SHORT COMMUNICATION

UHPLC-QTOF-MS/MS based characterization of anti-tumor constituents in *Ceratocarpus arenarius* L. and identification of EGFR-TK inhibitors by virtual screening

Le Pan, Li Li, Lu Xu, Jiaojiao Zhang, Jiashan Li, Ming Gao, JinMen Yu, Lu Jin and Dongyu Lei

*a*Chemical Engineering College, Xinjiang Agricultural University, Urumqi, China; *b*Department of Physiology, Preclinical School, Xinjiang Medical University, Urumqi, China

**ABSTRACT**

The constituents of *Ceratocarpus arenarius* L., as a traditional anti-cancer medicine of Kazakh, were firstly profiled with UHPLC-QTOF-MS/MS. The potential compounds against EGFR-TK were virtually screened. A result, forty-four compounds were analyzed, including 18 flavonoids, 8 steroids, 4 phenolic acids, 9 fatty acids, 1 coumarin and 4 other compounds. Among them, 9 flavonoids, *N*-trans-Feruloyltyramine (5), stigmasterol (11) and carthamone (38) were recognized as potential key anti-tumor constituents of *C. arenarius* through docking to active site of EGFR-TK. It indicated that the compounds formed moderate to strong interactions with EGFR-TK contributing to the antitumor activity through a synergetic actions. Besides, the anticancer effects of *C. arenarius* was verified with *in vitro* anti-tumor activity investigation against *A549*. Our results firstly reveals the active constituents basis of *C. arenarius* against cancer and provides novel insights into the further application of effective constituents and mechanism of *C. arenarius*.

**ARTICLE HISTORY**

Received 24 September 2021
Accepted 7 March 2022

**KEYWORDS**

*Ceratocarpus arenarius* L.; Amaranthaceae; UHPLC-QTOF-MS/MS; EGFR-TK inhibitors; docking

1. Introduction

Lung cancer has been estimated to cause 1.6 million deaths globally every year, in which non-small cell lung cancer (NSCLC) accounts for 80–90% (Iyikesici 2019). The high lung cancer mortality is due to the metastasis in most patients, therefore, effective therapies have always been emergency to the improvements in long-term survival. The epidermal growth factor receptor (EGFR) has been successfully validated as an anticancer target and epidermal growth factor receptor tyrosine kinase inhibitor...
EGFR-TKI has been proven to selectively inhibit both EGFR-TKI-sensitizing and EGFR T790M resistance mutations, which has played an important role on the current treatment of NSCLC (Herbst et al. 2018). Since gefitinib was introduced to cure lung cancer in 1990s, molecularly targeted therapy has developed to important chemotherapy tactics (Yang et al. 2019). Although some important achievements have been made over the past two decades, the overall survival rates for NSCLC remain low. Thus, the continued researches into novel candidates are required in cure of NSCLC with increasing understanding of the target structure (Chandrika et al. 2016).

*Ceratocarpus arenarius* L. (*C. arenarius*) is a desert annual plant which mainly grows in psammophytic or halophytic communities of the Gurbantunggut Desert in central Asia (Zhang et al. 2012). The plant is 5–30 cm high and has star hairs with leaves lanceolate (Figure S1). The high drought and salinity condition have endowed *C. arenarius* as a pioneer plant in colonization of harsh environments and consequently lead to the accumulation of specific secondary metabolites (Lamine et al. 2020). In traditional system of Kazakh medicine, *C. arenarius* is used to treat lung cancer. However, to date, the chemical characterization of *C. arenarius* was scarce, and the understanding of active constituents was too limited to provide scientific evidence for killing disease both from the chemical material basis and action mechanisms.

In the current work, the anti-tumor activity of the ethanol extract of *C. arenarius* was investigated by *in-vitro* investigation primarily, and UHPLC-QTOF-MS/MS was applied to identify the secondary metabolites of *C. arenarius*. Then, data mining was conducted by virtual screen with molecular docking to discover potential EGFR-TKIs in *C. arenarius* and predicted the possible action mode. Therefore, an active constituents mining and EGFR-TKIs discovery system from natural products were developed based on UPLC-QTOF-MS/MS combined with virtual screening.

2. Results and discussion

2.1. Extract yield

A high yield of 432.3 g crude extract (CE) was finally obtained after concentration under vacuum. The extract was sequentially fractionated by petroleum ether, chloroform, ethyl acetate to afford PE-fr (47.2 g), TCM-fr (108.5 g), EAC-fr (80.8 g) and AQU-fr (182.5 g) for further study.

2.2. In-vitro anti-tumor activity

In order to verify the possible anticancer activity of *C. arenarius*, the anti-tumor cytotoxicity against human non-small cell lung cancer A549 of crude extract (CE) of *C. arenarius* and four fractions (PE-fr, TCM-fr, EAC-fr, AQU-fr) were studied. The results showed that the crude extract, TCM-fr and EAC-fr exhibited significant anti-tumor activity at the concentration of 200 µg/mL. The crude extracts could inhibit the cell viability with the rate of 49.5%, and EAC-fr showed the strongest activity with the inhibitory rate of 60.7%, followed by TCM-fr (47.5%) and PE-fr (24.4%), whereas AQU-fr exhibited very weak effect (Figure S2). The IC$_{50}$ of crude extract, TCM-fr and EAC-fr were showed in the Table S1. The results showed that the extracts could inhibit the
growth of cell A549, which could support the anticancer application of C. arenarius in folk medicine.

2.3. Identification of chemical constituents with UHPLC-QTOF-MS/MS

UHPLC-QTOF-MS/MS was firstly applied to identify the chemical constituents in the total extract of C. arenarius. Full chromatogram of sample was shown as Figures S3 and S4. On the basis of comparison of retention time (RT) and mass spectral data with available standards and published data, a total of forty-four compounds was identified (Table S2). The compounds belonged to different classes, including 18 flavonoids, 8 steroids, 4 phenolic acids, 9 fatty acid, 1 coumarin and 4 other compounds.

2.4. Virtual screening and interaction analysis

The docking results indicated that the identified compounds have formed moderate to strong interactions with the active sites of EGFR. According to the docking and scoring results, top 12 out of 44 compounds were sorted, of which structures and docking scores are tabulated in Table S3. The content of 12 compounds were also analyzed and the quantitative results were shown in Table S4. Finally, 9 flavonoids (2, 4, 14, 16, 25, 26, 27, 32 and 33), N-trans-Feruloyltyramine (5), stigmasterol (11) and carthamone (38) were recognized as potential key anti-tumor constituents of C. arenarius.

The interaction analysis revealed that the identified compounds formed H-bonds, ion interactions with the residues, such as Asp 831 and Val 702. The representative binding modes of the identified compounds were shown in Figure S5. Two-dimensional interaction of the potential active compounds is shown in Figure S6.

Among the screened compounds, some flavonoids have been verified to be EGFR inhibitors at micromolar level in the previous study and proven to have anticancer activity (Huang et al. 2009). Besides, stigmasterol and N-trans-feruloyltyramine have also been found as potential compounds. The above compounds might show inhibitory activity against EGFR through a synergistic way, and finally contributed to anti-cancer activity of C. arenarius. Natural compounds were known to embrace significant activities on a host of disorders (Peng et al. 2019; Thanh Tin et al. 2019; Zingue et al. 2021). The discovered compounds supported the anti-cancer activity of C. arenarius, and also the various structures provided a guidance for further drug design. Therefore, the chemical profiling combined with virtual screening gave a deep understanding on the chemical basis of anti-tumor activity of C. arenarius, which will be helpful for the structure-based drug design and the anti-tumor mechanism study of C. arenarius.

3. Experimental

See supplementary material.
4. Conclusion

In this study, ethanol extract of *C. arenarius* and its subfractions were proven to be effective against cell A549, and EAC-fr and TCM-fr were most effective with IC\textsubscript{50} of 108.3 µg/mL and 205.4 µg/mL. UHPLC-QTOF-MS/MS combined with virtual screening was applied to understand the anticancer chemical basis of *C. arenarius*. A total of 44 compounds were profiled according to structural and fragment characteristic. By docking to EGFR, 12 metabolites were recognized as potential EGFR inhibitors. These compounds might be the key constituents responsible for the activity through synergistic actions. Overall, not only do our findings explore the anti-tumor chemical basis of *C. arenarius*, but also firstly provide whole insights into the constituents of *C. arenarius*.

Disclosure statement

No potential conflict of interest was reported by the authors.

Supplementary material

Experimental details are available online, alongside Figures S1–S6 and Table S1–S4.

Funding

The work was supported by Natural Science Foundation of Xinjiang Province, China (No. 2019D01B20 and 2021D01C288); National Natural Science Foundation of China (No. 31960547); National Natural Science Foundation of China (No. 21762043).

References

Chandrika BB, Steephan M, Kumar S, Sabu A, Haridas M. 2016. Hesperetin and Naringenin sensitize HER2 positive cancer cells to death by serving as HER2 Tyrosine Kinase inhibitors. Life Sci. 160:47–56.
Herbst RS, Morgensztern D, Boshoff C. 2018. The biology and management of non-small cell lung cancer. Nature. 553(7689):446–454.
Huang H, Jia Q, Ma JG, Qin GR, Chen YY, Xi YH, Lin LP, Zhu WL, Ding J, Jiang HL, et al. 2009. Discovering novel quercetin-3-O-amino acid-esters as a new class of Src tyrosine kinase inhibitors. Eur J Med Chem. 44(5):1982–1988.
Ilyikesici MS. 2019. Feasibility study of metabolically supported chemotherapy with weekly carboplatin/paclitaxel combined with ketogenic diet, hyperthermia and hyperbaric oxygen therapy in metastatic non-small cell lung cancer. Int J Hyperthermia. 36(1):446–455.
Lamine M, Gargouri M, Mliki A. 2020. Identification of the NaCl-responsive metabolites in Citrus roots: a lipidomic and volatolic signature. Plant Signal Behav. 15(8):1777376.
Peng W, Han P, Yu LY, Chen Y, Ye BZ, Qin LP, Xin HL, Han T. 2019. Anti-allergic rhinitis effects of caffeoylquinic acids from the fruits of Xanthium strumarium in rodent animals via alleviating allergic and inflammatory reactions. Rev Bras Farmacogn. 29(1):46–53.
Thanh Tin NN, Thanh Truc ND, Thu Hang HT, Nhat Trinh PT, Bach LG, Dung LT. 2019. Chemical constituents of the aerial parts of scoparia dulcis and anti-cancer, anti-inflammatory activities. KEM. 814:360–364.
Yang LX, Ying SL, Hu SM, Zhao XT, Li MC, Chen MQ, Zhu YR, Song P, Zhu LY, Jiang TT. 2019. EGFR TKIs impair lysosome-dependent degradation of SQSTM1 to compromise the effectiveness in lung cancer. Signal Transduct Target Ther. 4(1): 25.

Zhang T, Shi N, Bai DS, Chen YL, Feng G. 2012. Arbuscular mycorrhizal fungi promote the growth of Ceratocarpus arenarius (Chenopodiaceae) with no enhancement of phosphorus nutrition. PLoS One. 7(9):e41151.

Zingue S, Rutz J, Maxeiner S, Ndinteh DT, Chun FK-H, Jüngel E, Njamen D, Blaheta R. 2021. In vitro pro-apoptotic and anti-migratory effects of Treculia africana Decne. (Moraceae) and Entandrophragma angolense Welw (Meliaceae) extracts on prostate cancer cells. J Herb Med. 28(7):100443.