sciendo

*Polish Journal of Chemical Technology, 22, **4**, 31—35, 10.2478/pjct-2020-0036*

Influence of extraction technique on yield and antioxidant activity of extracts from *Moringa oleifera* leaf

Małgorzata Dzięcioł

West Pomeranian University of Technology, Szczecin, Faculty of Chemical Technology and Engineering, Department of Chemical Organic Technology and Polymeric Materials, Piastów Ave. 42, 71-065 Szczecin, Poland
**Corresponding author: e-mail: malgorzata.dzieciol@zut.edu.pl*

The article presents research on the exploring of extraction process of biologically active substances from the leaves of the *Moringa oleifera* tree using ethanol. Ethanolic extracts were obtained using three different techniques: maceration with shaking, ultrasound-assisted extraction and extraction in Soxhlet apparatus, in different time variants: 1, 2 and 4-hours. After solvent evaporating and drying, the yields of dry extracts obtained in particular processes were calculated. The antioxidant activity of extracts was analyzed spectrophotometrically using DPPH radical scavenging method, and total phenolic content (TPC) was determined by Folin-Ciocalteu method. By means of gas chromatography with mass selective detector (GC-MS), 11 biologically active compounds present in ethanolic extracts were identified, among which α-tocopherol had the greatest share. Based on the results, the influence of the extraction technique and time on the yield and antioxidant activity of *M. oleifera* leaf extracts were discussed.

Keywords: *Moringa oleifera*, extracts, antioxidant activity, DPPH radical scavenging method, total phenolic content.

INTRODUCTION

Moringa oleifera (*M. oleifera*) is the most widely known and cultivated tree from the family of *Moringaceae*. Its popular names are drumstick tree or horseradish tree, but also miracle tree, tree of life or a wonderful plant – due to the numerous benefits resulting from its use in both nutrition and natural therapies[1–3]. It originates from sub-Himalayan areas of India, Pakistan, Bangladesh and Afghanistan but currently is cultivated in many tropical countries[4]. Due to its valuable properties, it has been used by the indigenous people of Asia and Africa as a foodstuff and natural medicine. The leaves, seeds, pods and flowers of *M. oleifera* are traditionally used for its nutritional properties. The plant is rich in phytosterols, polyphenols, fatty acids, valuable amino acids, contains vitamin C, B, D, E, minerals including iron, calcium, and zinc[5, 6]. Moringa extracts are used in traditional folk medicines to cure various diseases[5]. Moreover, seeds of the plant are also used as effective eco-coagulant for water purification[7, 8]. Recently, the popularity of *M. oleifera* in Europe is increasing. It is classified into a group of adaptogenic plants – plants which can effectively increase resistance to stress and help to keep the body in homeostasis[9].

Leaves of *M. oleifera* in various forms, including raw material, juice and extracts, were subject to different studies *in vitro* and *in vivo*. They are known for increasing breast milk production during lactation due to the content of phytosterols such as stigmasterol and sitosterol[5, 10]. They also possess antidiabetic and antioxidant properties[5, 11, 12]. Studies on rats have shown that powdered *M. oleifera* leaves may effectively prevent renal impairment by increasing the total protein content in plasma and reducing urea and creatinine levels[13]. Extracts from *M. oleifera* leaves have also cytotoxic activity and significant anticancer potential[1, 3, 14–16]. It was confirmed that they decrease cell motility and colony formation in colorectal and breast cancer cell lines[14] and have apoptotic effect against prostate cancer cells[16]. Moreover, hydroethanolic leaf extracts significantly improved memory and reduced neurodegeneration in rats. Neuroprotective and memory enhancing effects may be the result of oxidative stress lowering and reduction of acetylcholine esterase activity, improving cholinergic function[17]. Gastroprotective activity of *M. oleifera* leaf extract against aspirin-induced ulcers, with evidence of mucus membrane enhancing activity, was also confirmed[18]. The antimicrobial activity of *M. oleifera* leaves and other tissues is also promising, and suggests the potential use of this plant in the control of different pathogenes, including Gram-negative and Gram-positive bacteria (e.g. *Staphylococus aureus*, *Enterococcus faecalis*, *Aeromonas caviae*) and popular viruses like Epstein-Barr virus (EBV) and herpes simplex virus (HSV)[19]. *M. oleifera* is also traditionally used in the treatment of HIV symptoms, possibly by improving the immune system[19]. The antiretroviral effect of powdered moringa leaves in combination with the root or bark was noticed during observation of HIV infected people in Zimbabwe[20].

Due to the content of numerous biologically active compounds, *M. oleifera* is increasingly used as a diet supplement and also tested for using a potential drug in diseases such as stomach ulcers, Alzheimer's disease, microbial diseases or even cancer[3, 5, 17, 18]. Extracts from moringa leaves, possessing high antioxidant activity, can be used not only as a dietary supplement but also as active additives in cosmetics, which may help to protect skin against damage caused by free radicals and reduce the signs of aging. Extracts can also have a protective function in cosmetic formulations, acting as effective antioxidants.

Most available in the literature reports on the extraction of active substances from moringa leaves are difficult to compare because of using plant materials from different countries and applying of various research methodologies. Therefore, the objective of the present study is to investigate the influence of extraction conditions on the yield, antioxidant activity and total phenolic contents of dry ethanolic extracts from *M. oleifera* leaf. Active compounds in extracts were analyzed using gas chromatography with mass selective detector (GC-MS) method.

EXPERIMENTAL

Extraction techniques

The aim of the research was comparison of different extraction techniques to determine the favorable conditions for obtaining of dry ethanolic extracts from *M. oleifera* leaf and to study their antioxidant activity. Studied plant material was powdered dry leaves of *M. oleifera* originated from India (producer: Minvita). Extracts were obtained using 96% ethanol by various techniques: maceration with constant shaking (MS), ultrasound-assisted extraction (UAE) and Soxhlet extraction (SE), in different time variants: 1, 2 and 4 hours. In each condition, three parallel experiments were carried out using 5 g of plant material and 150 cm^3 of 96% ethanol. After extraction and filtration through a Filtrak No. 390 paper, the solvent was evaporated using a rotary evaporator. The obtained concentrated ethanolic extracts were transferred to the watch glasses and left in a dark place at room temperature for 48 hours to evaporate the residual solvent. Next, it was dried using a laboratory dryer with air circulation at 35°C for 6 hours. After drying, the extracts were stabilized to constant weight in a desiccator with silica gel and weighed. For each extraction conditions the yield of the dry extract [mg/g] was calculated as an average of three parallel determinations.

Antioxidant activity

The antioxidant activity of the obtained extracts was measured using DPPH radical scavenging method. For this purpose, 0.01 g/cm^3 solutions of each dry extract in methanol were prepared and then diluted with methanol to obtain working solutions with concentrations in a range of 100–800 $\mu g/cm^3$. Directly before the analysis 0.002 $mmol/cm^3$ stock solution of DPPH (2,2-diphenyl-1-picrylhydrazyl) in methanol was prepared and diluted ten times with methanol to obtain a DPPH working solution. For determination of antioxidant activity, to 1.5 cm^3 of the extract solution, 3 cm^3 of DPPH working solution was added, mixed and left for 30 minutes incubation in darkness. A blank sample containing 1.5 cm^3 of solvent was prepared analogously. The analyses were carried out using a 1600PC UV–VIS spectrophotometer (VWR) in 1-cm cuvettes, by measurement of the absorbance at 517 nm, with methanol as a reference. The radical scavenging activity (RSA) of particular extracts was calculated from the obtained values of extract sample absorbance after 30 minutes (A_{30}) and blank sample absorbance (A_0), using the following formula[21]:

$$RSA = 100\ (A_0 - A_{30}) / A_0\ [\%]$$

Next, for the particular extracts obtained using different techniques, the plots of RSA (%) versus working solutions concentrations C ($\mu g/cm^3$) were prepared and the mathematical equations of these dependencies were determined. From the obtained equations, for the RSA = 50%, the concentrations of extracts causing a 50% inhibition of free radical activity (IC_{50}) were calculated. The IC_{50} values are inversely correlated with the radical scavenging activity and antioxidant properties of the sample.

Total phenolic contents

Total phenolic contents in dry *M. oleifera* extracts were determined by the method with Folin–Ciocalteu (F–C) reagent[22]. For this purpose, 0.5 cm^3 of the extract solution in methanol (C = 5 mg/cm^3), 0.5 cm^3 of F–C reagent (Chempur) and 1.5 cm^3 of sodium carbonate solution (C = 200 mg/cm^3) were placed in volumetric flask and made up to 25 cm^3 with redistilled water. The content of the flask was thoroughly mixed and kept at room temperature for 30 min for blue color development, shaking occasionally. After this time the absorbance was measured by using UV–VIS 1600PC spectrophotometer (VWR) in 1-cm cuvettes at 760 nm wavelength. Gallic acid (Sigma-Aldrich) was used as a reference standard (0.05 – 0.50 mg/cm^3) for calibration curve preparation. The total phenolic contents (TPC) were calculated using a linear regression equation obtained from the calibration curve of gallic acid and expressed as mg of gallic acid equivalent per 1 g of dry extract (mg GAE/g).

GC-MS analysis

The analysis of the obtained extracts was carried out by GC-MS method using a 6890N gas chromatograph with a 5973 Network Mass Selective Detector (Agilent Technologies). Separation was performed using HP--5MSI capillary column (Agilent 19091S-433I: 5%-Phenyl 95%-Methylpolysiloxane Inert column, 30 m x 0.25 mm x 0.25 μm) with the following temperature program: from 80°C to 320°C, at a rate of 5°C/min. The carrier gas was helium (1.2 cm^3/min). Tested samples (2.0 μl of dry extract solutions, C = 10 mg/cm^3) were dosed to the column in a split mode (10:1) using a 7683 Series Injector Autosampler. Electron impact ionization (70 eV) mass spectra were obtained and recorded in the range of 20–600 m/z. The detector temperatures were respectively: quadrupole 150°C, ion source 230°C.

Identification of the individual compounds present in the obtained extracts was carried out by comparison of their mass spectra with mass spectra of standards from the NIST 02 library. The identification was confirmed by comparison of the calculated linear retention indices (LRI) with the values found in the literature and also by comparison of retention times with standards when the standards were available. In order to determine linear retention indices, the standard mixture of the C_7-C_{40} *n*-alkanes was analyzed under the same chromatographic conditions[23, 24]. The quantitative analysis was performed by the internal normalization method. The relative contents of particular compounds identified in extracts using GC-MS method were evaluated as the percentages of a peak area in a total ion chromatogram (TIC) using the MestReNova 10.0.2 software.

Statistical analysis

The statistical analysis was performed using TIBCO Statistica 13.3 (TIBCO Software Inc.) and Microsoft Excel 2016 (Microsoft). The assays were performed in triplicate and the results were expressed as mean values ± s (standard deviation). To analyze the antioxidant parameters of the extracts, experimental data were subjected to analysis of variance (ANOVA) and the differences among mean values were evaluated by Tukey's HSD post-hoc test at a 5% significance level.

Results and discussion

For all studied extraction techniques, but especially in Soxhlet extraction (SE), the efficiency of the processes

increased with prolongation of the time. The ultrasound-assisted extraction (UAE) was the most effective process in all tested time variations. Conducting ultrasound-assisted extraction for 4 hours allowed to obtain the highest yield among all applied processes (198.0 mg/g). In the case of SE, the 1-hour process was not effective, because the obtained yield was the lowest among all processes (109.9 mg/g). Increasing the time of SE significantly improved the yield and allowed to obtain in a 4-hour process almost the same results (197.4 mg/g) as for a 4-hour UAE. Dependence of the obtained yields of dry extracts [mg/g] on the type and duration of the isolation process is shown in Figure 1.

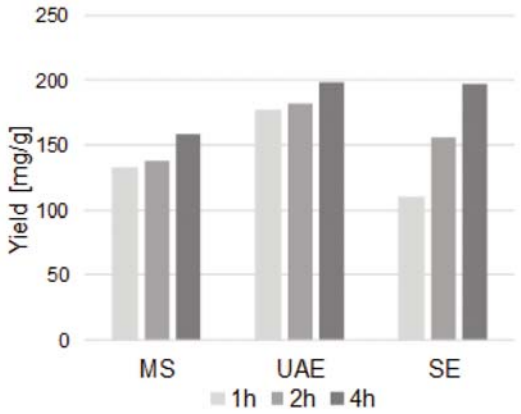

Figure 1. Comparison of yields of dry ethanolic extracts from *M. oleifera* leaf obtained by various extraction techniques: MS – maceration with shaking, UAE – ultrasound-assisted extraction, SE – Soxhlet extraction, with different duration: 1, 2 and 4 hours

The results of antioxidant activity for all obtained extracts, determined by DPPH radical scavenging method and expressed as IC_{50} values, are collected in Table 1, together with the results of total phenolic contents (TPC) analyses. The statistical analysis has shown that in most cases observed differences in antioxidant activity and total phenolic content for the particular techniques of extraction were significant, which was indicated by using different letters.

The antioxidant activity of the extracts expressed by the IC_{50} parameter varied in a range of 388–434 μg/cm^3. The results are in good correlation with the total phenolic contents (TPC), which increase with the lowering of IC_{50} values, as expected. The extract obtained in 1-hour ultrasound-assisted extraction showed the highest activity, represented by the lowest IC_{50} concentration (388.1 μg/cm^3) and the highest TPC value (76.3 mg GAE/g). In the case of ultrasound-assisted and Soxhlet extraction, some decrease of the antioxidant activity and total phenolic content was observed with the prolongation of the extraction time. It may be caused by possible decomposition of some biologically active compounds under the influence of ultrasounds and elevated temperature. Oppositely, time extension in maceration with shaking technique led to increasing of antioxidant activity and contents of phenolics. However, for MS technique the yield, antioxidant activity and TPC values obtained even in the longest time variant (4h) do not reach the results obtained for 1h UAE experiment.

Analyzing the available literature data and comparing them with data obtained in the presented study it can be seen that they show a quite big variation. It may result from the use of plant materials of various origin and different extraction techniques and conditions. Some results obtained by other authors indicate significantly lower antioxidant activity of the extracts. For example, Vats and Gupta[25] received extracts from *M. oleifera* leaf originated in India using 95% ethanol by maceration with shaking for 24 hours, for which the IC_{50} value was 610 μg/cm^3 and total phenolic content TPC = 9.58 mg GAE/g. Extracts obtained at present work using the same technique and solvent and raw material from the same country show significantly higher antioxidant activity and total phenolic content, despite the use of much shorter extraction time (from 1 to 4 hours). In turn, Wright et al.[26] studied *M. oleifera* leaf extract from plant material derived from Jamaica by means of 24-hour extraction in a Soxhlet apparatus using 80% ethanol, obtaining much lower antioxidant activity with a value of IC_{50} = 832.8 μg/cm^3. Higher values were reported by Vongsak et al.[27], who studied the extraction process of *M. oleifera* leaf from Thailand using several different techniques, including simple maceration, Soxhlet extraction, and percolation using 50 and 70% ethanol. The highest DPPH scavenging activity was found for extracts obtained with 70% ethanol during a 20-hour extraction in a Soxhlet apparatus (IC_{50} = 55.07 μg/cm^3) and during 72-hour simple maceration (62.94 μg/cm^3).

Applying of GC-MS method enabled the identification of 11 compounds present in *M. oleifera* leaf extracts. On the GC-MS chromatograms of extracts obtained using different techniques relative contents of particular compounds were similar. In Figure 2 chromatogram of the extract obtained using 96% ethanol during 1-hour ultrasound-assisted extraction is presented, while in Table 2 the retention times, retention indices and relative contents of identified compounds are summarized.

Table 1. Antioxidant activity and total phenolic contents of *M. oleifera* leaf extracts obtained using 96% ethanol by different techniques

Extraction technique	Extraction time [h]	IC_{50} ± s [μg/cm^3]	TPC ± s [mg GAE/g]
MS	1	433.5 ± 0.7^a	66.9 ± 0.2^a
	2	428.8 ± 1.0^b	71.5 ± 0.4^b
	4	413.5 ± 0.7^c	72.9 ± 0.6^{be}
UAE	1	388.1 ± 0.9^d	76.3 ± 0.6^c
	2	399.3 ± 0.8^e	75.1 ± 0.5^{cf}
	4	410.7 ± 0.6^f	69.8 ± 0.4^d
SE	1	400.4 ± 0.8^e	74.0 ± 0.5^{ef}
	2	418.9 ± 1.1^g	73.7 ± 0.3^{ef}
	4	426.0 ± 0.7^h	69.5 ± 0.8^d

MS – maceration with shaking; UAE – ultrasound-assisted extraction; SE – Soxhlet extraction; GAE – gallic acid equivalent; s – standard deviation (n = 3); different letters in the same column indicate significant differences among the results ($P < 0.05$)

Table 2. Retention parameters and relative contents of compounds identified in *M. oleifera* leaf extract obtained in 1-hour ultrasound-assisted extraction using 96% ethanol

No.	Compound	RT [min]	Linear retention index		Relative content ± s [%]
			LRI_{exp}	LRI_{lit}	
1	Phytol	26.13	2114	2116 ± 10	2.69 ± 0.11
2	Pentacosane	32.48	2496	2500	2.45 ± 0.08
3	Heptacosane	35.46	2696	2700	4.59 ± 0.10
4	Nonacosane	38.25	2896	2900	3.61 ± 0.14
5	β-Tocopherol	40.29	3052	3047 ± 4	0.94 ± 0.09
6	α-Tocopherol	41.33	3133	3129 ± 11	51.12 ± 0.97
7	β-Sitosterol	43.54	3316	3311	7.37 ± 0.10
8	Fucosterol	43.76	3335	3330	6.96 ± 0.08
9	β-Amyrin	43.90	3347	3312 ± 9	6.65 ± 0.11
10	Lupeol	44.46	3394	3383	3.42 ± 0.07
11	24-Methylenecycloartanol	45.22	3451	3447	2.17 ± 0.08

RT – retention time; LRI_{exp} – linear retention index determined on the HP-5MSI capillary column; LRI_{lit} – retention index from the literature[23, 24]; Relative content – percentage of the peak area in the total ion chromatogram; s – standard deviation (n = 3)

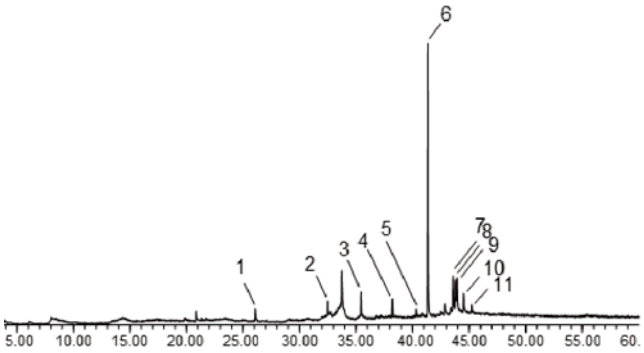

Figure 2. GC-MS chromatogram of *M. oleifera* leaf extract obtained in 1-hour ultrasound-assisted extraction (UAE) using 96% ethanol. Identified compounds: 1) phytol, 2) pentacosane, 3) heptacosane, 4) nonacosane, 5) β-tocopherol, 6) α-tocopherol, 7) β-sitosterol, 8) fucosterol, 9) β-amyrin, 10) lupeol, 11) 24-methylenecycloartanol

The main component, with share accounted for over 50%, was α-tocopherol – an important biologically active substance with strong antioxidant properties, necessary for the proper functioning of the human body. The other vitamin E analogue, β-tocopherol, was also detected but in small amounts (<1%). The analyzed extracts contained significant amounts of sterols (β-sitosterol, fucosterol), pentacyclic triterpenoids (β-amyrine, lupeol, 24-methylenecycloartanol) and phytol. Small amounts of aliphatic hydrocarbons: pentacosane, heptacosane and nonacosane were also found. Both tocopherols, sterols and pentacyclic triterpenoids are widely distributed in plants and are known to possess a number of bioactive properties and pharmacological effects. β-Amyrin and lupeol are bioactive compounds often found in medicinal plants, known for their anti-inflammatory, analgesic, anti-ulcerogenic, and hypoglycemic properties[28, 29]. Lupeol is also known as an effective antioxidant and anticancer agent, anticancer properties were also reported for 24-methylenecycloartanol[29, 30]. The presence of 24-methylenecycloartanol in *M. oleifera* is reported for the first time.

CONCLUSIONS

Comparing the efficiency of 3 various isolation techniques conducted in different time variants led to the conclusion, that extraction conditions significantly affect the yields of the ethanolic extracts from *M. oleifera* leaf. The highest yields of dry extracts were obtained in 4-hour ultrasound-assisted extraction and Soxhlet extraction processes (~198 mg/g), while the least effective was 1-hour Soxhlet extraction (~110 mg/g). The antioxidant activity of the extracts, expressed by the IC_{50} parameter, varied in the range of 388.1–433.5 μg/cm^3, while total phenolic content from 66.9 to 76.3 mg GAE/g. The highest antioxidant activity and the highest content of phenolics were found for the extract obtained by means of 1-hour ultrasound-assisted extraction. Among the compounds identified in extracts by using the GC-MS method, the main component was α-tocopherol with relative content over 50%. Other compounds found in smaller amounts, with antioxidant and other biological properties were: β-sitosterol, fucosterol, β-amyrin, lupeol, 24-methylenecycloartanol, phytol and β-tocopherol.

The research carried out using a uniform methodology, in relation to the same raw material, enabled to indicate the most preferred extraction conditions. Among all studied conditions, as the most favorable process 1-hour ultrasound--assisted extraction can be pointed out. This process was characterized by the highest extraction efficiency and allowed to obtain dry extract with the best antioxidant properties and the maximum total phenolic content in the shortest time. The obtained dry extracts of *M. oleifera* leaf proved to be a source of valuable biologically active compounds which could be potentially used in pharmacy. These extracts can also find application in cosmetics formulations for protection against reactive oxygen species and as an active anti-inflammatory agent.

LITERATURE CITED

1. 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, 12791–12835. DOI: 10.3390/ijms160612791.

2. Oyeyinka, A.T. & Oyeyinka, S.A. (2018). *Moringa oleifera* as a food fortificant: Recent trends and prospects. *J. Saudi Soc. Agric. Sci.* 17, 127–136. DOI: 10.1016/j.jssas.2016.02.002.

3. Karim, N.A., Ibrahim, M.D., Kntayya, S.B., Rukayadi, Y., Hamid, H.A. & Razis, A.F. (2016). *Moringa oleifera* Lam: Targeting chemoprevention. *Asian Pac. J. Cancer Prev.* 17(8), 3675–3686. DOI: 10.14456/apjcp.2016.155/APJCP.2016.17.8.3675.

4. Fahey, J.W. (2005). *Moringa oleifera*: A review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Part I. *Trees for Life Journal* 1:5. DOI: 10.1201/9781420039078.ch12.

5. Gopalakrishnan, L., Doriya, K. & Kumar, D.S. (2016). *Moringa oleifera*: A review on nutritive importance and its medicinal application, *Food Sci. Hum. Wellness* 5, 49–56. DOI: 10.1016/j.fshw.2016.04.001.

6. Sánchez-Machado, D.I., Núñez-Gastélum, J.A. & Reyes-Moreno, C. (2010). Nutritional quality of edible parts of *Moringa oleifera*, *Food Anal. Methods* 3, 175–180. DOI: 10.1007/s12161-009-9106-z.

7. Adesina, O.A., Abdulkareem, F., Yusuff, A.S., Lala, M. & Okewale, A. (2019). Response surface methodology approach to optimization of process parameter for coagulation process of surface water using *Moringa oleifera* seed. *S. Afr. J. Chem. Eng.* 28, 46–51. DOI: 10.1016/j.sajce.2019.02.002.

8. Camacho, F.P., Sousa, V.S., Bergamasco, R. & Teixeira, M.R. (2017). The use of *Moringa oleifera* as a natural coagulant in surface water treatment, *Chem. Eng. J.* 313, 226–237. DOI: 10.1016/j.cej.2016.12.031.

9. Pasha, S., Khaleel, M. & Som, S. (2010). Evaluation of adaptogenic activity of *Moringa oleifera* Lam. *Res. J. Pharmacol. Pharmacod.* 2(3), 243–247.

10. Kholif ,A.E., Gouda, G.A., T.A. Morsy, Salem, A.Z.M., Lopez, S. & Kholi, A.M. (2015). *Moringa oleifera* leaf meal as a protein source in lactating goat's diets: Feed intake, digestibility, ruminal fermentation, milk yield and composition, and its fatty acids profile. *Small Rumin. Res.* 129, 129–137. DOI:10.1016/j.smallrumres.2015.05.007.

11. Gupta, R., Mathur, M., Bajaj,V.K., Katariya, P., Yadav, S., Kamal, R. & Gupta, R.S. (2012). Evaluation of antidiabetic and antioxidant activity of *Moringa oleifera* in experimental diabetes. *J. Diabetes* 4, 164–171. DOI: 10.1111/j.1753-0407.2011.00173.x.

12. Moyo, B., Oyedemi, S., Masika, P.J. & Muchenje, V. (2012). Polyphenolic content and antioxidant properties of *Moringa oleifera* leaf extracts and enzymatic activity of liver from goats supplemented with *Moringa oleifera* leaves/sunflower seed cake. *Meat Sci.* 91, 441–447. DOI: 10.1016/j.meatsci.2012.02.029.

13. Adeyemi O.S. & Elebiyo T.C. (2014). Moringa oleifera supplemented diets prevented nickel-induced nephrotoxicity in Wistar rats, *J. Nutr. Metab.* 2014, Article ID 958621, 1–8. DOI: 10.1155/2014/958621.

14. Al-Asmari, A.K., Albalawi, S.M., Athar, M.T., Khan, A.Q., Al-Shahrani, H. & Islam, M. (2015). *Moringa oleifera* as an anti-cancer agent against breast and colorectal cancer cell lines. *PLoS ONE* 10(8), e0135814. DOI: 10.1371/journal.pone.0135814.

15. Edwinanto, L. Septiadi, E., Nurfazriah, L.R., Anastasya, K.S. & Pranata, N. (2018). Phytochemical features of *Moringa oleifera* leaves as anticancer - a review article. *J. Med. Health*. 2(1), 680–688. DOI: 10.28932/jmh.v2i1.745.

16. Khan, F., Pandeya, P., Jha, N.K., Jafri, A. & Khan, I. (2020). Antiproliferative effect of *Moringa oleifera* methanolic leaf extract by downregulation of Notch signaling in DU145 prostate cancer cells. *Gene Rep.* 19, 100619. DOI: 10.1016/j.genrep.2020.100619.

17. Sutalangka, C., Wattanathorn, J., Muchimapura, S. & Thukham-mee, W. (2013). *Moringa oleifera* mitigates memory impairment and neurodegeneration in animal model of age-related dementia. *Oxid. Med. Cell. Longev.* 2013, Article ID 695936, 1–9. DOI: 10.1155/2013/695936.

18. Ijioma, S.N., Nwaogazi, E.N., Nwankwo, A.A., Oshilonya, H., Ekeleme, C.M. & Oshilonya, L.U. (2018). Histological exhibition of the gastroprotective effect of *Moringa oleifera* leaf extract. *Comp. Clin. Pathol.* 27, 327–332. DOI: 10.1007/s00580-017-2594-0.

19. Wang, L., Chen, X. & Wu, A. (2016). Mini review on antimicrobial activity and bioactive compounds of *Moringa oleifera*. *Med. Chem. (Los Angeles)* 6(9), 578–582. DOI: 10.4172/2161-0444.1000402.

20. Monera, T.G. & Maponga, C.C. (2012). Prevalence and patterns of *Moringa oleifera* use among HIV positive patients in Zimbabwe: A cross-sectional survey. *J. Public Health Afr.* 3(6), 22–24. DOI: 10.4081/jphia.2012.e6.

21. Kedare, S.D. & Singh, R.P. (2011). Genesis and development of DPPH method of antioxidant assay. *J. Food Sci. Technol.* 48(4), 412–422. DOI: 10.1007/s13197-011-0251-1

22. Prior, R.L., Wu, X. & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J. Agric. Food Chem.* 53, 4290–4302. DOI: 10.1021/jf0502698.

23. Isidorov, V.A. & Szczepaniak, L. (2009) Gas chromatographic retention indices of biologically and environmentally important organic compounds on capillary columns with low-polar stationary phases. *J. Chromatogr. A* 1216(51), 8998-9007. DOI: 10.1016/j.chroma.2009.10.079

24. Babushok, V.I., Linstrom, P.J. & Zenkevich, I.G. (2011). Retention indices for frequently reported compounds of plant essential oils. *J. Phys. Chem. Ref. Data* 40, Article ID 043101, 1-47. DOI:10.1063/1.3653552.

25. Vats, S. & Gupta, T. (2017). Evaluation of bioactive compounds and antioxidant potential of hydroethanolic extract of *Moringa oleifera* Lam. from Rajasthan, *India Physiol. Mol. Biol. Plants* 23(1), 239–248. DOI: 10.1007/s12298-016-0407-6.

26. Wright, R.J., Lee, K.S., Hyacinth, H.I., Hibbert, J.M., Reid, M.E., Wheatley A.O. & Asemota, H.N. (2017). An investigation of the antioxidant capacity in extracts from *Moringa oleifera* plants grown in Jamaica. *Plants* 6, 1–8. DOI: 10.3390/plants6040048

27. Vongsak, B., Sithisarn, P., Mangmool, S., Thongpraditchote, S., Wongkrajang, Y. & Gritsanapan, W. (2013). Maximizing total phenolics, total flavonoids contents and antioxidant activity of *Moringa oleifera* leaf extract by the appropriate extraction method. *Ind. Crops. Prod.* 44, 566–571. DOI: 10.1016/j.indcrop.2012.09.021

28. Ivanov, I., Dincheva, I., Badjakov, I., Petkova, N., Denev, P. & Pavlov, A. (2018). GC-MS analysis of unpolar fraction from *Ficus carica* L. (fig) leaves. *Int. Food Res. J.* 25(1), 282–286

29. Neeraj, P., Vasudeva, N. & Sharma, S. (2019). Chemical composition of *Fagopyrum esceulentum* Moench seed through GC-MS. *Int. J. Pharm. Sci. Res.* 10(5), 2392–2396. DOI: 10.13040/IJPSR.0975-8232.10(5).2392-96.

30. Chia-Chen, L., Bao-Hong, L. & Ying-Jang, L. (2015). Antioxidation and antiglycation of *Fagopyrum tataricum* ethanol extract. *J. Food Sci. Technol.* 52(2), 1110–1116. DOI: 10.1007/s13197-013-1098-4.