Evaluation of the anticancer potential of secondary metabolites from *Pseudevernia furfuracea* based on epidermal growth factor receptor inhibition

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**ABSTRACT**

UHPLC/ESI/MS/MS profiling followed by bioactivity guided isolation of *Pseudevernia furfuracea* (*P. furfuracea*) extract yielded two polyphenolic molecules, Methyl haematommate (PF-1) and Atraric acid (PF-2). These molecules were evaluated for bioactivity against five cancerous cell lines. The results revealed that atraric acid showed significant activity against ovarian cancer cell line (PA-1) having GI50 at 16.42 μg/mL and moderate activity against the breast cancer cell line (MCF-7), having GI50 at 64.35 μg/mL. The results were further supported by *in silico* molecular docking studies of atraric acid with the epidermal growth factor receptor (EGFR) tyrosine kinase protein. The study revealed that atraric acid has the capacity to act as a potential EGFR inhibitor via occupying the ATP binding pocket of EGFR and making favourable electrostatic interactions and van der Waals interaction with its key residues. Our results highlight *P. furfuracea* and its polyphenolic compound, atraric acid as a promising candidate for ovarian cancer management.

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1. Introduction

Cancer is classified among the leading life-threatening diseases. Numerous biomolecules obtained from lichen have also been reported to show various bioactivities.
including anticancer potential (Cimmino et al. 2019; Goel et al. 2021). Pseudevernia furfuracea (P. furfuracea) is a foliose lichen having numerous bioactivities including cytotoxicity and used in various herbal mixture (Kalra et al. 2021). The major objective of the current investigation was to identify and separate any potential anticancer metabolites from the lichen P. furfuracea.

A comprehensive phytochemical investigation of lichen P. furfuracea was performed using UHPLC/ESI/MS/MS. Using bioactivity guided isolation, two compounds were isolated which were subsequently tested for bioactivity against five cancerous cell lines. Amongst the compounds tested, the initial results showed significant potential of PF-2 against ovarian cancer cell lines. Based on this, in silico molecular docking studies was used to investigate the interaction between bioactive isolated constituent with epidermal growth factor receptor EGFR tyrosine kinase protein, a highly expressed protein to analyse the potential anti-ovarian cancer effect.

2. Results and discussion

2.1. Anticancer screening

The extracts showed variable responses against five cancerous cell lines MDA-MB-231, MCF7, PA-1, HepG2 and SCC-40. The cytotoxic activity of three extracts i.e. P. furfuracea hexane (PFH), P. furfuracea ethyl acetate (PFET), P. furfuracea 70% methanol (PF70M) against all cell lines are shown in Table S1 and Figure S1. Among all the extract PFET found to be active against most of the cell lines and as such were selected for further isolation of bioactive molecules. The finding of this study was found in agreement with the previous anticancer studies of P. furfuracea (Šeklić et al. 2018; Aoussar et al. 2020).

2.2. UHPLC/ESI/MS/MS assisted tentative identification of P. furfuracea ethyl acetate extract

Considering the substantial anticancer activity of the ethyl acetate extract (PFET), an attempt was made to tentatively identify the biochemical composition of specified extract. Table S2 depicts the list of fifty compounds that were detected in negative
ion mode and tentatively identified based upon their m/z of the respective pseudomolecular ion [M – H]⁻ and MS/MS data. Out of these fifty, forty-eight compounds were categorised under depsidones, depsides, depsone, pulvinic acid derivative, lipids, dibenzofurans and diphenylether derivatives and one phenolic compound with a m/z 195.0505 was identified as atraric acid (Kalra et al. 2021).

2.3. Bioactivity guided isolation

Bioactivity guided isolation from PFET yielded two compounds (PF1 and PF2) (Figure 1). These compounds were identified and characterized by means of spectroscopic techniques, ¹H and ¹³C-NMR and mass spectrometry (HREI-MS) (Figures S2–S6) and identified based upon the spectroscopic data reported in the literature (Mallavadhani et al. 2019). The purity of the isolated compounds was confirmed by HPLC and representative chromatograms are presented in Figure S7.

2.4. Anticancer activities of compounds

Five concentrations (10–80 μg/mL) of isolated molecules were assessed against five cancerous cell lines. The antiproliferative effects of these compounds against all cell lines are depicted in Table S3 and Figure S8. Atraric acid (PF-2) displayed significant activity against PA-1 (ovarian cancer cell) with a GI₅₀ value at 16.42 μg/mL compared to Doxorubicin® as standard which achieved a GI₅₀ value of <10 μg/mL and moderate activity against the MCF-7 cell line with a GI₅₀ at 64.35 μg/mL. Methyl haematommate (PF-1) was observed to provide significant activity against the PA-1 cell line with a GI₅₀ at 25.28 μg/mL and moderate activity against the MCF-7 cell line with GI₅₀ at 70.92 μg/mL. These compounds showed no significant potential against the other cell lines (MDA-MB-231, HepG2 and SCC-40). This work highlighted the first report of anticancer potential of isolated compounds specifically atraric acid (PF-2) from P. furfuracea against PA-1 and MCF-7 cell lines.

2.5. Docking study, binding mode analysis and ADMET

The binding interaction of PF2 with the EGFR tyrosine kinase, a highly expressed in ovarian cancer was studied using in silico molecular docking analysis software (GOLD 5.5.0 software). The docked conformation of erlotinib was found to be similar to its co-crystallized conformation. The Root Mean Square Deviation (RMSD) between the docked and the co-crystallized conformation was observed as 0.651 Å which indicates that the adopted docking procedure involving the gold scoring function is appropriate for the docking of PF2 (Figure S9). The docking study showed that PF2 fits well into the binding cavity of EGFR and its conformation is stabilized via hydrogen bond (H-bond), polar and van der Waals interactions (Figure S10). The study suggests that PF2 may function as an EGFR inhibitor acting via the occupation of the ATP binding pocket of EGFR, thus making favourable electrostatic and van der Waals interactions with its key residues. Further PF2 was analysed for in silico ADMET parameters (Table S4) and oral toxicity (Table S5). It has been observed that it follows the Lipinski’s rule of five which indicates that the compounds can be sufficiently absorbed through the
gastrointestinal tract (Table S4). The predicted toxicity data profile indicated that PF2 is non-toxic in nature and can be used safely for potential therapeutics (Table S5).

3. Conclusion

The work highlighted the importance of lichen-based metabolites, specifically polyphenols as cancer therapeutic agent. A consolidated approach with the aid of UPLC-MS/MS analysis of P. furfuracea extract together with bio-guided isolation led to separation of one important bioactive molecule (atraric acid). The atraric acid showed potential activity against ovarian cancer cell line which was further supported by in-silico studies. This is the first report of the in-vitro bioactivity and in-silico simulations of atraric acid against ovarian cancer. The findings highlight that atraric acid is a potential inhibitor of EGFR. Also, prediction of drug ability through in silico studies affords illustrative evidence of the observed ranges with respect to the standard values of ADME parameters.

Disclosure statement

No potential conflict of interest was reported by the authors.

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