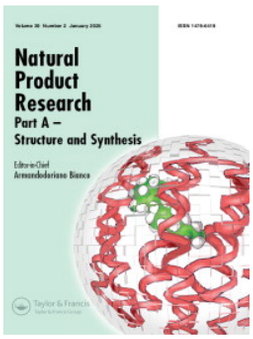

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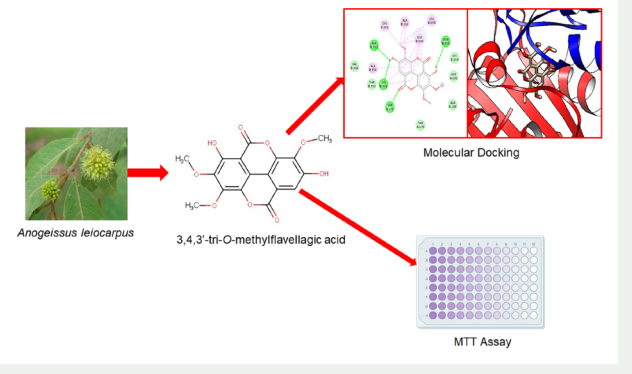
Isolation, cytotoxicity evaluation, and molecular docking of 3,4,3′-tri-*O*-methylflavellagic acid from *Anogeissus leiocarpus* (DC.) Guill. & Perr. root

Yemi A. Adekunle[a,b,c,d], Babatunde B. Samuel[a], Chinemenma M. Ezeude[d], Lutfun Nahar[e], Amos A. Fatokun[b] and Satyajit D. Sarker[b]

[a]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Ibadan, Ibadan, Oyo State, Nigeria; [b]Centre for Natural Products Discovery, School of Pharmacy and Biomolecular Sciences, Faculty of Science, Liverpool John Moores University, Liverpool, UK; [c]Department of Pharmaceutical and Medicinal Chemistry, College of Pharmacy, Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria; [d]Department of Pharmaceutical Chemistry, Dora Akunyili College of Pharmacy, Igbinedion University, Benin City, Edo State, Nigeria; [e]Laboratory of Growth Regulators, Palacký University and Institute of Experimental Botany, The Czech Academy of Sciences, Olomouc, Czech Republic

ABSTRACT

Cancer kills about 10 million people every year. Medicinal plants remain a major source in the global search for anticancer drugs. In this study, 3,4,3′-tri-*O*-methylflavellagic acid (MFA) was isolated from the methanol root extract of *Anogeissus leiocarpus*. The structure was determined by 1D- and 2D-NMR data. The cytotoxic effects of MFA were evaluated against human breast (MCF-7), colorectal (Caco-2), and cervical (HeLa) cancer cell lines using the 3-[4,5-dimethylthiazole-2-yl] 3,5-diphenyltetrazolium bromide assay. A multi-protein target screening *via* molecular docking was conducted against ten cancer-related proteins, and ADMET properties were evaluated. MFA exhibited the most potent activity against Caco-2 (IC_{50}: 46.75 ± 13.00 µM). Molecular docking analysis showed that MFA had a strong binding affinity for the colchicine-binding site of *αβ*-tubulin and polo-like kinase-1 (binding energies: −8.5 and −8.4 kcal/mol, respectively). MFA also satisfied the Lipinski's Rule of Five. MFA could, therefore, potentially serve as a scaffold for developing new anticancer molecules.



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CONTACT Yemi A. Adekunle y.a.adekunle@2022.ljmu.ac.uk, adekunleya@abuad.edu.ng; Babatunde B. Samuel bb.samuel@mail.ui.edu.ng; Lutfun Nahar nahar@ueb.cas.cz

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1. Introduction

Cancer is a leading cause of morbidity and death globally (Ferlay et al. 2021). It is characterised by abnormal growth of cells and tissues that ultimately leads to invasions and metastases (Meirson et al. 2020). In 2020, more than 19 million new cancer cases and almost 10 million cancer deaths were recorded (Sung et al. 2021). The burdens of cancer have increased in Africa due to the surge in population as well as the high prevalence of risk factors implicated in cancer, such as smoking, obesity, and physical inactivity (Wild et al. 2020).

Chemotherapeutic agents often have adverse effects, displaying activity against rapidly growing cells of the bone marrow, gastrointestinal tract, and hair follicles (Gyanani et al. 2021). This has constantly motivated the search for newer drugs. Nature, through medicinal plants, marine organisms and microbes, has produced molecules which are effective in combating human diseases, including cancer (Newman and Cragg 2020; Sarker et al. 2020). Research attention is being drawn to natural products (NPs) for newer anticancer agents. In the past, medicinal plants, including *Catharanthus roseus* (L.) G.Don, *Camptotheca acuminata* Decne, *Podophyllum pellatum* L., and *Taxus brevifolia* Nutt., produced leading drugs for cancer therapy (Sarker et al. 2020).

Anogeissus leiocarpus (DC.) Guill. & Perr., also known as African birch, is a large deciduous tree native to tropical Africa (Arbab 2014). Its height ranges from 15 to 18 metres, with a diameter of 1 metre (Singh et al. 2016). It is the only West African species of the genus *Anogeissus* and it is commonly distributed in tropical Central and East Africa (Arbab 2014). The stem bark, root, and leaf have traditional uses in the treatment of breast, head and neck, and skin cancers (Malami et al. 2020), diabetes mellitus (Adinortey et al. 2019), malaria (Sanogo et al. 2023), trypanosomiasis (Zongo et al. 2017), tuberculosis (Ofukwu et al. 2008), viral infections (Abubakar et al. 2022), as well as in maintaining mouth and teeth hygiene (Taiwo et al. 1999). The plant contains phytochemicals, including ellagitannins and ellagic acid derivatives (Shuaibu et al. 2008; Akande et al. 2022; Micheli et al. 2023), flavonoids (Attioua et al. 2011) and triterpenes (Chaabi et al. 2008; Akande et al. 2022). The bark predominantly contains ellagitannins and ellagic acid derivatives such as castalagin, di-methyl ellagic acid, di-methyl ellagic acid glucopyranoside and xylopyranoside, di-*O*-methylflavellagic acid, flavogallonic acid, punicalagin, tri-methyl ellagic acid, tri-*O*-methylflavellagic acid (Salih et al. 2017; Orlando et al. 2019; Akande et al. 2022). Most of these compounds have not been linked to biological activities (Adekunle et al. 2024).

Molecular docking has become an increasingly important drug discovery tool (Meng et al. 2011; Sarker and Nahar 2018). It is a simulation approach that models the interactions of small molecules (ligands) on the active sites of proteins (receptors) (Ainsley et al. 2018). It can predict the binding orientation of a ligand on the active site of a protein. It can also assess the binding affinity (Meng et al. 2011). To describe a drug's pharmacokinetic properties, its toxicity and drug-likeness are evaluated (Han et al. 2019). Consequently, drug-likeness and bioavailability predictions (Lipinski 2000) were evaluated for the ellagic acid derivative isolated and identified in the present study. The compound was tested on three cancer cell lines and was docked against ten selected cancer-related protein targets.

2. Results and discussion

Compound Q1, 2.5 mg, was obtained as a yellow flake which precipitated out of column-chromatographed *n*-hexane/ethyl acetate fractions (Figure 1). FTIR spectrum showed peaks at 3450 cm^{-1} (OH stretching), 3000 cm^{-1} and 2910 cm^{-1} (CH stretching), 1720 cm^{-1} and 1680 cm^{-1} (C = O stretching), 1610 cm^{-1} (C = C stretching), and 1010 cm^{-1} (C-O stretching) (Figure S10).^{1}H NMR spectrum (DMSO-d_6) exhibited one aromatic proton signal at δ_H 7.53 *s* (H-5′); and three methoxy protons at δ_H 3.89 *s* (CH_3-3), 4.04 *s* (CH_3-4) and 4.14 *s* (CH_3-3′) (Figure S7). Seventeen (17) carbon signals were observed in the ^{13}C-DEPT-Q spectrum (Figures S8 and S9), including one aromatic peak at δ_C 112.2 (C-5′); three methoxy groups at δ_C 61.5 (C-3), 61.7 (C-4), 62.2 (C-3′); and two carbonyl groups (lactone) at δ_C 161.4 (C-7) and 158.8 (C-7′) (Table S1). Other aromatic peaks were observed between δ_C 98.0 and 153.6. Comparing the NMR data with the literature values helped identification of the compound as 3,4,3′-tri-*O*-methylflavellagic acid (MFA) (Adigun et al. 2000; Serafin et al. 2007). MFA (Figure 1) is a flavellagic acid derivative that was first isolated from *Terminalia paniculata* Roth (Combretaceae) (Row and Raju 1967). Its occurrence and that of its 4′-*O*-glucoside has been reported in the stem bark extract of *A. leiocarpus* (Nduji and Okwute 1988; Adigun et al. 2000; Chaabi et al. 2008). Its glucoside inhibited the growth of *Candida albicans, Pseudomonas aeruginosa*, and *Staphylococcus aureus* (Adigun et al. 2000). It has also been reported in *Ruprechtia tangarana* Standl., (Polygonaceae) (Pettit et al. 2003) and *Plinia glomerata* (Myrtaceae) (Serafin et al. 2007).

The methanol extract of the root of *A. leiocarpus* and its dichloromethane fraction had previously been shown to possess activity against human breast adenocarcinoma and rhabdomyosarcoma cell lines (Adekunle et al. 2022). In this follow-up study, a compound (MFA) was isolated from the dichloromethane fraction and its cytotoxic effects were evaluated against human colorectal cancer cell line (Caco-2), human cervical cancer cell line (HeLa), and human breast cancer cell line (MCF-7) using the *in vitro* MTT assay. MFA showed most potent cytotoxic effect against Caco-2 as shown in Table S2. The second most sensitive cell line was MCF-7. Vincristine was used as the reference anticancer drug. MFA could be one of the secondary metabolites responsible for the anticancer activity

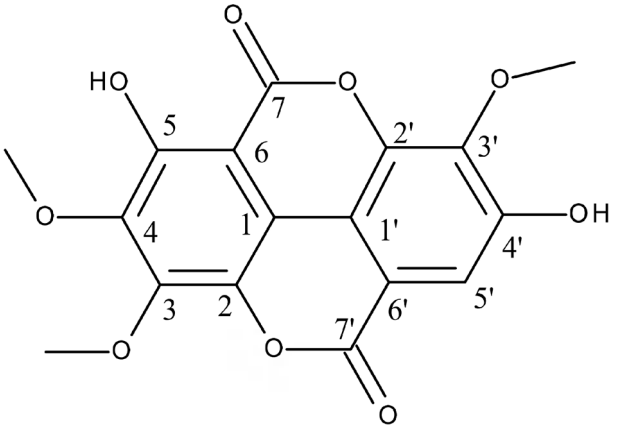

Figure 1. Structure of 3,4,3′-tri-*O*-methylflavellagic acid isolated from *A. leiocarpus* root.

of the root of *A. leiocarpus* (Salau et al. 2013; Hassana et al. 2018; Alqathama et al. 2020). MFA has been shown to have other biological activities. For example, a previous study noted the profound antinociceptive effects of MFA and its 4′-*O*-glucoside, with calculated ID_{50} value of 10.8 and 2.5 µmol/kg, respectively, when injected intraperitoneally, against writhing in mice. Comparison with the standard drugs aspirin, diclofenac, dipyrone, and paracetamol with ID_{50} values of 133.1, 38.0, 162, and 125.8 µmol/kg, respectively, showed MFA and its 4′-O-glucoside were more potent (Serafin et al. 2007).

Molecular docking was carried out to gain insights into the mechanisms of inhibitory potential of MFA against cancer-related targets (Oluyemi et al. 2022). MFA was observed to dock well with the selected proteins (Table S3). The compound showed highest binding affinity for *αβ*-tubulin (1SA0) at the colchicine-binding site, having a binding energy of −8.5 kcal/mol. The next two highest binding energies (–8.4 and −8.3 kcal/mol, respectively) were observed for polo-like kinase-1, PLK-1 (3FC2) and epidermal growth factor receptor tyrosine kinase, EGFR (1M17).

The *αβ*-Tubulin heterodimer is a subunit of the microtubule, a cyto-motive protein filament found in the cytoskeleton of nearly all eukaryotic cells (Tuszyński et al. 2005). Microtubules assemblage dynamics can be disturbed by various compounds, including vinca alkaloids (vincristine, vinblastine), colchicine, podophyllotoxin, and macrolides (Amos 2011). Critical residues that are involved in hydrophilic and hydrophobic interactions at the colchicine binding site include THR179, ALA180, ASN101, LEU248, ALA316, LYS352 (Wang et al. 2022). Analysis of the molecular docking result of MFA with *αβ*-tubulin showed multiple hydrogen bonding with SER178, ASN258, ALA317, and LYS352; π-alkyl interactions with CYS241, LEU248, LEU255, ALA316, and ALA354; and van der Waals interactions with THR179, ALA180, ASN101, LYS254, VAL318, and THR353 (Figure S2). These residues including CYS241 have been shown to be involved in the binding of colchicine to the receptor (Naaz et al. 2019).

MFA interacted with PK-1 *via* hydrogen bonding with CYS67, LYS82, GLU131, and ASP194. Carbon hydrogen bonding was observed with CYS133 and LEU59. MFA also showed π-π stacked interactions with PHE183. It formed π-alkyl interactions with two residues, ALA80 and LEU130 (Figure S3). The interactions of MFA with EGFR kinase include hydrogen bonding with MET769; carbon hydrogen bonding with GLU738 and ASP831; π-sigma bond with VAL702; and π-alkyl interactions with LEU694, ALA719, LYS721, and LEU820 (Figure S4).

Some physicochemical properties have been used in predicting the absorption and other pharmacokinetic parameters of small molecule drugs. These include the molecular weight, hydrogen bond acceptor, hydrogen bond donor, log *P* and topological polar surface area (Lipinski 2000). MFA did not violate Lipinski's Rule of Five as shown in Table S4. The bioactivity radar (Figure S5) shows that MFA bioavailability rating was slightly within the suitable physicochemical space for oral bioavailability (www.swissadme.ch).

Docking poses were visualised by BIOVIA Discovery Studio Visualiser (Baroroh et al. 2023).

4. Conclusions

Natural products have played a pivotal role in cancer drug discovery. A bioactive compound–3,4,3′-tri-*O*-methylflavellagic acid (MFA), which was isolated from the

methanol root extract of *Anogeissus leiocarpus*, had shown inhibitory activity against three human cancer cell lines. Molecular docking against ten cancer-related targets revealed that it had good binding affinity for the colchicine binding site of tubulin. Drug-likeness study also revealed that the compound conformed with Lipinski's drug pharmacokinetics parameters. Based on these findings, MFA could be considered a potential template for developing new anticancer agents.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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ORCID

Yemi A. Adekunle http://orcid.org/0000-0002-9392-3925
Babatunde B. Samuel http://orcid.org/0000-0001-5553-4438
Lutfun Nahar http://orcid.org/0000-0002-1157-2405
Amos A. Fatokun http://orcid.org/0000-0001-5183-7589
Satyajit D. Sarker http://orcid.org/0000-0003-4038-0514

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