



Conservation linkages of endangered medicinal plant and exploration of phytochemicals, pharmaceutical screening and in silico validation against diabetics using in vivo wild and in vitro regenerated plant *Boucerosia diffusa* Wight.

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Abstract

Boucerosia diffusa Wight. is an important endangered medicinal plant belonging to the family Asclepiadaceae. In this study, an efficient protocol has been developed for *B. diffusa* using nodal explants for callus induction and direct organogenesis. The optimal callus induction (83.7%) was observed on 0.6 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) in Murashige and Skoog medium. The shoot regeneration was observed on different concentrations and combinations of 6-benzylaminopurine (BAP) and 2,4-D using shoot induction (88.5%) was observed on 0.5 mg/L BAP and 0.6 mg/L 2,4-D. Maximum root induction frequency (85.6%) was obtained on 0.6 mg/L α -naphthalene-acetic acid (NAA) and 0.5 mg/L BAP. The fully developed plants were acclimatized (98.86% survival rate) and transferred to natural photoperiod conditions. The phytochemical and pharmacological activity was determined in in vitro-regenerated plants (IRP) and was compared to in vivo wild plants (IWP). The primary and the secondary metabolite contents of bioactive compounds were significantly higher in the methanolic extract of IRP. A comparative antioxidant activity study shows IRP exhibited better scavenging activity. The antidiabetic activity of α -amylase ($IC_{50} = 71.56 \pm 15.4 \mu\text{g/mL}$) and α -glucosidase ($IC_{50} = 82.94 \pm 12.84 \mu\text{g/mL}$) inhibitor activity also exhibited maximum in methanolic extract of IRP. Furthermore, chemical composition was analyzed using gas chromatography–mass spectroscopy (GC–MS). Antibacterial activity against human pathogenic bacteria, IRP methanolic extracts showed a maximum zone of inhibition (75 $\mu\text{g/mL}$) observed against *Salmonella typhi* ($23.5 \pm 0.5 \text{ mm}$) compared to the IWP. Molecular docking analysis of *B. diffusa* inhibition of antidiabetic activity showed better affinity in β -Sitosterol.

Keywords *Boucerosia diffusa* Wight. · *Caralluma diffusa* (Wight) N. E. Br. · Micropropagation · Phytochemical analysis · Antidiabetic activity · Antibacterial analysis

Abbreviations:

2,4-D 2,4-dichlorophenoxyacetic acid
BAP 6-benzylaminopurine
BSA Bovine serum albumin
DMSO Dimethyl sulfoxide
DPPH· 2,2-diphenyl-1-picrylhydrazyl

EDTA Ethylene-diamine-tetra-acetic acid
GC–MS Gas chromatography–mass spectroscopy
IAA Indole-3-acetic acid
IBA Indole-3-butyric acid
IRP In vitro-regenerated plants
IWP In vivo wild plants
KIN Kinetin
MHA Mueller–Hinton medium,
MS medium Murashige and Skoog medium
NAA 1-naphthaleneacetic acid
PVPP Polyvinyl pyrrolidone
TDZ Thidiazuron
ZOI Zone of inhibition

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