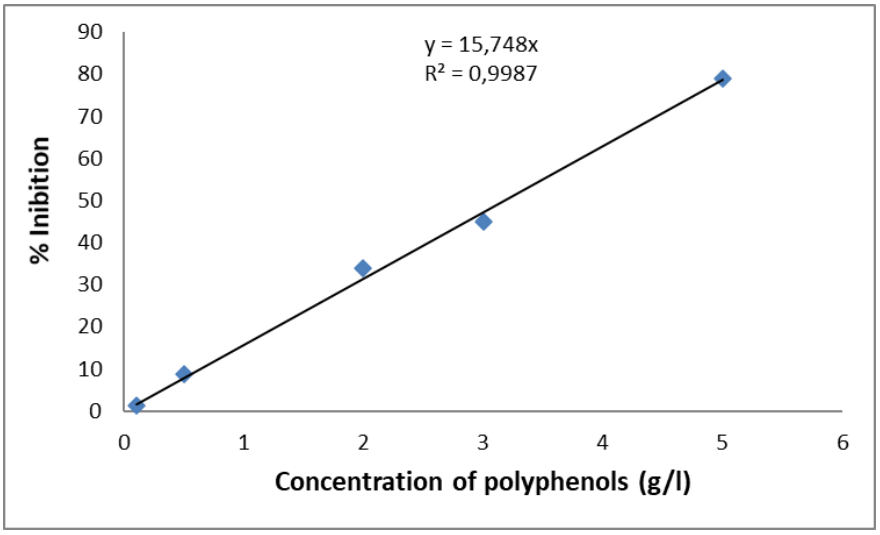

Figure 2. The variation of the concentration of polyphenols versus inhibition

Figure 2 Variation of the concentration of polyphenols versus inhibition. The EC50 of the polyphenol extract 3.17 g/l is higher than the EC50 of the aqueous and methanolic extracts which have 9.11 and 4.20 g/l. The result obtained may show that polyphenols are responsible for the antioxidant activity of Calendula officinalis (Shahidi & Zhong, 2015; Wiwekowati et al., 2017; Yadnya et al., 2016). The highest antioxidant activity correlated with polyphenol content was obtained for extracts prepared with methanole. The relationship between antioxidant activity and flavonoid content is non-linear, suggesting that antioxidant activity depends on the total polyphenol content (Butnariu & Coradini, 2012).

Chromatographic separations
Total alkaloids and TLC

Chromatographic analysis of total alkaloids was carried out using the chloroform/methanol (9/1) (v/v) system and revealed the presence of 4 spots, two of which were fluorescent: yellow and blue (Table 19, Figure 47). Following the same extraction protocol and chromatographic analysis (TLC) of total alkaloids from Berberis vulgaris bark as Mezouar et al. (2014), we obtained a yield of 31.7 % and the TLC reveals the presence of 4 spots of which two spots are fluorescent: yellow with Rf: 0.25 and a blue spot. They obtained a yield of 6.7% and chromatographic analysis of the total alkaloids showed the presence of 6 spots, including three fluorescent spots, one of which was yellow with an Rf of 0.25. The yellow spot could represent berberine, a very abundant alkaloid that fluoresces yellow according to the literature (Makkar et al, 2007).

Polyphenols and TLC

Chromatographic analysis of the polyphenols by using the ethyl acetate/formic acid/acetic acid/water system in the volume ratio (100/11/11/26) as the mobile phase (v/v) and revealed the presence of 6 spots, including three fluorescent spots: yellow, blue and violet. Using the table of Rf-values and spot colour of the methanolic extract of Ćetković et al. (2003), the violet spot observed under UV corresponds to the flavonoid glycoside, the blue is the equivalent of phenolic acid and the yellow corresponds to quercetin. Using the same protocol, Ćetković et al. (2003), found 2 fluorescent spots of blue and yellow colour, the yellow spot corresponds to phenolic acid and the blue spot corresponds to quercetin.

4 Conclusion

Medicinal plants are the source of the majority of natural antioxidants and are still under-exploited in the medical field. In this context, we are interested in the phytochemical study and evaluation of the antioxidant activity of different extracts of the plants Calendula officinalis (flowering part) and Berberis vulgaris (root part) and the analysis of the active compounds (polyphenols or alkaloids) of each plant. The phytochemical study revealed the presence of the following secondary metabolites: tannins, alkaloids, sterols and triterpenes, and saponosides but flavonoids, absent in both plants and coumarins are even absent in the petals of Calendula officinalis. The extraction of total alkaloids from the roots of Berberis vulgaris gave us a yield of 31.7 %. The extraction of polyphenols from calendula officinalis petals gives a yield of 26.5 %.

The antioxidant activity of total alkaloids of Berberis vulgaris shows higher percentages of inhibition at low concentrations with EC50 is 0.26 g/l. EC50 of polyphenol extract of Calendula officinalis is 3.17 g/l. Chromatographic analysis of total alkaloids from the roots of Berberis vulgaris showed 4 spots of which two spots are fluorescent: yellow with Rf: 0.25 and a blue spot. The yellow spot could represent berberine, a very abundant alkaloid. Chromatographic analysis of the polyphenols from petals of Calendula officinalis revealed the presence of 6 spots, including three fluorescent spots: yellow, blue, and violet. the violet spot observed under UV corresponds to the flavonoid glycoside, the blue is the equivalent of phenolic acid and the yellow corresponds to quercetin.

Acknowledgments
We are grateful to two anonymous reviewers for their valuable comments on the earlier version of this paper.

References

Adams, M., Gmünder, F., & Hamburger, M. (2007). Plants traditionally used in age related brain disorders—A survey of ethnobotanical literature. *Journal of ethnopharmacology*, *113*(3), 363-381. https://doi.org/10.1016/j.jep.2007.07.016

Alghazeer, R., Gao, H., & Howell, N. K. (2008). Cytotoxicity of oxidised lipids in cultured colonal human intestinal cancer cells (caco-2 cells). *Toxicology Letters*, *180*(3), 202-211. https://doi.org/10.1016/j.toxlet.2008.06.859

Brand-Williams, W., Cuvelier, M. E., & Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food science and Technology*, *28*(1), 25-30. https://doi.org/10.1016/S0023-6438(95)80008-5

Bruneton, J. (1999). Pharmacognosie, phytochimie, plantes médicinales, 3ème éd. *Lavoisier, Paris*, *1120*.

Butnariu, M., & Coradini, C. Z. (2012). Evaluation of biologically active compounds from Calendula officinalis flowers using spectrophotometry. *Chemistry central journal*, *6*, 1-7.

Ćetković, G. S., Đilas, S. M., Čanadanović-Brunet, J. M., & Tumbas, V. T. (2003). Thin-layer chromatography analysis and scavenging activity of marigold (Calendula officinalis L) extracts. *Acta periodica technologica*, (34), 93-102.

Chirinos, R., Rogez, H., Campos, D., Pedreschi, R., & Larondelle, Y. (2007). Optimization of extraction conditions of antioxidant phenolic compounds from mashua (Tropaeolum tuberosum Ruíz & Pavón) tubers. *Separation and Purification Technology*, *55*(2), 217-225. https://doi.org/10.1016/j.seppur.2006.12.005

Dahanukar, S. A., Kulkarni, R. A., & Rege, N. N. (2000). Pharmacology of medicinal plants and natural products. *Indian journal of pharmacology*, *32*(4), 81-118.

Ekwenye, U. N. (2006). Chemical characteristics of palm oil biodeterioration. *Biokemistri*, *18*(2).

El-Naili, M. A., Saleh, N. A., Ahmed, H. I., Rammash, B. K., & El-Buni, A. A. (2008). Phytochemical screening and in vitro antimicrobial activity of Arum cyrenaicum (Araceae) growing in Libya. *J. Egypt. Acad. Soc. Environ. Develop*, *9*, 1-12.

Fatehi, M., Saleh, T. M., Fatehi-Hassanabad, Z., Farrokhfal, K., Jafarzadeh, M., & Davodi, S. (2005). A pharmacological study on Berberis vulgaris fruit extract. *Journal of ethnopharmacology*, *102*(1), 46-52. https://doi.org/10.1016/j.jep.2005.05.019

Ivanovska, N., & Philipov, S. (1996). Study on the anti-inflammatory action of Berberis vulgaris root extract, alkaloid fractions and pure alkaloids. *International journal of immunopharmacology*, *18*(10), 553-561. https://doi.org/10.1016/S0192-0561(96)00047-1

Jabbari, M., & Gharib, F. (2012). Solvent dependence on antioxidant activity of some water-insoluble flavonoids and their cerium (IV) complexes. *Journal of Molecular Liquids*, *168*, 36-41. https://doi.org/10.1016/j.molliq.2012.02.001

Jebril, A. O. (2008). *A screening study on the antimicrobial and hepatoprotective effects of some medicinal plants in Libya* (Doctoral dissertation, MSc. Thesis, Faculty of Science, Al-Fateh University, Tripoli, Libya).

Kaur, P., Singh, N., & Mukherjee, D. (2015). Regulation of membrane leakage and activities of some antioxidant enzymes in petals of cut flowers of Calendula officinalis and Salvia splendens with metabolites and plant growth regulators. *Journal of Applied Horticulture*, *17*(1).

Makkar, H. P., Siddhuraju, P., & Becker, K. (2007). *Plant secondary metabolites* (Vol. 393, pp. 1-122). Totowa, NJ, USA:: Humana Press.

Meliani, N., Dib, M. E. A., Allali, H., & Tabti, B. (2011). Hypoglycaemic effect of Berberis vulgaris L. in normal and streptozotocin-induced diabetic rats. *Asian Pacific journal of tropical biomedicine*, *1*(6), 468-471. https://doi.org/10.1016/S2221-1691(11)60102-0

Mezouar, D., Lahfa, F. B., Djaziri, R., & Boucherit-Otmani, Z. (2014). Évaluation de l'activité antioxydante de Berberis vulgaris L. *Phytothérapie*, *12*(5), 297-301.

Moosmann, B., & Behl, C. (1999). The antioxidant neuroprotective effects of estrogens and phenolic compounds are independent from their estrogenic properties. *Proceedings of the National Academy of Sciences*, *96*(16), 8867-8872.

Parr, A. J., & Bolwell, G. P. (2000). Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. *Journal of the Science of Food and Agriculture*, *80*(7), 985-1012.

Santos-Buelga, C., & Scalbert, A. (2000). Proanthocyanidins and tannin-like compounds–nature, occurrence, dietary intake and effects on nutrition and health. *Journal of the Science of Food and Agriculture*, *80*(7), 1094-1117.

Shahidi, F., & Zhong, Y. (2015). Measurement of antioxidant activity. *Journal of functional foods*, *18*, 757-781. https://doi.org/10.1016/j.jff.2015.01.047

Shekarabi, S. P. H., Mehrgan, M. S., Ramezani, F., Dawood, M. A., Van Doan, H., Moonmanee, T., ... & Kari, Z. A. (2022). Effect of dietary barberry fruit (Berberis vulgaris) extract on immune function, antioxidant capacity, antibacterial activity, and stress-related gene expression of Siberian sturgeon (Acipenser baerii). *Aquaculture Reports*, *23*, 101041. https://doi.org/10.1016/j.aqrep.2022.101041

Wiwekowati, W., Astawa, P., Jawi, I. M., & Sabir, A. (2017). Antioxidant activity of Apis mellifera sp. Propolis extract from Java (Indonesia). *International Research Journal of Engineering, IT and Scientific Research*, *3*(5), 19-24.

Yadnya, T. B., Trisnadewi, A. A., Sukada, I. K., & Oka, I. G. L. (2016). The effect of fermented purple sweet potato (ipomoea batatas l) skin in diets on feed and anthocyanin consumption, carcass characteristics, anthioxidant profile and meat texture of Bali duck. *International Research Journal of Engineering, IT and Scientific Research*, *2*(9), 73-80.

Biography of Authors

Fatna Gougue
She is PhD, Teacher-researcher in Biology at the University of Djelfa, Algeria. Area of interest: medicinal plants and their biological activities.
Email: f.gougue@univ-djelfa.dz

Saad Boutaiba
He is PhD, Professor-researcher in Biology at the University of Djelfa, Algeria. Area of interest: microbiology, medicinal plants, and their biological activities.
Email: s.boutaiba@univ-djelfa.dz