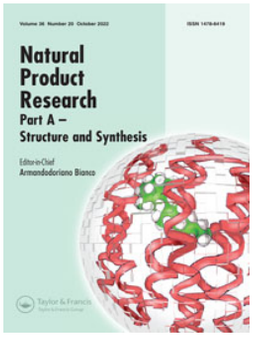

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SHORT COMMUNICATION

A new firewall in the fight against breast cancer: *in-vitro* and *in-silico* studies correlating chemistry to apoptotic activity of *Otostegia fruticosa*

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ABSTRACT

Breast cancer is the most devastating disease for women. There is a great demand for new sources to treat this disease. Medicinal plants are an indispensable source of bioactive compounds with wide range of pharmacological activities. *In-vitro* cytotoxic activity of *Otostegia fruticosa* methanolic extract against human breast cancer was studied using MCF-7 cell line. The extract showed mildly potent activity ($IC_{50} = 51 \pm 9.836\,\mu g/mL$) in comparison to the standard anticancer doxorubicin ($IC_{50} = 7.467 \pm 1.05\,\mu g/mL$). Potential compounds responsible for activity have been identified using Molecular Operating Environment (MOE) module on the major compounds detected by HPLC-MS/MS technique against estrogen alpha receptor (ERα+: PDB ID 2JF9). 3,5-di-*O*-dicaffeoyl-quinic acid, hyperoside and rutin showed similar binding and antagonistic interaction with the estrogen alpha receptor as tamoxifen in several poses. The retrieved results confirm that we can add this plant to a powerful arsenal that combats this insidious disease.

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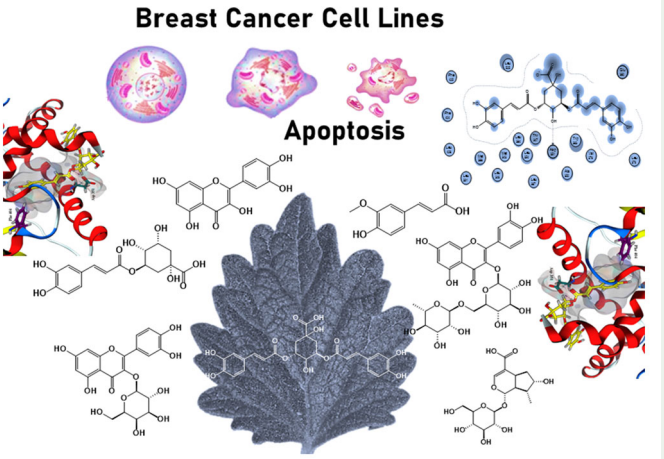

List of abbreviations: ROS: Reactive oxygen species; p53: Tumor-suppressor gene; p21: CDK-interacting protein 1; p27: Cyclin-dependent kinase inhibitor; NF-κb: Nuclear factor kappa-light-chain-enhancer of activated B cells; IGF1-R: insulin-like growth factor 1; His: Histidine; MCF-7: Michigan Cancer Foundation-7 = breast cancer cell line; Asp: Aspartic acid; MTT: (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay; IC_{50}: The half-maximal inhibitory concentration; HPLC-MS/MS: High Performance Liquid Chromatography- Mass spectrometry/mass spectrometry.; ERα: Estrogen receptor alpha; PDB: Protein Data Bank

1. Introduction

Breast cancer is one of the most common diseases in women, and it has become a global issue (Bray et al. 2018). Although chemotherapy is still widely used in breast cancer treatment, scientists have long been troubled by the resistance of breast cancer, a high rate of metastasis, and a poor prognosis (Sarosiek 2017). Breast cancer is not a single disease but rather a collection of molecularly distinct tumors arising from the epithelial cells of the breast (Comşa et al. 2015).

Tamoxifen, a selective estrogen receptor modulator (SERM), is the most used anti-estrogen adjuvant treatment for estrogen receptor alpha positive (ERα+) premenopausal women. SERMs have agonist and antagonist activity towards ER depending on the tissue (Sanyakamdhorn et al. 2016). Despite tamoxifen has a clear benefit in the treatment of breast cancer, it also has serious side effects on uterus because of its agonistic effect in this tissue. The risk of endometrial malignancy and hyperplasia increases due to cumulative and long-term use of tamoxifen by 1.5 to 6.9 folds (Subarnas et al. 2015). This risk is considerably increased in overweight menopausal women. Thus, alternative treatments are needed (Cohen et al. 2003; Muchtaridi et al. 2017).

Plant derived phenolic compounds showed many health benefits due to their anti-oxidant, anti-inflammatory, and anti-clastogenic properties (Lambert et al. 2005). The anti-carcinogenic effects of phenolic compounds are mediated through different mechanisms such as induction of cell cycle arrest, modulating ROS levels, promoting

tumour suppressor proteins such as p53, Phosphatase and Tensin Homolog (PTEN), p21, and p27 and controlling cell proliferation, apoptosis, and angiogenesis, by inhibiting oncogenic signaling cascades (Lambert et al. 2005).

Using phytochemical substances to fight cancer is an old practice that has contributed to the discovery of more than half of all modern medicine as an alternative and complementary drug either to eliminate or reduce many ailments in recent times (Rinaldi and Shetty 2015). Secondary metabolites from plants and microbes have been discovered to be a promising safer anti-cancer agents (Abdulazeem et al. 2018). Many plants have recently been evaluated for anti-cancer activity *in-vitro* and *in-vivo* as an alternative or adjuvant treatment with chemotherapy (Dholwani et al. 2008). They also have been shown to act on modulating key regulating genes such as oncogenic survival kinases; cell proliferation regulators, transcription factors, histone deacetylases and angiogenic factors (Dholwani et al. 2008).

Antitumor activity exerted by the sweet potatoes leaves extract on stomach cancer, colon cancer, and a promyelocytic leukemia cell was attributed to the presence of 3,5-di-*O*-caffeoylquinic acid, 4,5-di-*O*-caffeoylquinic acid and 3,4,5-tri-*O*-caffeoylquinic acid (Kurata et al. 2007).

Several *in-vivo* studies showed that a high intake of flavonoids may be associated with a reduced risk of cancer (Batra and Sharma 2013) such as breast cancer as they alters the endogenous activities of ER, which slow down or prevent the developments of breast tumors (Jarzabek et al. 2009).

Hyperoside showed an induction of apoptosis and inhibition of proliferation of breast cancer both *in-vitro* and *in-vivo* through deactivation of NF-κB pathway, induction of mitochondrial dysfunction, activation of caspase-3 and reduction of intracellular ROS levels (Park et al. 2016; Moradi-Marjaneh et al. 2019)

Rutin restrains the growth of breast cancer cells by regulating the miR-129-1-3p/Ca^{2+} signaling pathway (Li et al. 2021). Flow cytometry analysis showed that 20 μM and 50 μM rutin caused arrested cell cycle at G2/M and G0/G1 phases, respectively, significantly promoting cell apoptosis (Iriti et al. 2017).

Quercitrin exhibits significant cytotoxicity against MCF-7 at IC_{50}: 4.24 μM. This anticancer activity was attributed to the inhibitory activity of quercitrin against different kinases enzymes that secreted in various cancer diseases, namely, Aurora- B, CDK4/Cyclin D1, COT, FAK, and IGF1-R with IC_{50}: 4.78, 3.22, 25.65, 16.35, 21.21, respectively (Mostafa et al. 2019).

It is well known that Lamiaceae species have a unique, complex mixture of bioactive compounds, that contribute to their overall bioactivity. Their worth is derived from the production of a diverse range of secondary metabolites with potent antimicrobial, free radical scavenging, anti-inflammatory, and anticancer properties (Carović-Stanko et al. 2016). It is widely acknowledged that the powerful therapeutic properties of Lamiaceae species are attributed to their polyphenolic compounds that have many bioactive properties including anti-cancer activities (Prasad et al. 2009).

Otostegia fruticosa is a Lamiaceae plant that grows abundantly in certain Yemeni provinces (Al-Madhagy et al. 2022). Plants of the genus Otostegia are rich in volatile oils, terpenoidal and phenolic compounds (Khan and Syed 2013). They are widely used for their diverse activities in treating ulcers, spasms, depression, anxiety, sleep disorders, gum

diseases, ophthalmia, wounds, diabetes and infections (Aboutabl et al. 1995; Vural et al. 1996; Anwar et al. 2004; Tesso and Köing 2004). However, the cytotoxic activity of the Otostegia genus plants has not been investigated. In this study, we tested the activity of a methanolic extract of *Otostegia fruticosa* against the MCF-7 cell line and conducted an *in-silico* study to predict which compounds are responsible for its anti-tumor activity.

2. Results and discussion

2.1. Cytotoxic activity

Figures S1–S3 and Table S1 represents the data gathered from MTT assay used to evaluate the anticancer activity of the methanolic extract of *Otostegia fruticosa* leaves versus the standard doxorubicin using MCF-7 Human Breast Cancer cell lines. The standard doxorubicin was tested at different concentrations (100, 50, 25, 12.5, 6.25, 3.125 μg/mL), while the methanolic extract of *Otostegia fruticosa* leaves was tested at the following concentrations (1000, 500, 250, 125, 65.5, 31.25 μg/mL). As depicted from the results of Figures S1–S3 and Table S1, the extract of *Otostegia fruticosa* leaves showed a modest cytotoxic activity towards the MCF-7 cancer cells and attained an IC_{50} of 51 ± 9.836 μg/mL which is about 10 times the value of the standard doxorubicin which resulted in an IC_{50} of 7.467 ± 1.05 μg/mL [95% Confidence interval: 27.68 to 59.39]. A previous research work on the essential oil of *Otostegia fruticosa* also showed cytotoxicity against MCF-7 with IC_{50} = 55.1 μg/mL (Ali et al. 2017).

2.2. In silico docking study

Results of the docking simulation of ER and our 14 compounds detected by HPLC-MS/MS technique (Figure S4 and Table S2) are shown in the Table S3, Figures S5 and S6. Compounds were ordered according to their docking score. No Hydrogen bonds were formed between our compounds and His 524 residue which supports the antagonist potential of the compounds towards ER. However, by analyzing all docking interactions, we noticed that only 3,5-di-*O*- dicaffeolyquinic acid, hyperoside, rutin, 3-*O*-caffeoylquininc acid, and loganic acid, respectively showed similar binding and antagonist interaction with the receptor as the tamoxifen in several poses (Zainab et al. 2021; Durcik et al. 2022). Therefore, these compounds were considered as the best hits that might be responsible for the antagonist activity of *Otostegia fruticosa* methanolic extract on MCF-7 cell line (Figure S6). Interestingly, the distance of hydrogen bond formed between these compounds and the Asp 351 were 2.98, 3.12, 3.26, 3.13, and 3.07, respectively which suggest more selectivity towards breast tissue than uterus and thus less side effects on long term use.

3. Conclusion

Results of the MTT assay clearly highlights the anticancer activity of the methanolic extract of *Otostegia fruticosa* which abridged the cells viability and proliferation and induced a potent cytotoxic activity as denoted by the presence of an IC_{50} = 51 ± 9.836 μg/mL as compared to the standard doxorubicin which attained IC_{50} =

7.467 ± 1.05 μg/mL. Potential compounds responsible for this activity have been identified by in-silico studies using Molecular Operating Environment (MOE) module on the major compounds detected by HPLC-MS/MS technique against estrogen alpha receptor (ERα+:PDB ID 2JF9). 3,5-di-*O*-dicaffeoylquinic, hyperoside, rutin, 3-*O*- caffeoylquinic acid, ferulic acid, quercetin and loganic acid showed similar binding and antagonistic interaction with the estrogen alpha receptor as tamoxifen in several poses.

Disclosure statement

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