The Pharma Innovation Journal 2023; 12(9): 2801-2806

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(9): 2801-2806

www.thepharmajournal.com
Received: 03-07-2023
Accepted: 07-08-2023

G Kishore Kumar
Department of Processing and Food Engineering, AEC & RI, TNAU, Kumulur, Tamil Nadu, India

Dr. IP Sudagar
Department of Processing and Food Engineering, AEC & RI, TNAU, Kumulur, Tamil Nadu, India

Dr. R Arulmari
Department of Processing and Food Engineering, AEC & RI, TNAU, Kumulur, Tamil Nadu, India

Dr. P Rajkumar
Department of Processing and Food Engineering, AEC & RI, TNAU, Kumulur, Tamil Nadu, India

Dr. P Krishnakumar
Department of Processing and Food Engineering, AEC & RI, TNAU, Kumulur, Tamil Nadu, India

Dr. K Gurusamy
Department of Food Process Engineering, AEC &RI, TNAU, Coimbatore, Tamil Nadu, India

Dr. VP Santhanakrishnan
Department of Medicinal and Aromatic Plants, HC&RI, TNAU, Coimbatore, Tamil Nadu, India

Corresponding Author:
Dr. IP Sudagar
Department of Processing and Food Engineering, AEC & RI, TNAU, Kumulur, Tamil Nadu, India

Optimization of oil extraction method for isolation of glucosinolates from *Moringa oleifera*

G Kishore Kumar, Dr. IP Sudagar, Dr. R Arulmari, Dr. P Rajkumar, Dr. P Krishnakumar, Dr. K Gurusamy and Dr. VP Santhanakrishnan

Abstract
Oil extraction methods from moringa seeds using solvent extraction (hexane, petroleum ether), screw press oil extraction and rotary press oil extraction to enhance the quality and value of oil and seed meal were investigated in this study. The glucosinolates extraction from the moringa seed meal was carried out using microwave assisted extraction and ultrasound assisted extraction. Results show that rotary press oil extraction increased the yield of glucosinolates content from moringa seed meal compared to other oil extraction methods. The methanolic extraction was followed for extraction of glucosinolates, column chromatography was performed for purification and HPLC analysis was carried out for quantification of glucosinolates. The predominent glucosinolates present in *Moringa oleifera* of glucomoringin was quantified from the moringa seed meal. The concentration of glucomoringin 290.12 µg / g and 250.65 µg / g was obtained from UAE and MAE methods from the seed meal.

Keywords: Glucomoringin content, *Moringa oleifera*, rotary press, seed meal

1. Introduction
Moringa oleifera Lam. (Family: Moringaceae) is a medicinal plant native with antioxidant and anti-inflammatory properties, geographically distributed in tropical and subtropical climatic regions. However, it is now cultivated in other regions of the world. *M. oleifera* is considered the storehouse of plant natural products (PBNP) and has been recognized as an economically and nutritionally important plant due to its health benefits (Vergara-Jimenez *et al.*, 2017) [12]. *Moringa oleifera* contains several phytoconstituents of alkaloids, protein, quinine, saponins, flavonoids, tannin, steroids, glycosides, glucosinolates anthocyanins etc. (Kandeepan *et al.*, 2022) [8].
Glucosinolates are sulphur containing plant secondary metabolites with nutritional effects and biologically active compounds. *Moringa oleifera* is particularly rich in 4 - O - (α – L - rhamnopyranosyloxy) - benzyl glucosinolates including glucomoringin (GMG), which is found mainly in the seeds and leaves (Maldini *et al.*, 2014) [10]. From the moringa seeds, moringa oil is extracted for edible purposes and also for medicinal purposes. The seed meal is an important by product of oilseed processing (Chitra, 2021) [4]. It was used as a feed for animals as also used as a fertilizer. It can also be used as an edible ingredient or for non-edible applications due to its fatty acid composition, tocopherols composition, thermal stability and its excellent resistance to autoxidation (Cheikhyoussef *et al.*, 2020)[2]. The seed meal is a good source of bioactive components and the oil extraction method is having significant influence on the bioactive component retention. Different oil extraction methods of solvent extraction (using hexane, petroleum ether), screw press oil extraction and rotary press oil extraction was carried out and seed meal is obtained. In this study, the extraction of glucosinolates from the seed meal was carried out and oil extraction method was optimized by comparing the yield of glucosinolates from different methods.

2. Materials and Methods
2.1 Materials and standard procurement
Solvents used for extraction and purification such as HPLC-grade methanol, ethanol, ethyl acetate, and water were purchased from M/s. Precision Scientific Ltd. Coimbatore. Glucomoringin potassium salt standard was purchased from M/s. Ponmani and Co., Coimbatore.

2.2 Sample collection
Moringa oleifera seeds of the PKM-1 variety were procured from the Institute of Agriculture, Kumulur, Trichy. The seeds were ground using a mixer, seived, and the fine powder was stored in an airtight container. Solvents for oil extraction such as hexane and petroleum ether were purchased from M/s. Ponmani & Co.

2.3 Moringa oil extraction
Different extraction methods such as solvent extraction, screw press oil extraction, and rotary press oil extraction, have been used for the extraction of moringa oil from seeds.

2.3.1 Moringa oil extraction by solvent extraction
Moringa seed oil extraction by solvent extraction was carried out using a Soxhlet apparatus at the Food Processing Laboratory in AEC &RI, TNAU, Kumulur. Powdered moringa seeds (40 g) were placed in the siphon tube of a Soxhlet extractor, and the oil was extracted using hexane and petroleum ether. Extraction was carried out for 2 h at 65 °C. The solvent was evaporated and passed through the seeds again, and the oil was extracted. The solvent - oil mixture is vacuum concentrated in rotary evaporator and oil is obtained and seed meal is collected and stored safely (Mani *et al.*, 2007) [11].

2.3.2 Screw press oil extraction
Moringa seed oil extraction was performed in a screw press expeller (Model: SARDAR 240552, Mini master – 12 CR) available at the Food Processing Workshop at AEC & RI, TNAU, Kumulur. The screw press expeller consisted of an electric gear motor, feed hopper, expression chamber, worm shaft, shaft housing, belts and pulleys, bearings, oil outlet, cake outlet, and machine frame. Seeds (400 g) were fed into the machine through a feed hopper. The machine conveys, grinds, and presses the seeds in a cylindrical drum with the help of a worm shaft until the oil is pressed out of the seeds. The oil obtained is drained through the oil outlet trough into the oil pan where it is collected, while the residual cake is drained into the cake pan at the cake outlet and collected (Fakayode and Ajav, 2016) [6].

2.3.3 Rotary press oil extraction
Moringa seed oil extraction was carried out using a rotary press available at the Pulses and Millets Processing Center, AEC & RI, TNAU, Kumulur. The rotary press consisted of a large mortar and pestle, with the mortar anchored in the base, and the pestle was moved in the mortar by a motor. Moringa seeds (2 kg) were placed in a mortar, and the pestle was grounded to remove the oil. Oil flowed through the bottom of the mortar, and the cake was removed manually. Motorized rotary presses are faster than manual or animal models, but they are more expensive, and their higher capital and operating costs require a larger production volume to be profitable. The width of this gap, which can be varied using an adjustable pressure cone, controls the operating pressure of the press. It works out through a stout upright pestle that descends from an upper curved or angled part, where the pestle rests in a hollowed recess that allows the pestle to rotate (Kate *et al.*, 2014) [9].

2.4 Microwave assisted extraction and ultrasound assisted extraction
Extraction of glucosinolates from *Moringa oleifera* leaves and seeds was performed using microwave-assisted extraction and ultrasound-assisted extraction methods. Ultrasound-assisted extraction was carried out using a probe sonicator (LABMAN, PRO 650). A laboratory microwave system (Ethos-X) equipped with a 12-vessel carrousel that operates in closed - vessel mode was used for microwave -assisted extraction. The temperature, microwave power, and extraction time were monitored in a single extraction vessel during operation using an automatic control system. For each extraction, moringa seed meal samples were placed in the extraction vessel, and the extraction solvent (methanol) was added to the samples. The vessel was then shaken gently for a few minutes. Microwave treatment (250 W, 80 °C) and ultrasonic treatment (400 W, 20 kHz) were applied to the samples according to the experimental design. After extraction, samples were centrifuged in a refrigerated centrifuge. The supernatant was collected and concentrated in a rotary evaporator. The crude extracts were further purified, then analysed in HPLC and LC-MS for quantification and identification of glucosinolates and other bioactive components (Maldini *et al.*, 2014) [10].

2.5 Purification of glucosinolates
Silica gel column chromatography was performed to purify the glucosinolates from the crude leaf and seed extracts. Ethyl acetate was used to prepare the column, silica gel was added to ethyl acetate and mixed well, and the silica gel was poured into the column with the solvent in the form of a slurry. The column was allowed to settle for a few minutes. After packing the column, the crude extract of the sample was added to the column. The solvent fractions ethyl acetate: hexane 5:5 (100 ml), ethyl acetate (100 ml) and ethyl acetate: methanol 99:1 (100 ml) were used to elute the glucosinolates. The fractions were eluted drop wise and collected in a separate Erlenmeyer flasks (50 ml each). The fraction containing the component was identified by TLC analysis and concentrated in a rotary evaporator. Subsequently, the concentrated purified seed and leaf extract was analysed by LC-MS and HPLC (Chen *et al.*, 2019) [3].

2.6 High performance liquid chromatography (HPLC) analysis
Extracts from the intact glucosinolate extraction method were qualitatively and quantitatively analyzed using an HPLC system (Make: Shimadzu, Model: LC 20 AD). Ten microliters of sample was
(LaChrom L - 7200 Auto Sampler) injected onto a 4.6 x 250 mm SB - C18 column (Zorbax 5 lm, Agilent) and separated using the following gradient program: 0-2 min : 0-1 B, 2-20 min :1 - 50% B,
20-24 min: 50-100% B, 24-26 min : 100% B, 26-27 min: 100-1% B, and 27-35 min:
1 - 0% B at a flow rate of 1.5 ml/min. Buffered eluents for intact extracts with solvent A: 100% ammonium acetate (0.1 M), solvent B : 40% acetonitrile / 0.1 M ammonium acetate. Detection was performed at
229 nm using a photodiode array detector (LaChrom L-7455), and the components were determined based on retention time (intact glucosinolates) and UV spectra (desulfoglucosinolates) and quantified against the standard. Retention times were 10.1

min for rhamno-benzyl - GS for the analysis of intact glucosinolates in buffered eluents. The relative response factors (RF) used to correct for the difference in absorption between the reference standard glucomoringin and the glucosinolates found in the *M. oleifera* extracts were determined to be 0.68 for rhamno - benzyl - GS (Förster *et al.*, 2015) [7].

2.7 Optimization of oil extraction method for glucosinolates extraction

The seed meal from obtained after oil extraction contains bioactive components and glucosinolates. The oil extraction process had significant effect on glucosinolates present in it. Hence the glucosinolates extraction was carried out from seed meal obtained in all the oil extraction processes at the optimized ultrasound and microwave assisted extraction conditions from the previous researches (Extraction time of 10 mins, Solvent sample composition of 5:1) and the oil extraction method which retain higher glucosinolates content was compared and optimized.

2.8 Calibration and quantification of glucomoringin

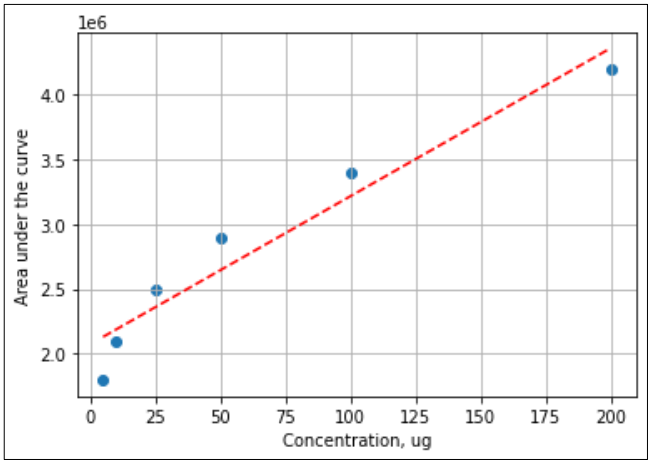


Fig 1: Calibration curve for glucomoringin quantification

The calibration curve was plotted for various concentrations of glucomoringin standard to quantify the glucomoringin content present in the plant extracts. As a starting point, a stock solution of glucomoringin (10 mg/ml) in methanol was prepared. The application volume of the aliquots was 1 ml. From the stock solution, the reference solutions containing 5, 10, 25, 50, 100 and 200 µg/ml were prepared by diluting stock solution with methanol. The concentration peak of glucomoringin was plotted against area. y = 11433.692 * x + 2073476.70152 is the linear regression line (R^2 = 0.987). The regression results showed a good linear relationship between 5-200 µg/ml concentrations. Beer's law was used to construct the curve (Figure 1).

2.9 Statistical analysis

The statistical analysis ANOVA was performed for analysing the significance of oil extraction method on yield of glucosinolates in Microsoft excel (Version 2019).

3. Results and Discussion

3.1 Optimization of oil extraction method for isolation of glucosinolates

The oil extraction from moringa seeds was carried out using different oil extraction methods such as solvent extraction, screw press oil extraction and rotary press oil extraction. The seed meal also obtained from all extraction processes that is an important by-product of oilseed processing and contains the highest amount of glucosinolates. It was served as a feed for animals as well as used as a fertilizer. It can also be used as an edible ingredient or for non-edible applications due to its fatty acid composition, tocopherols composition, thermal stability and its excellent resistance to autoxidation (Cheikhyoussef *et al.*, 2020) [2]. The seed meal is a good source bioactive components and the oil extraction method is having significant influence on the bioactive component retention. So, the extraction of glucosinolates from the seed meal was carried out and oil extraction method was optimized by comparing the yield of glucosinolates from different methods.

3.2 Oil yield

Moringa seed oil 19 g (40%) and seed meal 32 g were obtained after solvent extraction using hexane in soxhlet apparatus (Plate 1 a,e), and the oil yield (35%) of 17 g and seed meal (65%) of 33 g were obtained in solvent extraction using petroleum ether from 50 grams of Moringa seeds (Plate 1 b,f). Crude oil (15%) of 60 g and seed meal (85%) of 340 g was obtained after screw press oil extraction from 400 g of moringa seeds (Plate 1 c,g). Also oil yield (17.5%) of 350 g and seed meal (83.73%) of 1.760 kg were obtained after pressing 2 kg of moringa seeds in rotary press oil expeller (Plate 1 d, h). Figure 1 shows oil yield from different oil extraction methods.

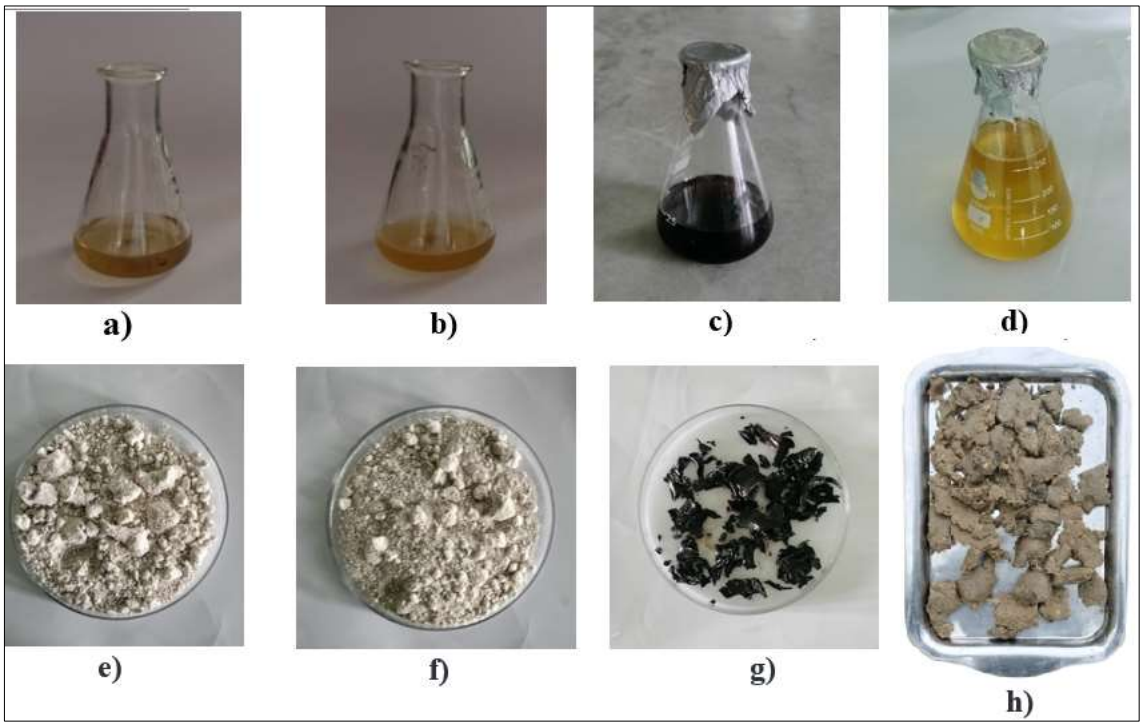
3.3 Quantitation of glucosinolates from seed meal

Table 1 lists glucomoringin yield obtained from microwave assisted extraction and ultrasound assisted extraction from seed meal samples. The predominent glucosinolate glucomoringin was extracted from the seed meal obtained from various oil extraction processes at the optimized ultrasound and microwave assisted extraction conditions (Extraction time 10 min, solvent sample composition of 5:1). The seed meal obtained from screw press was not suitable for glucomoringin extraction as bioactive components present in the seed meal were degraded due to the heat developed in the screw press oil extraction process.

There are moderate to large variations in accumulation levels of the glucosinolates between the seed meal from different oil extraction methods. These levels are affected by oil extraction conditions of rise in temperature and the type of solvent used in solvent extraction methods followed in the process. Similarly the level of glucosinolates extracted from *Camelina sativa* seed meal was affected by environmental conditions (Berhow *et al.*, 2013). The results indicates the rotary press oil extraction was found to retain the highest glucomoringin concentration of 290.12 µg /g in MAE and 250.65 µg /g in UAE from moringa seeds followed by solvent extraction using hexane and petroleum ether. Figure 2 and Figure 3 shows the HPLC chromatogram of seed meal extract (rotary press oil extraction) from UAE and MAE. This may be correlated with that, the process doesn't develop heat during the process and bioactive components were preserved in the process. The seed meal often being used as a feed for cattles. Though this method yields less oil content than solvent extraction, this method is mostly preferred as this method gives less impact towards environmental pollution and in

terms of preservation of bioactive components. Table 2 shows the statistical results of ANOVA. The ANOVA results indicates that oil extraction method had significant ($p< 0.05$) influence on the yield of glucomoringin content as shown in the Figure 4. Also the glucosinolates extraction methods (UAE and MAE) doesn't have significant influence ($p> 0.05$) on the yield of glucosinolates. Zhan *et al.* (2020) reported that the ultrasonic assisted extraction conditions at 150 W, 70% ethanol, 4:1 (ml/g) solvent sample composition, 60 °C temperature and extraction frequency of 5 times/15 min, the glucosinolate yield of 62.1 mg/g can be reached from the rapeseed meal.

Citeau *et al.* (2019) [5] also reported that hydroalcoholic extraction of oil from rapeseed significantly increased the glucosinolates extractability from the seed meal while increasing the water content in the solvent.



a) Oil from solvent extraction (hexane), b) Oil from solvent extraction (petroleum ether)
c) Oil from screw press oil extraction, d) Oil from rotary press oil extraction
e) Seed meal after hexane solvent extraction, f) Seed meal after petroleum ether solvent extraction
g) Seed meal after screw press oil extraction, h) Seed meal after rotary press extraction

Plate 1: *Moringa oleifera* seed processing products

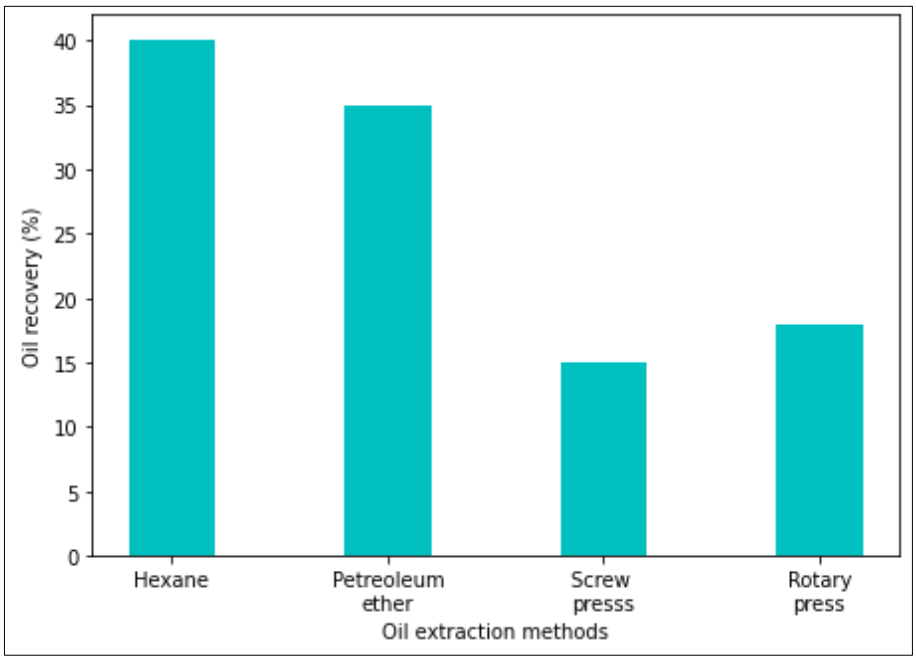


Fig 1: Oil yield from different oil extraction methods

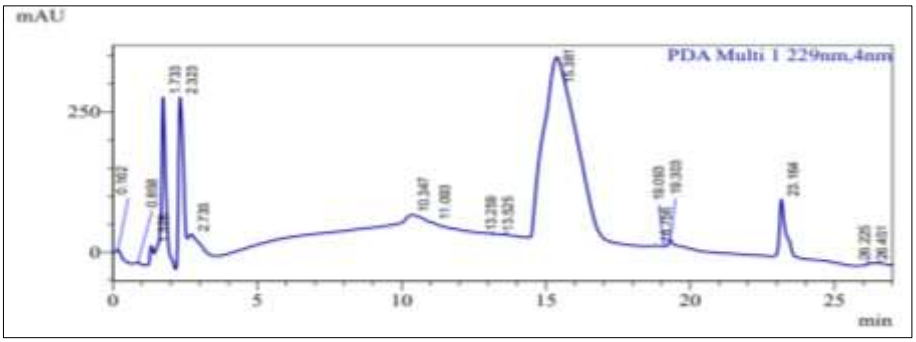


Fig 2: HPLC chromatogram of seed cake extract of *Moringa oleifera* (Rotary press oil extraction) from UAE

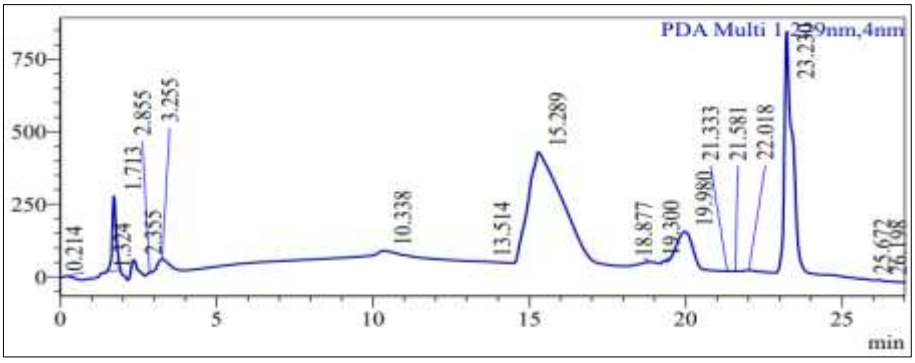


Fig 3: HPLC chromatogram of seed cake extract of *Moringa oleifera* (Rotary press oil extraction) from MAE

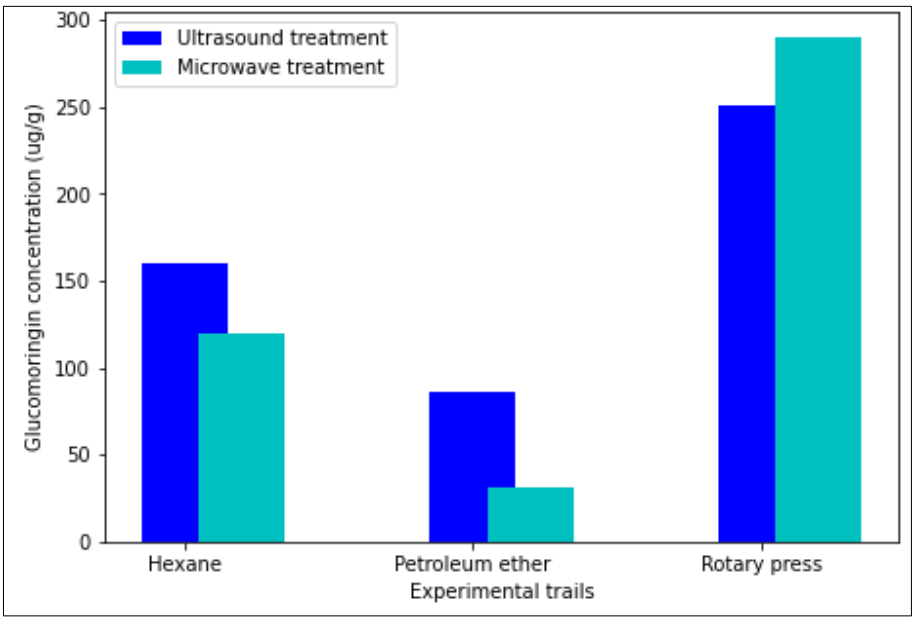


Fig 4: Glucomoringin yield using UAE and MAE from seed meal of different oil extraction processes

Table 1. Glucomoringin concentration from seed meal obtained from different oil extraction methods.

S. No	Treatments	Oil extraction from seed meal	Concentration of glucomoringin (µg / g)
1	Microwave	Rotary press extraction	290.12
2	Microwave	Solvent extraction seed meal (Hexane)	120.55
3	Microwave	Solvent extraction seed meal (Petroleum ether)	30.777
4	Ultrasound	Rotary press extraction	250.65
5	Ultrasound	Solvent extraction (Hexane)	160.46
6	Ultrasound	Solvent extraction (Petroleum ether)	86.29

Table 2: Two way ANOVA for effect of oil extraction method and glucosinolates extraction method on glucomoringin yield

Source of Variation	SS	df	MS	F - Value	P-value	F crit
Oil extraction method	969.1375	1	969.1375	1.222777	0.384031	18.51282
Treatment	41293.13	2	20646.57	26.05013	0.036968	19
Error	1585.141	2	792.5707			
Total	43847.41	5				

4. Conclusion

In this study, the focus was on optimizing the oil extraction method to get seed meal with good quality containing

glucosinolates from *Moringa oleifera*. The influence of two different solvent extraction methods, screw press oil extraction method and rotary press oil extraction method on quality of seed meal in terms of extractability of glucosinolates was investigated. The two glucosinolates extraction methods of ultrasound assisted extraction and microwave assisted extraction were followed. Among the oil extraction methods rotary press oil extraction was found to retain more glucosinolates and phytoconstituents. The glucomoring in concentration of 290.12 µg / g and 250.65 µg / g was obtained from UAE and MAE from the moringa seed meal.

5. References

1. Berhow MA, Polat U, Glinski JA, Glensk M, Vaughn SF, Isbell T, Ayala-Diaz I, *et al*. Optimized analysis and quantification of glucosinolates from *Camelina sativa* seeds by reverse-phase liquid chromatography. Industrial Crops and products. 2013;43:119-125.
2. Cheikhyoussef N, Kandawa-Schulz M, Böck R, Cheikhyoussef A. Cold pressed *Moringa oleifera* seed oil. In Cold pressed oils; c2020. p. 467-475.
3. Chen R, Wang XJ, Zhang YY, Xing Y, Yang L, Ni H, *et al*. Simultaneous extraction and separation of oil, proteins, and glucosinolates from *Moringa oleifera* seeds. Food Chemistry. 2019;300:125162.
4. Chitra P. Potential and Utilization of By-Products of Oilseeds in Animal Feed Industry. Biotica Res. Today. 2021;3(8):655-657.
5. Citeau M, Regis JP, Carré F. Value of hydroalcoholic treatment of rapeseed for oil extraction and protein enrichment. 2019;26:1.
6. Fakayode OA, Ajay EA. Process optimization of mechanical oil expression from Moringa (*Moringa oleifera*) seeds. Industrial Crops and products. 2016;90:142-151.
7. Förster N, Ulrichs C, Schreiner M, Müller CT, Mewis I. Development of a reliable extraction and quantification method for glucosinolates in *Moringa oleifera*. Food Chemistry. 2015;166:456-464.
8. Kandeepan C, Sabitha M, Parvathi K, Senthilkumar N, Ramya S, Boopathi N. Phytochemical screening, GCMS profile, and In-silico properties of bioactive compounds in methanolic leaf extracts of *Moringa oleifera*. Journal of Drug Delivery and Therapeutics. 2022;12(2):87-99.
9. Kate A, Lohani U, Pandey J, Shahi N, Sarkar A. Traditional and mechanical method of the oil extraction from wild apricot kernel: A comparative study. Res. J Chem. Environ. Sci. 2014;2:54-60.
10. Maldini M, Maksoud SA, Natella F, Montoro P, Petretto GL, Foddai M, *et al*. *Moringa oleifera*: study of phenolics and glucosinolates by mass spectrometry. Journal of Mass Spectrometry. 2014;49(9):900-910.
11. Mani S, Jaya S, Vadivambal R. Optimization of solvent extraction of Moringa (*Moringa oleifera*) seed kernel oil using response surface methodology. Food and Bioproducts Processing. 2007;85(4):328-335.
12. Vergara-Jimenez M, Almatrafi MM, Fernandez ML. Bioactive components in *Moringa oleifera* leaves protect against chronic disease. Antioxidants. 2017;6(4):91.
13. Zhan Q, Yan J, Bian J, Tan C, Huang H, Lin H. Study on Ultrasonic Extraction and Stability of Glucosinolate in Rapeseed Cake. IOP Conference Series: Earth and Environmental Science; c2020.