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ORIGINAL ARTICLE

Methyl Elaidate: A Major Compound of Potential Anticancer Extract of *Moringa oleifera* Seeds Binds with Bax and MDM2 (p53 Inhibitor) *In silico*

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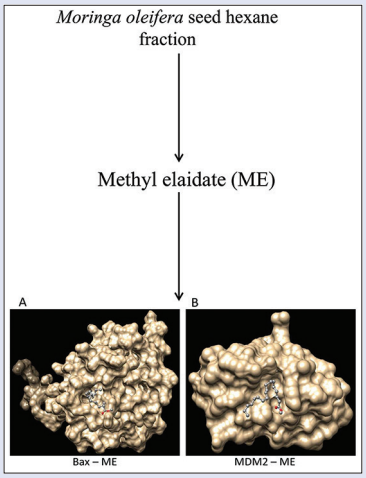
ABSTRACT

Background: Apoptosis is an energy-required programmed cell death process that is vital for normal homeostasis. This is because aged, malfunctioning, and genetically deficient cells such as cancer cells are eliminated through this process to avoid any abnormality in the function of the body system. Earlier, we have reported that the Lipophilic hexane fraction of crude ethanolic extract (HF-CEE) of *Moringa oleifera* seeds inhibited MCF7 breast cancer cell growth. **Objectives:** The aims of this study are to identify the esters responsible for HF-CEE cytotoxicity and to determine the ability of the compound to trigger Bax and p53 apoptotic proteins. **Materials and Methods:** Gas chromatography–mass spectrometry (GC-MS) analysis of the HF-CEE was performed. Methyl elaidate, the major compound identified, was molecularly docked with Bax and MDM2 (p53 inhibitor) using AutoDock 4.2. Drug suitability of methyl elaidate was determined by the rule of "5." **Results:** Eleven different esters were identified in HF-CEE by GC-MS. Methyl elaidate was the major anticancer agent among the esters. Methyl elaidate bound with Bax and MDM2 at their active sites that can trigger apoptosis in cancer cells. Finally, methyl elaidate has potential drug properties according to the rule of "5." **Conclusion:** Methyl elaidate of HF-CEE could be responsible for its anticancer activity and it is a potential apoptotic agent.

Key words: Apoptosis, Bax, MDM2 (p53 inhibitor), methyl elaidate, *Moringa oleifera* seeds

SUMMARY

- In this study, we identified methyl elaidate as a major compound of lipophilic hexane fraction of *Moringa oleifera* seeds extract using GC-MS method. Molecular docking analysis revealed the methyl elaidate could bind with Bax and MDM2 (inhibitor of p53) to induce apoptosis. Hence, it is concluded that methyl elaidate is a potential apoptosis inducer in cancer cells.



Abbreviations used: HF-CEE: Hexane fraction of crude ethanolic extract of *Moringa oleifera* seeds; GC-MS: Gas chromatography mass spectrometry; MDM2: Mouse double minute 2 homolog.

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INTRODUCTION

Apoptosis is an energy-required programmed cell death process that is vital for normal homeostasis. This is because aged, malfunctioning, and genetically deficient cells such as cancer cells are eliminated through this process to avoid any abnormality in the function of the body system. The pathways through which apoptosis takes place are many which include extrinsic, intrinsic, p53 signaling, and nuclear factor-κB pathways. These pathways are activated when they receive cell signals such as hypoxia, DNA damage, and activation of oncogenes to serially activate the biomarkers and proteins along their pathways to bring about apoptosis.[1]

Bax and p53 are from the important proteins that must be activated to induce apoptosis. Bax is a member of Bcl-2 family proteins and it has proapoptotic activity when activated.[2] It usually resides in mitochondria or cytosol of the cell. When Bax is activated, it undergoes conformational changes to trigger apoptosis cell death through mitochondrial-mediated (intrinsic) pathway.[3,4] This leads to the release of cytochrome c from the mitochondria to the cell cytosol. The cytochrome c forms apoptosome complex together with Apaf-1 and caspase-9, wherein caspase-9 will be activated by induced proximity. The activated caspase-9 will cleave and activate caspase-3 and caspase-7 which will subsequently result in the death of the cell.[2]

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p53 tumor suppressor protein activates transcription of proapoptotic genes encoding proteins in major apoptotic pathways, induces proapoptotic proteins, and represses antiapoptotic genes. p53 can activate proapoptotic genes encoding members of the Bcl-2 family such as Bax, also the death domain receptor for TNF-related apoptosis-inducing ligand DR5.[5] p53 induces Apaf-1 expression to activate apoptosome complex.[6,7] p53 antagonizes antiapoptotic effects of Bcl-X_L and Bcl-2 by interacting with them and induces Bak oligomerization to trigger apoptosis.[8] Apoptosis is also triggered by p53 when it represses survivin (antiapoptotic protein) to enhance caspase activation.[9] However, all these proapoptotic functions of p53 can only be realized when it is activated by dissociation of it from its inhibitor MDM2. This is the reason why MDM2 inhibitors are considered proapoptotic agents.[10]

Earlier, we have reported that the nonpolar hexane extract-crude ethanolic extract (HF-CEE) of *Moringa oleifera* seeds inhibited MCF7 breast cancer cell growth.[11] Hence, in this study, we profiled the phytochemical compounds of HF-CEE to identify potential anticancer agents. The major compound found in HF-CEE that had been reported to have antiproliferative effect was docked with Bax and MDM2 *in silico*.

MATERIALS AND METHODS

Gas chromatography–mass spectrometry analysis for identification of compounds in hexane extract-crude ethanolic extract

Gas chromatography–mass spectrometry (GC MS) analysis of the sample (HF-CEE) was performed using Agilent GC 7890A equipped with splitless/split injector coupled with 5 MS (5% phenyl methyl silox) capillary column that has 30 µm × 250 µm film dimension and 0.25 µm thickness, respectively. The column is attached to a mass detector. The flow rate of helium (He) as the carrier gas was 10 ml/min. The injector and detector temperatures were both maintained at 280°C. The column temperature was initially set to 70°C and held for 2 min followed by a raise to 200°C at a rate of 8°C/min for 2 min. Finally, the temperature was further raised to 250°C at 10°C/min and held for 2 min. Total run time was 27.2 min. Mass spectra were acquired by mass spectrometer in scan mode at 70 eV in a range of 30–800 m/z. The injection volume was 1 µl of 2.5 mg/ml. Sample was intermittently run with methanol as blank, and the run for the extract was in triplicate.

Identification of phytochemical compounds

Phytochemical compounds in HF-CEE were identified by matching mass spectra acquired with mass spectra of known compounds available in the library of the National Institute of Standards and Technology. Matchings with 90% quality were considered to be possible identity.

Molecular docking analysis

To study possible structural interactions that activate Bax and inhibit p53-MDM2 complex (a complex that inactivates p53 apoptotic action) action, protein–ligand molecular docking of methyl elaidate (9-octadecenoic acid, methyl ester) that was identify as the major anticancer agent in HF-CEE and proteins, Bax, and MDM2 was differently performed using AutoDock 4.2 (Molecular Graphics Laboratory, The Scripps Research Institute, La Jolla, USA).[12] Mol file of methyl elaidate structure was retrieved from ChemSpider (chemspider.com) and subsequently optimized and converted to pdb file using open babel. Bax (pdb id: 1F16) and MDM2 (pdb id: 1YCR) were downloaded from Protein Data Bank. The ligand (methyl elaidate) was made flexible to make it adaptable to rigid target (receptor) conformation. Unbounded atoms and water molecules were removed from receptor (Bax/MDM2) by AutoDock tools. Then, hydrogen atoms and charges by Gasteiger computation were assigned to the receptor. Docking calculations were performed by Lamarckian genetic algorithm (LGA) method. Grid box with grid map of dimensions (100 Å × 100 Å × 100 Å for Bax; 70 Å × 70 Å × 70 Å for MDM2) was placed to cover receptor's surface with grid spacing of 0.375 Å. One hundred LGA runs of 150 individuals each were performed. The rest parameters were set to default. Upon completion of docking, conformation with lowest binding energy was chosen for analysis. The interaction of the compound with protein (Bax/MDM2) was visualized by Chimera 1.9, and hydrogen bond formation and hydrophobic interactions between the two molecules were observed and analyzed by LigPlot+.

Drug-likeness prediction of methyl elaidate by rule of "5"

The SMILES of methyl elaidate was retrieved from ChemSpider (http://www.chemspider.com/) and submitted to molinspiration (www.molinspiration.com/cgi-bin/properties) for determination of drug-likeness of the compound by the rule of 5.

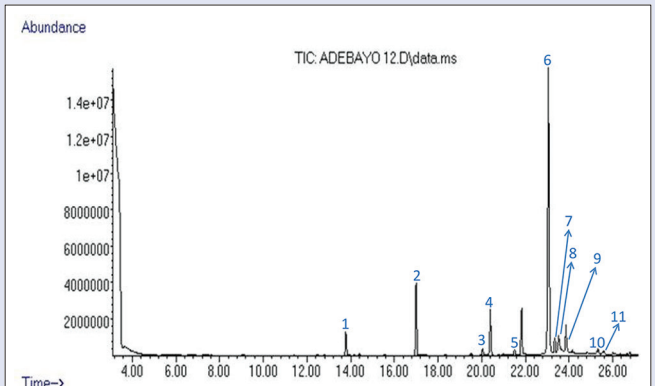
RESULTS

Identification of 9-octadecenoic acid, methyl ester (methyl elaidate), and other esters in hexane extract-crude ethanolic extract by gas chromatography–mass spectrometry

GC-MS analysis of HF-CEE was performed to identify compounds in the fraction because hexane usually extracts esters from plant which are easily detected by the method.[13] Methyl elaidate (55.36%) was identified as the major compounds together with other esters. Figure 1 displays the chromatogram, names, and other properties of the esters identified in HF-CEE, respectively.

Methyl elaidate binds to Bax and p53 inhibitor MDM2 *in silico*

Molecular docking of methyl elaidate (ligand) and Bax and MDM2 proteins was separately performed by AutoDock 4.2, and the results were visualized by Chimera 1.9 and LigPlot+. Methyl elaidate binds to Bax with the best conformation that has a minimum free binding energy



2-Butenedioic acid (Z)-, monododecyl ester (1). Dodecyl acrylate (2). 9-Hexadecenoic acid, methyl ester (Z) (3). Pentadecanoic acid, 14-methyl-, methyl ester (4). Hexadecanoic acid, ethyl ester (5). 9-Octadecenoic acid, methyl ester, (E) (6). Octadecanoic acid, methyl ester (7). 6-Octadecenoic acid (Z) (8). Ethyl oleate (9). 11-Eicosenoic acid, methyl ester (10). Eicosanoic acid, methyl ester (11).

Figure 1: Chromatogram of gas chromatography–mass spectrometry analysis of hexane extract-crude ethanolic extract showing the peaks of identified esters with their names below the figure

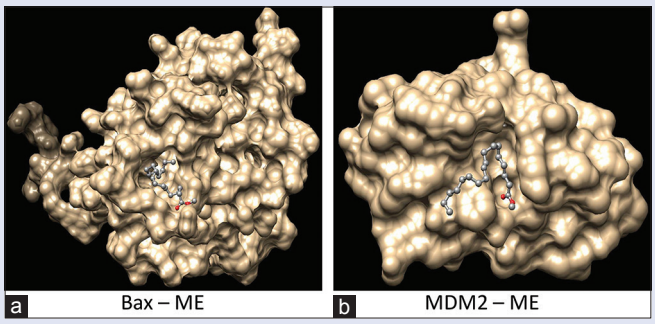


Figure 2: Protein-ligand interactions between methyl elaidate and Bax/MDM2. (a) Methyl elaidate–Bax complex and (b) Methyl elaidate–MDM2 complex as displayed by Chimera 1.9. Methyl elaidate ligand is showed as ball and stick at the active sites of Bax and MDM2

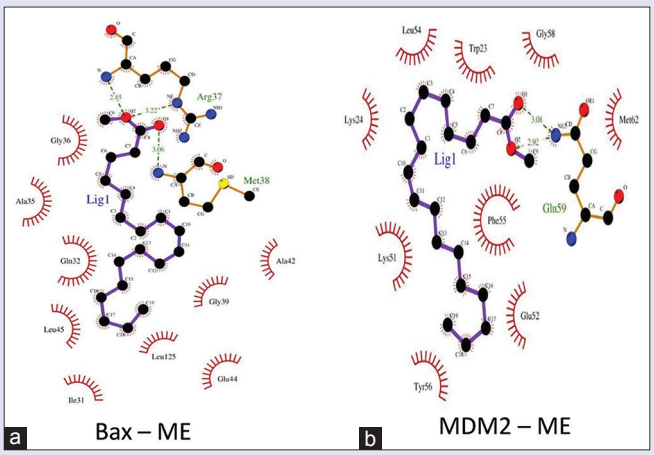


Figure 3: Hydrogen bonds and hydrophobic interactions formed between methyl elaidate ligand and Bax (a) and between methyl elaidate ligand and MDM2 (b) as analyzed by LigPlot+. Methyl elaidate is labeled as Lig1

of −4.44 kcal/mol [Figure 2a]. Met38 and Arg37 of Bax bound to 1-oxygen and 2-oxygen atoms of methyl elaidate, respectively. Other interactions between atoms and compounds of the two molecules (methyl elaidate and Bax) are hydrophobic [Figure 3a].

Interestingly, we found that methyl elaidate binds to p53 inhibitor MDM2 at the same binding pocket as p53.[10] The best conformation of methyl elaidate that binds to MDM2 has −5.05 kcal/mol binding energy [Figure 2b]. Gln59 of MDM2 forms hydrogen bond with the two oxygen atoms of methyl elaidate. Leu54, Phe55, Lys51, Gly58, and other amino acid residues of MDM2 in the binding pocket form hydrophobic interactions with methyl elaidate carbon atoms [Figure 3b].

Methyl elaidate passes drug-likeness test

Drug-likeness of methyl elaidate (major compound in HF-CEE) was determined by the rule of "5" using web-based server (www.molinspiration.com/cgi-bin/properties), and the results are displayed in Table 1. As displayed in the table, methyl elaidate did not violate more than one of the rules, suggesting that it could be suitable for oral administration.

Table 1: Drug-likeness prediction of methyl elaidate

Methyl elaidate property	Score/value
Molecular weight	296.50
Number of hydrogen bond acceptors	2
Number of hydrogen bond donors	0
LogP	7.89
Number of violation	1

DISCUSSION

To identify which HF-CEE ester is responsible for its antiproliferative effect, GC-MS analysis was performed. Eleven ester compounds were identified in HF-CEE by GC-MS, and the chromatogram showing their peaks is shown in Figure 1. Most of the compounds are methyl ester derivatives, and 9-octadecenoic acid, methyl ester (methyl elaidate) is present at 55%, equivalent to five times higher than the next methyl ester. Fatty acids and derivatives in plant extracts have been widely reported to inhibit cancer cell proliferation by induction of apoptosis.[14-16] A clear example of this was the extract from *Euphorbia kansui*, which contained a high amount of octadecenoic acid, methyl ester as the major compound that triggered apoptosis in human gastric cancer cell line (SGC-7901). Similarly, in this study, the same compound (methyl elaidate) is the most abundant in HF-CEE[17] which showed the extract could have apoptotic activity on cancer cells.

Hence, we studied the apoptotic ability of methyl elaidate *in silico*. Protein–ligand interactions of methyl elaidate and Bax/MDM2 obtained by docking are visually presented in Figure 2. Intermolecular bonds involved in the interactions were determined by LigPlot+ [Figure 3]. The best conformation of methyl elaidate for the formation of complex with Bax has a minimum free binding energy of −4.44 kcal/mol which is far lesser than that of known small-molecule Bax agonist SMBA1 that has −0.5464 kcal/mol. Methyl elaidate interacts with Bax by the formation of hydrogen bonds between Arg38 and Met38 of Bax and its oxygen atoms. The hydrogen bond distances are within 2.5–3.5 Å which suggests that the complex has high stability.[18] Hydrophobic interactions also occurred between carbon atoms of methyl elaidate and many residues of Bax [Figure 3a]. Seven of Bax protein residues (Ile31, Gln32, Ala35, Gly36, Gly39, Ala42, and Leu125) that interacted with methyl elaidate fall within the active sites of Bax potential modulator needed to bind for activation of its apoptotic action.[19] This clearly depicts that methyl elaidate could act as an inducer of apoptosis in cancer cells through Bax activation. In addition, a relatively stable complex was formed by methyl elaidate and a p53 inhibitor, MDM2 with a conformation that has the lowest free binding energy, and the highest binding affinity (−5.05 kcal/mol).[20] Although hydrogen bonds exist between methyl elaidate and MDM2, most interactions between them are hydrophobic. The interactions involved many residues of MDM2 such as Lys24, Leu54, and Gly58. [Figure 3b]. Similar to nutlin-3a (a known inhibitor of MDM2), methyl elaidate forms complex by hydrophobic interactions with three important MDM2 amino acids that bind to p53 which are Trp23, Leu54, and Gly58.[21,22] This strongly suggests that methyl elaidate could activate p53 by inhibiting formation of p53-MDM2 complex. Finally, drug-likeness of methyl elaidate was determined, and it violated only the drug permeability rule (logP >5, poor absorption) in the standard drug-likeness rule of "5," but its poor membrane permeability can be significantly improved by various well-known methods.[23,24]

CONCLUSION

In silico experiment showed that methyl elaidate (from *M. oleifera* seeds) could bind to Bax and MDM2 to induce apoptosis. However, it will be interesting to investigate the antiproliferative effect of methyl elaidate *in vitro* and *in vivo* as well.

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Conflicts of interest

There are no conflicts of interest.

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