Science World Journal Vol. 15(No 3) 2020
www.scienceworldjournal.org
ISSN 1597-6343
Published by Faculty of Science, Kaduna State University
https://doi.org/10.47514/swj/15.03.2020.026

Full Length Research Article

CHARACTERIZATION OF GMG-ITC ISOLATED FROM AERIAL PARTS OF *MORINGA OLEIFERA* TREE

Richard Auta, Peter Waziri, Jaafaru Sani Mohammed, Rabiat Idrees Abubakar, Samson Wayah and Yusuf Alhassan

Department of Biochemistry, Kaduna State University, PMB 2339 Tafawa Balewa Way, Main Campus, Kaduna, Nigeria.

*Corresponding Author's Email Address: zautarichard2004@yahoo.com Phone: +2348035877395

ABSTRACT
Isothicyanate is a major bioactive compound in *Moringa oleifera* Lam. There are numerous literatures that report the therapeutic effects of isothiocyanates. It is for this reason that the local consumption of the plant is increasing. In the current study, we devised a rapid protocol for the extraction of ITC from the aerial parts of *M. oleifera* and also determined the plant part with the highest yield. The purity of the ITCs was confirmed by High Performance Liquid Chromatography analysis. Our findings revealed that the seeds contain the highest proportion of isothiocyanate. This implies that the seeds of *M. oleifera* could serve as invaluable feedstock for large scale extraction of the ITCs for pharmaceutical and food industries.

Keywords: HPLC based Characterization, Glucomoringin Isothiocyanate, *Moringa oleifera*

INTRODUCTION

Isothiocyanates (ITC) are a class of Sulphur containing secondary metabolites produced via the hydrolysis of glucosinolates (Jaafaru *et al.,* 2018). The hydrolysis of the glucosinolates occurs under the action of the enzyme myrosinase in plants. This process is usually triggered by tissue damage, chopping or chewing of the plant tissues that enables the release of myrosinase and its consequent hydrolytic activity (Dinkova-Kostova and Kostov, 2012). Regular consumption of plants that are rich in glucosinolates provides the body with a pool of ITCs that exert beneficial therapeutic effects in the body. ITCs, the active form of glucosinolates are predominantly found in Brassicaceae, Capparaceae and Moringaceae plant families (Sharma and Kapoor, 2015). *Moringa oleifera* is reported to contain significant proportion of important phytochemicals including GMG-ITC (Glucomoringen-ITC) that have been used for the treatment of cancer, inflammation, neurodegenerative diseases and bacterial, viral and fungal infections (Sharma and Kapoor, 2015). The seeds of *M. Oleifera* contain essential oils, rhamnose, glucose and relatively high quantity of glucosinolates and their derivatives (isothiocyanates) (Jaja-Chimedza *et al*., 2017; Tripathi *et al*., 2017). The seeds also contained certain prominent substances like benzylamine (moringinine), thiocarbamate (moringin or [4-(α-l-rhamnopyranosyloxy) benzyl isothiocyanate]), glycosides (niaziminin and niazinin), β-sitosterol and lutein (Mbikay, 2012; Rajan *et al*., 2016). The ash content of *M. oleifera* Lam is rich in miringinine and moringin (alkaloids), and the purely extracted gum from the plant has been reported to possess galactose, arabinose, mannose, glucuronic acid, rhamnose and xylose, all in L form (Lalas *et al*., 2017; Singh, 2017). These monosaccharides are the mildly acidic products produced from the hydrolysis of the entire gum (Singh, 2017). Furthermore, the flower part of the plant contains traces of alkaloids, D-glucose, quercetin, kaempferitrin, wax and sucrose; with calcium and potassium abundantly present in the ash (Maizuwo *et al*., 2017; Oyeyinka and Oyeyinka, 2018). The leaves of the plant have been reported to be good source of antioxidants as they contains flavonoids, ascorbic acid, carotenoids and phenolics in addition to proteins, β-carotene, vitamin C, potassium and calcium (Nouman *et al*., 2016). Miracle tree, as it is popularly called, the entire plant has been associated with a number of medicinal activities ranging from anti-inflammatory, antioxidant, antibacterial, antiulcer, anticancer, antidiabetic and neuroprotective properties (Daba, 2016; Vaknin and Mishal, 2017, Jaafar *et al*., 2019). Generally, *M oleifera* Lam is known for its economic value and versatile uses as functional food, medicinal agent, water purifying potential and nutraceutical among others (Daba, 2016). Being a repository of ITCs, we extracted and characterized ITCs from the seeds, leaves, stem bark and root of *Moringa oleifera* Lam. We also estimated the percentage yield from the aerial parts of the plant, as this would enable large scale extraction for commercial purposes by pharmaceutical and food industries.

MATERIALS AND METHOD

Plant material: The seeds, leaves, stem bark and root of *Moringa oleifera* Lam. were collected from Zaria, Kaduna State and identified by Malam Umar Ghali of the Department of Biological science (resident botanist), Kaduna State University. The voucher specimen no, 1121 was assigned to the plant material and deposited in the herbarium.

Extraction of Glucomoringin-isothiocyanate: The plant materials were shade dried for 4 weeks and ground to fine powder prior to the extraction using a modified protocol of Jaafaru *et al*. (2018). About 10 g each of the dried *M. oleifera* seed, leaf, stem bark and root powder were soaked separately in 200 mL of 50% methanol (Analytical grade) in 500 mL conical flasks. The solutions were vortexed for 15 s and incubated at room temperature for 1 hour and then sonicated for 35 mins at 30°C. The yield of crude extract was calculated as follows:

$$\text{Yield (\%)} = \frac{\text{Weight of dried powdered seeds (mg)}}{\text{Weight of dried powdered seeds (mg)}} \times 100$$

Isolation of Glucomoringin-isothiocyanate: The mixture above were spun at 5000 rpm for 30 mins and the supernatant was collected and loaded on to a column parked with DEAE Sephadex (A-25). A mixture of 50% methanol, acetic acid and water in the ratio, 1:1:3 was prepared and used to flush the column. To elute the GMG-ITC, 50 mL of 5% ethanol in 0.5 M K_2SO_4 was used to run the column. The GMG-ITC was purified further using cold ethanol, and dried again on a rotary evaporator at 40°C. However,

Science World Journal Vol. 15(No 3) 2020
www.scienceworldjournal.org
ISSN 1597-6343
Published by Faculty of Science, Kaduna State University
https://doi.org/10.47514/swj/15.03.2020.026

the yield of compound with high purity was calculated as follows:

$$\text{Yield (\%)} = \frac{\text{Weight of dried eluent (mg)}}{\text{Weight of initial material (mg)}} \times 100$$

High performance liquid chromatography (HPLC): The purity of the isolated compound was ascertained on (1290 Infinity II LC System, USA) equipped with C18 column (250 mm × 3.0 mm × 5 µM). The isocratic program of 100% milliQ at 0.5 ml/min flow rate was performed at 299 nm, and a clear peak with a retention time between 1.8 to 4.0 minutes were observed as described by Jaafaru *et al*. (2018).

RESULTS AND DISCUSSION

Following extraction and isolation of the compound under study, the yields from starting material for crude extract and that of isolated compound of interest are displayed in Table 1. HPLC analysis using 299 nm wave length as described above, showed the elution of a phytochemical between 1.8 to 4.0 minutes with an intensity of about 7000 µV (Figures 1 to 4), indicating that the eluted compound was a sulphur containing principle as reported by de Graaf *et al*. (2015).

Table 1 : Yield of extract and isolated compound from *M. oleifera* Lam seeds, leaves, stem bark and root in (mg) and (%)

Sample	Initial weight (mg)	Yield of extract		Yield of compound	
		(mg)	(%)	(mg)	(%)
Seeds	10000	2121.85	21.22±0.33	335.33	3.353±0.13
Leaves	10000	1811.43	18.11±0.51	252.15	2.522±0.21
Stem bark	10000	1322.24	13.22±0.23	135.42	1.354±0.24
Root	10000	424.11	4.24±0.11	87.36	0.874±0.23

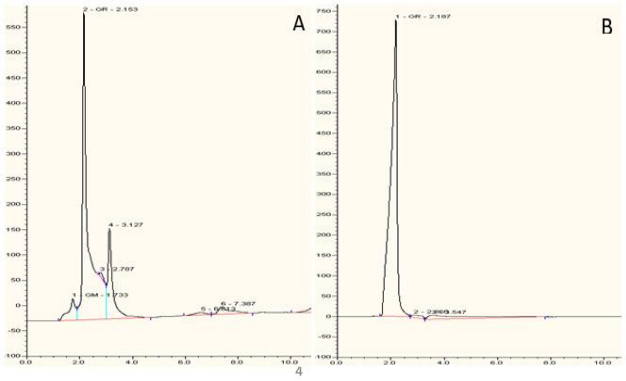

Figure 1: HPLC chromatogram of extracted isothiocyanate from the leaves of *M. oleifera* (A) and standard sinigrin (B).

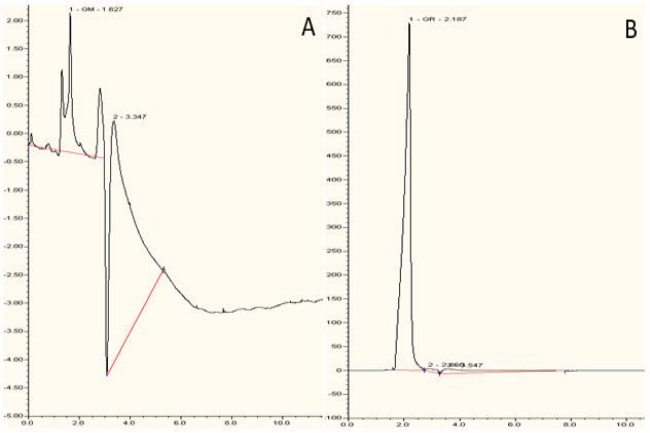

Figure 2: HPLC chromatogram of extracted isothiocyanate from the stem of *M. oleifera* (A) and standard sinigrin (B).

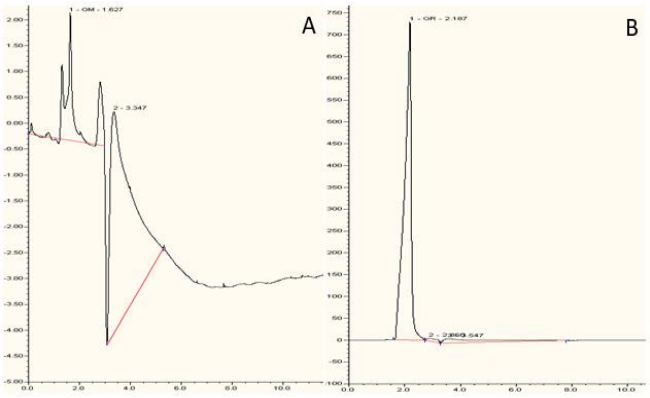

Figure 3: HPLC chromatogram of extracted isothiocyanate from the root of *M. oleifera* (A) and standard sinigrin (B).

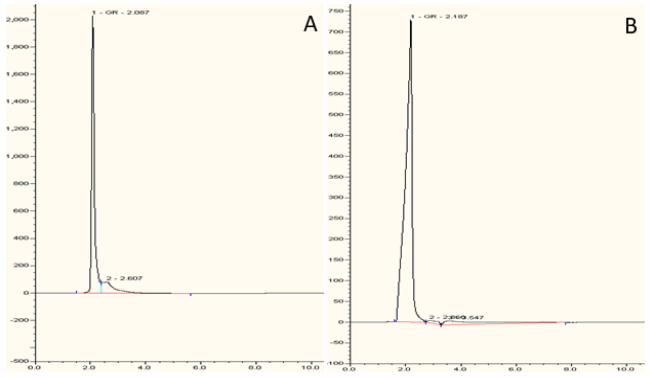

Figure 4: HPLC chromatogram of extracted isothiocyanate from the seed of *M. oleifera* (A) and standard sinigrin (B).

The use of *M. oleifera* in folklore medicine in Africa and Asia has been intensified in recent years, the aerial parts of the plant particularly the seeds and the leaves have been used to cure a number of disorders including but not limited to cancer, cardiac, infectious disease, diseases of the nervous system and metabolic diseases (Giacoppo *et al.,* 2017). Among the phytochemical content of the plant, glucomoringin isothiocyanate hold high

therapeutic potential against several disease conditions (Giacoppo *et al.,* 2017). Thus, the need to identify the plant part containing the highest percentage of GMG-ITC is a prerequisite for large scale extraction of the active component by pharmaceutical companies. The objective of this study was to isolate, characterize GMG-ITC from aerial parts of *M. oleifera Lam* using HPLC and to identify the GMG-ITC rich regions of the plant.

Of all the aerial parts of the plant used for the extraction, the seeds of *Moringa oleifera* produced the purest form of isothiocyanate and the percentage yield of the compound is much higher in the seeds compared to other parts. This was closely followed by that of the leaves, stem bark and finally the root which contained the least amount among them. Similar findings reported that the seeds of *M. oleifera* contain high content of Glucomoringin (GMG) which is the precursor of the isothiocyanate (Galuppo *et al.,* 2015). The elution time of the compound on HPLC system is in agreement with that reported by De Graaf *et al.* (2015) as observed on 1290 Infinity II LC System, where GMG-ITC was eluted between 2 to 4 minutes while its corresponding precursor was eluted between 5 to 8 minutes.

Based on literatures reviewed, the seeds and leaves of *M. oleifera* are the main parts used for the extraction of GMG-ITC. In a recent study by Jaafaru *et al*. (2018) on GMG-ITC isolated from *M. oleifera* pods, it was reported that the GMG-ITC rich fraction induced apoptosis and prevented the proliferation of human prostrate adenocarcinoma cells. Similarly, the phenylisothiocyanate has been reported to induce chemopreventive properties in cancer stem cells (Yuan *et al*., 2016).
Oral doses of the ITCs for clinical studies have been formulated and used successfully (Fahey *et al*., 2019). More importantly, ITCs have been used as an antiviral, antifungal, antibacterial, antidiabetic and anti-inflammatory agents (Sharma and Kapoor, 2015). The method described in this study is a rapid protocol that enabled the isolation of pure forms of ITCs. In addition, the study showed the seeds and leaves of *M. oleifera* produce purer and better yields of ITCs. Thus, the collective outcomes of the above analysis and supporting data from literature, enabled us to unequivocally confirm the isolated compound as 4-(α-L-rhamnosyloxy) benzyl glucosinolate known as glucomoringin (GMG).

REFERENCES

Daba, M. (2016). Miracle tree: A review on multi-purposes of Moringa oleifera and its implication for climate change mitigation. *Journal of Earth Science and Climate Change*, *7*(4).

Dinkova-Kostova, A. T., & Kostov, R. V. (2012). Glucosinolates and isothiocyanates in health and disease. *Trends in Molecular Medicine, 18*(6), 337-347.

De Graaf, R. M., Krosse, S., Swolfs, A. E., te Brinke, E., Prill, N., Leimu, R., Van Galen, P. M., Wang, Y., Aarts, M. G., & Van Dam, N.M. (2015). Isolation and identification of 4-α-rhamnosyloxy benzyl glucosinolate in Noccaea caerulescens showing intraspecific variation. *Phytochemistry*, *110*, 166-171.

Fahey, J. W., Zalcmann, A. T., & Talalay, P. (2019). The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry, 56*(1), 5-51.

Galuppo, M., Giacoppo, S., Iori, R., De Nicola, G., Milardi, D., Bramanti, P., & Mazzon, E. (2015). 4 (α-L-Rhamnosyloxy) benzyl isothiocyanate, a bioactive phytochemical that defends cerebral tissue and prevents severe damage induced by focal ischemia/reperfusion. *Journal of Biological Regulators* & *Homeostatic Agents, 29*, 343-356.

Giacoppo, S., Rajan, T. S., De Nicola, G. R., Iori, R., Rollin, P., Bramanti, P., & Mazzon, E. (2017). The isothiocyanate isolated from moringa oleifera shows potent anti-inflammatory activity in the treatment of murine subacute parkinson's disease. *Rejuvenation Research*, *20*(1), 50-63.

Jaafaru, M. S., Abd Karim, N. A., Enas, M. E., Rollin, P., Mazzon, E., & Abdull Razis, A. F. (2018). Protective effect of glucosinolates hydrolytic products in neurodegenerative diseases (NDDs). *Nutrients*, *10*(5), 580.

Jaafaru, M. S., Nordin, N., Rosli, R., Shaari, K., Noor, N. M., & Razis, A. F. A. (2019). Prospective role of mitochondrial apoptotic pathway in mediating GMG-ITC to reduce cytotoxicity in H2O2-induced oxidative stress in differentiated SH-SY5Y cells. *Biomedicine & Pharmacotherapy*, *119*, 109445.

Jaafaru, M. S., Nordin, N., Shaari, K., Rosli, R., & Abdull Razis, A. F. (2018). Isothiocyanate from Moringa oleifera seeds mitigates hydrogen peroxide-induced cytotoxicity and preserved morphological features of human neuronal cells. *PLoS One*, *13*(5), e0196403.

Jaja-Chimedza, A., Graf, B. L., Simmler, C., Kim, Y., Kuhn, P., Pauli, G. F., & Raskin, I. (2017). Biochemical characterization and anti-inflammatory properties of an isothiocyanate-enriched moringa (Moringa oleifera) seed extract. *PloS One*, *12*(8), e0182658.

Lalas, S., Athanasiadis, V., Karageorgou, I., Batra, G., Nanos, G. D., & Makris, D. P. (2017). Nutritional characterization of leaves and herbal tea of *Moringa oleifera* cultivated in greece. *Journal of Herbs, Spices & Medicinal Plants*, *23*(4), 320-333.

Maizuwo, A. I., Hassan, A. S., Momoh, H., & Muhammad, J. A. (2017). Phytochemical constituents, biological activities, therapeutic potentials and nutritional values of moringa oleifera (zogale): a review. *Journal of Drug Design and Medicinal Chemistry*, *3*(4), 60.

Mbikay, M. (2012). Therapeutic potential of Moringa oleifera leaves in chronic hyperglycemia and dyslipidemia: a review. *Frontiers in Pharmacology*, *3*, 24.

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. *Industrial Crops and Products*, *83*, 166-176.

Oyeyinka, A. T., & Oyeyinka, S. A. (2018). Moringa oleifera as a food fortificant: Recent trends and prospects. *Journal of the Saudi Society of Agricultural Sciences*, *17*(2), 127-136.

Rajan, T. S., De Nicola, G. R., Iori, R., Rollin, P., Bramanti, P., & Mazzon, E. (2016). Anticancer activity of glucomoringin isothiocyanate in human malignant astrocytoma cells. *Fitoterapia, 110*, 1-7.

Sharma, P., & Kapoor, S. (2015). Biopharmaceutical aspects of Brassica vegetables. *Journal of Pharmacognosy and Phytochemistry, 4*(1).

Singh, R. B. (2017). Degraded gum polysaccharide structure from Moringa oleifera Lam. gum polysaccharide by methylation studies. *IJCS*, *5*(3), 46-48.

Tripathi, M. K., Sharma, A. K., Giri, S. K., Deshpande, S. S., & Jadam, R. S. (2017). Use of Moringa oleifera as a complementary food fortificant. *Octa Journal of Biosciences*, *5*(2).

Vaknin, Y., & Mishal, A. (2017). The potential of the tropical "miracle tree" Moringa oleifera and its desert relative Moringa peregrina as edible seed-oil and protein crops under Mediterranean conditions. *Scientia Horticulturae*, *225*, 431-437.

Yuan, T. F., Gu, S., Shan, C., Marchado, S., & Arias-Carrión, O. (2015). Oxidative stress and adult neurogenesis. *Stem Cell Reviews and Reports*, *11*(5), 706-709.