Characterization of Brassinosteroid Isolated from Bacopa monnieri L. and their Free Radical Scavenging Activity

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Abstract: Brassinosteroids are a steroidal plant growth hormone. Recent reports show their stress protective potential like antiviral, antitumor and antigenotoxic properties. In the present work, an objective was made to isolate 24-epibrassinolide from Bacopa monnieri L. leaves which was characterized by UPLC analysis. The UPLC data of isolated BRs was compared with the standard. The antioxidant activity of isolated BRs were also investigated through in vitro model systems such as 1,1-diphenyl-2-picryl hydrazyl (DPPH), reducing power and total antioxidant capacity scavenging assays.

Keywords: Brassinosteroids, Ultra performance liquid chromatography, Antioxidant Activity, Bacopa monnieri

1. Introduction

Brassinosteroids are growth promoting hormone which ubiquitously distributed in plant kingdom. They are a class of polyhydroxysteroids that structurally related to their animal counterparts. These compounds are classified as C₂₇, C₂₈ or C₂₉ types depending on length of their side chain [1]. Besides other phytohormones, the presence of brassinosteroids (BRs) have been reported in a number of families like Brassicaceae [2], Arecaceae [3], Gramineae [4], Liliaceae [5], Typhaceae [6] and some member of Scrophulariaceae [7], but no report is available regarding their presence in Bacopa monnieri, a medicinal herb. Bacopa monnieri is widely used revitalizing herb that strengthens nervous function and also possesses antiepileptic anti-inflammatory antioxidative, and properties. Keeping in mind the medicinal importance of Bacopa monnieri and presence of BRs in plant kingdom, the present study was attempted to isolate and characterize 24-epibrassinolide from B. monnieri leaves and determine their free radical scavenging activity.

2. Material and Methods

Fresh leaves of Bacopa monnieri L. was collected from Sunrise Nursery, Pune, Maharashtra, India for extraction, isolation and characterization of 24-epibrassinolide. Analytical grade chemicals were purchased from Fisher Scientific (UK) and Merck (Germany). High performance liquid chromatography (HPLC) grade chemicals and standards, acetonitrile, 24-epibrassinolide was obtained from Sigma Aldrich (UK). Ultrapure water used was purified using the Milli-Q-plus filter system by Millipore (USA).

2.1 Extraction, Purification and Bioassay

Fresh leaves of Bacopa monnieri L. (500g) was ground by using liquid nitrogen and extracted in 80% methanol (3x500ml). The methanol extract was dried in vacuum using rotary evaporator (Buchi R-210, Switzerland) and partitioned between CHCl₃ and water. The CHCl₃ extract

was further partitioned between 80% MeOH and nhexane. The resulting 80% MeOH extract was again dried under vacuum and re-dissolved in methanol and used for further purification. The methanolic extract of B. monnieri L. was subjected to silica gel (60-120 mesh) column chromatography two times with step-gradient elution of MeOH in CHCl₃ i.e 0%, 2%, 5%, 7%, 10%, 15%, 20%, 40%, 60% and 100% of methanol in chloroform (each 150 ml). All the fractions were subjected to radish hypocotyl bioassay to find bioactive fraction [8]. The biologically active fractions were then purified with Sephadex LH-20 (Fig.1). Further, these active fractions were pooled, concentrated and subjected to HPLC for purification.

2.2 Characterization of Brassinosteroids

The bioactive fractions obtained from HPLC column chromatography and standard EBL was dried and derivatized using methaneboronic acid and subjected to UPLC (Shimadzu Lab solution). The derivatized sample was dissolved in acetonitrile and further used for characterization. The UPLC separation was performed following the method of Huo et al. (2012) using analytical column C18 (2 µm, 2.1 mm x100 mm).

2.3 In vitro studies for free radical scavenging activity:

The radical scavenging activity of the isolated BRs was determined by using the DPPH (1, 1-diphenyl-2 picrylhydrazyl) assay as described by [9] with a slight modification. The DPPH radical scavenging activity was expressed as:

% DPPH scavenging activity (%) = [(Abs control - Abs sample)] / (Abs control)] \times 100, where Abs control is the absorbance of DPPH + DMSO; Abs sample is the absorbance of DPPH radicals + sample extract.

The ferric reducing power of plant extract was determined using a modified version of the FRAP assay of Benzie and Strain [10]. This method is based on the reduction, at low pH, of a colorless ferric complex (Fe³⁺⁻tripyridyltriazine)

to a blue-colored ferrous complex (Fe²⁺ tripyridyltriazine) by the action of electron-donating antioxidants. The reduction is monitored by measuring the change of absorbance at 593 nm. A standard curve was prepared using various concentrations of FeSO₄ × 7H₂O. In this assay, the reducing capacity of the extracts tested was calculated with reference to the reaction signal given by a Fe²⁺ solution. FRAP values were expressed as mmol Fe^{2+/}g of sample.

3. Results

3.1 Isolation and characterization of EBL

Bioactivity of the purified fractions after each column chromatography was measured by radish hypocotyls bioassay. In first silica gel column 5, 7, 10, 20, 60 and 100 % fractions were found active respectively (Fig. 1A). These fractions were pooled and passed through second silica gel column which revealed bioactivity in 5, 10, 15, 20 and 60 % fractions (Fig. 1A) and these fractions were pooled and subjected to purification by Sephadex LH-20 column. In Sephadex LH-20, all the fractions were found biologically active (Fig. 1B). The HPLC purified fractions were analysed by UPLC for the presence of EBL. Our results showed the presence of active EBL compound in *B. monnieri* leaves. This was confirmed when compared with retention time of the standard EBL (Fig. 2A & B).

DPPH radical scavenging activity is one of the most widely used method for screening the antioxidant activity of plant extract. In the present study extract of isolated BRs was found to possess significant DPPH radical scavenging activity with an IC₅₀ value of 0.65 mg/ml (Fig. 3A) against the standard ascorbic acid. The straight relationship among antioxidant activity and reducing power of plant extracts were reported. However, with the increasing concentration of plant extract, the reducing power increased (Fig.4).

4. Discussion

Since brassinosteroids (BRs) were first isolated and purified from Brassica napus pollen in 1979 by Grove et al. [11] and widely recognized as a new phytohormone in the 1990s. Earlier reports showed that brassinosteroids are presented ubiquitously in whole plant kingdom [1]. But till now there are no report on isolation of BRs from Bacopa monnieri L., an important Indian medicinal herb. Therefore the present piece of work led to the isolation and characterization of EBL (one of bioactive compound) from B. monnieri. Our results revealed the presence of EBL in B. monnieri leaves after characterising with UPLC. The reported compound belongs to the C_{28} group of BRs. They are intermediates of the early and late C-6 oxidation pathway for synthesis of BL. The C₂₈ BRs carrying either a-methyl, b-methyl or methylene group might be derived from campesterol, dihydrobrassicasterol or 24-methylene-cholesterol [12]. Bhardwaj et al [13] also characterized brassinosteroid compounds from different plant species like Camellia sinensis (L.) O. Kuntze, Aegle marmelos Corr. (Rutaceae) and Centella asiatica [7].

In addition to this, radical scavenging assays performed were demonstrated strong impact of EBL on the abilities to scavenge ROS. Reduced values of DPPH and reducing power assays were recorded for different concentration of isolated BRs (Fig. 3 & 4). It is possible that the phytochemicals present in the extract/fractions provide the essential factor as a radical scavenger and are responsible for the high scavenging activity. The above assays confirmed the antioxidant potential of the plant. According to some previous reports, the brassinosteriod compounds are responsible for the antioxidant activity [14]. All biological systems have devised unique strategies to combat oxidative stress, an outcome of excessive ROS. Active participations brassinosteroid on antioxidant system, PC, MT and several osmolytes in metal detoxification have been widely established [14, 15]. BRs have become a popular tool for oxidative stress amelioration [16].

5. Conclusions

Our study evidently showed the presence of biologically active plant steroidal compound 24-epibrassinolide in the leaf extract of *Bacopa monnieri* L. Their presence was confirmed after UPLC analysis of standard compound. In addition, anti-stress and antioxidant potential of 24epibrassinolide was seen by studying the ROS scavenging assays. Our results confirmed from the results that they have antioxidant potential.

6. Future Scope of the Study

The compound isolated from Bacopa monnieri, in future may serve the model compound as for formulating/designing the drugs to treat various diseases associated with free radicals induced damage like cancer, diabetes etc. Further progress and research will be required for making clear the active principles and for substantiating the claim as presented in this research work. Dietary intake of the food rich in these steroidal compounds can cure steroidal imbalances in the body which particularly cause prostrate and breast cancers. The present work is in step in this direction, which would explore the potential of brassinosteroids and related compounds from these medicinal plants as for their modulatory role against antioxidative, anticancer and their eventual use as drug therapeutics.

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References

- [1] A. Bajguz, A. Tretyn, "The chemical characteristic and distribution of brassinosteroids in plants," Phytochemistry, 62, pp. 1027-1046, 2003.
- [2] M.K. Kanwar, R. Bhardwaj, P. Arora, S.P. Chowdhary, P. Sharma, S. Kumar, "Plant steroid hormones produced under Ni stress are involved in the regulation of metal uptake and oxidative stress in *Brassica juncea* L.," Chemosphere, 86 pp. 41–49, 2012.
- [3] A.K. Zaki, J. Schmidt, F.M. Hammouda, G. Adam, "Steroidal constituents from pollen grains of *Phoenix dactylifera*," Planta Medica, 59, pp. 613, 1993.
- [4] P.E. Taylor, K. Spuck, P.M. Smith, J.M. Sasse, T. Yokota, P.G. Griffiths, D.W. Cameron, "Detection of brassinosteroids in pollen of *Lolium perenne* L. by immunocytochemistry" Planta, 189, pp. 91– 100,1993.
- [5] E. Yasuta, T. Terahata, M. Nakayama, H. Abe, S. Takatsuto, T. Yokota, "Free and conjugated brassinosteroids in the pollen and anthers of *Erythronium japonicum* Decne." Bioscience, Biotechnology and Biochemistry, 59, pp.2156–2158, 1995.
- [6] J.A. Schneider, K. Yoshihara, K. Nakanishi, N. Kato, "Typhasterol (2-deoxycastasterone): a new plant growth regulator from cat-tail pollen," Tetrahedron Letter, 24, pp.3859–3860, 1983.
- [7] N. Sondhi, R. Bhardwaj, S. Kaur, M. Chandel, N. Kumar, B. Singh, "Inhibition of H2O2-induced DNA damage in single cell gel electrophoresis assay (comet assay) by castasterone isolated from leaves of *Centella asiatica*," Health, 2, pp. 595-602, 2010.
- [8] S. Takatsuto, N. Yazawa, N. Ikekawa, T. Takematsu, Y. Takeuchi, M. Koguchi, "Structure-activity relationship of brassinosteroids," Phytochemistry, 22, pp. 2437–2441, 1983.
- [9] L.I. Mensor, F.S. Menezes, G.G. Leitao, A.S. Reis, T. dos Santos, C.S. Coube, S.G. Leitao, "Screening of Brazillian plant extracts for antioxidant activity by the use of DPPH free radical method," Phytotherapy Research, 15, pp. 127-130, 2001.
- [10] I.F.F. Benzie, J.J. Strain, "The Ferric Reducing Ability of Plasma (FRAP) as Measure of 'Antioxidant Power': The FRAP Assay," Analytical Biochemistry, 239, pp. 70-76, 1996.
- [11] M.D. Grove, G.F. Spencer, W.K. Rohwedder, N.B. Mandava, J.F. Worley, J.D. Warthen, G.L. Steffens, J.L. Flippen-Anderson, J.C. Cook, "Brassinolide, a plant growth-promoting steroid isolated from *Brassica napus pollen*," Nature, 281, pp. 216–217, 1979.
- [12] T. Yokota, "Chemical structures of plant hormones," Chemical Regulation Plants, 30, pp.185–196.
- [13] R. Bhardwaj, S. Kaur, P.K. Nagar, H.K. Arora, "Isolation and characterization of brassinosteroids from immature seeds of *Camellia sinensis* (O) Kuntze," Plant Growth Regulation, 53, pp. 1–5, 2007.
- [14] S.P. Choudhary, M. Kanwar, R. Bhardwaj, J.O. Yu, L. S. P. Tran, "Chromium Stress Mitigation by

Polyamine-Brassinosteroid Application Involves Phytohormonal and Physiological Strategies in *Raphanus sativus* L.," PLoS ONE, 7, e33210. doi:10.1371/journal.pone.0033210, 2012.

- [15] C.M. Andre, L. Yvan, E. Daniele, "Dietary antioxidants and oxidative stress from a human and plant perspective: a review," Current Nutrition and Food Science, 6, pp. 2–12, 2010.
- [16] G.E. Gudesblat, E. Russinova, "Plants grow on brassinosteroids," Current Opinion of Plant Biology," 14, pp. 530–537, 2011.

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Fig.1 A Biological active fractions of *B. monnieri* L. after purifying by silica gel I & II (A) Sephadex LH-20 (B) chromatography employing radish hypocotyls bioassay



Fig.2 UPLC analysis chromatogram (280nm) of 24-Epibrassinolide in purified *B. monnieri* L. extract (A) and standard 24-Epibrassinolide (B).

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Fig.3 DPPH radical scavenging activity A) Isolated BRs extract of *B. monnieri* b) Standard Ascorbic acid



Fig.4 DPPH radical scavenging activity A) Isolated BRs extract of *B. monnieri* b) Standard Ascorbic acid