



Phytochemical Screening And Antioxidant Capacity Of *Berberis asiatica* Root

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Abstract: Medicinal plants have bioactive compounds which may be used to curing of various diseases. In the present investigation, a lesser-known medicinal plant *Berberis asiatica* root extracts were studied. Phytochemical screening for the metabolites was accomplished with extracts prepared in five different solvents methanol, ethanol, chloroform, ethyl acetate, and deionized water. The methanolic extract was found good for extraction. Therefore, it was subjected to antioxidant capacity measurement. Phytochemical screening revealed the occurrence of tannins, alkaloids, saponins, and flavonoids and the absence of steroids, cardiac glycosides, anthraquinones, phenols, and terpenoids. Antioxidant measurements showed *Berberis asiatica* root contains significant amounts of antioxidant compounds.

Keywords: *Berberis asiatica* • Phytochemical screening • Antioxidant • Kingoda.

Introduction

Reactive compounds containing free oxygen and nitrogen are termed free radicals. They are considered essential for humans to retain good health and metabolic balance, but their unregulated and additional concentrations may lead to the deterioration of limbs and lead to chronic diseases such as cognitive impairment, macular degeneration, glaucoma, arthritis, osteoporosis, and gastric disorders [Sergun et al., 2022; Pham-Huy et al., 2008]. Different types of antioxidants can play a major role in counterbalancing free radicals. Either by a reduction in their concentration or converting them into another harmless form such as a reduction of H₂O₂ into H₂O; or neutralization of metal ions into molecules [Singh et al., 2004; Sharma et al 2014]. It is also well evident that not only fruits and vegetables but also the other parts of the plants such as root, bark, stem and leaves are good sources of natural antioxidants [James et al., 2014; Adedapo et al., 2014]. Therefore, the free radical scavenging ability or the antioxidant capacity assessment is obvious to the plants. Over-the-counter, Antioxidant based

herbal formulations and dietary supplements are popular these days which primarily contain plant-derived products seeds, root, flowers, leaves, fruits, stem and bark. Medically they are prescribed to manage or suppress disease conditions [Sergun et al., 2022; Mathew et al., 2014; Sacan et al., 2017].

Berberis asiatica is a well-known medicinal plant among the population of the *Garhwal Himalayan* region. Its vernacular names are *kingoda*, *kilmora* and *daruhaldi* [Munesh et al., 2011]. Its fruits are eaten as a dessert. Its decoction is used to cure ear, and eye infections and heal wounds same has been described in older medicinal texts Ayurveda also. Previous ethnomedicinal studies revealed that the tribal population of the *Kumaun* region also use decoction for the same ailments [Joshi et al., 1971]. Tibetans use the same decoction for the cure of gastric disorders, piles and other related complications [Srivastav et al., 2004]. Several studies have reported that the plant contains *berberine* an important medicinal bioactive compound that showed activity against cholera,



diarrhea, malaria, and oriental sore [Singh et al., 2012; The Wealth of India 1988].

It may be concluded from the previous reports that the plant *Berberis asiatica* can be utilized to develop either herbal formulation or supplementary functional food items to manage or suppress liver, skin related disease conditions. Scattered reports legs on the screening of the plant for bioactive compounds such as steroids, cardiac glycosides, etc. that can hinder such beneficial development. Therefore, the present study has been carried out to fill this gap with the objectives to assess the antioxidant capacity and the presence of bioactive compounds in the root of the plant *Berberis asiatica* and the therapeutic potential of the plant can be revealed.

Materials and methods

The plant material was collected from the *Himalayan* forest near the village *Khola*, block *Khirsu* (Longitude: 78.8679886 Latitude: 30.1722334 and Altitude: 1766 m), *Pauri Garhwal*, *Uttarakhand*, India in August 2021. The collected plant root was washed, shade-dried for 15 days and chopped into small pieces, and further dried for another 15 days. Then these small pieces turned into coarse powder of the root using the household grinder and stored in an airtight container till further use.



Figure 1: *Berberis asiatica* leaves

A concise taxonomical depiction of the plant:

It is an evergreen shrub, with a stem possibly 1 – 2 m high and 15 cm in diameter. Its bark may be rough, wrinkled, and a little corked. Hairless twigs or shortly puberulent, pale chromatic. Elliptical leaves 2 - 7 by 2 - 4 cm in the 2D plane, generally accompanied by big distant pointed teeth, dark green accompanied by the noted primary and secondary light reticulate provision. Depicted in Fig. 1

The outer and inner appearance of the root:

Fig. 2 depicts the outer and Fig. 3 inner parts of the root respectively. From the outside it may be corked, dark brown in hue; very hard, rounded, and up to 6 cm or more in diameter. The root bark may be 1 to 1.5 mm thick. The wood underneath is lemon yellow like *Curcuma longa*. Bitter in taste and very hard to crack.



Figure 2: *Berberis Asiatica* root



Figure 3: Outer bark and inner wood of root



Chemicals and Reagents

Iodine crystals (99%), Chloroform (99%), Sulphuric acid (98%), Hydrochloric acid (35%) (Himedia), Methanol (99%), Ethyl acetate (99%), Ethanol (99%) (MERK); Potassium iodide (99%), Ammonia solution (25%), Ferric chloride (98%), Lead acetate (99%), butylated hydroxyl toluene (BHT) (99%), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (99%) (Fisher Scientific); Deionized distilled water (DI water) (Milli Q); Benedict's quantitative reagent (Himedia); freshly prepared Wagner's reagent obtained by dissolving 6 gm of potassium iodide and 2 gm of iodine crystals. Similarly, the ferric chloride solution was prepared by dissolving 2 gm of ferric chloride in 50 ml DI water.

Preparation of root extracts

Previously prepared coarse powder of the root was turned into a fine powder and 5 gm of prepared powder was extracted via five separate solvents (100 ml of each) methanol, ethanol, chloroform, ethyl acetate, and DI water. Each extraction was accomplished through magnetic stirring at the temperature of 75°C for approx. 1 hour. All extracts were preserved in the refrigerator at a temperature of 10°C till further use.

Phytochemical screening

Each of the prepared extracts was treated as per the standard procedures to identify phytochemical components [Markham et al., 1978; Sati et al., 2018].

Screening of the tannins (Ferric chloride test): In a test tube holding 2 ml of the prepared extract was treated with 4-5 drops of freshly prepared ferric chloride solution. The appearance of a brownish-green layer confirmed the presence of tannins.

- 1) **Screening of the alkaloids (Wagner's test):** A test tube filled with 2 ml of extract, and 5-6 drops of Wagner's reagent were added slowly.

The formed reddish-brown acceleration confirmed the acquaint of alkaloids.

- 2) **Screening of the steroids (Salkowski's test):** 2 ml of chloroform was mixed in 2 ml of extract; a further similar volume of concentrated H₂SO₄ was added. The top layer revolved red and the bottom layer revolved yellow with green glare, confirming the presence of steroids.
- 3) **Screening of the saponins:** To identify the existence of saponins, 2 ml of extract was liquefied in 2 ml of Benedict's reagent. Blue-black acquainting indicated the presence of saponins.
- 4) **Screening of the cardiac glycosides (Keller killiami test):** To identify the presence of cardiac glycosides, 2 ml of extract was liquefied with 2 ml of glacial acetic acid and 5% FeCl₃ each in a test tube, after that 1 ml of sulphuric acid was added in it. Deep reddish-brown color confirmed the presence of cardiac glycosides.
- 5) **Screening of the flavonoids (lead acetate solution test):** 2 ml of extract was liquefied with 2 ml of 10% lead acetate. Yellowish green color confirmed the presence of flavonoids.
- 6) **Screening of the anthraquinones:** Firstly, 1 ml of extract was simmered with 1 ml of 10% HCl for a few moments in a water bath. Upon cooling to room temperature, the same amount of chloroform and a few drops of Ammonia solution (10%) were added to it. The rose pink color of the solution confirmed the presence of anthraquinones.



- 7) **Screening of the phenols (Ferric chloride test):** To identify the existence of phenols, 1 ml of extract and 2 ml Milli Q, water were combined in a test tube then a few drops of 10% ferric chloride solution were added in it. The appearance of blue or green color indicated the presence of phenols.
- 8) **Screening of the terpenoids (Salkowski's test):** 2 ml of extract was liquefied with 2 ml of chloroform after that few drops of sulphuric acid were consciously added to it. The presence of reddish brown color specified the presence of terpenoids.

Antioxidant capacity estimation

The antioxidant capacity of prepared methanolic extract was assessed against the standard BHT solution in methanol according to the procedure mentioned by B. Nickavar et al [Nickavar et al., 2012] with slight modification. In brief, firstly, the five concentrations of plant extract 20, 40, 60, 80, and 100 µg/ml were taken in test tubes and made up to 1 ml volume by adding 90% methanol. 1 ml of 0.01 mM DPPH dissolved in 90% methanol was added to all the test concentrations and kept in the dark for 30 minutes, at room temperature. The absorbance of the sample was looked through at 517 nm using a spectrophotometer. Secondly, in separate test tubes, each concentration of plant extract was taken and made up to 1 ml volume by adding 90% methanol. Further, 1 ml of each 0.01 mM DPPH and 0.01 mM BHT solution was

added. The prepared solution was termed the control solution. The absorbance at 517 nm was measured for each control solution. The antioxidant capacity percentage was calculated using the formula given below.

$$\text{Antioxidant capacity (\%)} = \left(\frac{A(\text{Control}) - A(\text{Sample})}{A(\text{Control})} \right) \times 100$$

Where A(Control) and A(Sample) are the absorbance measured for the control and sample solution respectively. To avoid errors in measurements the whole experiment was repeated three times on alternate days and the average of the observed values were used.

Results and discussion

The screening of *Berberis asiatica* root powder was accomplished with the five types of extraction solvents. Table -1 shows the results of different tests carried out on each extraction solvent. Methanol and Chloroform were found to be equally good extraction solvents. The outcomes showed that the crude extracts had tannins, alkaloids, saponins and flavonoids. All possess the antioxidant capacity. Due to the health risks involved with chloroform, the methanolic extract was appropriate for further antioxidant capacity estimation. Results also supported that methanol is a good selection for the descent of bioactive compounds from *Berberis Asiastica* root. Further, the methanolic extract offers the possibility of testing biological actions such as antimicrobial and antitumor effects. Outcomes have shown the presence of vital components having considerable therapeutic values namely tannins, alkaloids, saponins, and flavonoids.

Table 1: Phytochemical examination of *Berberis asiatica* Roots

Phytochemicals	Methanol extract	Ethanol extract	Chloroform extract	Ethyl acetate extract	Aqueous extract
Tannins	+	+	+	+	+



Alkaloids	+	-	+	-	+
Steroids	-	-	-	-	-
Saponins	+	+	+	-	-
Cardiac glycosides	-	-	-	-	-
Flavonoids	+	+	+	-	+
Anthraquinones	-	-	-	-	-
Phenols	-	-	-	-	-
Terpenoids	-	-	-	-	-

+ component is present; - component is absent

Tannins possess many phenolic hydroxyl groups, therefore can be useful in scavenging free radicals (antioxidants), antimicrobial, and improving bowel movements [Santos-Buelga et al., 2000]. Alkaloid can reform the neurotransmitting scheme and thus finds their utilization in the treatment of epilepsy, seizures, Alzheimer and peroxynitrite scavenging [Hussain et al. 2018; Konarth et al., 2013]. Saponins have been reported for the treatment of organ inflammation, tumors, obesity, and diabetes [Yang et al. 2015]. Flavonoids may improve memory. Researchers have reported flavonoids as anti-inflammatory, antioxidant, anti-cold, and anti-cancer [Spencer et al. 2010; Joseph et al. 2011]. Moreover, the compounds that may interfere with the therapeutic potential of beneficial compounds extracted via root extracts namely steroids, cardiac glycosides, anthraquinones, phenols, and terpenoids were absent in all extracts [Khan et al. 2016; Liu et al., 2010; Parida et al. 2019; Proshikina et al. 2020].

Antioxidant capacity may be termed as the capacity to provide an electron or hydrogen atom to the free DPPH [Sies et al., 2007]. By accepting the hydrogen DPPH either gets regenerated into 2,2-diphenyl-1-hydrazine (DPPH-H) or in its reverse conjugate DPPH-R [Njoya et al., 2021]. The results of Antioxidant capacity estimation represented in a percentage that has been calculated from the absorbance are shown in Figure 4. It has been observed that with an increase in the concentration of the root extract, absorbance measured at 517 nm and the antioxidant capacity increases almost linearly. A closer look at Fig. 4 (a) in comparison with Fig. 4 (b) indicates that the root extract at the concentration of 100 µg/ml showed a 72% ±1% antioxidant capacity whereas the BHT at the same concentration showed 68% ±1%. This may be due to the presence of other compounds with a higher ability to scavenge free radicals such as phenols and flavonoids [Asha et al., 2012].

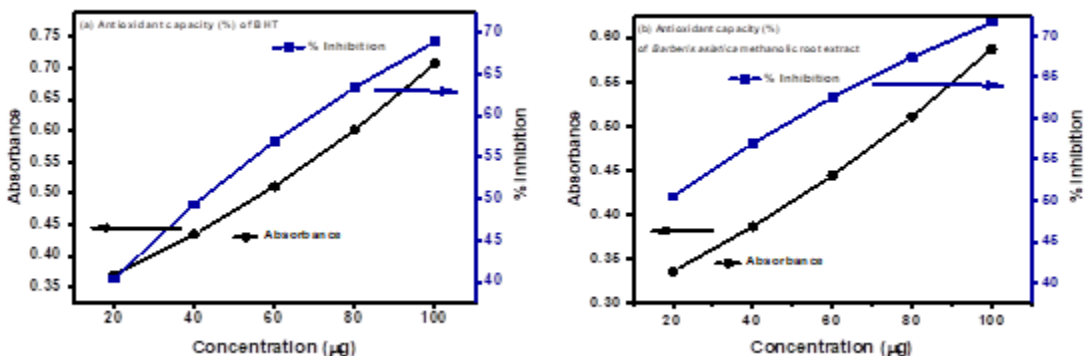


Figure 4: Absorbance measured for different concentrations of methanolic solution (root extract and BHT) and calculated Antioxidant capacity represented as % inhibition. **4 (a)** Antioxidant capacity of BHT solution; **4 (b)** Antioxidant capacity of *Berberis asiatica* root extract

Conclusion

The carried-out work has confirmed the therapeutic potential of *Berberis asiatica*. Methanol is a good extraction solvent for root. Moreover, the root of *Berberis asiatica* is rich in antioxidants, along with secondary metabolites tannins, alkaloids, saponins, and flavonoids. Metabolites that can confer health benefits, particularly steroids, cardiac glycosides, anthraquinones, phenols, and terpenoids were absent in all extracts. A significant presence of beneficial metabolites suggests the great potential of plant utilization in developing herbal formulations. Alternatively, the food industry can use it as an ingredient in supplementary food products for accomplishing nutritional needs and managing disease conditions.

Declaration Statement

The authors declare that they have no conflict of interest.

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References:

- Adedapo, A.A., Jimoh, F.O., Afolayan, A.J. and Masika, P.J., 2009. Antioxidant Properties of the Methanol Extracts of the Leaves and Stems of *Celtis africana*. *Records of Natural Products*, 3(1).
- Asha, K.K., Mathew, S. and Lakshmanan, P.T., 2012. Flavonoids and phenolic compounds in two mangrove species and their antioxidant property.
- Hussain, G., Rasul, A., Anwar, H., Aziz, N., Razzaq, A., Wei, W., Ali, M., Li, J. and Li, X., 2018. Role of plant derived alkaloids and their mechanism in neurodegenerative disorders. *International journal of biological sciences*, 14(3), p.341.
- James, D.B., Sheneni, V.D., Kadejo, O.A. and Yatai, K.B., 2014. Phytochemical screening, and in-vitro antioxidant activities in different solvent extracts of *Vitex doniana* leaves, stem bark and root bark. *American*



- Journal of Biomedical and Life Sciences, 2(1), pp.22-27.
- Joshi, M.C., 1971. An ethnobotanical study of the Kumaon region of India. *Economic botany*, 25(4), pp.414-422.
- Khan, B.A., Mena, F., Reich, A., Caldeira, E. and Bakhsh, S., 2016. Potential phytotherapy of atopic dermatitis, acne, psoriasis, vitiligo.
- Konrath, E.L., Passos, C.D.S., Klein-Júnior, L.C. and Henriques, A.T., 2013. Alkaloids as a source of potential anticholinesterase inhibitors for the treatment of Alzheimer's disease. *Journal of Pharmacy and Pharmacology*, 65(12), pp.1701-1725.
- Liu, T., Brown, D.A. and O'Rourke, B., 2010. Role of mitochondrial dysfunction in cardiac glycoside toxicity. *Journal of molecular and cellular cardiology*, 49(5), pp.728-736.
- Markham, K.R., Ternai, B., Stanley, R., Geiger, H. and Mabry, T.J., (1978). Carbon-13 NMR studies of flavonoids—III: Naturally occurring flavonoid glycosides and their acylated derivatives. *Tetrahedron*, 34(9), pp.1389-1397.
- Mathew, M. and Subramanian, S., 2014. In vitro evaluation of anti-Alzheimer effects of dry ginger (*Zingiber officinale* Roscoe) extract.
- Munesh, K., Rainer, W.B., Joshi, M. and Pramod, K., 2011. Ethnomedicinal uses of plants close to rural habitation in Garhwal Himalaya, India. *Journal of Medicinal Plants Research*, 5(11), pp.2252-2260.
- Nickavar, B. and Esbati, N., 2012. Evaluation of the antioxidant capacity and phenolic content of three *Thymus* species. *Journal of Acupuncture and Meridian Studies*, 5(3), pp.119-125.
- Njoya, E.M., 2021. Medicinal plants, antioxidant potential, and cancer. In *Cancer* (pp. 349-357). Academic Press.
- Joseph, A., Mathew, S., Skaria, B.P. and Sheeja, E.C., 2011. Medicinal uses and biological activities of *Argyrea speciosa* sweet (Hawaiian baby woodrose)-an overview.
- Parida, R.C. and Ghosh, P.K., 2019. *Cassia occidentalis*: Medicinal or Poisonous?.
- Pham-Huy, L.A., He, H. and Pham-Huy, C., 2008. Free radicals, antioxidants in disease and health. *International journal of biomedical science: IJBS*, 4(2), p.89.
- Proshkina, E., Plyusnin, S., Babak, T., Lashmanova, E., Maganova, F., Koval, L., Platonova, E., Shaposhnikov, M. and Moskalev, A., 2020. Terpenoids as potential geroprotectors. *Antioxidants*, 9(6), p.529.
- Sacan, O., Akev, N. and Yanardag, R., 2017. In vitro inhibitory effect of *Aloe vera* (L.) Burm. f. leaf extracts on the activity of some enzymes and antioxidant activity.
- Santos-Buelga, C. and 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), pp.1094-1117.



- Sati, A., Sati, S.C., Negi, J.S. and Sati, O.P., (2018). Mineral Composition of *Berberis aristata* DC Roots. *Analytical Chemistry Letters*, 8(3), pp.361-367.
- Sergun, V., Gorbushina, I., Valentina, B., Poznyakovsky, V., Tokhiriyon, B. and Lapina, V., 2022. Plant-Based Dietary Supplements and Antler Products for Prevention and Treatment of Age-Related Diseases: Efficacy Study. *International Journal of Pharmaceutical Research & Allied Sciences*, 11(3).
- Sergun, V., Gorbushina, I., Valentina, B., Poznyakovsky, V., Tokhiriyon, B. and Lapina, V., 2022. Plant-Based Dietary Supplements and Antler Products for Prevention and Treatment of Age-Related Diseases: Efficacy Study. *International Journal of Pharmaceutical Research & Allied Sciences*, 11(3).
- Sharma, N., 2014. Free radicals, antioxidants and disease. *Biology and Medicine*, 6(3), p.1.
- Sies, H., 2007. Total antioxidant capacity: appraisal of a concept. *The Journal of nutrition*, 137(6), pp.1493-1495.
- Singh, R., Tiwari, S.S., Srivastava, S. and Rawat, A.K.S., 2012. Botanical and phytochemical studies on roots of *Berberis umbellata* Wall. ex G. Don.
- Singh, R.P., Sharad, S. and Kapur, S., 2004. Free radicals and oxidative stress in neurodegenerative diseases: relevance of dietary antioxidants. *J Indian Acad Clin Med*, 5(3), pp.218-225.
- Spencer, J.P., 2010. The impact of fruit flavonoids on memory and cognition. *British Journal of Nutrition*, 104(S3), pp.S40-S47.
- Srivastava, S.K., Singh Rawat, A.K. and Mehrotra, S., 2004. Pharmacognostic evaluation of the root of *Berberis asiatica*. *Pharmaceutical biology*, 42(6), pp.467-473.
- The Wealth of India: A Dictionary of Indian Raw Materials and Industrial Products—Raw Materials Series, Publications and Information Directorate, CSIR, New Delhi, India, Revised Series, 1988, Vol. 2B, pp. 114-118.
- Yang, Y., Lee, J., Rhee, M.H., Yu, T., Baek, K.S., Sung, N.Y., Kim, Y., Yoon, K., Kim, J.H., Kwak, Y.S. and Hong, S., 2015. Molecular mechanism of protopanaxadiol saponin fraction-mediated anti-inflammatory actions. *Journal of Ginseng Research*, 39(1), pp.61-68.