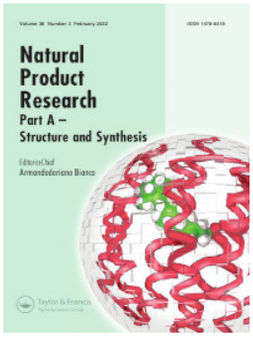


Taylor & Francis
Taylor & Francis Group

Natural Product Research

Formerly Natural Product Letters

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/gnpl20

UPLC-MS/MS and GC-MS based metabolites profiling of *Moringa oleifera* seed with its anti-*Helicobacter pylori* and anti-inflammatory activities

Abeer M. El Sayed, Farghaly A. Omar, Marwa Mostafa Abdel-Aziz Emam & Mohamed A. Farag

To cite this article: Abeer M. El Sayed, Farghaly A. Omar, Marwa Mostafa Abdel-Aziz Emam & Mohamed A. Farag (2022): UPLC-MS/MS and GC-MS based metabolites profiling of *Moringa oleifera* seed with its anti- *Helicobacter pylori* and anti-inflammatory activities, Natural Product Research, DOI: 10.1080/14786419.2022.2037088

To link to this article: https://doi.org/10.1080/14786419.2022.2037088

NATURAL PRODUCT RESEARCH
https://doi.org/10.1080/14786419.2022.2037088

Taylor & Francis
Taylor & Francis Group

SHORT COMMUNICATION

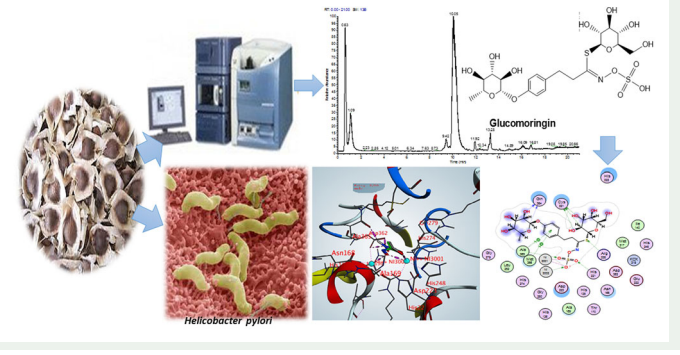
UPLC-MS/MS and GC-MS based metabolites profiling of *Moringa oleifera* seed with its anti- *Helicobacter pylori* and anti-inflammatory activities

Abeer M. El Sayed[a], Farghaly A. Omar[b], Marwa Mostafa Abdel-Aziz Emam[c] and Mohamed A. Farag[a,d]

[a]Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt; [b]Medicinal Chemistry Department, Faculty of Pharmacy, Assiut University, Assiut, Egypt; [c]Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt; [d]Department of Chemistry, School of Sciences and Engineering, American University, Cairo, Egypt

ABSTRACT

Compared to its leaf, few studies have been reported on the seeds of *Moringa oleifera* Lam. Metabolites profiling analysis of *M. oleifera* seed methanolic extract (ME) and its fixed oil (MO) was attempted *via* LC/MS and GC/MS. LC/MS analysis of *M. oleifera* seeds annotated 84 peaks of which glucosinolates and their corresponding acetyl isomers were abundant. GC/MS of seed oil revealed the abundance of fatty acids with oleic acid at 34.3%. ME exhibited significant anti-*Helicobacter pylori* activity with MIC_{50} 0.92 μg/mL, nearly one-half that of Clarithromycin. Fixed oil (MO) showed a nonselective anti-inflammatory effect with IC_{50}= 24.4 ± 0.8 μg/mL correlated to Ibuprofen. To unravel the mechanism of the anti-*H. pylori* activity a molecular docking study of the principal components of the ME has been performed, using H. pylori urease enzyme. Interactions with Ni^{2+} ions and amino acid residue in the active site, which are crucial for the enzyme's biochemical role, are evidenced.



ARTICLE HISTORY
Received 11 September 2021
Accepted 25 January 2022

KEYWORDS
Fixed oil; glucosinolates; GC/MS; *Helicobacter pylori*; LC/MS; molecular docking

CONTACT Abeer M. El Sayed abeer.ali@pharma.cu.edu.eg

Supplemental data for this article can be accessed online at https://dx.doi.org/10.1080/14786419.2022.2037088.

1. Introduction

Moringa oleifera leaves are traditionally used as nutritious foods and likewise conventional medicine. *Moringa oleifera* rich in polyunsaturated fatty acids, and phytonutrients i.e., polyphenols and glucosinolates (Amaglo et al. 2010) account for its nutritive value and several health benefits. Moringa seeds are rich in glucosinolates (Shanker et al. 2007). Recently, (Adege et al. 2016) identified Moringa seed oil as of high yield at 30–40% w/w in addition to its, high-level of monounsaturated fatty acids. Haristoy et al. (2005) confirmed that 4-α-L-rhamnosyloxy benzyl isothiocyanate, has a potential anti- *Heliobacter pylori* effect and is derived from the hydrolysis of 'glucomoringin' of *M. oleifera*.

Previous reports confirmed moringa parts i.e., leaf, seed, root, and oil anti-inflammatory along with antiulcer effects (Meireles et al. 2020). *Moringa oleifera* displayed certain level of protection against the *H. pylori* strain of bacteria mostly attributed to its benzylisothiocyanate contents (Peixoto et al. 2011). Data on *M. oleifera* was limited to specific germplasm Malaysian or Ghanaian cultivars (Nouman et al. 2016) and plants grown in Sahrawi Refugee Camps, Chad, and Haiti (Leone et al. 2015) mostly evaluated for polyphenolic contents. Few studies were reported concerning fatty acid composition of moringa seed oil (Aly et al. 2016; Barakat and Ghazal 2016). Whilst few report has been made on *Moringa* seed of the Egyptian plant and its detailed metabolites composition using advanced analytical tools. Abdelsayed et al. (2021) reported a quantification of niazimicin, thiocarbamate glycoside in leaves and seed of *M. oleifera* which measured as valuable drug in potential pharmaceutical application. Aim of work was to assess the metabolites profile of *M. oleifera* seed. First, UPLC-MS was used for the profiling secondary metabolites i.e., phenolics and glucosinolates. Second, GC/MS was used for characterisation of the fixed oil derived seeds in terms of its fatty acid composition. Seed extracts subjected to anti- *Helicobacter pylori* and anti-inflammatory activities. Furthermore, docking study for principal components identified in Moringa seeds was attempted to decipher the underlying mechanism of *H. pylori* antibacterial effect.

2. Results and discussion

2.1. Profiling of M. oleifera seed secondary metabolites through UPLC-MS

A total of 84 peaks were annotated in both ESI^+ and ESI^- ionisation modes (Table S1 and Figure S1). As of the base peak intensity chromatograms, it appears that the positive was better than the negative ionisation mode. Metabolite classes identified include thiocarbamates, glucosinolates, nitriles, phenolic acids, flavonoids, alkaloids, glycosides, and steroids with the chemical structures (Figure S1-2).

Ultraperformance liquid chromatography-tandem mass spectrometry (UPLC-MS) assessments of ME showed a varied array of phenolics with flavanols as the major subclass (Table S1). Hydroxycinnamic acids were identified in several of which were previously reported in leaves of moringa (Vergara-Jimenez et al. 2017). Based on molecular masses, along with fragmentation patterns 7 flavanols and 13 cinnamates were tentatively known by association with literature. Distinct flavonoids were characterised by

quercetin and kaempferol derivatives present mostly as *O*-glycosides including kaempherol-3-*O*-glucoside, kaempherol-3,7-*O*-diglucoside **(5)**, kaempherol-7-*O*-glucoside **(7)**, afromosin (isoflavone) (**25**), quercetin-3-*O*-acetylglucoside **(44),** isorhamntin **(50)**, luteolin-3-*O*-pentoside **(52)** and kaempferide-3-*O*-(2,3-di-*O*-acetyl-β-D-glucopyranoside **(59)** (Table S1 and Figure S1).

With respect to hydroxycinnamic acids, UPLC-MS study recognised the resulting compounds: *O*-caffeoyl-γ-quinic acid lactone **(9)**, *O*-acetyl-4-*O*-caffeoylquinic acid **(13)**, malonylcaffeoylquinic acid **(24)**, 4-*O*-caffeoyl- quinic acid butyl ester (**28**), caffeic acid **(29)**, *O*-feryloy-γ-quinide-methylether (**32**), trihydroxycinnamoyl gallic alcohol derivatives **(36),** *p*-coumaric acid -*O*-hexoside **(58)**, cryptochlorogenic acid **(62)** and 3-*p*-coumaroyl quinic acid **(69)** (Table S1). The main hydroxylcinnamic acids were *O*-caffeoylquinic acids suggestive that seeds of the Egyptian *M. oleifera* is a great supply of these bioactives compared to leaves as reported (Nouman et al. 2016). Major Phenolics identified in *M. oleifera* seeds were crypto-chlorogenic acid **(62)**, quercetin-*O*-acetyl glucoside **(44)** and astraglin **(7)** (Figure S1) which, opposing to findings in India and Ghana, which depicted the primary compounds like chlorogenic acid and rutin (Amaglo et al. 2010).

Next to phenolics, Egyptian *M. oleifera* seeds ME showed peaks for glucosinolates and isothiocynates eluted in the range 3.8–8.5 and 10.1 − 14.4 min, respectively. UPLC/MS identified 9 glucosinolates noticed from their even molecular ion detected in peaks. Glucosinolates detected in seeds hydromethanolic included desulfated glucomoringin (hydroxybenzyl thiocarbamic acid-4′-*O*-β-L-rhamnoside **(1)**, glucsinalbin(4′-hydroxybenzyl-glucosinolate)**(2)**, Niazimin A **(3)**, Niazirinin**(11)**, Glucomoringin **(12)**, Niazidin **(14)**, Niazinin A **(19)**, Marumoside B **(20)**, Niazimicin **(22),** Marumoside A **(33),** Niazicin A **(45)**, 4-hydroxybenzylisothiocyanate-*O*- α-L-rhamnoside **(49)**, Niaziminin A **(53)** and Niazicinin A **(60)**. The most plentiful glucosinolate in *M. oleifera* is recognised as glucomoringin (Amaglo et al. 2010). The profile of glucosinolate in seeds mostly analogies that of those in matured leaves (Fahey et al. 2018). Whereas, niazimicin was detected in the seed of *M. oleifera* as twofold its amount in leaves (Abdelsayed et al. 2021). Niazimicin has demonstrated an antimicrobial activity (Jeon et al. 2014). Our results of LC-MS screening of *M. oleifera* seeds extract had identified five representative components of the methanolic extract (glucomoringin; niazimicin, niazirinin, niazidin and 4-Hydroxycoumarinenolate (Figure S2) which were selected for the molecular docking study.

Moringa seed is highly nutritious as commercial leaves. Major differences were noted for the content of polyphenols among leaves and seeds of moringa. Kaempferol-3,7-diglucoside exhibited comparable existence in both leaves and seeds of moringa. Whereas kaempferol-7-*O*-glucoside was emphasised in seed than in leaves (Nouman et al. 2016). The flavonols profile documented in the current work as steady with the reported by (Amaglo et al. 2010) with close variations that could be ascribed to the diverse genetic background and the difference of the agro-climatic conditions. Manguro and Lemmen (2007) reported galloylated and other flavonoids contrasted with the results obtained herein.

2.2. Profiling of moringa seed primary metabolites via GC-MS

Oil derived from Moringa seeds cultivated in Egypt using solvent extraction with *n*-hexane reached 40% is comparable to that in previous reports (Abdelkarim et al. 2007). Gas chromatography–mass spectrometry (GC/MS) was used to characterise chemicals in the fixed oil with a total of 55 compounds amounting for 96.9%. Table (S2) represents the content of the main fatty acids (stated as percentage of total fatty acids in the oil) relates it with records of (Nguyen et al. 2011). These results suggest that moringa oil is a rich source of omega-9 fatty acids like that of olive oil. The unsaturated fatty acid presented by oleic acid as major makeup by 34.3% nearly double concentration in comparison to the previous report (Adege et al. 2016).

2.3. Anti- Helicobacter pylori activity

Results clearly confirm *M. oleifera* seeds as potent growth inhibitor of *H. pylori* (Figure S3A and Tables S3, S4). Results presented in Figure S3 A revealed that in general the ME was more effective than that of MO, and suggestive that polar metabolites extracted using methanol in ME mediate for that effect rather the nonpolar nature of fixed oil in MO. Glucosinolates and their breakdown products, isothiocyanates, previously reported to display an inhibitory effect for urease from *H. pylori* (Fahey et al. 2013, Romeo et al. 2018). Consequently, it was crucial to have an insight about the potential mechanism of the anti-*Helicobacter pylori* activity.

2.4. Anti-inflammatory activity

2.4.1. In vitro cyclooxygenase (COX) inhibition assay

Non-specific COX inhibitory reaction was observed for MO (IC_{50}=24.4 ± 0.84 μg/mL) is nearly half that of Ibuprofen (IC_{50}= 12.7 ± 1.2 μg/mL) which are concurrent with the previously reported data of Moringa leaves (Mittal et al. 2017). Percentage of COX-2 inhibition of ME and MO are represented in Table S5. Results presented (Figure S3B) demonstrated that the MO showed significantly higher activity than ME of the seed, and opposite to that of the anti-*H. pylori* assay. The abundance of omega-9 fatty acid i.e., oleic acid in seed oil mediate for its anti-inflammatory action has yet to be determined. Moringa seed has a dual action; it exerts an anti- *Helicobacter pylori* and anti-inflammatory effect. So, it could be used for protection of stomach against the inflammation results from *H-pylori* infection.

2.5. Molecular docking study

The docking results illustrated in Figure S4 B(a-e) demonstrates that all the studied compounds (Figure S2) were well fitted in the active pocket of the enzyme (Table S6). Analysis of the data revealed that all the studied compounds coordinate to the Ni^{2+} ions, in addition to H-bonding or hydrophobic interactions to several amino acid deposits inside the active site of the urease enzyme. Specific function groups appear to be substantial in the coordination with Ni^{2+} ions and binding to the essential amino acid residues. Examples are the oxosulfonate group ($-OSO_3^-$) in glucomoringin,

the thiocarbamate group (niazimicin and niazidin), CN group (Niazirinin), and the enolate group of 4-hydroxychromenenolate. The docking scores surpassing that of the native inhibitor (AHA) by $\sim 2-3$ times. The values ranging from -21.2077 to -11.6139 Kcal/mol suggesting that the binding varies as the functional group is different. The uppermost compound is glucomoringin (lowest score value -21.2077 Kcal/mol), it implies that this is the greatest binding inhibitor. Various natural ingredients also show an inhibitory activity against *H. pylori* urease (Hassan and Žemlička 2016).

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

The author(s) reported there is no funding associated with the work featured in this article.

ORCID

Abeer M. El Sayed http://orcid.org/0000-0002-1553-5919
Mohamed A. Farag http://orcid.org/0000-0001-5139-1863

References

Abdelsayed EM, Abdel Motaal A, Hanafi RS. 2021. Novel UPLC-MS/MS method for standardization of niazimicin content in edible seeds and leaves of *Moringa Oleifera* Lam. J Food Drug Anal. 29 (1):128–143.

Abdelkarim SM, Long K, Lai OM, Muhammad SKS, Ghazali HM. 2007. Frying quality and stability of high-oleic *Moringa oleifera* seed oil in comparison with other vegetable oils. Food Chem. 105(4):382–1389.

Adege AA, Larayetan RA, Omojuwa TJ. 2016. Proximate analysis, physicochemical properties, and chemical constituents' characterization of *Moringa oleifera* (Moringaceae) seed oil using GC-MS analysis. Am J Chem. 6(2):23–28.

Aly AA, Maraei RW, Ali HGM. 2016. Fatty acids profile and chemical composition of Egyptian *Moringa oleifera* seed oils. J Am Oil Chem Soc. 93(3):397–404.

Amaglo NK, Bennett RN, Lo Curto RB, Rosa EAS, Lo Turco V, Giuffrida A, Curto AL, Crea F, Timpo GM. 2010. Profiling selected phytochemicals and nutrients in different tissues of the multipurpose tree *Moringa oleifera* L., grown in Ghana. Food Chem. 122(4):1047–1054.

Barakat H, Ghazal G. 2016. Physicochemical properties of *Moringa oleifera* seeds and their edible oil cultivated at different regions in Egypt. Food Nutr Sci. 7(06):472–484.

Fahey JW, Olson ME, Stephenson KK, Wade KL, Chodur GM, Odee D, Nouman W, Massiah M, Alt J, Egner PA, et al. 2018. The diversity of chemoprotective glucosinolates in Moringaceae (Moringa spp.). Sci Rep. 8(1):7994.

Fahey JW, Stephenson KK, Wade KL, Talalay P. 2013. Urease from *Helicobacter pylori* is inactivated by sulforaphane and other isothiocyanates. Biochem Biophys Res Commun. 435(1):1–7.

Haristoy X, Fahey JW, Scholtus I, Lozniewski A. 2005. Evaluation of the antimicrobial effects of several isothiocyanates on *Helicobacter pylori*. Planta Med. 71(4):326–330.

Hassan STS, Žemlička M. 2016. Plant-derived urease inhibitors as alternative chemotherapeutic agents. Arch Pharm (Weinheim)). 349(7):507–522.

Jeon SR, Lee KH, Shin DH, Kwon SS, Hwang JS. 2014. Synergistic antimicrobial efficacy of mesoporous ZnO loaded with 4-(α-L-rhamnosyloxy)-benzyl isothiocyanate isolated from the *Moringa oleifera* seed. J Gen Appl Microbial. 60:251–255.

Leone A, Spada A, Battezzati A, Schiraldi A, Aristil J, Bertoli S. 2015. Cultivation, genetic, ethnopharmacology, phytochemistry and pharmacology of *Moringa oleifera* leaves: An overview. Int J Mol Sci. 16(6):12791–12835.

Manguro LO, Lemmen P. 2007. Phenolics of *Moringa oleifera* leaves. Nat Prod Res. 21(1):56–68.

Meireles D, Gomes J, Lopes L, Hinzmann M, Machado J. 2020. A review of properties, nutritional and pharmaceutical applications of *Moringa oleifera*: integrative approach on conventional and traditional Asian medicine. Adv Tradit Med (Adtm)). 20(4):495–515.

Mittal A, Sharma M, David A, Vishwakarma P, Saini M, Goel M, Saxena K. 2017. An experimental study to evaluate the anti-inflammatory effect of *Moringa oleifera* leaves in animal models. Int J Basic Clin Pharmacol. 6(2):452–457.

Nguyen HN, Gaspillo PD, Maridable JB, Malaluan RM, Hinode H, Salim C, Huynh HKP. 2011. Extraction of oil from *Moringa oleifera* kernels using supercritical carbon dioxide with ethanol for pretreatment: optimization of the extraction process. Chem Eng Process. 50 (11-12): 1207–1213.

Nouman W, Anwar F, Gull T, Newton A, Rosa E, Domínguez-Perles R. 2016. Profiling of polyphenolics, nutrients, and antioxidant potential of germplasm's leaves from seven cultivars of *Moringa oleifera* Lam. Ind Crop Prod. 83:166–176.

Romeo L, Iori R, Rollin P, Bramanti P, Mazzon E. 2018. Isothiocyanates: an overview of their antimicrobial activity against human infections. Molecules. 23(3):624.

Peixoto JRO, Silva GC, Costa RA, de Sousa Fontenelle JL, Vieira GHF, Filho A. d, Fernandes Vieira RHS. 2011. In vitro antibacterial effect of aqueous and ethanolic Moringa leaf extracts. Asian Pac J Trop Med. 4(3):201–204.

Shanker K, Gupta MM, Srivastava SK, Bawankule DU, Pal A, Khanuja SPS. 2007. Determination of bioactive nitrile glycoside(s) in drumstick *(Moringa oleifera*) by reverse phase HPLC. Food Chem. 105(1):376–382.

Vergara-Jimenez M, Almatrafi MM, Fernandez ML. 2017. Bioactive components in Moringa oleifera leaves protect against chronic disease. Antioxidants (Basel, Switzerland). 6(4):91.