Effect of different oil extraction techniques for *Moringa Peregrina* seed on its meal content of glucosinolates

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Background
Moringa peregrina (M.P) is a multipurpose promising tree that grows widely in Asia and Africa's tropical and subtropical regions. It is generally rich in nutrients especially oil which can be used for edible or nonedible purposes and also rich in protein. However, some anti-nutritional substances, namely glucosinolates (GSLs), remain in M.P meal preventing its utilization in animal or human feeding. These substances though good for the plant to defend against herbivores, cause deleterious effects or are even toxic to animals and humans. GSLs are the most important anti-nutritional substances in M.P seed and meal.
Objective
Accordingly, this investigation aims to use different techniques for oil extraction from M.P seed and their effect on the GSLs content of the meal remaining after oil extraction, then the meal is detoxified to be suitable for using in animal or human feeding.
Materials and methods
In this study, M.P meal was obtained as a by-product left over after oil extraction from moringa seed by different extraction procedures including (hydraulic press, screw pressing, extraction by solvent via Soxhlet, and prepress – solvent extraction at room temperature).The sinalbin, moringin, and total GSLs were determined and identified by HPLC. Detoxification of GSLs was then applied for M.P meal remaining after oil extraction by hexane, using soaking in water followed by a water-washing treatment.
Results and conclusion
The results indicated that sinalbin and moringin are the major dominant GSLs in M. P seed and meal. Moreover, the total GSLs content in the meal that remained after all different oil extraction techniques was significantly higher when compared with their content in the seed. Also, the results showed that there was a significant decrease in the level of total GSLs in M.P meal as a result of the water treatment. It decreased from 941.8±34.14 nmol/mg in defatted meal before water treatment to 23.97±18.31 nmol/mg in residues left after treatment with water for 15 min. Therefore, soaking in water followed by a water-washing treatment is the simplest and most efficient process to remove the GSLs from M.P meal to be suitable for animal feeding.

Keywords:
glucosinolates, meal, *Moringa peregrina*, oil extraction

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Letter to the Editor of Egyptian Pharmaceutical Journal

Introduction

Moringa peregrina (MP) belongs to the sole genus of Moringaceae which has about 10 xerophytic species of the genus, namely Moringa [1]. MP is native to the region extending from the Dead Sea to Southern Arabia and northern Somalia [2]. In Egypt, it is confined to the south Sinai mountains and its wadis [3,4], moreover the higher elevations of the north-facing slopes of the mountains around the Red Sea, from 1360 to 2187 m. above sea level [5]. M.P is an extremely fast-growing tree with 5-15 m height, diameter of 20–40 cm, and grayish-green bark. It has 20–70 cm leaves with several tiny leaflets that drop when the leaf matures. The flowers (10–15 mm long) are generally yellowish white to pink, bisexual, and harbor insect pollination characteristics e.g. large, showy, slightly scented, and zygomorphic [6–8]. The fruit size is 10.25×1–1.5 cm and has 5–15 ovoid, tigonous, hard-coated seeds [9]. The seed kernel is rich in oil (42–54%), with up to 23% protein. Oleic acid is the main fatty acid (>70%) [10]. The high oleic acid content of its seeds has a role in lowering blood lipids,

systolic blood pressure, and serum cholesterol [11]. In industry, there are two major techniques of oil extraction from seeds: first by mechanical pressing carried out either by (hydraulic or screw pressing) and second by direct extraction with solvent (hexane), [12]. In some cases of oil seeds with a high percentage of oil content these techniques can be a combined, in what is called prepress-solvent extraction, to raise the efficiency of the oil extraction process from the seed and to reduce the oil percentage that remains in the cake after oil extraction.

Screw pressing has some advantages over hydraulic pressing including higher oil yield, low labor cost, and elimination of the cost of filter cloth. However, screw pressing produces darker oils due to the heat developed by a mechanical fraction as well as a meal of less nutritive value. Solvent extraction is considered an effective technique for the recovery of oil from its bearing material in the industry and it is most advantages with materials low in oil such as soybean [13]. There are two major advantages of solvent extraction method over mechanical methods. First, the lower labor cost of the processing costs in solvent extraction compared with in case of hydraulic press system and higher yield of oil where solvent extraction produces a meal containing 1-1.5% oil compared with about 8% in the meal after efficient pressing. Second, the low temperature in solvent extraction of oil seeds produces better oil than oil from the high temperature of screw pressing. However, solvent extraction has some disadvantages compared with extraction by pressing. The extraction equipment is more expensive and there is a risk of fire and explosion due to the use of flammable solvents. Also, with some types of oilseeds such as cottonseed, oil obtained by solvent extraction is darker than the oil obtained by normal hydraulic press because the solvent extracts pigments matter including fixed pigments as effectively as the oil. While, the last previous technique for oil extraction "prepress-solvent extraction" is considered a combination between hydraulic pressing and solvent extractions. This technique is usually used for oil seeds that contain a high percentage of oil such as sunflower seed (traditional oil seeds) as well as untraditional oil seeds (M.P seed). Such types of seeds are difficult to flake before solvent extraction in the oil industry. The reduction of oil content to around 18–20% by a prepressing step makes the flaking step much easier and increasing the efficient of solvent extraction in the second step to get a higher yield of oil from the seed. On the other hand, GSLs, haemagglutinins, and alkaloids are considered to be the main antinutritional factors in the seed and meal of Moringa species. Particularly in M.P, they are likely to have adverse effects on animal health and production if found at high levels in the feed that the animal consumes on a daily basis. By water treatment for meal left after oil extraction, these three components were virtually absent [14]. The levels of GSLs observed for kernel and meal samples were similar to those in rapeseed meal [15,16] and *Camelina sativa* seeds [17]. Some of these GSLs can make an important contribution to the flavor and aroma of the food, the others have been shown to be potentially harmful, so it is generally unacceptable to have high levels of GSLs in foods for human and animal consumption. [18]. GSLs are metabolized by the enzyme myrosinase to their biologically active, cognate isothiocyanates [19–21]. However, isothiocyanates have long been known for their herbivore deterrent, fungicidal, bactericidal, nematocidal, and allelopathic properties [22–27]. Accordingly, the aim of this study was to investigate the effect of using different techniques for oil extraction from M.P seed on the GSLs content in the meal remaining after oil extraction, then the meal is detoxified from the GSLs to be suitable for use in animal or human feeding.

Materials and methods

Plant materials: The fully mature dried seeds of M.P samples were collected from the Red Sea region and Gabel Elba, Egypt.

Plan of work: This study was completed in two stages:

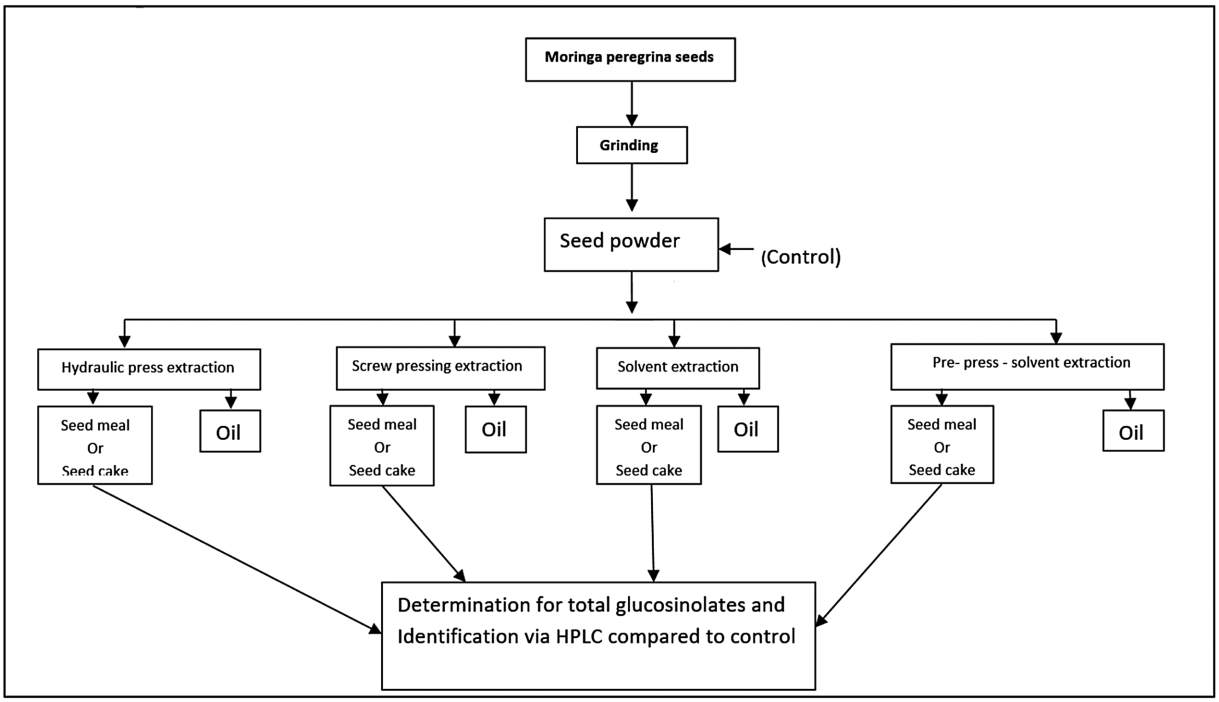
The first stage was done at labs of Fats and Oils Department, Food Industries and Nutrition Research Institute, National Research Centre, Dokki, Cairo, Egypt. Including oil extraction from M.P seed by different extraction procedures and collecting the remaining cake after each extraction process separately. As well as, detoxification of GSLs for M.P meal remaining after oil extraction by hexane.

The second stage was done at labs of the German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Institute of Biodiversity, Friedrich Schiller University Jena, Germany. Including determination and identification of GSLs in the meal leftover after oil extraction by HPLC.

Extraction procedures of *Moringa peregrina* oil

The oil was extracted from the seed by different extraction procedures including Hydraulic press, Screw pressing, Extraction by solvent via Soxhlet,

Figure 1



Extraction procedures of *Moringa Peregrina* oil.

and prepress solvent extraction at room temperature; as described in Figure 1.

Extraction by hydraulic pressing

M.P oil was extracted from the corresponding seeds by using hydraulic pressing at room temperature as follows: 750 g of the dried seeds were milled into a fine powder, then batches of the powder were wrapped in a thick heavy-duty cloth and the oil was extracted using an in-house made hydraulic laboratory scale pressing machine and operated at its maximum pressure (3500 psi) for 1 h at room temperature [28].The extracted oil was collected and the meal that remained after oil extraction was milled into a fine powder and packed in polyethylene bags and stored in a deep freezer at −20±2°C until analyzed. All extractions were performed in triplicate.

Extraction by screw pressing

M.P oil was also extracted from the same batch of seed by using komet expeller screw pressing model DD85G. 100 g of the seed was exhaustively screw pressed for two successive cycles at 60°C and an optimum screw-speed of 40 rpm [29]. The extracted oil was collected and the meal that remained after oil extraction was milled into a fine powder and packed in polyethylene bags and stored in a deep freezer at −20±2°C until analyzed. All extractions were performed in triplicate.

Extraction by solvent (n-hexane)

M.P oil was exhaustively extracted from the same batch of seed by using a Soxhlet extractor. 100 g of the milled sample was placed in extraction thimbles and extracted with n-hexane at 60°C for 6 h repeatedly using the Soxhlet apparatus [30]. After extraction the solvent was evaporated at 40°C by a rotary evaporator to obtain the crude oil then the meal which remained after oil extraction was dried to get rid of the traces of hexane and packed in polyethylene bags and stored in a deep freezer at −20±2°C until analyzed. All extractions were performed in triplicate.

Prepress – solvent extraction at room temperature

In this procedure the oil extraction from Moringa seed was performed in two steps: In the first step: The oil extracted from the corresponding seeds by using hydraulic pressing at room temperature as described previously, and the meal which remained after oil extraction from the seed of M.P was milled into a fine powder.

In the second step: the residual oil in M.P meal which remained after oil extraction in the first step was extracted as follows: 100 g of the ground M.P meal was mixed with a solvent (n-hexane) at a ratio of solvent-to-solid 5/1 (ml/g) in several consecutive multi-stages until no oil was left in the seed. The number of extraction stages needed to achieve a

specific percentage of oil recovery from Moringa cake was three consecutive multi-stages and in each extraction stage the Moringa cake powder was intimately mixed with the solvent at room temperature with gentle stirring for 15 min; a time which was sufficient to achieve physical equilibrium between the solvent rich phase (upper one) and the solid rich phase (lower one). The seed meal extracted in each stage was delivered to the next stage together with a fresh quantity of solvent. The quantity of the latter was adjusted in each case so that the ratio of solvent to solid is kept constant all-over the whole process. The solvent- rich phase is usually referred to as the overflow while the solid-rich phase is usually referred to as the underflow [31]. The extracted oil was kept in sealed bottles under refrigeration (0–4°C) for further processing and analysis and the meal which remained after oil extraction from the seed of M.P were milled into a fine powder and packed in polyethylene bags and stored in deep freezer at −20 ±2°C until analyzed. All extractions were performed in triplicate.

Detoxification of glucosinolates

In this study, the detoxification of GSL was applied for M.P meal which remained after oil extraction by solvent (hexane). In industry, the direct solvent extraction technique is considered the most effective, common, and widespread in Egypt and the Middle East. The method of GSLs detoxification was carried out after modification of the described methods [32,33]. 50 g of glucosinolate-rich, ground M.P meal which remained after oil extraction by solvent was soaked in distilled water at a ratio of liquid-to-solid 5/1 (ml/g). The mixture was gently stirred for 15,30, and 45 min. to choose the most efficient duration that was sufficient to achieve GSL removal from M.P meal depending on the time of exposure, followed by sieving to recover the residue then water-washing treatment one time was performed for M.P residue. The samples of M.P residue were dried at 70°C packed in polyethylene bags and stored in a deep freezer at −20±2°C until analyzed. All extractions were performed in triplicate.

Determination for total glucosinolates and identification via HPLC

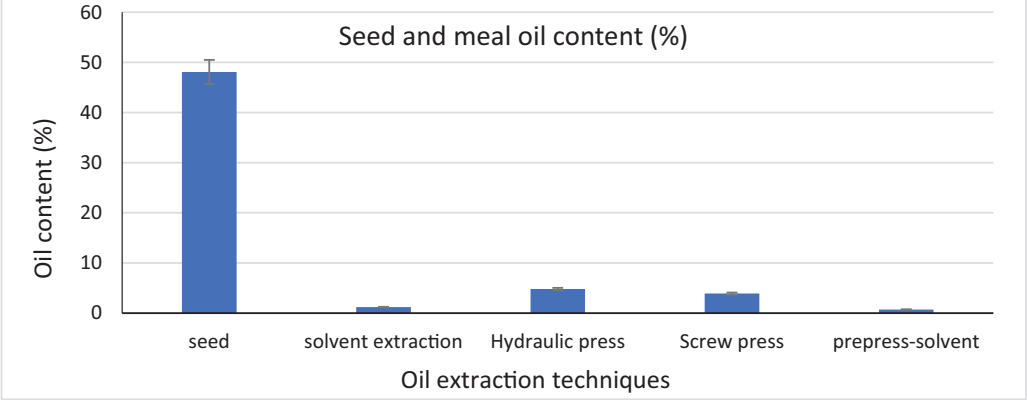
GSL extraction was performed following the described method [34]. In brief, the ground seed and cake of M.P which remained after oil extraction from the seed by different extraction procedures, as well as the detoxified M.P residues were freeze-dried, after which ±100 mg of material was used for extraction from three technical replicates in experimental work. GSLs were extracted in 70% methanol at 90°C, after which the supernatant was transferred to an ion-exchange column with Sephadex G-25 (Merck, Darmstadt, Germany) as column material. After washing the extracts with 70% methanol and adding a NaOA buffer to the column, sulfatase (from Helix pomatia type H-1, Merck, Darmstadt, Germany) was pipetted onto the extracts to remove the sulfate group from the GSLs. The desulfo-GSLs that were released from the ion-exchange column as a result of sulfatase activity were eluted in ultrapure water and collected. Next, the samples were freeze-dried and re-dissolved in 1 ml of ultrapure water. The GSLs in the samples were separated using a reversed phase high-pressure liquid chromatography (HPLC) set-up equipped with a photodiode array detector (PDA; ThermoScientific Ultimate 3000 series) at wavelengths of 229 nm and 272 nm. A reversed-phase Acclaim300 C18 column (4.6×150 mm, 3 mm, 300 °A, Acclaim 300, and Thermo Fisher Scientific) was used for separation with 100% H_2O (solvent A) and 99%acetonitrile in water (solvent B) as solvents. The separation conditions were as follows: equilibration took place at agradient profile of 98% of solvent A for 4.3 min, followed by a gradient to 35% solvent B within 24.3 min, and a hold until 29°min at 35% solvent B. Next, the gradient went back to the initial98% of solvent A within 1 min and held at initial conditions for 10 min at a flow of 0.6 ml/min. desulfo-GSLs were identified based on retention time and UV spectra compared with commercially available reference standards (Phytoplan, Heidelberg, Germany). Peaks with a characteristic UV spectrum for GSLs that could not be matched to our library were annotated as unknown GSLs. Sinigrin was used as an external standard for GSLs quantification. The resulting data were processed using Chromeleon 7.2 SR5 MUa (9624; ThermoFisher Scientific, Waltham, MA, USA).

Results and discussions

The M.P oil was extracted from the seed by using the most popular traditional procedures for oil extraction that used in industry including (hydraulic press, screw pressing, extraction by solvent and pre- press–solvent extraction at 25°C). In order to determine the best and most efficient method for extracting moringa oil, which was clearly demonstrated by the percentage of oil remaining in the cake after each method of extracting the oil from M.P seed.

From the data summarized in (Fig. 2), it could be observed that the pre-press solvent extraction technique is considered the most efficient method

Figure 2



The remaining oil content in *Moringa Peregrina* meal leftover after oil extraction by different techniques compared with its content in seed.

for extracting the oil from M.P seed, due to the remained cake after oil extraction by this technique contained the lowest amount of oil (0.72%) compared with the other extraction methods. Whereas the oil extraction from M.P seed recorded 1.2, 3.91, and 4.8% for the cakes remained after oil extraction by solvent, screw pressing, and hydraulic press extraction techniques, respectively.

According to the obtained data (Table 1), sinalbin and moringin were found to be the major dominant GSLs in M.P seed and meal. The sinalbin content in M.P meals left over after oil extraction was similar, independent from the previous different techniques used to extract the oil. Whereas, the moringin content in M.P meals which remained after oil extraction was much higher than in the M.P seed. The highest level of moringine was found in the meal left after oil extraction from the seed by pre-press solvent extraction technique which recorded 1159.38 ±77nmol/mg. It should be noted that the total GSLs content in the meal that remained after each of the different techniques was significantly higher when compared with their contents in the seeds and also higher than the permissible level of the food/feed. The variation in GSL levels in M.P seeds before and after oil extraction can be attributed to a decrease in the seed's oil content due to the extraction process. GSLs are polar, do not dissolve in oil and remain in the meal. The reduction of biomass by the oil results in an increase in the concentration of other seed constituents, including GSLs, compared with their initial levels in the unprocessed seeds. The efficiency of the extraction process directly correlates with the rate of increase in the level of GSLs in the remained cake. A higher efficiency of oil extraction from seed (a lower percentage of remaining oil in the cake), leads to a higher level of GSLs in the cake. From the above results in (Fig. 2 and Table 1), it can be indicated that the highest level of total GSLs 1295.89±85.92 nmol/mg was found in the meal left after oil extraction from the seed by prepress solvent extraction technique which is considered the more effective technique for oil extraction from M.P seed compared with other techniques used in the present study which recorded the least amount of oil (0.72%) in the meal. These GSLs can undergo chemical and enzymatic hydrolysis to produce a range of products that possess antinutritional properties leading to reduced growth and impaired reproduction. For swine, the limiting value above which sows fertility may be impaired is 4 μmol of total GSLs$\bar{g}^{1}$diet and 8 mmol of daily intake

Table 1 Glucosinolates content in *Moringa Peregrina* meal leftover after oil extraction by different techniques

	GSLs content in M.P				
		Meal leftover after oil extraction by			
Type of GSLs (nmol/mg)	Seed	Solvent extraction	Hydraulic press extraction	Screw press extraction	Prepress-solvent extraction
Sinalbin	17.50±2.5	17.65±3.7	23.20±5.3	14.28±10.11	21.72±3.10
Moringin	537.6±109.73	941.8±34.14	917.8±241.86	896.13±69.73	1159.38±77.00
Unknown-GSLs	52.74±9.90	98.74±1.9	92.71±23.90	85.91±5.87	114.79±5.89
Total GSLs	607.8±118.32	1058±34.49	1033.70±271.02	996.33±84.69	1295.89±85.92

*Values are expressed as means of triplicate determination±SD.

Table 2 The Effect of treating the defatted *Moringa Peregrina* meal (meal remained after oil extraction by solvent via Soxhlet) by soaking in water on its content of the residual glucosinolates

	Concentration of the residual GSLs in defatted M.P meal treated by soaking in water at different times		
Type of GSLs (nmol/mg)	15 min.	30 min	45 min.
Sinalbin	0.00±00	0.00±00	4.49±3.18
Moringin	23.97±18.31	40.31±4.06	87.44±5.75
Unknown-GSLs	0.00±00	0.00±00	3.68±2.61
Total GSLs	23.97±18.31	40.31±4.06	95.61±11.25

*Values are expressed as means of triplicate determination±SD.

of these compounds. In rats, a diet with GSL levels greater than 2.7 μmol $\bar{g}^1$ feed might increase the mortality of pups, possibly due to the transfer of GSL breakdown products to milk. In cows, a significant increase in days from caving to conception was observed when the daily intake of GSLswas 75 mmol/cow [35].

From the results presented in (Table 2), it could be observed that the water treatment of defatted M.P meal that remained after oil extraction by solvent via Soxhlet for 15, 30 min led to the disappearance of sinalbin from the meal. While, treating the meal with water for 45 min led to the reappearance of sinalbin again, but with a lower content at a level of 4.49±3.18 nmol/mg compared with its content in the defatted meal before treated with water which recorded 17.50±2.5 nmol/mg. The water treatment for defatted meal for 15 min. led to a decrease in the moringin content at a level of 23.97 ±18.31 nmol/mg in meal compared with its level before the water treatment which was 941.8±34.14 nmol/mg. From the above can be noted that by increasing the time of water treatment, a gradual increase was observed in the level of the residual moringin in the M.P cake as well as the reappearance of sinalbin again. It is likely due to a dynamic equilibrium occurring between the solid phase (defatted M.P meal) and the liquid phase (water) by the gradual increase in the duration of the water treatment for the cake by soaking. In general, the results showed that there was a significant decrease in the level of total GSLs in M.P meal as a result of the water treatment which decreased from 941.8±34.14 nmol/mg in defatted meal before water treatment to 23.97±18.31 nmol/mg in residues left after treatment with water for 15 min, which is considered within range of the recommended level (30 μ mol/g) for canola meal [36]. Furthermore, the level of total GSLs rose again in the cake by increasing the duration of the water treatment by soaking, which increased from 23.97±18.31 nmol/mg when soaked for 15 min to recorded 95.61±11.25 nmol/mg by increasing the soaking period to 45 min. These results differ somewhat from those reported by [37] which indicated that GSLs were virtually absent in residues left after water treatment. Therefore, an attractive approach to detoxify meal for animal feeding and removing the better taste from the meal is by soaking in water for 20–30 min, followed by sieving to recover the residue for animal feeding. This difference is likely due to the difference in sensitivity and nature of the method used to estimate GSLs in the current study and the comparative study, as well as the difference in the two cultivars of Moringa seed and the concentration of GSLs in both cultivars used in both studies.

Conclusion

Moringa peregrina is considered one of the most promising multi-purpose trees in Asia and Africa because it is regarded as a good source of edible oil as well as the possibility of using the remaining meal after extracting the oil from M.P seed for animal feed, provided the GSLs and other anti-nutritional factors present in the meal are removed. It is very important to test which processing methods used to treat the meal resulted in the safest end product. Soaking in water for 15 min followed by a water-washing treatment is the simplest and most efficient process to get rid of GSLspresent in M.P meal to be suitable for animal or human feeding.

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Conflicts of interest

No competing inte.rests are disclosed by the authors.

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