Phytochemical analysis, in-vitro and in-silico study of antiproliferative activity of ethyl acetate fraction of Launaea cornuta (Hochst. ex Oliv. & Hiern) C. Jeffrey against human cervical ca

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Introduction: Cervical cancer is one of the leading causes of death among women globally due to the limitation of current treatment methods and their associated adverse side effects. Launaea cornuta is used as traditional medicine for the treatment of a variety of diseases including cancer. However, there is no scientific validation on the antiproliferative activity of L. cornuta against cervical cancer.

Objective: This study aimed to evaluate the selective antiproliferative, cytotoxic and antimigratory effects. L. cornuta and to explore its therapeutical mechanisms in human cervical cancer cell lines (HeLa-229) through a network analysis approach.

Materials and methods: The cytotoxic effect of L. cornuta ethyl acetate fraction on the proliferation of cervical cancer cells was evaluated by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) bioassay and the antimigratory effect was assessed by wound healing assays. Compounds were analysed using the qualitative colour method and gas chromatographymass spectroscopy (GC-MS). Subsequently, bioinformatic analyses, including the protein-protein interaction (PPI) network analysis, Gene Ontology (GO), and Kyoto Encyclopaedia of Genes and Genomes (KEGG) analysis, were performed to screen for potential anticervical cancer therapeutic target genes of L. cornuta. Molecular docking (MD) was performed to predict and understand the molecular interactions of the ligands against cervical cancer. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was performed to validate the network analysis results.

Introduction

Cervical cancer is a major global health disease that affects women (Drokow et al., 2022). It is one of the leading causes of cancer death among women and the fourth most common cancer worldwide (Revathidevi et al., 2021). Human papillomavirus is the primary aetiological driver in carcinogenesis (Balasubramaniam et al., 2019). Not all human papillomavirus (HPV) infections in women result in cervical cancer. High-risk HPV genotypes induce a normal cell to transform into a precancerous lesion, which subsequently becomes an invasive lesion (Kasi et al., 2021). HPV infection causes the overexpression of viral oncogenes, which may inhibit a number of cellular proteins and affect biological processes such as cell proliferation, cell cycle, and apoptosis (Martínez-Rodríguez et al., 2021).

Globally, in 2020, a total of 604127 cases of cervical cancer were estimated, and 341831 deaths were recorded (Olthof et al., 2024). The incidence rate was 13.3 cases per 100,000 women, and the mortality rate was 7.2 deaths per 100,000 women (Singh et al., 2023). Over 58% of cervical cancer cases globally were estimated in Asia,

followed by Africa (20%), Europe (10%) and Latin America (10%) (Singh et al., 2023). However, the incidence and mortality rates for cervical cancer are exceptionally high in Sub-Saharan Africa at 19.59% and 24.55% annual global burden, respectively (Black and Richmond, 2018).

Botanicals' secondary metabolites and their derivatives have been evaluated in oncology-related clinical research and trials (Omara et al., 2022). The comprehensive knowledge on the mechanism of action for some investigated plant compounds provided the basis to develop novel, efficacious, safer botanicalderived compounds or their semi-synthetic analogues. Such had additional therapeutic advantages over the convectional chemotherapeutic drugs, which are associated with high toxicity not only to cancer cells but also to noncancerous cells (Scatchard et al., 2012; Vordermark, 2016; Johnson et al., 2018; Paken et al., 2023). Cervical cancer has become resistant to conventional therapeutic drugs, including cisplatin, paclitaxel, and carboplatin (Small et al., 2017), thus rendering them less effective. Hence, it was imperative to speed up research on botanical plants as an alternative source of less toxic, more tolerable and effective anticancer drugs for cervical cancer treatment.

The rich biodiversity has contributed significantly to traditional medicine and medicinal systems since ancient times (Omara, 2020). Approximately 1,200 medicinal plants are used in Kenya, 41 of which have anticancer properties, which account for only 10% of the medicinal plants (Onyancha et al., 2018). Botanical-based drugs are gaining attention due to their low toxicity, availability, costeffectiveness, and tolerability compared to conventional drugs (Tariq et al., 2017; Ochwang'i et al., 2014; Misonge et al., 2016). In this study, Launaea cornuta (Hochst. ex Oliv. & Hiern) C. Jeffrey also known as "mchunga" was investigated. L. cornuta is an erect herbaceous perennial plant belonging to the Asteraceae family, characterised by a hollow leafy and stem, slightly succulent, glabrous, with milky juice and grows to a height of about 1.5 m (J et al., 2015). It is indigenous to Kenya, Burundi, Cameroon, Uganda, Nigeria, Rwanda, Chad, Djibouti, Eritrea, Ethiopia, Malawi, Mozambique, Somalia, Sudan, Tanzania, Zambia, Central African Republic, Zaïre, and Zimbabwe (Machocho et al., 2014; Omara et al., 2022). In Kenyan communities, this plant is used as a wild vegetable

and a source of vitamin C (Musila et al., 2013). The localised budding root concoction known as 'Kipche' is used to cure throat cancer ("Koroitab mokto"), typhoid, gonorrhoea, benign prostate hyperplasia, and breast cancer, among others (J et al.,

2015; Fatuma Some, 2014; Khan et al., 2016; Chemweno et al., 2022). Regardless of the extensive use of L. cornuta plants in traditional medicine, their therapeutic efficacy, and toxicity have not been empirically validated, especially in cervical cancer.
In this study, we systemically evaluated the anticancer activity of the ethyl acetate fraction of L. cornuta, analysed and identified compounds using the GC-MS approach and explored the relevant targets and pathways involved in eliciting the anticervical effects through a network analysis approach.

Modified Eagle Medium (EMEM) supplemented with 1% L-glutamine (200 mM), 10% fetal bovine serum (FBS), 1.5% sodium bicarbonate, 1% HEPES (1M), 1% penicillinstreptomycin and 0.25 µg/mL amphotericin B. The cell culture was conducted at 37°C under a humidified atmosphere and 5% CO2 to achieve 75% – 80% confluence.

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