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RESEARCH ARTICLE

Evaluation of total phenolic and flavonoid content and antioxidant activity of *Ophiorrhiza ochroleuca* Hook.f.

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Abstract

The present study was carried out to evaluate total phenolic content, total flavonoid content, and antioxidant activity from methanolic extracts of the stem and leaves of *Ophiorrhiza ochroleuca* Hook.f. (Rubiaceae). The total phenolic content of both leaf and young stem was determined using the Folin-ciocalteu reagent method. Leaf displayed the highest phenolic content (43.97 ± 0.71 mg gallic acid equivalent (GAE)/g of dry sample extract followed by the stem with 16.24 ± 0.76 mg GAE/g). while the highest flavonoid content was observed in leaf extract with the result 90.40 ± 1.20 mg rutin equivalent (RE)/g of dry sample. The antioxidant activity was determined using DPPH (2,2-dyphenyl-1-picrylhydrazyl) assay with ascorbic acid as standard (IC_{50} value 6.65 ± 0.22). Leaf reported the highest antioxidant activity with IC_{50} value 508.81 ± 17.60 µg/ml followed by the stem with IC_{50} value of 581.62 ± 45.22 µg/ml.

Keywords: Phenolic Content; Flavonoid Content; Antioxidant; *Ophiorrhiza ochroleuca*

1. Introduction

The free radicals are highly reactive and unstable with a lone unpaired electron. The generation of highly reactive free radicals such as reactive oxygen species (ROS), reactive nitrogen species (RNS), super-oxides, etc. can induce oxidative stress within our body. The immune system of our body protects cells from oxidative damage and immature aging conditions nevertheless the accumulation of high levels of free radicals can change the membrane fluidity and plays a major role in introducing various metabolic disorders, numerous neurodegenerative diseases, cancer, aging, cellular injury, cardiovascular and renal disorders (Aryal et al., 2019; Preethamol and Thoppil, 2020). The antioxidant system present in our body scavenges these free radicals by pairing with its lone unpaired electron and regulates the oxidation and anti-oxidation reactions (Xu et al., 2017). But in the present time high alcohol consumption, smoking, exposure to radiation, and environmental toxins induce the production of ROS and RNS in excessive amounts that can disrupt the balance between oxidation and anti-oxidation processes within our body (Li et al., 2015). The human body can obtain antioxidants from natural food sources such as medicinal plants, fruits, vegetables, spices, mushrooms, etc. in their daily diet. The natural antioxidants are mainly polyphenolic compounds (phenolics, flavonoids, anthocyanins, carotenoids, vitamins) (Cai et al., 2014). So, in recent times, analysis of phenolics and flavonoids with antioxidant activity has been studied in nutrition and food science. Phenolic compounds are good electron donors and can directly contribute to the anti-oxidation reaction (Lee et al., 2015; Aryal et al., 2019).

The genus *Ophiorrhiza* L. is widely distributed in tropical wet forests to subtropical Asia, Australia, New Guinea, and the Pacific Islands, with more than 400 species (Schanzer, 2005). A total of 52 species have been recorded from India (Nayar, 2014), hitherto with addition to *Ophiorrhiza ripicola* Craib to the flora of India from Arunachal pradesh the species increased to 53 species, and out of 53 species 25 species are endemic to India 6 species from Northeast India (Hareesh et al., 2015, 2017), however, with the addition of one species i.e. *Ophiorrhiza ripicola* Craib, and nevertheless a total of 8 species from Assam (Brooah and Ahmed, 2014), Consequently the number of *Ophiorrhiza* L. species will be a total of 9 species from North East India. The genus *Ophiorrhiza* L. The genus *Ophiorrhiza* (Rubiaceae) has wide applications in traditional medicine, The majority of the species are used for the treatment of wounds, inflammation, body pain, cancer, bacterial infections, snakebite, etc. (Krishnan et al., 2015; Taher et al., 2020), and several species have been revealed the presence of secondary metabolites like alkaloids, flavonoids phenols, terpenoids, steroids, etc., in addition, several biological activities, including anticancer, anti-fungal, anti-malarial, cytotoxic activity, and antiviral activity obtained from the extract plants parts (Aryal et al., 2019; Preethamol and Thoppil, 2020). although assessment of the total phenolic content, total flavonoid content, and antioxidant activity of *Ophiorrhiza ochroleuca* Hook.f. have no reported in earlier works, therefore, the present study was aimed to assess the total phenolic content, total flavonoid content, and antioxidant activity of *Ophiorrhiza ochroleuca* Hook.f.

2. Material and method

2.1. Collection and identification of plant samples

Through the critical observation and morphological study of the collected plant samples and compared with the prologue and digitalized herbarium of Kew herbarium and digitalized herbarium Specimens of JSTOR Global Plants, the plant samples were identified

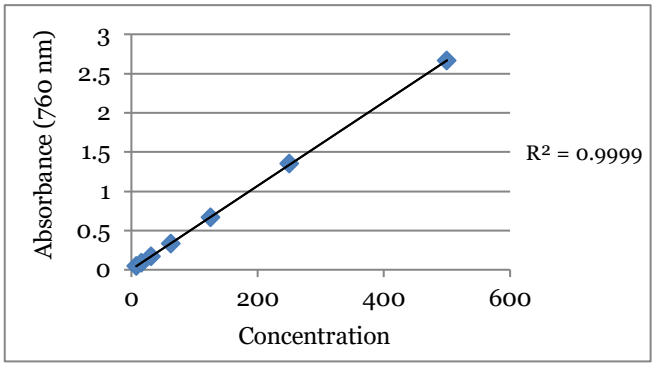

Figure 2. Gallic acid (concentration v/s absorption) calibration curve (TPC).

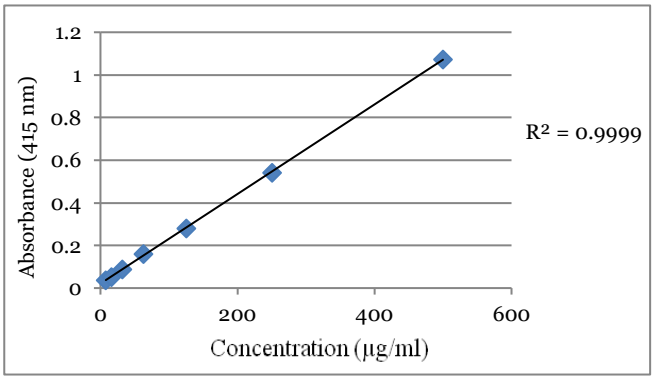

Figure 3. Rutin (concentration v/s absorption) calibration curve (TFC)

as *Ophiorrhiza ochroleuca* Hook.f. The plant sample was collected from the forest margin of the Tinsukia district and planted in the college botanical garden for ex-situ conservation and the multiplication of the plant species was done. However, for estimation of phenolic and flavonoid content and antioxidant activity, the separate plant was planted in a polyhouse and collected for analysis.

2.2. Preparation of extract

The extract was prepared using a cold maceration process. After collection leaf and stem were separated, washed properly to remove visible dust, shade dried, grounded into powder, and macerated in 95% methanol for 7 days. Filter the samples after 7 days of maceration and concentrate the filtrates using a rotary evaporator and keep the concentrated extract at cold temperature for further phytochemical analysis.

2.3. Estimation of total Phenolic Content (TPC)

The total phenolic content of the sample was estimated using Folin-Ciocalteu reagent method with little modification (Singleton et al., 2019). Gallic acid was taken as standard. Briefly, 100µl of samples (1mg/ml in methanol), blank (methanol), and standard in different concentrations were mixed with 500 µl of Folin-ciocalteu reagent. After 5 minutes incubation at room temperature 400 µl of 7.5 % sodium carbonate was added and further incubated for half an hour at room temperature. The absorbance was taken at 760 nm using a Thermo Scientific Multiscan Spectrophotometer against blank. Results are calculated from the gallic acid calibration curve and expressed as milligrams (mg) of gallic acid equivalent per gram (g) of the dry sample extract.

2.4. Estimation of total Flavonoid Content (TFC)

The total flavonoid content of the sample was estimated by using aluminum chloride colorimetric method (Goswami et al., 2023). Rutin was taken as standard. Briefly, 25 µl of sample (1mg/ ml, in methanol), blank and standard (different concentrations, 500 – 7.81 µg/ml range) were mixed with 100µl of distilled water followed by the addition of 15 µl of 10 % $AlCl_3$ solution and incubated the mixtures for 5 minutes at room temperature. After 5 minutes, 50 µl of 1M sodium acetate solution was added. After 40 minutes of incubation at room temperature absorbance was measured at 415 nm using Thermo Scientific Multiscan Spectrophotometer. The results are calculated from the rutin calibration curve and expressed as milligram (mg) rutin equivalent per gram (g) of dry sample.

Figure 1. *Ophiorrhiza ochroleuca* (A) Seedling stage (B) Flowering stage.

2.5. Antioxidant activity

The antioxidant activity was determined by using DPPH (2,2-dyphenyl-1-picrylhydrazyl) radical scavenging earlier method (Cai et al., 2014; Xu et al., 2017), with slight modification. 0.1 mM DPPH stock solution was prepared in methanol. 200 µl DPPH solution mixed with 100 µl sample (different concentrations 2000 – 31.25 µg/ml range), standard ascorbic acid (in different concentrations 100 – 1.56 µg/ml range) and control (DPPH + methanol) in a 96 well plate. Incubate the sample mixture in the dark for 30 minutes at room temperature and absorbance was taken at 517 nm using a Thermo Scientific Multiscan Spectrophotometer. The radical scavenging activity was calculated using the formula:

$$\text{Inhibition \%} = \frac{Control\ absorbance - sample\ absorbance}{Control\ absorbance} \times 100$$

Results are expressed as IC_{50} (50 % inhibition concentration by the sample) value.

3. Results

3.1. Total phenolic and flavonoid content

The total phenolic content of *Ophiorrhiza ochroleuca* was calculated from the regression equation of gallic acid calibration curve (y = 0.0053x + 0.0033) and results are expressed as milligram gallic acid equivalent per gram of dry plant sample extract (mgGAE/g). The total phenolic content of methanolic crude extract of leaf and stem of *O. ochroleuca* is to be found 43.97 ± 0.71 mgGAE/g and 16.24 ± 0.76 mgGAE/g dried extract respectively. Figure 2 depicts the concentration v/s absorbance curve of standard gallic acid for determination of Total Phenolic Content (TPC) of samples.)

The total flavonoid content of *O. recurvipetala* was calculated from the regression equation of rutin calibration curve (y = 0.0021x + 0.0211) and results are expressed as milligram rutin equivalent per gram of dry plant sample extract (mgRE/g). The total flavonoid content of methanolic crude extract of the leaf and stem of *O. ochroleuca* is to be found 90.40 ± 1.20 mgRE/g and 20.86 ± 3.27 mgRE/g dried extract respectively. Figure 3 depicts the concentration v/s absorbance curve of standard rutin for determination of Total Flavonoid Content (TFC) of samples.)

3.2. Antioxidant activity

The antioxidant activity of methanolic leaf and stem extracts of *O. ochroleuca* was determined by one assay only – DPPH radical scavenging assay. The main principle of this assay is the capacity to inhibit 50% (IC_{50}) of DPPH free radical by the crude extracts. Results are expressed as IC_{50} value and compared with standard ascorbic acid (IC_{50} = 6.65 ± 0.22). Lower the value of IC_{50} higher will be the antioxidant activity i.e. radical scavenging potentiality by the crude sample. Both leaves showed higher radical scavenging activity with IC_{50} value of 508.81 ± 17.60 µg/ml followed by the stem with IC_{50} 581.62 ± 45.22 µg/ml.

4. Discussion

The Rubiaceae family is renowned for its ability to generate bioactive substances like iridoids, indole alkaloids, anthraquinones, terpenoids, flavonoids, and various other forms of phenolic compounds. These compounds exhibit notable pharmacological effects, contributing to the family's significant pharmacological profile (Martins D and Nunez, 2015). Scientific literature on Ophiorrhiza plants highlights their extensive presence throughout Asia and its neighboring regions, where they have been traditionally employed in medicinal practices to combat a range of ailments including cancer, viral infections, and microbial diseases (Taher etal., 2020). Several species of *Ophiorrhiza* such as *O. radicans, O. mungos, and O. nicobarica*, have been found to contain significant quantities of alkaloids, flavonoids, and terpenoids. (Chattopadhyay et al., 2007; Krishnan et al., 2014; Prabha and Karuppusamy, 2018; Taher etal., 2020;). The antioxidant activity, total phenolic content and total flavonoid content of many species of Ophiorrhiza like *O. pectinata* (Preethamol and Thoppil, 2020), *O. jacobii* (Preethamol and Thoppil, 2022), *O. mungos* (Krishnakumar etal., 2012) have also been assessed, yielding promising results.

5. Conclusion

Phenolic compounds (phenolic acid, flavonoid, etc.) are bioactive compounds that have health benefits. These compounds have antioxidant, anti-inflammatory, anti-microbial, etc. properties. Earlier studies on plants of the Genus *Ophiorrhiza* reveal that it possesses anti-tumor and anti-cancer activities. The plants of the Genus contain an important secondary metabolite called Camptothecin. The present studies on methanolic crude leaves and stem extract of *O. ochroleuca* show the presence of phenolic and flavonoids with a maximum in the leaves and radical scavenging activity (anti-oxidant properties). Further, detailed studies on the phytochemicals, their extraction and isolation, molecular structures, role in the biochemical pathways, and mechanism of actions with special emphasis on Camptothecin an important potent anti-cancer plant-derived metabolite are needed using suitable biochemical and molecular analytical techniques.

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Authors contributions
All the authors have equally contributed in research design, data generation and manuscript draft.

Conflicts of interest
The authors declare that they have no conflict of interest.

Reference

Aryal S, Baniya MK, Danekhu K, Kunwar P, Gurung R, and Koirala N. 2019. Total Phenolic Content, Flavonoid Content and Antioxidant Potential of Wild Vegetables from Western Nepal. Plants (Basel) 8(4): 96.

Brand-Williams, W, Cuvelier ME, Berset C. 1995. Use of a free radical method to evaluate antioxidant activity. LWT-Food Science and Technology 28: 25-30.

Brooah C and Ahmed I. 2014. *Plant Diversity of Assam (A checklist of Angiosperms & Gymnosperm)*. Director of Assam Science Technology and Environment Council, Govt of Assam, India.

Cai Y, Luo Q, Sun M and Corke H. 2014. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sciences 74(17): 2157–2184.

Chattopadhyay D, Das S and Mandal AB. 2007. Arunachalam G, Bhattacharya SK. Evaluation of analgesic and anti-inflammatory activity of *Ophiorrhiza nicobarica*, an ethnomedicine from Nicobar Islands, India. Advances in Traditional Medicine 7 (4): 395-408.

Goswami MJ, Dutta U, Tage S, Bharali SJ, Yanka H, Tag H, Bharali P, Kakati D.2023. Antioxidant and Antidiabetic Properties of Extracts from Three Underutilized Food Plants of North East India. Chem. Biodiversity 20 (2): e202200718.

Hareesh VS, Joe A and Sabu M. 2017. *Ophiorrhiza debiana* (Rubiaceae, Rubioideae), a new species from North-East India 309 (3): 291-294.

Hareesh VS, Sreekumar VB, Prabhukumar KM, Sabu M and Sreejith KA. 2015. Lectotypification of *Ophiorrhiza heterostyla* Dunn and the new record of O. *rugosa* var. *angustifolia* (Thwaites) Ridsdale (Rubiaceae) for India. Webbia 70: 109-112

Krishnakumar G, Rameshkumar KB, Srinivas P, Satheeshkumar K and Krishnan PN. 2012. Estimation of camptothecin and pharmacological evaluation of *Ophiorrhiza prostrata* D. Don and *Ophiorrhiza mungos* L. Asian Pacific Journal of Tropical Biomedicine 2(2): S727-31.

Krishnan SA, Dileepkumar R, Nair AS and Oommen OV. 2014. Studies on neutralizing effect of *Ophiorrhiza mungos* root extract against Daboia russelii venom. Journal of Ethnopharmacology 151(1): 543-7.

Krishnan SA, Dileepkumar R, Nair AS and Oommen OV. 2015. Studies on neutralizing effect of *Ophiorrhiza mungos* root extract against Daboia russelii venom. Journal of Ethnopharmacology 151(1): 543-7.

Lee YH, Choo C, Watawana MI, Jayawardena N and Waisundara VY. 2015. An appraisal of eighteen commonly consumed edible plants as functional food based on their antioxidant and starch hydrolase inhibitory activities. Journal of the Science of Food and Agriculture 95(14): 2956–2964.

Li S, Tan HY, Wang N, Zhang ZJ, Lao L, Wong CW and Feng Y. 2015. The Role of Oxidative Stress and Antioxidants in Liver Diseases. International Journal of Molecular Sciences 16(11): 26087–26124.

Martins D and Nunez CV. 2015. Secondary metabolites from Rubiaceae species. Molecules 20(7): 13422-95.

Midhu CK, Hima S, Binoy J and Satheeshkumar K. 2019. Influence of incubation period on callus tissues for plant regeneration in Ophiorrhiza pectinata Arn. through somatic embryogenesis. Proceedings of the National Academy of Sciences 89.

Nayar TS. Beegam AR and Sibi M. 2014. *Flowering Plants of the Western Ghats India, Dicots*. Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Kerela, India.

Prabha G and Karuppusamy S. 2018. Phytochemical profile and radical scavenging activity of alcoholic extract of *Ophiorrhiza radicans* Gardner (Rubiaceae)—A rare plant of Southern Western Ghats of India. BioScience Trends 11: 1572-1576

Prabha G and Karuppusamy S. 2018. Phytochemical profile and radical scavenging activity of alcoholic extract of Ophiorrhiza radicans Gardner (Rubiaceae)-a rare plant of southern Western Ghats of India. Trends in Biosciences 11 (7): 1572-1576

Preethamol SN and Thoppil JE. 2020. Phenolic Content, Flavonoid Content and Antioxidant Potential of Whole Plant Extract of *Ophiorrhiza pectinata* Arn. Indian Journal of Pharmaceutical Sciences 82(4): 712-718.

Preethamol SN and Thoppil JE. 2022. Antioxidant Potential and Chemical Composition of Ophiorrhiza jacobii: A Promising Medicinal Plant Endemic to Western Ghats. Indian Journal of Pharmaceutical Sciences 1 (84): 2.

Preethamol SN and Thoppil JE.2020. Phenolic Content, Flavonoid Content and Antioxidant Potential of Whole Plant Extract of Ophiorrhiza pectinata Arn. Indian Journal of Pharmaceutical Sciences 1(82): 4.

Schanzer IA. 2005. Three new species of Ophiorrhiza (Rubiaceae–Ophiorrhizeae) from Thailand. Thai Forest Bulletin 33: 161-170.

Singleton VL, Orthofer R and Lamuela-Raventós RM. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. In: Jameson D, Verma M, Westerhoff HV, editors. Methods in Enzymology: Academic Press 299: 152-178.

Taher M, Shaari SS, Susanti D, Arbain D and Zakaria ZA. 2020. Genus Ophiorrhiza: A Review of Its Distribution, Traditional Uses, Phytochemistry, Biological Activities and Propagation. Molecules 25(11): 2611.

Taher M, Shaari SS, Susanti D, Arbain D and Zakaria ZA. 2020. Genus Ophiorrhiza: A review of its distribution, traditional uses, phytochemistry, biological activities and propagation. Molecules 25(11): 2611.

Xu DP, Li Y, Meng X, Zhou T, Zhou Y, Zheng J, Zhang JJ and Li HB. 2017. Natural Antioxidants in Foods and Medicinal Plants: Extraction, Assessment and Resources. International Journal of Molecular Sciences 18(1): 96.